



AQUATIC ECOLOGY STUDIES



Current Ecological Knowledge of the Critically Endangered Stocky Galaxias *Galaxias tantangara*

Report to EMM Consulting Pty Ltd

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Cover: A large adult Stocky Galaxias (Photo: Hugh Allan)

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Surveys of distribution and abundance

Stocky galaxias *Galaxias tantangara* was first collected in 2003, and subsequently identified as a separate taxon by Raadik (2014). The known distribution was only a 60 m section of Tintangara Creek, where it was the only fish species present (Raadik 2014). Surveys undertaken as part of a postgraduate study have confirmed its presence 3 km upstream of the site originally surveyed by Raadik (2014). Greater than 3 km upstream stream size diminishes, and sampling efficiency reduces due to dense vegetation, reduced light penetration and difficulties in locating water of sufficient depth. It is unlikely that many, if any fish would persist here year-round. The downstream distribution of *G. tantangara* is limited by trout presence immediately downstream of a large natural waterfall barrier described by Raadik (2014)(Figure 1). The adult population size of the species is unknown but by using the mean densities of fish captured during backpack electrofishing surveys from 2016-2018, the adult population is tentatively estimated at approximately 2000 individuals (but the quantitative detection capacity of backpack electrofishing is unknown).

Several other tributaries in the Tintangara Creek catchment have been sampled for galaxiids in 2016 and 2017, although no galaxiids were caught and trout were present at all sites. All these sites were similar in size and stream morphology to Tintangara Creek, and galaxiids would be expected to inhabit these areas were it not for trout.



Figure 1. The main waterfall barrier on Tintangara Creek

Reproductive ecology

Reproductive development of *G. tantangara* was monitored monthly (excluding winter months due to difficulties associated with site access) between November 2016 and May 2018 by visual inspection of fish during electrofishing surveys, and from sacrificial samples analysed at the university laboratory. All fish collected were measured and weighed alive in the field. A range of fish sizes and sexes were sacrificed to better estimate the onset of sexual maturity, fecundity, egg size and seasonal gonad development. Gonad development was determined according to the size and appearance of gonads when viewed through the translucent underbelly of each fish, and gonad weight relative to body weight (sacrificed fish only). As gonads of mature fish could be clearly seen when in the field and sexual products could be manually extruded with light pressure of the abdomen (ripe fish ready to spawn) the sex of mature fish could be determined visually in the field.

Field observations

Ripe females were detected between November and March, and spent females only occurred in November and December. Ripe males however, were observed in almost all months of the year. Males were typically more abundant in surveys and were generally smaller than females. It is likely that males appeared more populous because they maintained gonads at a further stage of development throughout the year, and as a result were more easily identified than females. Mature and ripe gonads were observed in smaller males than in females.

GSI development

Gonad development peaked once per year for fish of both sexes – in October for females and in April/May for males. At peak maturation females had relatively larger gonads than males. Females quickly lost gonad condition between October and November, while males maintained higher gonad condition for longer and declined over several months.

Egg searches

Spent females were first observed in Nov 2017, and the stream was searched for eggs on four subsequent occasions in November and December. Egg searches involved lifting and inspecting rocks in the stream. Over 2000 rocks were inspected in the four visits in November and December. A single spawning site was found eight days after spent fish were first observed in monthly surveys. Eggs were adhered to the underside of a cobble. An estimated 50 eggs were adhered to the stone, in multiple clusters of 2-25 eggs per cluster (Figure 2). The spawning site was marked and revisited on two more occasions in December 2017. On the first revisit fish in the 'eyed' stage could be seen moving inside the eggs, and by the next visit the eggs no longer remained.



Figure 2. Egg mass of *G. tantangara* found in November 2017

Fecundity and egg size

The number of eggs in the spawning site was substantially less than the observed mean fecundity of mature females which were sacrificed. Fecundity varied with fish size, with larger fish generally being more fecund than smaller fish. Fecundity ranged from 200 to 800 eggs per fish.

Oocyte (unfertilised egg) diameter varied throughout the year with oocytes increasing in size upon approach of the spawning season but did not appear to be related to fish size. The largest oocytes examined were from fish collected in November 2017, when the spawning site was also located.

Appearance of larvae & growth of cohort

The earliest larvae were observed in field electrofishing surveys in Dec 2017. Schools of up to 20 larvae were observed free-swimming in slow sections of the stream. Collected larvae were approximately 10 mm in length, transparent, and did not have a yolk sac. Fish of this cohort grew substantially by July of the following year, although relative abundance of fish of this size declined over winter indicating reasonably mortality of juveniles.

Cohorts of fish the same size and age become difficult to distinguish after what is assumed to be their first year, although based on findings from O'Connor and Koehn (1991) and Shirley and Raadik (1997) for other species in the Mountain galaxias (*G. olidus*) complex, it is likely that fish live to 3 or more years of age. Longevity of closely-related *Galaxias* species has been recorded at 4-7 years (*G. olidus*, Cowden 1988) and up to 15 years (*G. fuscus*, Raadik et al. 2010).

Summary of reproductive ecology

Based on a combination of observations of reproductive stages of fish in surveys, GSI development, discovery of a spawning site and occurrence of larvae in surveys, it is likely that spawning of *G. tantangara* occurs in November. Sampling in 2016 also recorded ripe females in early November, and an absence of ripe females by late November/early December, again suggesting that spawning occurred in November 2016. This same pattern was repeated in the summer of 2017/18.

As males were observed with developed gonads all year round, it is likely that female maturation determines when spawning occurs (Hardie et al. 2007). Like other galaxiids, it seems that *G. tantangara* spawns only once per year over a relatively short period (Stoessel et al. 2015), during a time of increasing photoperiod and mean daily water temperatures (Humphries 1989, Shirley and Raadik 1997, Moore et al. 1999, Stoessel et al. 2015). Typical of galaxiids adapted to a wholly-freshwater life history, *G. tantangara* produces relatively few, but large eggs (McDowall 1970). Exact egg development time is unknown although it is likely that eggs incubate for a relatively long period given the cold mean daily water temperatures in late November.

By adopting the reproductive strategy of a small number of large eggs, size of larvae is generally maximised, promoting greater individual survival (Ware 1975, Closs et al. 2013). This is especially beneficial for fish subject to harsh conditions such as those in Tantangara Creek during summer and winter, and in environments where larval dispersal is restricted (McDowall 1970). The use of large rocky substrates is known for other galaxiids in the Mountain Galaxias complex (Cowden 1988, O'Connor and Koehn 1991, Stoessel et al. 2015). Adhering eggs to large heavy substrates reduces the chance of disturbance during the incubation period (Stoessel et al. 2015), and likely increases egg survival. The location of a spawning site in a fast-flowing riffle may be a mechanism to minimise likelihood of sedimentation and ensure eggs are not smothered. The number of eggs in the single spawning mass located did not correspond with mean fish fecundity and was substantially smaller than the minimum fecundity recorded, suggesting fish may possibly spawn at multiple sites (Stoessel et al. 2015). Given that no female fish were found partially spent, and spawning season appears to be relatively short, it is likely that if *G. tantangara* does spawn at multiple sites, spawning is completed quickly over several days, like suggested for *G. fuscus* (Stoessel et al. 2015). Like other species in the Mountain Galaxias complex, it is unlikely that *G. tantangara* exhibits any parental care of eggs (O'Connor and Koehn 1991, Stoessel et al. 2015). Based on the growth of larvae and age classes of closely-related species (O'Connor and Koehn 1991, Shirley and Raadik 1997), it is unlikely that fish would spawn in their first year. Male fish likely spawn in their second year, while female fish probably don't spawn until their third year.

Monitoring using PIT telemetry

Movement

Investigating movement of individual small-bodied fish has been historically problematic as usually the identification of individuals has been limited to batch marking using either fin-clipping or other marks such as visual implant elastomers (Skalski et al. 2009). The deployment of telemetry options for small individuals (e.g. radiotelemetry or acoustic telemetry) has been limited by tag size and battery life (Clark 2016, Allan et al. 2018). In recent years the ability to mark small-bodied individuals with Passive Integrated Transponder (PIT) tags which have no internal battery, has allowed the movement ecology of small fish to be investigated.

Following a successful trial of the use of 9 mm PIT tags in the closely related *Galaxias olidus* (Allan et al. 2018), a sample of wild *G. tantangara* were PIT tagged in January 2018 in a 200 m focal reach of Tintangara Creek, using the same method as in the trial study. Fish were tagged and released in their original section of capture on the same day. Tagged fish were monitored using portable PIT telemetry involving a long-range antenna and tag reader, manually operated by a researcher walking along the stream in daylight hours (Figure 3). Two passes of the study reach were conducted in each sampling occasion, with at least one hour between passes. Based on observations when sampling, read-range of the antenna is estimated at up to 20 cm. Data was collected at 2-4-week intervals, on seven separate occasions between January and May 2018. In total 80% of fish were redetected at least once, and 34% of fish were located four times or more. 14% of fish were never detected.

Preliminary results from the redetection data indicate that like *G. olidus* (Berra 1973), most *G. tantangara* exhibit a small home range of about 30 lineal metres of stream or less. In 50% of observations fish had moved <5 m since their last detection, and in 75% of observations movement was 15 m or less. Movements of >170 m were recorded indicating that although infrequent, fish may undertake large-scale movements. No fish were collected upstream of the focal reach, and only a single fish was detected downstream of the focal reach.

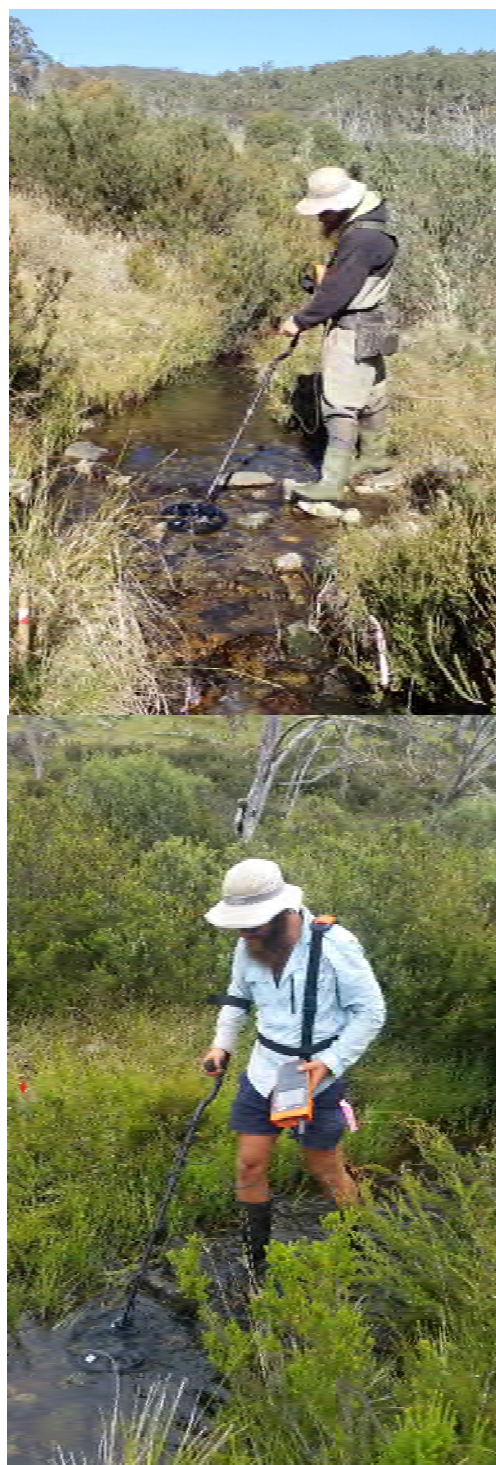


Figure 3. Portable PIT monitoring in Tintangara Creek

Habitat use

Data on habitat both used by fish and habitat availability in the study area has been collected on each sampling occasion during the movement study. This data is currently being processed and analysed.

Diet analysis

Stomachs of sacrificed individuals have been preserved at the University of Canberra. Time constraints has meant their analysis is not currently planned as part of the Master's degree, but analysis could be completed if further funding becomes available.

The main threats to *Galaxias tantangara*

The major threat to the continued persistence of *G. tantangara* remains invasion past the waterfall (Figure 1) by trout. Trout of sufficient size to predate on galaxiids have been collected immediately below the waterfall, and the complete elimination within 3-18 months of other threatened *Galaxias* populations following the introduction of even small numbers of trout has been documented (Raadik et al. 2010). The most likely scenarios that allow trout invasion past the waterfall are deliberate illegal introduction by anglers or drown-out of the barrier during extremely high flows.

Critical Knowledge Gaps

Assessment of potential translocation streams

A preliminary search for potential translocation sites has been conducted in the sub-catchment surrounding the known distribution of the species, but a comprehensive search in the entire Tantangara Creek catchment is needed. The preliminary search assessed sites for their suitability based on the presence of trout or galaxiids, stream morphology and the presence of either a natural or augmentable barrier to trout invasion. From the preliminary survey, translocation options were limited, with a single potential site located in a nearby tributary of Tantangara Creek. A small rocky cascade provides vertical relief of about 1.5 metres over about 5 horizontal metres. Trout (in very low numbers) were caught upstream of this cascade, and it was considered that only during high flows would trout be able to breach this structure from downstream. Upstream of the cascade, the creek is very similar in morphology to Tantangara Creek, and the catchment is of similar size. In the absence of trout, it is expected that this stream would be suitable for galaxiids.

The proximity of this nearby stream to the catchment where *G. tantangara* is present ensures likely climatic conditions to the existing population occur. However, the close proximity of the stream to the existing population means that identified threats such as bushfire may impact both sites. The identification of additional translocation sites is desirable.

Establishment of additional wild populations of *G. tantangara*

To spread the risk of stochastic extinction of the species in its very small, single stream distribution, the establishment of additional populations is required. There is only one currently known viable translocation site, and three priority actions are required at this site:

1. Augmentation of the existing partial barrier with additional structures (e.g. large boulders, logs and/or an engineered structure such as a vertical metal grill) to provide a complete barrier to trout movement upstream, even during periods of high flow.
2. The subsequent removal of trout from the stream by electrofishing and/or rotenone treatment would render it fishless and allow the experimental introduction of *G. tantangara*.
3. If a population establishes, then fencing to exclude feral horses from the creek would be required.

As all reintroduced populations are likely to be small in spatial extent (as a result of the widespread distribution of trout), ideally, number of additional wild populations are required to ensure long-term survival of the species in the event of local stochastic extirpations of reintroduced populations.

Research into captive husbandry of the species

It is desirable to establish a captive-bred insurance population of *G. tantangara* to enable reintroductions in the event of extinction in the wild. To achieve this aim, research is needed into captive-breeding and husbandry, as has recently been done with *Galaxias fuscus* (Raadik et al. 2010).

Impacts of feral horses

Within the 3 km section of stream currently occupied by *G. tantangara*, there is a high abundance of feral horses. A pilot field survey of horse crossing frequency and impacts was conducted in November 2018, but detailed investigation is required to quantify impacts, and monitor potential change in impacts across years (Allan and Lintermans 2018). Feral horses are responsible for extensive destruction of riparian vegetation and erosion of stream banks on Tantangara Creek and elsewhere in the high country (Evans 2018, Paull 2018, Driscoll et al. 2019, Robertson et al. 2019). The November 2018 pilot field survey identified a large number of feral horse crossings of Tantangara Creek within the known range of *G. tantangara*. The damage at each crossing was variable, but severe in many instances. This damage is clearly seen in places, where the destruction of stable banks has led to widening of the stream from 1 m to up to 5 m wide (Figure 4). Depth of the stream has decreased as the stream has widened, to less than 5 cm in places and flow velocity appears considerably less than in undamaged parts of the stream.

Stream width within a horse crossing was on average more than double the stream width immediately up and downstream, with the widest crossing more than five times wider than the adjacent stream. Water depth within crossings was substantially shallower than the stream immediately upstream or downstream. It is likely that crossings act as a source of fine sediment which washes downstream and is responsible for the observed decreased stream depth immediately downstream. The amount of silt present in non-crossing areas is likely a result of particle mobilisation from damaged stream banks, i.e. horse crossings. Bare ground on stream banks is infrequent, if not rare, in areas outside of crossings, with small-medium shrubs covering the majority of the stream bank.

Reduced stream velocity facilitates sedimentation of substrate interstices, in both the immediate area of the damage, but also downstream (Figure 4). Large substrates and clean interstices between particles appear important for spawning of *G. tantangara*. The spawning site located during this

study was in a fast-flowing riffle, and eggs were adhered to the underside of a cobble raised slightly off the stream bed. Sedimentation of these substrate complexes reduces the amount of available spawning habitat and may smother and kill eggs of *G. tantangara* if it occurs with eggs are incubating. The potentially long incubation time of eggs of *G. tantangara* means eggs are vulnerable to the effects of sedimentation for a longer period. Unlike other Australian fishes which exhibit parental care for eggs and nest sites (Gowns 2004) which often includes fanning to remove sediment or improve oxygenation (Lintermans 1998), it is considered that like other galaxiids, *G. tantangara* does not have parental care, leaving eggs particularly susceptible to sedimentation and smothering during incubation. Direct disturbance such as trampling by feral horses also has the potential to impact egg and larvae survival.



Figure 4. A section of Tintangara Creek where bankside vegetation and structure have been destroyed (right). Note the stream width and vegetation cover of the unaffected section in the top left corner of the image. And right, fine sediments suspended by wading downstream of this section. Note the otherwise large cobbles and boulders which dominating the substrate. Photo: Mark Lintermans.

Reduction of the impacts of feral horses in the immediate catchment should be investigated as a priority for the protection of *G. tantangara*. The very restricted geographic distribution of *G. tantangara* also means that quite localised threats from wildfire followed by heavy rainfall could deliver considerable quantities of sediment to the stream, impacting reproduction. Identification of potential translocation sites to establish new populations (in addition to nearby tributary) within the broader Tintangara Creek/upper Murrumbidgee catchment is therefore of high importance.

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research for a sustainable future



Fish transfer risk associated with Snowy 2.0 pumped hydro scheme Report No.107

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Executive summary

Snowy 2.0 is a pumped hydro project with the potential to provide storage for large scale, reliable renewable energy to Australia. During the pumping phase, Snowy 2.0 will draw water from Talbingo Reservoir, on the Tumut River, and discharge it into Tantangara Reservoir, on the Murrumbidgee River. During the generation phase, the direction of water flow will reverse. An unintended consequence of this operating scheme is that aquatic fauna could be entrained and transferred between these storages. Indeed, Redfin perch (*Perca fluviatilis* Linnaeus), a NSW-listed Class 1 noxious species, are present in Talbingo Reservoir, but not in Tantangara Reservoir.

Rainbow trout (*Oncorhynchus mykiss* Walbaum) and Brown trout (*Salmo trutta* Linnaeus) were introduced to the Snowy Mountains region of New South Wales in the late 1800s to establish recreational fisheries. The recreational fishery in this region expanded following the construction of 16 impoundments during the 1950s, 60s and 70s associated with the Snowy Mountains Hydroelectric Scheme. The impoundment fisheries have been hugely successful, and over a decade ago were worth an estimated AU\$70 million to the local economy. Tantangara Reservoir itself supports a strong trout fishery, is connected to the upper Snowy and is indirectly connected to several threatened species populations. The introduction of Redfin perch into Tantangara Reservoir could have unintended adverse consequences for these species.

All hydropower projects have fish-related impacts and it is good practice to ensure these are considered during the design phase. Most fish-related issues can be mitigated using engineering solutions. A review of existing data confirmed the presence of Redfin perch in Talbingo Reservoir, and their absence from the Murrumbidgee River upstream of Tantangara Reservoir and the Snowy River upstream of Jindabyne Dam. A likelihood assessment then determined that it is likely that live Redfin perch would be transferred from Talbingo Reservoir to Tantangara Reservoir, if the Snowy 2.0 project was to proceed without mitigative controls. Furthermore, a tunnel connecting Tantangara Reservoir to Lake Eucumbene introduces a credible risk that colonisation of the Upper Snowy River by Redfin would also occur if controls are not put in place at this point. Redfin perch can also carry the Epizootic Haematopoietic Necrosis Virus (EHNV), which can be lethal to rainbow trout and selected native fish species and survives for extended periods outside its host. As its presence in Talbingo has not been confirmed, the likelihood of transfer was assessed as possible.

The consequences of the introduction of Redfin perch into Tantangara Reservoir would be further expansion of a Class 1 noxious species within the footprint of the Snowy Hydro Scheme. Although it may establish a new recreational fishery within Tantangara Reservoir (and possibly other connected reservoirs), existing trout populations would be significantly reduced by increased predation and competition. Existing populations of threatened species such as the Stocky galaxias (*Galaxias tantangara* Raadik) should remain unaffected if Redfin colonise Tantangara; however, a population of Macquarie perch (*Macquaria australasica* Cuvier) in the Murrumbidgee River downstream of Tantangara could be impacted by both increased predation and competition, as well as infection by EHNV if Redfin are able to colonise the section of river below the dam following introduction to the reservoir.

If the Snowy 2.0 project is to proceed, options for avoiding Redfin perch (and EHNV) incursion into Tantangara Reservoir include eradication from Talbingo Dam, the design and installation of a combination of UV treatment systems, to destroy entrained fish and virus particles, and fine-mesh screens to physically exclude fish.

The introduction of Redfin perch into Tantangara Reservoir would have a range of undesirable outcomes. The development of engineering solutions to eliminate this risk will be a critical component of the feasibility study.

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Background

Snowy 2.0 Pumped Hydro Scheme

Snowy 2.0 is a proposed pumped hydropower electric storage project with the potential to provide storage for large scale, reliable renewable energy to Australia.

All hydropower projects have fish-related impacts and it is good practice to ensure that these are considered during the design phase. To this end, Snowy Hydro have engaged Charles Sturt University and the NSW Department of Primary Industries to provide advice on fisheries related matters, when required, during the feasibility and design process.

The purpose of this report was to investigate the likelihood that a viable population of Redfin perch (*Perca fluviatilis* Linnaeus), a NSW-listed Class 1 noxious species, could establish in Tantangara Reservoir (and possibly beyond) following transfer through the proposed Snowy 2.0 Pumped Hydropower Electric Storage Facility from Talbingo Reservoir.

A desktop study was undertaken to:

1. assess the likelihood of entrainment into the pipe and survival of Redfin perch once inside the pipeline (all life stages)
2. assess the suitability of Tantangara Reservoir and upstream tributaries as habitat for Redfin perch i.e. to assess habitat suitability for breeding, temperature tolerances etc.
3. assess the likelihood of Redfin perch establishing in Tantangara Reservoir via other pathways i.e. to determine the likelihood of these fish establishing in the reservoir in the future without the construction of this pipeline
4. provide a list of existing deterrents and controls that could be used to exclude Redfin perch, and other fish, from the new scheme.

Fisheries-related issues regarding hydropower and pumping

Conventional knowledge about fish passing through a hydro plant turbine demonstrates that fish experience hydraulic conditions that may impact their welfare. The three main processes studied so far relate to when fish move from upstream to downstream. Specifically:

1. During an initial approach, there is an increase in pressure as the fish approaches the deepest point in the weir pool or dam.
2. The fish then either enters a draft tube or moves under a spillway gate, where it experiences a rapid decompression and gains velocity. The velocity often exceeds the critical swimming ability, rendering the fish susceptible to physical strike.
3. The fish then enters the river downstream and can be subjected to shear stress, where discharged water collides with the tailwater.

The reverse is true for fish travelling from downstream to upstream.

1. During the pumping phase in pumped hydropower schemes, water is pumped under high pressure back from a shallow and narrow part of the downstream reservoir to the upstream reservoir.
2. Therefore any fish entrained within water being pumped may be subjected to high water velocities and shear stresses, as well as pressure changes associated with the difference in head between the downstream and upstream reservoirs (Foye & Scott 1965).

The barotrauma-, physical strike- and shear stress-causing processes associated with hydropower generation and pumping could lead to fish injury or mortality, although the operating head, regulator or hydro plant design, site hydrology, and individual species tolerances influence the severity of the impact. The relative importance of these mechanical stressors (physical strike, shear stress, and decompression) varies among fish species. The effectiveness of various mitigation measures are also likely to vary based on the project design and the fish species of interest. Many existing mitigation measures have been

developed largely for salmon smolt, but these may not be readily transferrable to other freshwater species, which have different physiological tolerances.

The design of the Snowy 2.0 system incorporates unprecedented pressure profiles uncommon in Australian freshwater systems, because there are no other systems 700 m deep. It will also move water bi-directionally. If entrained during pumping, fish may be compressed to pressures in excess of 6,800 kPa, and subjected to a range of unknown shear stresses. If entrained during generation, fish will be compressed, and then rapidly decompressed as they move through the turbine. These hydraulic characteristics could be potentially lethal and fish may die during passage. However, the extent of that risk is presently unknown due to a lack of hard data. Further detailed modelling of the pressure profile and travel times of water through the pipeline would determine fish exposure to a range of hydraulic parameters and improve the understanding of potential impacts.

Existing hydropower systems in Australia rarely have operating head's exceeding 100 m. In addition, these systems do not pump water upstream. Instead they rely on water in the reservoir being replenished through natural rainfall. Pumped hydro replenishes the reservoir via mechanical means, drawing water previously used to generate power, back upstream. Australia currently has 1,490 MW of pumped hydroelectricity storage in operation (Geoscience Australia, 2010). The largest facilities exist at Tumut-3 (integrated with conventional hydropower; 158 m head), Shoalhaven (Bendeela station; 120 m head) and Wivenhoe (78 m operating head) (Hearps *et al.* 2014). However, all of these systems have functional operating heads far less than expected at Snowy 2.0. The largest pumped hydro systems globally are the Ingula hydroelectric scheme (South Africa; 480 m operating head) and the Bath County Pumped Hydro Scheme (380 m elevation, USA).

Importance of the Snowy Region trout fishery

Rainbow trout (*Oncorhynchus mykiss* Walbaum) and Brown trout (*Salmo trutta* Linnaeus) were introduced to the Snowy Mountains region of New South Wales in the late 1800s to establish recreational fisheries (Tilzey 1976). The recreational fishery in this region expanded following the construction of 16 impoundments during the 1950s, 60s and 70s associated with the Snowy Mountains Hydroelectric Scheme (Faragher *et al.* 2007; Snowy Hydro Limited 2016). The impoundment fisheries have been hugely successful, and over a decade ago were worth an estimated AU\$70 million to the local economy (Dominion Consulting 2001). Lake Eucumbene is the largest impoundment in the Snowy Mountains and contains a popular recreational fishery for Rainbow and Brown trout (Faragher *et al.* 2007; Tilzey 2000a; Tilzey 2000b). The fishery is regulated by harvest restrictions and the waterway is stocked annually with 150,000 fingerling Rainbow trout, whereas the Brown trout population is considered to be self-supporting, and thus Brown trout are not stocked (Faragher *et al.* 2007).

There are concerns that the operation of the proposed Snowy 2.0 development may inadvertently introduce Redfin perch (*Perca fluviatilis* Linnaeus) into Tantangara Reservoir. Redfin perch are known to prey upon juvenile and adult trout. Therefore, any introduction would be expected to adversely impact the valuable Snowy region fisheries, thus creating economic and social concerns. Tantangara Reservoir is also directly connected to Lake Eucumbene via a gravity fed tunnel (Figure 1). From Lake Eucumbene, water can be transferred via the tunnel to the Eucumbene River downstream of the dam, Geehi Reservoir on the Geehi River, a tributary of the Murray River, and Tumut Pond Reservoir on the Tumut River. Consequently, if Redfin perch are introduced into Tantangara Reservoir, it is possible that further expansion of their distribution will occur via the existing tunnel system.

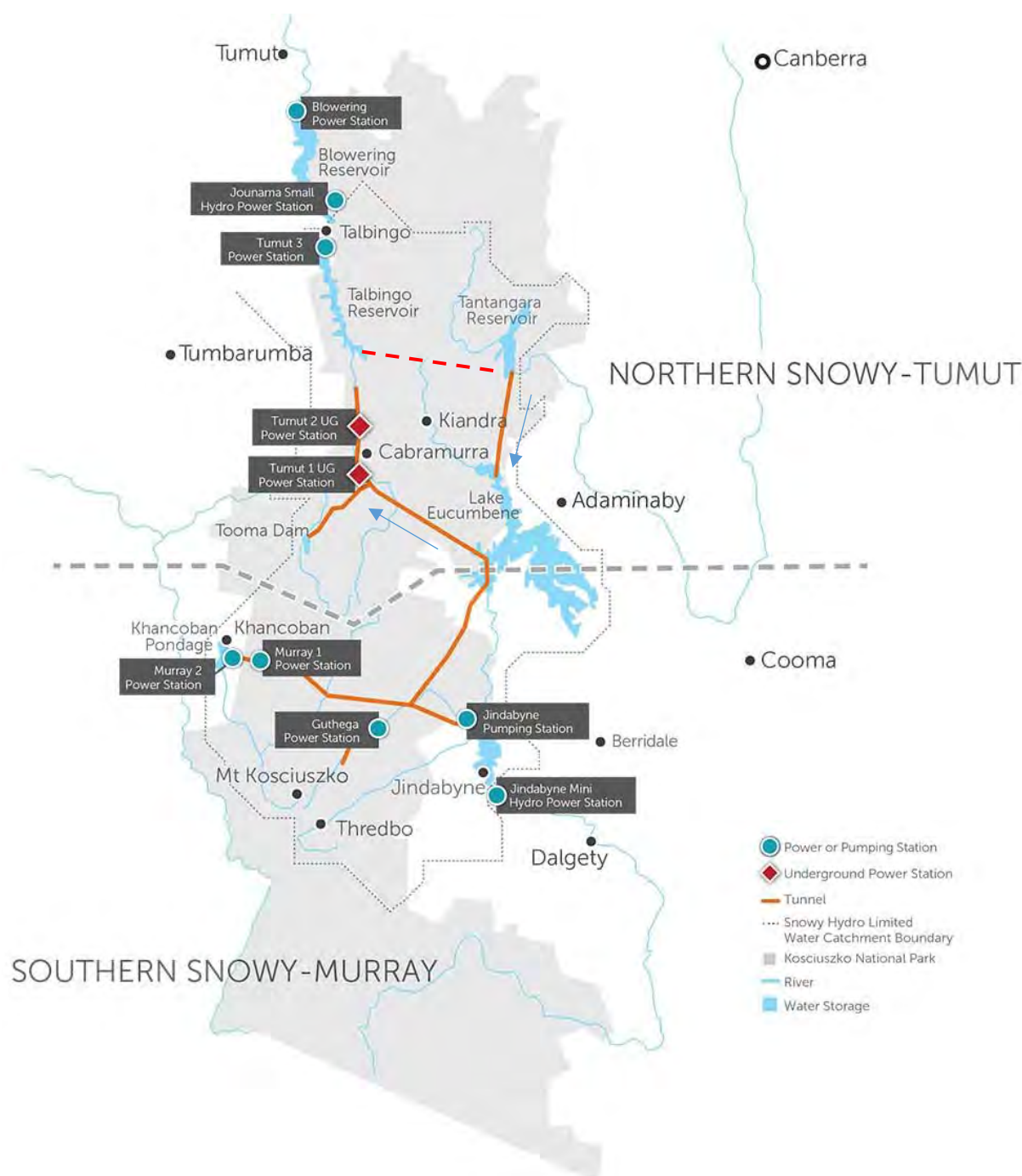


Figure 1. Overview of the Snowy Hydro Scheme showing existing tunnels (orange). Dotted red line represents the proposed Snowy Hydro 2.0 Scheme. Blue arrows denote flow down gravity fed tunnels. If Redfin perch colonise Tantangara Reservoir, then in the absence of further controls, water flowing via the tunnel to Lake Eucumbene could also contain entrained fish.

Issues related to Redfin perch and Snowy 2.0

Preventing the establishment and spread of invasive species is a key objective of many management programs. This is because once such species are established, it is extremely difficult to eradicate them or reduce their numbers (Braysher 2017). Strategies to prevent invasion by undesirable species are therefore crucially important, particularly in areas where they may have considerable environmental, economic or social impacts. In most

cases, the best way to minimise the spread of an invasive species is to exclude it completely from the waterbody (Vander Zanden & Olden 2008).

Presently, there are concerns that unless mitigation measures are put in place, Redfin perch, an invasive species present in Talbingo Reservoir, may be transferred to Tantangara Reservoir where they are currently absent, via the proposed Snowy 2.0 pumped-storage hydroelectricity station (Figure 2). Redfin perch are classed as noxious in NSW and translocations are illegal (Anon 2015). Excluding Redfin perch from Tantangara Reservoir and other locations within the Scheme where they are currently absent would be beneficial because:

1. the Snowy Lakes trout fishery is worth an estimated \$70M per annum to the local economy (Dominion Consulting 2001). The introduction of Redfin perch will threaten the long term sustainability of this important resource.
2. there are populations of the threatened Macquarie perch (*Macquaria australasica* Cuvier) present in the upper Murrumbidgee River below Tantangara Reservoir (Lintermans 2007). If introduced into Tantangara Reservoir, in the absence of further controls, Redfin perch may be able to colonise the Murrumbidgee River below the dam as well owing to their invasiveness (Knight 2010). Protecting this species from Redfin perch is a major challenge for fisheries management.
3. if introduced, Redfin perch will be very difficult eradicate (Knight 2010).

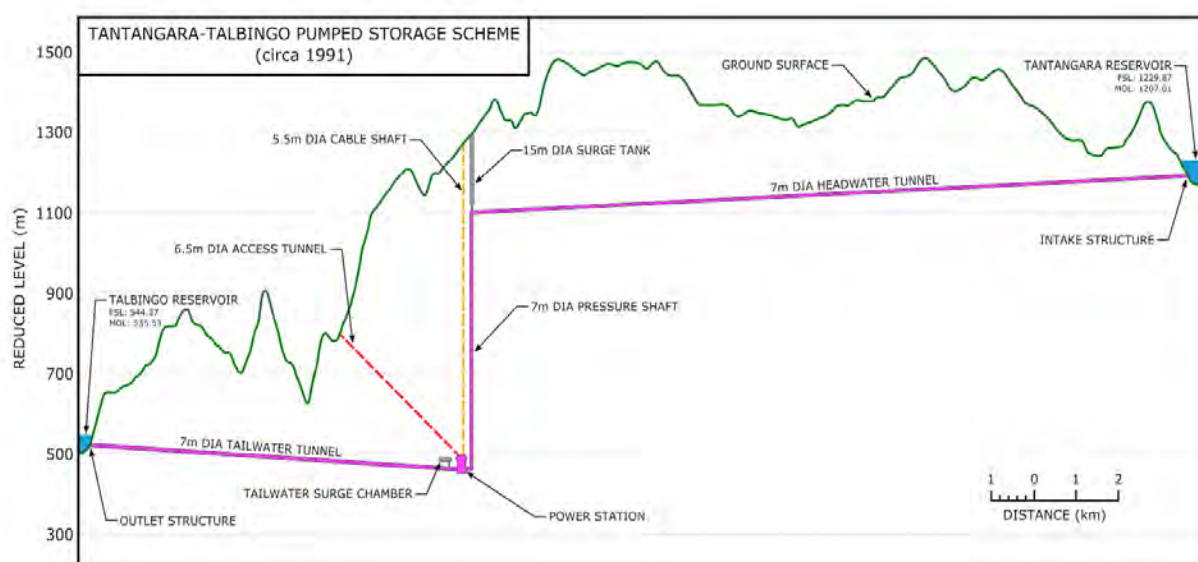


Figure 2. Conceptual layout of the Snowy Hydro 2.0 Scheme detailing the proposed connection between Talbingo Reservoir and Tantangara Reservoir.

Chapter 1: Assessing the risk of Redfin perch establishment in Tantangara Reservoir

Ecology of Redfin perch

Redfin perch are a moderate-sized, freshwater fish native to Europe and parts of Asia (McDowall 1996). Redfin were introduced into Australia in the 1860s, and their numbers rapidly increased not long after (Lintermans 2004). The species' rapid initial population increase was evidenced by recreational fishermen, who reported large catches (up to 200 fish per hour) during that period (NSW Fisheries, unpublished data). Whilst welcomed by many fishers, Redfin perch have helped to facilitate the decline of many native fish species owing to a number of factors (Arthington & McKenzie 1997; McDowall 1996). Firstly, Redfin perch are carnivorous, and regularly consume native fish and invertebrates. Secondly, they often destroy recreational fisheries in enclosed waters by building up large numbers of stunted fish and eliminating other species (Knight 2010). Thirdly, being predatory, they also compete with native species, and may influence the behaviour and resource utilisation of native prey (Closs *et al.* 2006; Shirley 2002). Finally, Redfin perch are known to carry the Epizootic Haematopoietic Necrosis Virus (EHNV), which can adversely affect many native species (Lintermans 2007) (Figure 3). EHNV is a somewhat resistant virus that can retain its infectivity after long periods of desiccation (113 days (Langdon 1989)), and may be transferred mechanically (by fomites) (Langdon & Humphrey 1987). It has also been suggested that regurgitation of ingested material by piscivorous fish, transfer on nets and boats may also potentially contribute to the transmission of EHNV (Peeler *et al.* 2009; Whittington *et al.* 1996). Once the virus is in a waterbody, it is thought to be impossible to remove (Lintermans *et al.* 2014). The presence of EHNV is yet to be confirmed in Talbingo Reservoir but could be determined through a series of simple water quality tests.

Habitat and breeding

Redfin perch occur in a wide range of habitats, including estuarine lagoons, lakes of all types, and various streams and rivers (Freyhof & Kottelat 2008). They generally inhabit slow-flowing or still water habitats, particularly those with abundant plant life (McDowall 1996). By contrast, they tend to avoid cold, fast-flowing waters, or extremely warm waters and/or waters with elevated salinity levels (Lintermans 2007; McDowall 1996). Furthermore, the habitat usage patterns of Redfin perch vary throughout their stages of ontogenetic development (Persson 1988; Eklöv 1997). For instance, larvae live in open water areas, where they feed on pelagic organisms (especially zooplankton), and are partly dispersed by currents (Coles 1981; Freyhof & Kottelat 2008). As they increase in body size, they move from the pelagic zone to the benthic zone — mainly in the littoral habitats, where they start feeding on larger invertebrates and fish (Coles 1981; Eklöv 1997; Persson 1988). Eventually they inhabit both the littoral and pelagic zones, once they become large enough to feed mainly on fish (Eklöv 1997; Persson 1988). According to Freyhof and Kottelat (2008), the switch to piscivory (i.e. feeding on fish) typically occurs when individuals reach about 120 mm standard length (SL). Nevertheless, populations with different life-histories may co-inhabit some river or lake habitats (e.g. pelagic zooplankton feeding, benthic feeding, littoral feeding), occasionally with varying spawning times and sites (Freyhof & Kottelat 2008). Spawning site depths, in particular, have been shown to range from 0.2 m (Smith *et al.* 2001) to between 4 and 12 m (Gillet & Dubois 1995).

Redfin perch reach sexual maturity during the period from autumn through winter, and spawn annually in spring (Guma'A 1978; Hokanson 1977). Females typically spawn with several males, once each year (Freyhof & Kottelat 2008; Treasure 1981). The female initiates the spawning act by circling a spawning substrate, such as the stem of a submerged plant or a fallen tree branch (Treasure 1981). A single male usually follows the female, while several other males look on but remain stationary (Freyhof & Kottelat 2008; Treasure 1981). The female eventually releases a single egg strand while swimming in spiral clockwise

movements (Freyhof & Kottelat 2008; Treasure 1981). All eggs in the single egg strand are quickly fertilised, and the egg strand becomes entangled around the spawning substrate (Treasure 1981). Egg strands deposited on the lake/river bottom typically suffer higher mortalities than those attached submerged vegetation, potentially due to their increased exposure to currents, smothering and/or microorganisms (Smith *et al.* 2001). The eggs are typically 2–3 mm in diameter, and develop and hatch in 1–2 weeks, with the juveniles forming large schools to avoid predation (Lintermans 2007). Males typically reproduce for the first time at 1–2 years of age, while females are usually between 2 and 4 years old (Freyhof & Kottelat 2008).

Current distribution of redfin and other key native fish in the Snowy Scheme

Redfin perch were initially introduced to the Canberra region in 1959 for the purposes of legal stocking by a fisheries agency, but are now present in the majority of the major waterways in the upper Murrumbidgee catchment (Lintermans 2004). Outputs from the New South Wales (DPI) freshwater fisheries database confirm that Talbingo Reservoir contains a large resident Redfin perch population (Figure 5). Talbingo Reservoir was completed in 1971; and at the time Redfin perch were already distributed throughout the Murrumbidgee and Tumut Rivers. It is likely that construction of the impoundment trapped existing populations in the upstream reservoir. There are no formal records of Redfin perch being deliberately introduced into Talbingo Reservoir at this time. Fish surveys undertaken by NSW DPI at four sites in Talbingo Reservoir from 2004–16 have also recorded the presence of Goldfish (*Carassius auratus* Linnaeus), Rainbow trout (*Oncorhynchus mykiss* Suckley) and native Two-spined blackfish (*Gadopsis bispinosus* Sanger) (NSW DPI, unpublished data).

There are no known Redfin perch in and above Tantangara Reservoir or downstream of the dam in the upper Murrumbidgee catchment upstream of the ACT border. There are likely to be low densities of Redfin perch in the Murrumbidgee upstream of this point, but only to the base of Bredbo Falls (which they cannot pass; Luke Pearce, pers comm). There is a single record of an individual in the Cooma Weirpool from 1993. However, none have been collected since then, despite regular and ongoing sampling. In the Snowy River catchment, Redfin perch are restricted to the Bombala River sub-catchment. There have been no observations of Redfin perch in the catchment upstream of Jindabyne Dam, which includes Guthega, Island Bend and Eucumbene reservoirs.

If a resident population of Redfin perch established in Tantangara Reservoir, it is expected that it would affect trout fisheries. The critically endangered Stocky galaxias (*Galaxias tantangara* Raadik) also occurs in this area, but is restricted to a small segment of Tantangara Creek upstream of Tantangara Falls. The presence of Redfin perch in Tantangara Reservoir would not represent any additional threat to this population fragment as Tantangara Falls is also likely to be a barrier to Redfin as it is to trout. Nevertheless, it amplifies the inhibition of Stocky galaxias population expansion and recovery already posed by salmonids. Further, the endangered Macquarie perch population in the Murrumbidgee River downstream of Tantangara Reservoir would be significantly impacted if Redfin perch were to establish in Tantangara Reservoir and then disperse downstream through the dam into the Murrumbidgee River.

Temperature thresholds

Temperature thresholds play a pivotal role in determining the geographic distribution of fish species', since such thresholds strongly influence their growth, reproduction and survival (Böhling *et al.* 1991; Hokanson 1977; Persson 1986). Redfin perch are considered to be a eurythermal species, meaning that they are to tolerate a wide range of temperatures (Hokanson 1977). They commonly inhabit fresh and brackish waters with temperatures ranging between 0 and 30 °C (Christensen *et al.* 2017; Thorpe 1977). Corollary evidence indicates that Redfin perch can even persist in environments subjected to sub-freezing

temperatures (Vainikka *et al.* 2012), and they are highly targeted by ice fishers in some European countries like Finland (Vainikka *et al.* 2012).



Figure 3. Redfin perch infected with Epizootic Haematopoietic Necrosis Virus (EHNV). The virus is known to be transmitted to trout as well as native species. Avoiding transmission of the virus into Tantangara Reservoir should be considered a priority.

Redfin perch have been shown to undertake spring spawning events throughout their known geographical ranges in North America where the temperatures have ranged from 2–26 °C (Hokanson 1977), and throughout such ranges in England where the temperatures have ranged from 9–18 °C (Guma'A 1978). They become sexually mature at temperatures below 12 °C (Hokanson 1977), and their immature egg cells (oocytes) develop from autumn through winter in preparation for the spring spawning event (Hokanson 1977). Guma'A (1978) undertook a laboratory experiment examining the effects of temperature on the development and mortality of Redfin perch eggs, and noted that the longest and healthiest larvae hatched at 14 °C. At temperatures above or below this value, the size of the larvae at hatching declined (Guma'A 1978).

The optimum temperature to support growth beyond these early life stages appears to be greater than that for hatching (Mélard *et al.* 1995). Mélard *et al.* (1995) undertook growth–food ratio–temperature experiments in Redfin perch of 3–300 g body weight, and reported that maximum growth rates occurred at 23 °C. Percid species may begin to die once they experience prolonged exposure to temperatures in excess of 29 °C, with their ultimate upper incipient (i.e. chronic) lethal temperatures believed to range from 29 to 35 °C, depending on the species and environmental conditions (Hokanson 1977). Thus, *Perca* spp. are geographically distributed in areas with temperature thresholds between 16 and 31 °C over summer months, as these thresholds respectively dictate the lower temperature for the growth of young and maturation of adults, and the upper temperature for their survival and reproduction (Hokanson 1977).

Table 1. Temperature thresholds reported in association with various life-history processes and/or attributes for Redfin perch. References: Thorpe (1977)¹; Christensen et al. (2017)²; Hokanson (1977)³; Guma'A (1978)⁴; M  lard et al. (1995)⁵.

Life-history process/attribute	Temperature threshold(s) reported
General distribution of the species	0–30 °C ^{1,2}
Reaching sexual maturity and the development of immature eggs (oocytes)	Autumn through winter; temperatures < 12 °C ³
Spawning	Spring; temperatures 2–26 °C in North America ³ ; 9–18 °C in England ⁴
Hatching	Optimum temperature 14 °C ⁴
Growth	Maximum growth rates at 23 °C ⁵
Incipient (i.e. chronic) lethal temperature	29–35 °C for Percid species ³

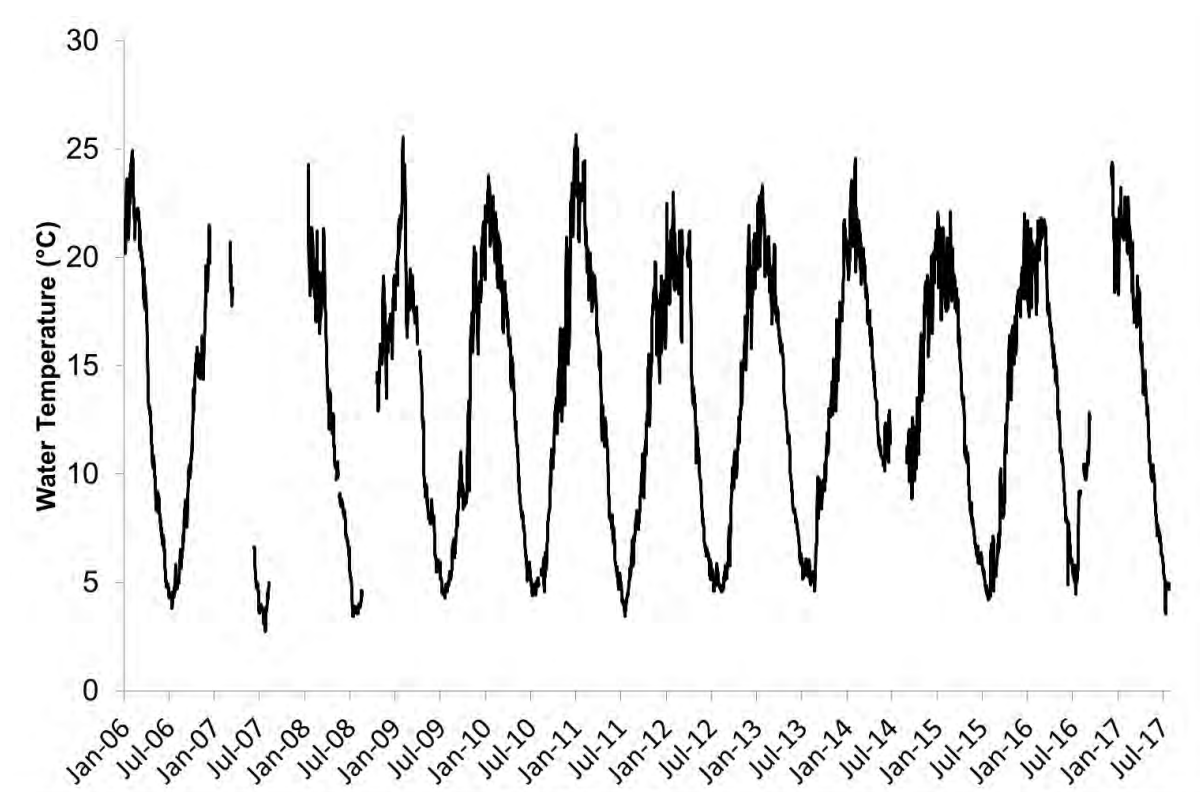


Figure 4. Thermal profile of Tantangara Reservoir from 2006–17. Data represents changes in minimum daily temperatures between 0–2 m depth. Only the surface water has been plotted because it will be subjected to the extreme variations in upland regions. Deep water in reservoirs has a more stable profile. The thermal tolerances of Redfin perch range from 0 °C to 31 °C.

Thermal profile of Tantangara reservoir

The thermal profile of Tantangara reservoir is typical of an upland lake. Surface water temperatures (0–2 m deep) reach a minimum of 3–4 °C in winter, but generally do not exceed 25–26 °C during summer (Figure 4). These tolerances are well within the thermal ranges of adult, juvenile, and larval Redfin perch (Table 1). As such, it would be expected that, based on thermal profile alone, Redfin perch would be able to survive if introduced into Tantangara Reservoir.

MaxEnt modelling of likelihood for redfin establishment

Following on from the examination of the thermal conditions of Tantangara Reservoir, maximum entropy (MaxEnt) modelling was deployed to provide a more detailed assessment of the capacity for streams and waterbodies within the footprint of the Snowy Hydro Scheme to support Redfin perch should they be introduced. MaxEnt is used for modelling species niches and distributions (Phillips *et al.* 2006). It takes a list of species presence locations as input, as well as a set of environmental predictors (e.g. rainfall, temperature, habitat characteristics) across a user-defined landscape that is divided into grid cells. From this landscape, MaxEnt extracts a sample of background locations that it contrasts against the presence locations (Phillips *et al.* 2006). Redfin perch presence was defined by distributions within the NSW Freshwater Fisheries Research Database, which includes all known observations of Redfin perch from standardised sampling. These observations were then modelled against known habitat variables in the region to assess the probability that each stream habitat could support Redfin perch (Figure 5).

Only the most upland portions of tributary streams were associated with values that would not support Redfin perch. Over 70% of waterways within the Snowy Hydro footprint could support Redfin perch if introduced. The Tumut River, both upstream and downstream of Talbingo Reservoir, and the Murrumbidgee River, downstream of Bredbo, have the most suitable habitat for Redfin perch; and the species is already established in these locations. MaxEnt further determined that whilst habitat suitability is marginal, Redfin could survive in Tantangara Reservoir and in many of the surrounding tributaries (Figure 5), thus adding support to the findings from the initial thermal suitability assessment in the preceding section. Also, Tantangara Reservoir contains some submerged and emergent vegetation suggesting that there is suitable spawning substrata available for Redfin perch.

MaxEnt also determined that Lake Eucumbene (Snowy River catchment) contains habitat that would support Redfin perch. As such, the potential for a secondary introduction via the existing tunnel system would require mitigation to prevent expansion into Lake Eucumbene and from there to other locations within the Snowy Scheme.

Likelihood of transfer by anglers

Humans have translocated fish both alien and native fish species extensively since the mid-1800s (Clements 1988). Acclimatisation societies and government agencies moved fish between and among catchments to improve recreational fisheries over many decades (NSW Fisheries, unpublished data).

Water diversions and inter-basin transfers have also led to translocations of many native species in Australia. Translocations can have both positive and negative impacts. For instance, translocations of the Pedder galaxias (*Galaxias pedderensis* Frankenberg) prevented localised extinctions in Tasmania (Sanger 2001). However, the translocations of alien species, such as Redfin perch, have been implicated in the widespread declines of Silver perch (*Bidyanus bidyanus* Mitchell), Mountain galaxias (*Galaxias olidus* Günther), Macquarie perch and Murray cod (*Maccullochella peelii peelii* Mitchell) in some areas (Arthington & McKenzie 1997; Lintermans 1991). The risk from disease and parasites can also pose threats to existing species where the translocated populations are carriers (Gillanders *et al.* 2006).

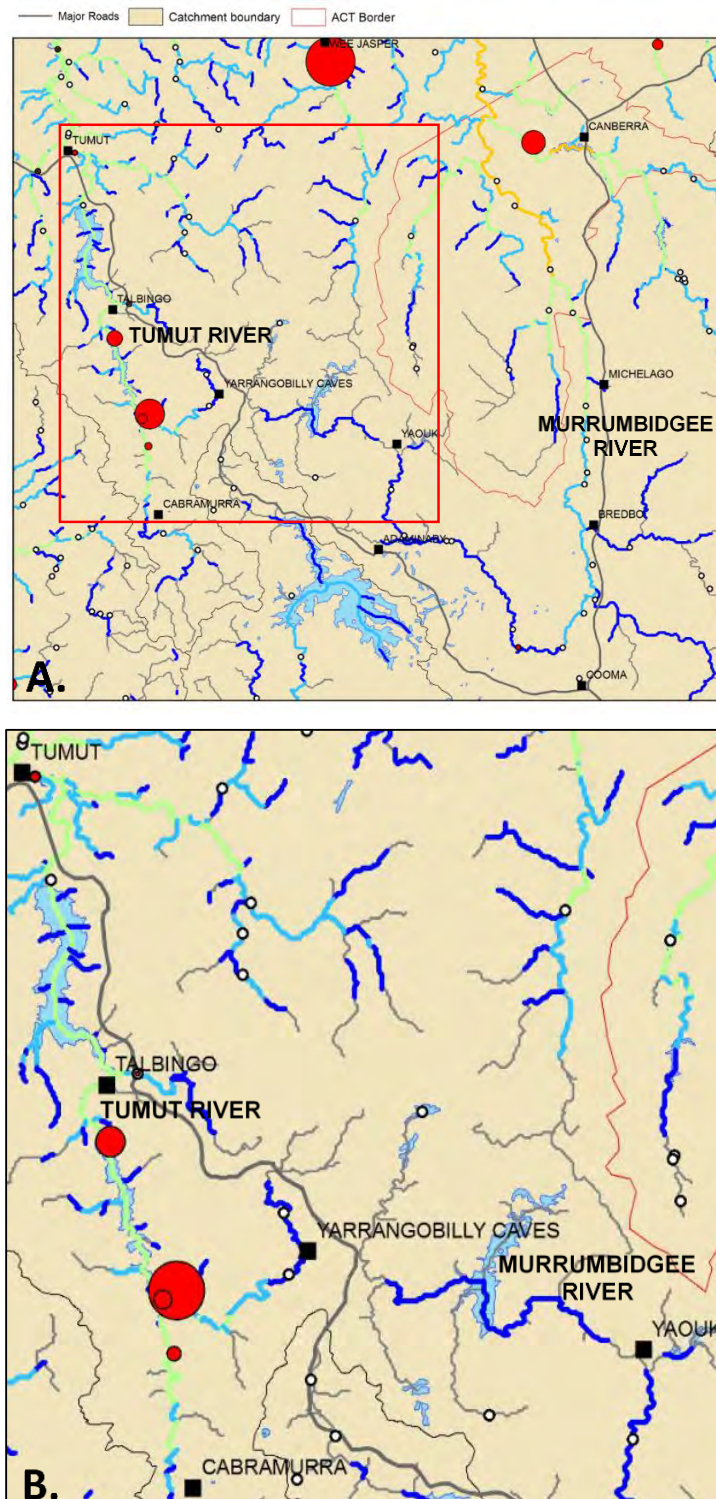


Figure 5. Map of the Snowy region (with the study area denoted by the red box) (A.) and the study area itself (B.) showing where Redfin perch have been recorded (red circles scaled to total catch on a logarithmic scale; small 1 individual; medium 10 individuals, large 1,000 individuals). Sampling locations where no Redfin perch have been caught are denoted as small white circles. MaxEnt modelling has been used to predict waters that could support Redfin perch if introduced. Grey areas are unlikely to support; dark blue denotes more marginal habitats (<0.2 chance of survival), pale blue (0.2-0.4), green (0.4-0.6), yellow/orange (0.6-0.8), red (0.8-1.0).

Redfin perch are capable of rapidly populating new waterways, and can form very dense populations in stable water bodies such as lakes and dams (Knight 2010). The species was first introduced to Tasmania for angling between 1858 and 1862, followed by Victoria in 1861, and to NSW and the ACT not long after (Lintermans 2007). It has been largely transferred by anglers both intentionally, and unintentionally via eggs attached to boats, trailers and other fishing gear (Anon 2017). For example, the transfer of Redfin perch around Southern Australia was largely intentional. Similarly, the spread of Redfin perch throughout the Canberra region occurred in response to the illegal transport and release of individuals from Lake George into the Molonglo River drainage (Lintermans 2004).

The predatory nature of Redfin perch combined with the ability to carry EHNV have led to a listing as a Class 1 noxious species in New South Wales (from December 2010 — Anon 2017). Indeed, one report indicated that Redfin perch were responsible for eliminating 20,000 newly released rainbow trout fry from a reservoir in south-western Australia in less than 72 hours (Anon 2017). Thus, any introduction of Redfin perch into Tantangara Reservoir and Lake Eucumbene will likely result in a rapidly expanding population, which will impact the local trout fishery and native fish species.

Existing regulation and compliance frameworks have prevented the expansion of Redfin perch populations so far. Selling, importing or translocating a live Class 1 noxious species carries heavy fines. Fisheries officers have the main responsibility for preventing translocations via any of these mechanisms.

Chapter 2: Factors influencing potential introduction into Tantangara Reservoir

Risk of entrainment of Redfin perch into the Talbingo Reservoir intake

Entrainment refers to the risk of transfer by trapping fish in transferred water. Thus, entrainment risk is the probability that fish will move into the pumped hydro system via the intake. The ability for a fish to escape or avoid entrainment typically relates to the magnitude of velocities generated perpendicular to the intake (approach velocity) relative to that along the intake (sweeping velocity) (Swanson *et al.* 2004). Reducing approach velocities relative to sweeping velocities minimises entrainment risk. To achieve this, an intake is usually made larger in terms of its surface area and/or angled to increase cross flow, but this is difficult to do in a reservoir (Swanson *et al.* 2004).

Criteria to determine appropriate sweeping and approach velocities can be determined from laboratory studies of fish swimming capabilities. The ability to control hydraulic variables in a replicated and experimentally robust way allows specific aspects of intake design to be refined for a variety of flow conditions in a rapid and cost-effective manner (Peake 2004; Swanson *et al.* 2005; White *et al.* 2007; Zydlewski & Johnson 2002). Generic design criteria developed from these studies can then be implemented and field-validated (McMichael *et al.* 2004). Field-validation is important because intake performance and fish behaviour can differ between laboratory and riverine settings. Nevertheless, the most commonly-applied mitigation measure to prevent fish entrainment is the installation of physical screens. If no physical screens are included in the design for Snowy 2.0, it must be expected that fish of any size could enter the system.

The tolerances of fish to stressors associated with conventional hydropower schemes have been examined in a number of studies (see Pracheil *et al.* 2016 for a review). Most of these studies have focussed on the tolerances of fish to barotrauma (e.g. Sebert & Macdonald 1993), shear stress (e.g. Baumgartner *et al.* 2017; Boys *et al.* 2014; Deng *et al.* 2005; Neitzel *et al.* 2004; Neitzel *et al.* 2000), and mechanical injury (e.g. Čada 2001). Turbine entrainment mortality for some fish species has been found to be as low as 3–5% for some turbine types and velocities (Čada 2001), but greater than 25% for other types (GeoSense 2011). Comparatively few studies have considered the tolerances of fish to the stressors associated with pumped hydropower schemes, although pressure effects are again thought to be highly important for fish in such schemes (Foye & Scott 1965). Indeed, a high pressure is needed at the pumping site of a pumped hydropower scheme to overcome the difference in head between the upper and lower reservoirs, and thus, the survival of any fish entrained within water being pumped would strongly depend on its pressure tolerances (Foye & Scott 1965).

Similar studies at the Ingula Hydropower project were investigated in South Africa (Gordon O'Brien, *pers comm*). There were substantial concerns that species transfers would occur between the head and tail waters. Investigations were performed to determine the overall transfer risk and it was deemed that fish survival was likely. Following operation of the scheme, which has an overall operating head of 480m, there are anecdotal reports that fish were able to survive passage and establish viable populations in both head and tailwater regions, but this has yet to be confirmed.

Pressure tolerances of Redfin perch

Based on the minimal research conducted on shallow water fish exposed to compression (Sebert 2002), there is a potential risk that Redfin perch may survive passage through the Snowy 2.0 System. Mortality has been observed in many fish species exposed to high pressures (Sebert & Macdonald 1993); however, in most cases, fish were able to survive pressures in excess of 8000 kPa (Sebert & Macdonald 1993). A comparison of these studies indicates that the effects of compression can vary depending on the species and life stage, rate at which the compression occurs, time held at pressure, and water temperature (Belaud

& Barthelemy 1973; Sebert & Macdonald 1993). The longer an egg is held at pressure, the greater the chance of injury, or tripoidy. Eggs are the life stage most likely to survive compression exposure, as shown through the production of the chromosomal abnormality, triploidy, in which foetuses are born with an extra set of chromosomes. Specifically, it has been shown that triploidy can be induced in Yellow perch (*Perca flavescens* Mitchill) by exposing their eggs to approximately 75,000 kPa for 5 to 12 minutes (Malison & Garcia-Abiado 1996).

Currently the effects of compression have not been examined in species from the Percidae family (apart from eggs for triploid production), and the rates at which the compression is likely to occur in the Snowy 2.0 pump storage facility are considerably greater than what has been examined on any fish species in the available literature (Figure 6). Without any information on these two subjects, it is difficult to provide an accurate estimate regarding the response of Redfin perch to compression. Therefore, based on the current literature, which has demonstrated that shallow water fish can potentially survive exposure to pressures greater than 8000 kPa (Sebert & Macdonald 1993), there is a credible risk that Redfin perch could potentially survive and be transferred to Tantangara Reservoir. To provide a more accurate estimate, further research will be required, and a simple laboratory study could determine the pressure thresholds for Redfin perch eggs, larvae, juveniles and adults.

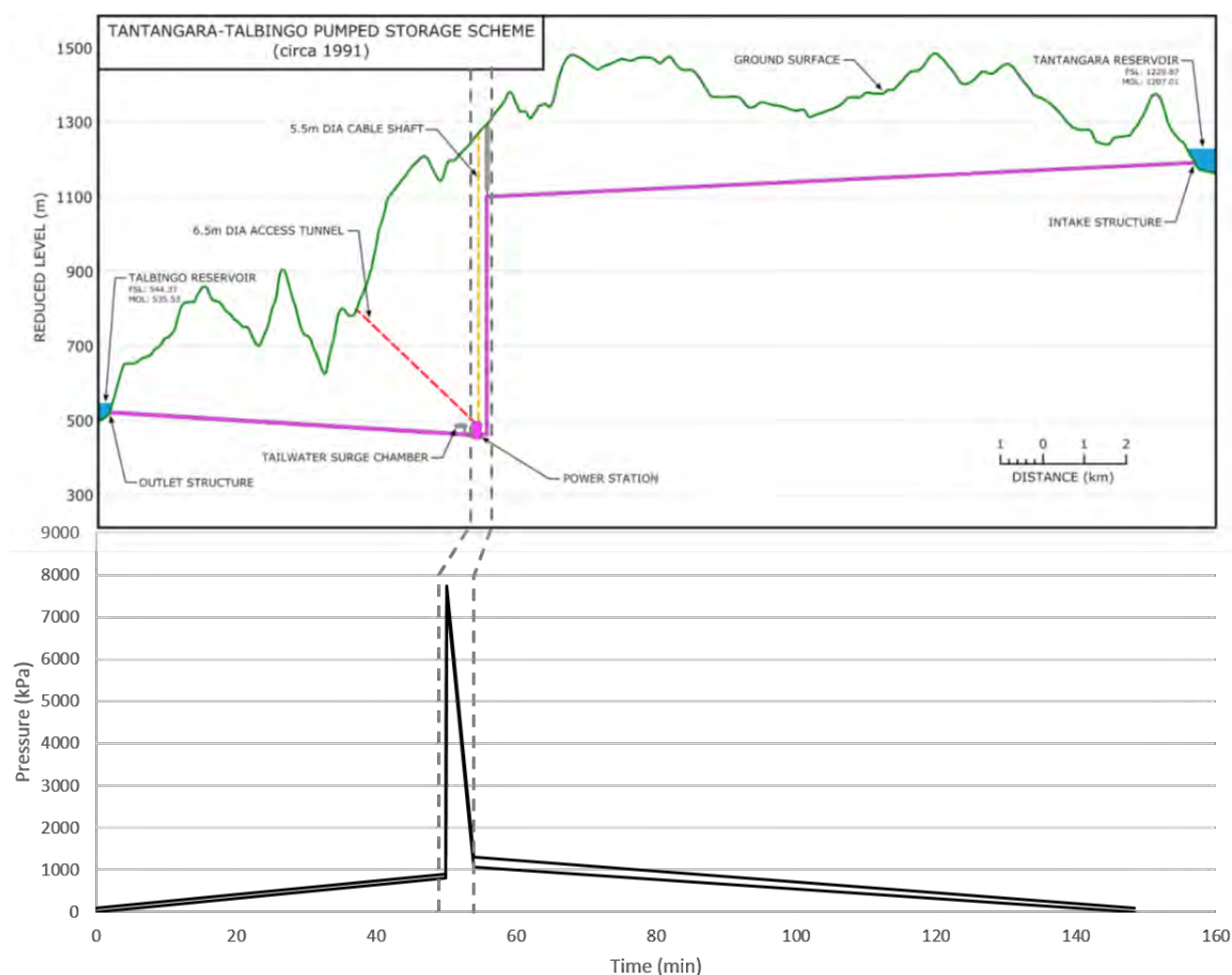


Figure 6. Preliminary modelled pressure gradient along the pumped hydro system. Modelling is very coarse and does not account for lower pressures expected on the downstream side of the turbine blades. Travel time is based on the assumption that flow will be 3 m/s (as advised by Snowy Hydro Corporation).

Fluid shear tolerances of Redfin perch

The susceptibility of Redfin perch to shear flows has not been determined, and in-depth computational fluid dynamics (CFD) analysis is necessary to determine the shear exposure range that Redfin perch may encounter when passing the Snowy 2.0 pump storage facility. Until this exposure range is determined, it is difficult to estimate the likelihood that Redfin perch will die due to shear exposure. However, based on research from other species and measurements taken from other Francis turbines (which have been extensively modelled), there is a credible risk that Redfin perch will survive any exposure to shear flows, and could potentially be transported through the Snowy 2.0 system. Several species have undergone laboratory analysis of exposure to varying degrees of shear stress (Baumgartner *et al.* 2017; Boys *et al.* 2014; Deng *et al.* 2005; Neitzel *et al.* 2004; Neitzel *et al.* 2000). Susceptibility was found to vary between species and life-stages (Table 2), and a percentage of the representatives for several of these species/life-stages was able to withstand exposure to strain rates exceeding 1000 cm/s/cm (Baumgartner *et al.* 2017; Boys *et al.* 2014; Neitzel *et al.* 2004).

In addition to varying susceptibility between species and life stages, the degree to which fish are exposed to shear can vary greatly within a turbine or pump. Most sensor fish deployed through Francis turbines recorded significant acceleration spikes (>95 G) attributed to strike or shear (Duncan & Carlson 2011; Duncan 2011). However, a small percentage of sensor fish were able to pass the turbine without encountering a significant shear event. This suggests that shear profiles are not uniform and that the turbine/pump within the Snowy 2.0 system will likely expose fish to a range of shear conditions that may cause mortality on the higher end, but, on the lower end, allow fish to pass relatively unharmed. Therefore, shear flows are not likely a means to prevent the transport of live Redfin perch within the Snowy 2.0 system. Nevertheless, it is likely that eggs and larvae will be more susceptible to shear stress than other life history stages. If a confirmed threshold could be identified, then mitigation strategies could focus on preventing tolerant life stages from being entrained. But in order to progress to such a planning stage, a knowledge of shear stress susceptibility, and expected stress values within the Snowy 2.0 system, would be required.

Table 2. Previously reported susceptibility of different fish species and life stages to shear, reported as strain rate.

Species	Life stage	Orientation to water jet	Strain rate (cm/s/cm)*	Mortality	Reference
Fall Chinook salmon	Pre-smolt	Head first	1008	10%	Neitzel <i>et al.</i> 2004
	Smolt	Tail first	1008	3%	Neitzel <i>et al.</i> 2004
	Smolt	Head first	1008	40%	Neitzel <i>et al.</i> 2004
Spring Chinook salmon	Smolt	Head first	1008	10%	Neitzel <i>et al.</i> 2004
		Tail first	1008	0%	Neitzel <i>et al.</i> 2004
Rainbow trout	Yearling	Tail first	1008	0%	Neitzel <i>et al.</i> 2004
Steelhead	Smolt	Head first	1008	0%	Neitzel <i>et al.</i> 2004
		Tail first	1008	0%	Neitzel <i>et al.</i> 2004
American shad	Yearling	Head first	1008	100%	Neitzel <i>et al.</i> 2004
Golden perch	Egg	N/A	148	100%	Boys <i>et al.</i> 2014
	Larval 12 dph	N/A	1297	55%	Boys <i>et al.</i> 2014
	Larval 18 dph	N/A	1297	35%	Boys <i>et al.</i> 2014
	Larval 26 dph	N/A	1297	10%	Boys <i>et al.</i> 2014
Silver perch	Egg	N/A	629	100%	Boys <i>et al.</i> 2014
	Larval 13 dph	N/A	1297	100%	Boys <i>et al.</i> 2014
	Larval 19 dph	N/A	1297	15%	Boys <i>et al.</i> 2014
	Larval 27 dph	N/A	1297	10%	Boys <i>et al.</i> 2014
Murray cod	Larval 9 dph	N/A	1267	100%	Boys <i>et al.</i> 2014
	Larval 19 dph	N/A	1297	15%	Boys <i>et al.</i> 2014
	Larval 29 dph	N/A	1297	60%	Boys <i>et al.</i> 2014
Silver shark	Mature	N/A	1297	50%	Baumgartner <i>et al.</i> 2017

*Highest strain rate examined or the strain rate at which 100% mortality was observed, whichever was attained first.

Chapter 3: Deterrents and controls that could be applied to Snowy 2.0

Overview of suitable deterrence systems

Fish deterrence systems aim to prevent fish entrainment into unfavourable environments. In the instance of Snowy 2.0, the main management aim is to ensure that Redfin perch from Talbingo Reservoir, will not be transferred to Tantangara Reservoir through the tunnel; then further onto Lake Eucumbene and the upper Snowy system. Fish deterrence systems can be effective for this purpose and are classified into non-physical or behavioural and physical barriers.

Non-physical or behavioural barriers emerged as a promising field to enhance effectiveness of fish protection systems at water infrastructure facilities in the 1990s, and they have since come to the forefront of fish protection/passage research (Popper & Carlson 1998). These barriers use behavioural mechanisms to affect fish navigational systems (Lemasson *et al.* 2008), stimulating the sensory system to elicit attractive or repulsive responses (Schilt 2007), guiding fish to a desired location and preventing their entrainment into hazardous areas (Lemasson *et al.* 2008).

Light (mainly strobe lights), sound (low and high frequency sound), electrical stimuli and bubble curtains all represent behavioural systems tested to improve fish protection (Bullen & Carlson 2003; Dawson *et al.* 2006; Schilt 2007; Vowles & Kemp 2012). These systems are also considered as non-physical screens because they can obstruct fish from an undesirable location, without influencing the waterway like physical barriers, such as traditional screens, do (Noatch & Suski 2012).

Physical barriers are often associated with screening systems to guide fish to safe routes, by deflecting or impeding the entrance in turbines or water intakes at dams, weirs or water diversion systems (Vowles & Kemp 2012). Furthermore, screens (Andrade *et al.* 2012) have been used and mechanical gates (Faria *et al.* 2010) have been trialled in laboratory as barriers to impede fish from entering the draft tubes of hydropower facilities.

The effectiveness of these barriers to deter fish entrainment in harmful areas in hydropower facilities is directly influenced by flow conditions (Vowles & Kemp 2012) and biological variables (e.g. migration triggers, depth of migration/movement, and behavioural response to the barrier) related to the species of interest (Boubée & Haro 2003). For hydropower plants, most of these systems have been tested at dams in the Columbia river, USA (see the compilation in Coutant 2001). Therefore, the control systems outlined below are indicative of measures that have been tested, in laboratory or field trials, as fish deterrents in hydropower plants. The suitability of these systems for the Snowy Hydro Scheme is not exhaustively discussed in this report and should be interpreted as suggestive only. As the effectiveness of these systems can be influenced by several variables, it would be unwise to support their use without a careful approach to analyse Redfin behavioural responses to them, under both laboratory and field conditions, as suggested by Taft (2000), Boubée and Haro (2003) and Amaral *et al.* (2005).

Electric barriers

Passing moderate to low voltage currents through the water column can create a barrier that prevents movement. Electrical barriers have been tested for controlling several fish problems including escapement and prevention of entrainment (Clarkson 2004). As technological developments allowed for the design of electrical barriers using pulsed-DC current, the potential for electrical barriers to impede fish movement at different water infrastructure facilities emerged (Clarkson 2004). However, little information is available in the literature to evaluate the effectiveness of the electrical barriers to deter fish (Dawson *et al.* 2006; Noatch & Suski 2012). Recently, Faria *et al.* (2010) showed the potential for an electromechanical barrier to impede movements of the Yellow catfish (*Pimelodus maculatus* Lacépède) into the draft tube of hydropower turbines using laboratory trials. Large-scale field trials have been conducted in the Chicago Sanitary and Ship Canal, USA, showing that an electric barrier

system was effective to reduce entrainment of Asian carp at certain conditions (Parker *et al.* 2016). As flow plays an important role in determining the efficiency of electric barriers, it is unlikely that electric barriers, alone, could be used for the Snowy Hydro Scheme. They could, nonetheless, potentially be combined with other systems, such as screens, to improve efficiency.

Acoustic systems

Some fish species actively avoid sound, so mitigation techniques which generate pulses that deter target species can be a solution for certain conditions. Low and high frequency sound have also been used as a possible non-physical screen for fish. Tests with low frequency sound, generally below 50 Hz, need to be further developed (Taft 2000), with a few experiments demonstrating attractive or repulsive responses for different species (Schilt 2007). High frequency sound has been shown to repel fish (Taft 2000), but its effectiveness was also variable depending on the species tested. Gibson and Myers (2002) showed that a high frequency sound for fish protection was not effective for eight species among 11 tested at the Annapolis tidal hydroelectric generating station (Nova Scotia). Considering Snowy 2.0 will have a bi-directional flow and given some examples from the literature, a sound system is very unlikely to be effective for preventing Redfin entrainment into the turbine intake.

Bubble curtains

Another visual cue that may deter fish is the bubble curtain, which is a fence or a curtain of bubbles emitted from the bottom of and perpendicular to the channel (Noatch & Suski 2012). To be effective, bubble curtains have to be visually detected by fish from a certain distance that would allow them to change direction and move away from the undesired area. Therefore, the effectiveness of bubble curtains is highly influenced by reduced light penetration in water and increased turbidity. Field trials have demonstrated the potential for the application of bubble curtains to deter downstream moving carp in Kohlman creek, USA, although the authors suggested that the curtains be used as an alternative approach for projects where reduction, and not elimination, of entrainment is the primary goal (Zielinski & Sorensen 2015). Therefore, this system would not be recommended for the Snowy Hydro Scheme.

Light

According to Königson *et al.* (2002), light is a primary stimulus to fish and several species have very well developed visual systems. Thus, constant light and strobe lights have been used to concentrate or guide fish for different reasons (Ploskey & Johnson 2001). Light can elicit varying attraction or repulsion responses from fish depending on the species, light intensity, flash rate and diel period (Ploskey & Johnson 2001), and laboratory experiments are of paramount importance to determine fish behaviour in response to this stimulus before field trials can be undertaken. Generally, results on the use of strobe lights are controversial, showing high (Johnson *et al.* 2001; Sager *et al.* 2000a; Sager *et al.* 2000b) or low efficiency (Johnson *et al.* 2005; Vowles & Kemp 2012), and environmental conditions, such as flow regime and turbidity, appear to have an important influence on the effectiveness of strobe light barriers. The suitability of a light system to eliminate Redfin entrainment within the Snowy Hydro Scheme would be very low, considering the numerous variables that would influence its effectiveness. Moreover, this system has thus far never proven to be 100% effective at any site.

Pheromones

Fish can release pheromones in the water, which will either attract them to or deter them from different areas. Pheromones are chemical odours that elicit behavioural responses from conspecifics, playing an important role in reproduction (attraction) or predator avoidance (deterrence) (Noatch & Suski 2012). Deterrence behaviour is elicited by an alarm substance formed from the chemical compound, hypoxanthine-3-N-oxide. This compound is produced by the club cells, located in the skin superficial layer (epidermis) and is released to the environment when the cell is ruptured. Thus, when the skin becomes damaged, the alarm substance is released, eliciting a fright reaction on fish. To be used, the alarm substance

would have to be collected or synthesised, which prevents its application for large-scale projects, considering the amount that would be required to elicit evasive responses.

UV treatments

Concerns over microbial contamination of potable water pipelines has led to the development of in-line ultraviolet treatments options. These generally work by constricting flow within a section of pipe where a high intensity ultraviolet ray is applied to effectively 'sterilise' the water and destroy pathogens. Multiple treatment points may be installed along a given pipeline. The practice is becoming increasingly accepted in the wastewater treatment industry and can effectively destroy up to 78–100% of contaminants (Sassi *et al.* 2005). Existing commercial systems can achieve effective sterilisation at flows up to 4,000 gallons per minute (16,000 litres per minute). If combined with effective screening options (see below), UV treatment has potential application in Snowy 2.0, particularly for removing EHN particles during the transfer process. A risk in relying solely on ultraviolet treatment as a solution is that it would need to work effectively during all pumping and generating operations. Any operational lapse introduces the risk of fish or virus particles being transferred. As such, further development of an ultraviolet option would require a nuanced design approach to ensure that:

- (a) effectiveness could be achieved under the full range of proposed flow rates
- (b) the UV treatment destroys all biota, including fish
- (c) sufficient redundancy is included to ensure operational reliability during periods of breakdown, maintenance or power outages.

Screens

Screens aim to create a physical barrier that direct fish away from the diversion point and back to the river (Neitzel *et al.* 1990). Screens come in a variety of designs, but the appropriateness of any specific design depends largely on the species to be protected, volume of flow, type of diversion system and ongoing maintenance requirements. The swimming ability of target species is the primary factor ultimately determining the design and size of a screen (Anon 1997). A combination of focussed research, adaptive management and incentive programs has facilitated large-scale recovery programs targeted primarily towards freshwater fish (Kepshire 2000).

A range of screen-types have been applied and/or proposed for use at hydropower stations, including close-spaced and angled bar racks, low-velocity fish screens, high-velocity fish screens, barrier nets and louvers (Federal Energy Regulatory Commission 1995). The Electric Power Research Institute (EPRI) (1986) also described six other screen-types: rotary drum screens, bar racks, infiltration intakes, cylindrical wedge-wire screens, traveling and stationary screens, and barrier nets. However, many of the screen-types described by EPRI (1986) are probably more appropriate for hydropower intakes with relatively small flow volumes. Indeed, a number of the facilities described in EPRI (1986) are used mainly at industrial intakes and thermal power stations.

Any screening program to exclude Redfin perch from the Talbingo intake will require consideration of different life-history stages (adults, juveniles and larvae). Fish screens operate in hostile environments and are continually exposed to water, sediment and debris. Interference with screen operation through fouling or damage can substantially reduce optimal function (McMichael *et al.* 2004). Ongoing maintenance is therefore a critical aspect of screen effectiveness (Neitzel *et al.* 1990), often necessitating the development of ongoing monitoring and evaluation programs to ensure maximum screen efficiency, compliance with guidelines and fish protection at times of peak fish migration (McMichael *et al.* 2004). Screen maintenance is seen one of the biggest challenges faced by screening programs in France (Larinier 2008; Larinier & Travade 2002). Oregon State law requires that government agencies be responsible for screen repair at diversions less than 30 cfs (73.4 ML.day⁻¹) (Rusak & Mosindy 1997) (ODFW 2010). In New Zealand, screening guidelines acknowledge

the need for maintenance and outline requirements for ensuring continual operation performance, but no specific agency or person is responsible for implementation (Jamieson *et al.* 2005). Any fish screening program that might be considered to exclude Redfin perch will need an appropriately designed operations and maintenance program. Failure to develop and implement these appropriate safeguards will decrease screen effectiveness and increase the probability that Redfin perch will be transferred.

Screens represent an effective mechanism to prevent fish entrainment into the proposed pipeline, and also to prevent transfer from Tantangara Reservoir to Lake Eucumbene. Screen design would need to target very early life-history stages (i.e. eggs and larvae) right up to adult stages. Redfin perch larvae are pelagic, and eggs are less than 2 mm in diameter. Therefore, the main criteria for a fully effective screen would be a very large surface area incorporating mesh size smaller than the minimum egg diameter. Preferably the screen would be self-cleaning to ensure operability without the constant need for human intervention. Considering mesh size would need to be less than 2 mm, a robust operation and maintenance program would be needed to avoid fouling and maintain efficient operation under all proposed operational regimes.

Eradication

The best control option would obviously be to eradicate all Redfin perch from Talbingo Reservoir, but this would be extremely difficult to achieve owing to the high level of invasiveness of the species (Knight 2010). For example, Rayner and Creese (2006) investigated the success of an attempt to eradicate another invasive fish species (*Phallocheros caudimaculatus*) using rotenone, from a system of freshwater pools in Sydney's Northern Beaches district. While this eradication program was undertaken in a closed system, the presence of dense aquatic vegetation prevented adequate mixing of rotenone and enabled the target fish to persist in areas where the concentration of rotenone remained low. Similarly, Knight (2010) explored the effectiveness of a range of physical and behavioural techniques for excluding Redfin perch from Macquarie perch habitat in the Hawkesbury-Nepean Catchment, and found that none of the techniques were 100% effective apart from a velocity barrier (which is a physical device).

Likelihood matrix with mitigating controls

This review has determined that Redfin perch could potentially colonise Tantangara Reservoir. Operation of Snowy 2.0 will substantially increase this likelihood beyond existing levels. A suitable program to reduce the likelihood of translocation will require a range of interventions. For instance, screens could be installed to reduce entrainment risk, but the mesh size would need to be small enough to exclude eggs and larvae (Table 4). Turbines designed to generate high shear stress above traditional thresholds could be another mechanism to prevent transfer. However, these options only focus on ensuring no transfer of live fish or eggs. Redfin perch also carry EHN, a lethal disease that infects trout and native species (Table 4). Screens and fluid shear would not impact live virus particles which survive outside the host for up to 113 days (and there is potential for these particles to be transmitted mechanically by fomites (Langdon and Humphrey 1987) — see Chapter 1). In that instance, options such as high intensity UV treatment points along the pipeline could effectively 'sterilise' water (Table 4). It was beyond the scope of this project to design such interventions, but they are mentioned here for potential consideration by design engineers.

Table 3. Matrix used for the likelihood ratings.

Likelihood	Definition
Almost certain	Very likely to occur
Likely	Likely to occur
Possible	May occur about half of the time
Unlikely	Unlikely to occur
Rare	Very unlikely to occur

Table 4. Matrix outlining the key mechanisms by which Redfin perch could be introduced into Tantangara Reservoir, and their likelihood of occurrence. A mitigated likelihood is given for each mechanism, which represents an adjusted prospect rating if the recommended mitigation strategy was implemented.

	Likelihood
1. Risk that Redfin Perch will be entrained and survive pumping from Talbingo to Tantangara in the absence of additional controls: Data from the literature for other species at similar sites suggest that severe compression won't be sufficient to kill Redfin perch. Other species are able to cope with substantial compression during egg stages, as no gas bubble is present in these species. In addition, adults of other species are known to survive significant compression.	Likely
2. Risk that current shear profile (as per existing design) <u>won't</u> kill Redfin perch: Existing work on other species has demonstrated high survival. Thus, it must be assumed that there is a substantial chance that eggs, larvae, juveniles or adults will make it through the system if entrained in water.	Unlikely
3. Risk that fishermen will introduce Redfin perch into Tantangara Reservoir: There are substantial concerns that Redfin perch may be intentionally introduced by members of the public. Redfin were transferred around Southern Australia largely via this route.	Unlikely
4. Risk that Redfin perch are already present in Tantangara: There are no known Redfin perch in the upper Murrumbidgee catchment upstream of the ACT border, but there are likely to be low densities upstream to at least the base of Bredbo Falls (which they cannot pass). There are no records from Tantangara and recreational fishermen do not report them in catches.	Rare
5. Risk that Redfin perch will colonise Tantangara upstream via the Eucumbene pipeline or via the existing Tantangara spillway: The risk of invasion via the Eucumbene pipeline is currently zero as there are no Redfin perch known from the upper Snowy. There are fish downstream of Tantangara Reservoir in the Murrumbidgee, but they	Rare

	Likelihood
are prevented from reaching the spillway due to excessive velocities and downstream natural barriers.	
6. Risk that Redfin perch will colonise Eucumbene (and possibly to other locations) if they enter Tantangara: The unscreened pipeline between Tantangara Reservoir and Eucumbene, although gravity fed, directly links the two reservoirs. Flow rates through the pipe exceed the swimming velocities of most species, so it is unlikely that any fish would move from Eucumbene to Tantangara. However, if no further controls are put in place, fish would be able to freely pass from Tantangara to Eucumbene through the system. There are no significant pressure or shear stress issues, so survival would be likely.	Almost certain
7. Risk that Tantangara Reservoir contains suitable habitat for Redfin perch: The thermal profile of Tantangara Reservoir is warmer than some of the places Redfin perch are found in Europe. Redfin perch are a popular ice-fishing species and have been located in water less than 2 °C. The most critical threshold for Redfin perch is exposure to high temperatures. Anything exceeding 31 °C is considered lethal. Temperature profiles obtained from Tantangara suggest that Redfin perch would easily survive if introduced. Tantangara Reservoir also contains habitat rocks, timber and vegetation which is preferred spawning substrate of Redfin perch.	Possible
8. Risk that Redfin-specific diseases (i.e. EHNV) will be transferred from Talbingo Reservoir into Tantangara Reservoir (and downstream): If Redfin perch in Talbingo Reservoir are exposed to EHNV, then virus particles will be present throughout the reservoir. These passive particles would be drawn into the pumped hydro intake and be translocated into Tantangara Reservoir, then subsequently via gravity lines into Lake Eucumbene. EHNV is known to impact trout and native species present in the upper Murrumbidgee and upper Snowy and thus they could be expected to be infected.	Possible (unknown if present)

Conclusion

A multiple lines of evidence approach has determined that there is a substantial risk of Redfin perch incursion into Tantangara Reservoir under present design parameters associated with Snowy 2.0. Survival through the Snowy 2.0 system is likely because:

- there is a strong, and actively recruiting, Redfin perch population in Talbingo Reservoir
- in the absence of further controls, it is expected that all life stages of Redfin perch could be entrained into the pipeline
- expected pressure profiles within the Snowy 2.0 pipeline will not exceed known lethal ranges for the eggs of other species within the *Perca* genus
- expected shear strain rates are, based on modelling within other Francis-style turbines, unlikely to exceed ranges that destroy all eggs, larvae, juveniles and adults
- the thermal profile in Tantangara Reservoir is well within known tolerance limits for Redfin perch
- MaxEnt modelling of habitat suitability determined that Redfin perch could survive in Tantangara Reservoir if introduced
- existing regulatory controls have been, so far, sufficient to prevent Redfin perch introduction into Tantangara Reservoir via human translocation.

The consequences of Redfin perch introduction in the Tantangara reservoir are:

- further expansion of a Class 1 noxious species into Tantangara Reservoir and in the absence of further controls, possibly into other areas within the footprint of the Snowy Hydro Scheme
- establishment of a new recreational fishery for Redfin perch within Tantangara Reservoir (and possibly to other connected reservoirs)
- a significant reduction of existing trout populations through increased predation and competition
- additional stresses on known Macquarie perch populations downstream of Tantangara through increased predation and competition if the subsequent transfer of Redfin perch is allowed downstream of Tantangara
- the introduction of EHN (if present in Talbingo) into Tantangara Reservoir and all downstream tributaries, which will lead to increased mortality among trout and native fish populations
- the introduction of Redfin perch (via existing gravity-fed tunnel systems) into Lake Eucumbene and then possibly on to connected reservoirs and waterways.
- that if Redfin perch entered Tantangara Reservoir, they could (without separate additional controls) enter Lake Eucumbene and introduce a new incursion elsewhere within the Snowy Water Catchment.

If the Snowy 2.0 Pumped Hydro Scheme proceeds, and the translocation of Redfin perch and EHN (if present in Talbingo) to Tantangara is to be prevented, then the most effective way to reduce the overall introduction risk is to:

- install multiple high intensity ultraviolet treatment stations within the pipeline to effectively destroy any Redfin perch, and associated viral particles. Such a solution will require detailed assessment to guarantee effectiveness and will destroy all biota and must be demonstrated to work under all operating scenarios. UV treatment should not be the sole solution and must be combined with the construction of effective self-cleaning screens (see below) to prevent Redfin perch (adult, juvenile, larval and egg) entrainment into the proposed pipeline.

If the Snowy 2.0 Pumped Hydro Scheme proceeds, and Redfin perch transfer is to be prevented, but EHNV transmission is allowed (or it is deemed not present in Talbingo); then the most effective way to reduce overall introduction risk is via the construction of fine mesh (less than 2 mm diameter) self-cleaning screens on the:

- Talbingo intake to prevent introduction into Tantangara Reservoir; and/or
- Lake Eucumbene pipeline intake to prevent introduction into Lake Eucumbene and possibly beyond.

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Determining the presence or absence of invasive *Perca fluviatilis* (redfin) at Tantangara Reservoir using environmental DNA

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Abbreviations

Abbreviations	Description
eDNA	environmental DNA
qPCR	quantitative polymerase chain reaction
EHNV	epizootic haematopoietic necrosis virus

Executive Summary

As part of the possible future expansion of Snowy Hydro's hydro-electric capacity, a new pipeline is proposed connecting Talbingo and Tantangara Reservoirs. Invasive *Perca fluviatilis* (redfin) are currently known to exist in Talbingo but are thought to be absent in Tantangara. Using highly sensitive environmental DNA (eDNA) techniques, Snowy Hydro sought to confirm the absence of *P. fluviatilis* in Tantangara in order to assess the risks of possible incursions as a result of the proposed pipeline. Water samples were collected and filtered on-site from both reservoirs and screened using species-specific *P. fluviatilis* markers.

No *P. fluviatilis* eDNA was detected from extensive sampling undertaken throughout Tantangara Reservoir ($n = 124$). Conversely, *P. fluviatilis* was detected in every sample collected from Talbingo Reservoir ($n = 30$) where the species is known to occur. The results indicate that the eDNA assay is highly sensitive and effective at detecting *P. fluviatilis* and provides a high level of confidence that *P. fluviatilis* was not present in Tantangara Reservoir at the time of sampling for this study.

Background

Invasive species are one of the most significant threats to biodiversity worldwide (Simberloff et al. 2013), particularly in aquatic ecosystems (Dudgeon et al. 2006). Invasive *Perca fluviatilis* (redfin perch) were introduced to Australia in the 1860's for angling and are now widespread throughout much of south-eastern Australia. They are highly fecund and aggressive predators that have been implicated in the decline of native fish species including Murray Cod, Macquarie Perch and the pygmy perch (Allen et al. 2002). In addition, the species are carriers of the epizootic haematopoietic necrosis virus (EHNV), which can devastate native and recreational fish populations. For these reasons, the NSW Department of Primary Industries declared *P. fluviatilis* a noxious species in 2010.

Snowy Hydro is conducting a feasibility study into the expansion of its hydro-electric storage capacity (Snowy 2.0). As part of this expansion, a new water pipeline is proposed between Talbingo and Tantangara Reservoirs. Both reservoirs are accessible by the public and are popular recreational fishing locations. *Perca fluviatilis* are known to exist in Talbingo but are not known to be present in Tantangara. In this project, Snowy Hydro sought to confirm the absence of *P. fluviatilis* in Tantangara in order to assess the risks of possible incursions as a result of the proposed development.

Environmental DNA (eDNA) is a non-invasive sampling technique that detects traces of genetic material from a target species secreted into its surrounding environment (water). Quantitative comparisons with traditional sampling methods have demonstrated that eDNA methods can be superior in terms of sensitivity and cost efficiency, particularly for scarce, elusive or cryptic species (Biggs et al. 2015; Smart et al. 2015; Weeks et al. 2015), enabling effective detection of species at low densities and at all life stages. During this project, eDNA techniques were used to investigate the presence of *P. fluviatilis* in Tantangara Reservoir.

Methods

Perca fluviatilis spawn in late winter and spring and this period would be expected to yield the highest detection probability for eDNA due to increased activity, and the presence of gametes or juveniles in the water. During early October 2017, water samples were collected by boat from nine areas of Tantangara Reservoir (Figure 1a) using two filtration methods to maximize filtration volume and DNA retention to improve detection probability. Two areas of Talbingo Reservoir were also sampled to provide a positive control (Figure 1b). Sampling areas primarily focused on shallower water near the banks where *P. fluviatilis* are expected to be spawning. However, one sampling area of Tantangara Reservoir encompassed the reservoir outflow, which originates from deeper water near the dam wall to increase sampling coverage within the target reservoir.

Within each area, water samples (1-1.5 L) were collected from ten sites and passed through a 1 µm filter (EnviroCheck) using a drill-powered vampire sampler (Watson-Marlow, UK) positioned upstream of the filter for a total volume of 10-16 L per filter. Additional water samples (150-420 mL) were collected and passed through a 0.22 µm filter (Sterivex) using a sterile 60 mL syringe at 4-5 sites in each area (Figure 1). An exception to this sampling regime was area 4 in Tintangara Reservoir that only used 0.22 µm filters (n = 10). A total of 124 and 30 sites were sampled in Tintangara and Talbingo Reservoirs respectively.

Samples were kept on ice for a maximum of 72 hrs before being transported to the laboratory for processing. DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen DNeasy Blood and Tissue Kit). A species-specific *P. fluviatilis* TaqMan® assay targeting the 12S region of the mitochondrial genome was developed using previously published data (Furlan and Gleeson 2016) and verified using *P. fluviatilis* tissue sourced from populations local to the study area. The TaqMan® assay was undertaken in a quantitative PCR on all DNA extractions in triplicate. Negative controls were included for the DNA extraction and qPCR steps.

Results and Discussion

The presence of *P. fluviatilis* eDNA was detected in all samples collected from the positive control site (n = 30; Table 1), Talbingo Reservoir, despite no fish being caught at the time. Discussions with local anglers on-site indicated very few *P. fluviatilis* had been captured around the time of sampling. Target eDNA was detected in every sample from Talbingo Reservoir, highlighting the power of this technique to detect target species in a large body of water with limited sampling effort. The target DNA appears relatively homogeneous throughout the sampling area, and both sampling/filtration methods picked up the *P. fluviatilis* eDNA in all qPCRs undertaken.

Conversely, no *P. fluviatilis* eDNA was detected from the more extensive sampling undertaken in Tintangara Reservoir (Table 1). Given the results from Talbingo Reservoir, this provides high confidence that *P. fluviatilis* was not present in Tintangara Reservoir at the time of sampling. The assay has previously been demonstrated as highly sensitive to detect *P. fluviatilis* at low densities (Furlan and Gleeson 2016). These results support anecdotal accounts from several long-term anglers who stated they have never caught the species in Tintangara Reservoir.

As a highly fecund species, *P. fluviatilis* are capable of rapid population growth and can reach very high abundance in enclosed waterbodies. Therefore, unless the species had been very recently introduced we would expect relatively high numbers within Tintangara at the time of sampling and be readily detectable. Anglers have been implicated in facilitating incursions of *P. fluviatilis*, particularly in isolated waterbodies, either through the use of juveniles as bait or deliberate attempts to establish new populations. It is possible that this can or has previously occurred in

Tintangara Reservoir as the area is open to public access. A visual inspection of the reservoir suggests habitat quality is relatively poor for *P. fluviatilis* due to the lack of aquatic vegetation and other substrate complexity (e.g. rocky outcrops) required for spawning. In addition, the colder water temperatures in Tintangara Reservoir (although not investigated in this project) may inhibit colonization and establishment of *P. fluviatilis* as the species spawns when water temperatures exceed 12 °C (Lintermans 2007) and the species does not seem to occur in other surrounding alpine reservoirs of similar altitude. Therefore it is possible that occasional incursions may occur but local conditions have prevented *P. fluviatilis* from establishing. However, this does not preclude the species establishing in the future if conditions change.

Incursions of an invasive species into a new environment can pose a significant threat to that ecosystem and the endemic species. Such impacts can include predation, competition for resources, habitat degradation, and the introduction of parasites or diseases (Ross 1991). *Perca fluviatilis* has the potential to have a significant impact on the native and recreational fishing species in Tintangara Reservoir if the species were to become established as a highly fecund and aggressive predator and a vector of EHNIV.

Conclusion

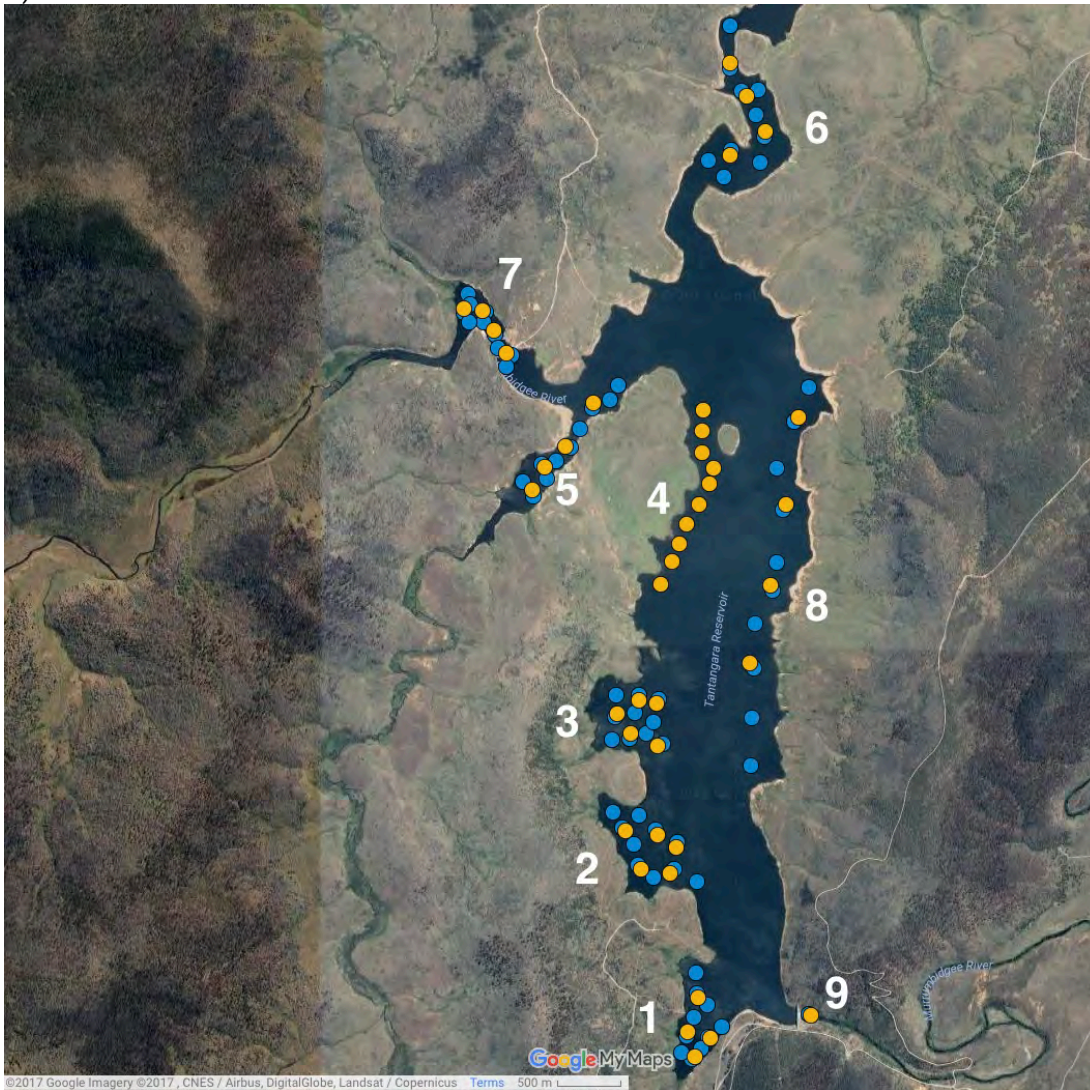
Conclusive detection of *P. fluviatilis* eDNA in all samples collected from Talbingo Reservoir indicates that the eDNA assay is highly sensitive and effective at detecting the species. Therefore the negative results from comprehensive sampling in Tintangara Reservoir provides a high level of confidence that *P. fluviatilis* was not present at the time of sampling for this study.

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Determining the presence or absence of *P. fluviatilis* in Tantangara Reservoir

a)



b)

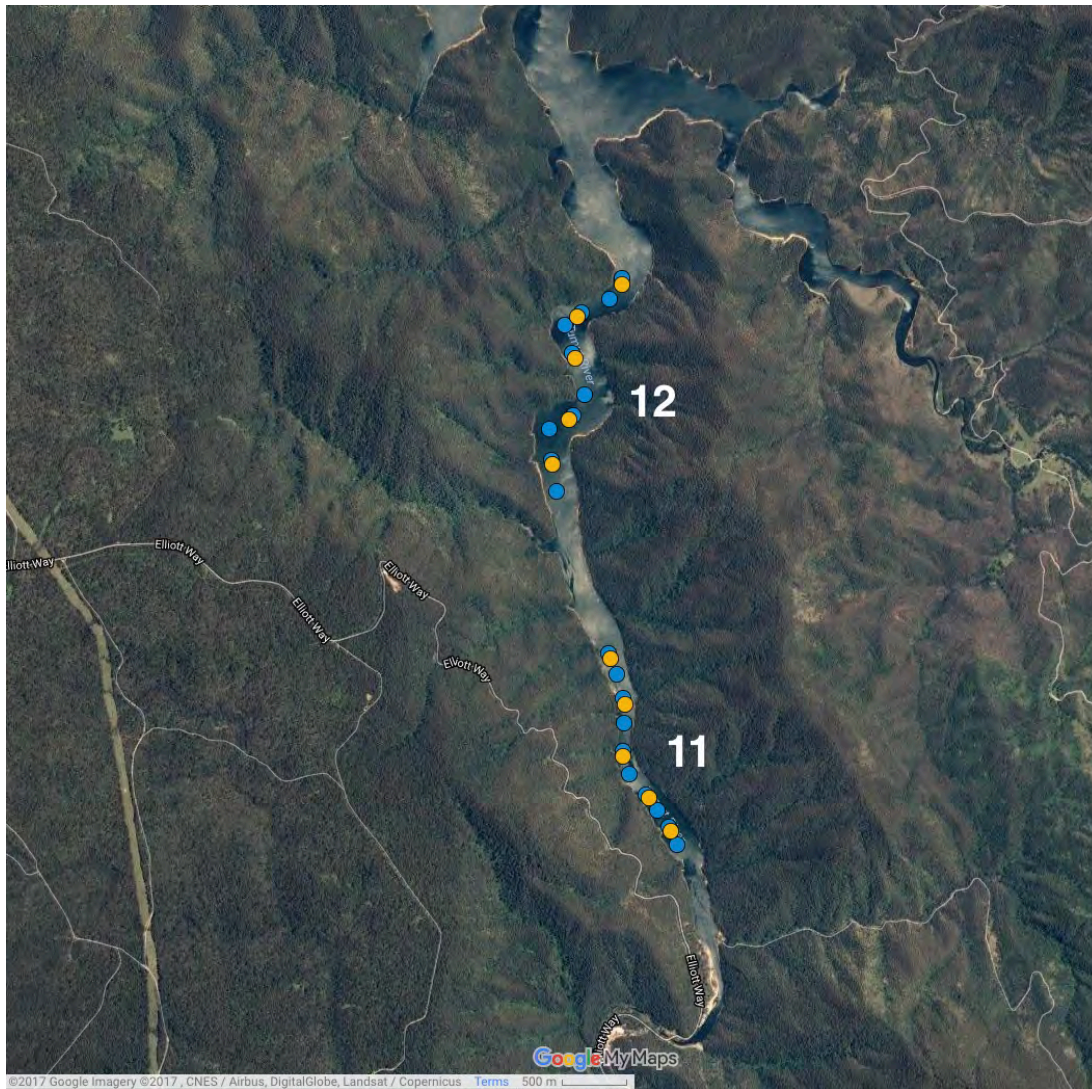


Figure 1. Location of water sampling sites in Tantangara (a) and Talbingo (b) Reservoirs. Blue symbols represent sites sampled with the 1 μ m EnviroCheck filters and orange symbols represent sites sampled with the 0.22 μ m Sterivex filters.

Table 1. Summary of results for *P. fluviatilis* eDNA surveys. Note: 1µm filters are pooled water from 10 sub-sampled sites.

Reservoir	Site Code	Filter size	Volume (mL)	No. positive PCR (3)	Av. DNA conc. (pg/L)	Result
Tantangara	1.0	1.00µm	16000	0	0	Negative
Tantangara	1.1	0.22µm	420	0	0	Negative
Tantangara	1.2	0.22µm	420	0	0	Negative
Tantangara	1.3	0.22µm	360	0	0	Negative
Tantangara	1.4	0.22µm	360	0	0	Negative
Tantangara	2.0	1.00µm	12000	0	0	Negative
Tantangara	2.1	0.22µm	300	0	0	Negative
Tantangara	2.2	0.22µm	300	0	0	Negative
Tantangara	2.3	0.22µm	300	0	0	Negative
Tantangara	2.4	0.22µm	300	0	0	Negative
Tantangara	2.5	0.22µm	300	0	0	Negative
Tantangara	3.0	1.00µm	10000	0	0	Negative
Tantangara	3.1	0.22µm	360	0	0	Negative
Tantangara	3.2	0.22µm	300	0	0	Negative
Tantangara	3.3	0.22µm	300	0	0	Negative
Tantangara	3.4	0.22µm	300	0	0	Negative
Tantangara	3.5	0.22µm	300	0	0	Negative
Tantangara	4.1	0.22µm	300	0	0	Negative
Tantangara	4.2	0.22µm	300	0	0	Negative
Tantangara	4.3	0.22µm	300	0	0	Negative
Tantangara	4.4	0.22µm	300	0	0	Negative
Tantangara	4.5	0.22µm	300	0	0	Negative
Tantangara	4.6	0.22µm	300	0	0	Negative
Tantangara	4.7	0.22µm	300	0	0	Negative
Tantangara	4.8	0.22µm	300	0	0	Negative
Tantangara	4.9	0.22µm	340	0	0	Negative
Tantangara	4.10	0.22µm	300	0	0	Negative

Determining the presence or absence of *P. fluviatilis* in Tantangara Reservoir

Tantangara	5.0	1.00µm	10000	0	0	Negative
Tantangara	5.1	0.22µm	360	0	0	Negative
Tantangara	5.2	0.22µm	360	0	0	Negative
Tantangara	5.3	0.22µm	360	0	0	Negative
Tantangara	5.4	0.22µm	220	0	0	Negative
Tantangara	6.0	1.00µm	10000	0	0	Negative
Tantangara	6.1	0.22µm	240	0	0	Negative
Tantangara	6.2	0.22µm	160	0	0	Negative
Tantangara	6.3	0.22µm	150	0	0	Negative
Tantangara	6.4	0.22µm	150	0	0	Negative
Tantangara	7.0	1.00µm	10000	0	0	Negative
Tantangara	7.1	0.22µm	300	0	0	Negative
Tantangara	7.2	0.22µm	300	0	0	Negative
Tantangara	7.3	0.22µm	300	0	0	Negative
Tantangara	7.4	0.22µm	300	0	0	Negative
Tantangara	8.0	1.00µm	10000	0	0	Negative
Tantangara	8.1	0.22µm	300	0	0	Negative
Tantangara	8.2	0.22µm	300	0	0	Negative
Tantangara	8.3	0.22µm	300	0	0	Negative
Tantangara	8.4	0.22µm	300	0	0	Negative
Tantangara	9.0	1.00µm	10000	0	0	Negative
Tantangara	9.1	0.22µm	360	0	0	Negative
Tantangara	9.2	0.22µm	360	0	0	Negative
Tantangara	9.3	0.22µm	360	0	0	Negative
Tantangara	9.4	0.22µm	360	0	0	Negative
Talbingo	11.0	1.00µm	13000	3	0.00057	Positive
Talbingo	11.1	0.22µm	360	3	0.043	Positive
Talbingo	11.2	0.22µm	360	3	0.129	Positive
Talbingo	11.3	0.22µm	360	3	0.159	Positive
Talbingo	11.4	0.22µm	360	3	0.039	Positive

Determining the presence or absence of *P. fluviatilis* in Tantangara Reservoir

Talbingo	11.5	0.22µm	360	3	0.031	Positive
Talbingo	12.0	1.00µm	13000	3	0.00003	Positive
Talbingo	12.1	0.22µm	360	3	0.013	Positive
Talbingo	12.2	0.22µm	360	3	0.027	Positive
Talbingo	12.3	0.22µm	360	3	0.058	Positive
Talbingo	12.4	0.22µm	360	3	0.013	Positive
Talbingo	12.5	0.22µm	360	3	0.015	Positive



Fish and decapod environmental DNA biodiversity surveys in the Snowy 2.0 project area

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Abbreviations

Abbreviations	Description
eDNA	environmental DNA
EIA	Environmental Impact Assessment
EPBC	Environment Protection and Biodiversity Conservation
PCR	Polymerase Chain Reaction
OTU	Operational Taxonomic Unit
NGS	Next Generation Sequencing

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Executive Summary

As part of the possible future expansion of Snowy Hydro's hydro-electric capacity, significant new infrastructure is proposed including the construction of an underground power station and tunnels connecting the existing Tantangara and Talbingo Reservoirs (Snowy 2.0). The project will also include the construction of new roads and upgrades to existing tracks. As part of an Environmental Impact Assessment for the proposed works, Snowy Hydro sought to use environmental DNA techniques to complement traditional electrofishing techniques. Water samples were collected and filtered from sites in the project area in both Talbingo and Tantangara Reservoirs. Multispecies metabarcoding eDNA techniques were used to assess the occurrence of fish and decapod species in all collected water samples. Twenty fish species were detected (nine native and eleven non-native) across all sites. Non-native species dominated the fish communities; redfin (*Perca fluviatilis*), which was restricted to the Talbingo Reservoir and tributaries, rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salmo trutta*). Goldfish (*Carassius auratus*) and eastern mosquitofish (*Gambusia holbrooki*) were also prevalent throughout Talbingo Reservoir and Jounama Pondage. Native fish species were generally detected at few sites, with localised distribution and low DNA abundance. Despite being recently stocked by NSW DPI, Murray cod (*Maccullochella peelii*) and golden perch (*Macquaria ambigua*) were only detected at low levels in Jounama Pondage. The threatened trout cod (*Maccullochella macquariensis*) and Macquarie perch (*Macquaria australasica*) were not detected from water samples at any sites. Other notable native species included two-spined blackfish (*Gadopsis bispinosus*) in the Yarrangobilly system and several galaxias species across the sampling area. The decapod assay detected three spiny crayfish species, the common yabby (*Cherax destructor*) and a freshwater glass shrimp (*Paratya australiensis*). Reiks crayfish (*Euastacus reiki*) was relatively widespread in the Eucumbene and Upper Murrumbidgee catchment while the vulnerable Murray crayfish (*Euastacus armatus*) was restricted to the Yarrangobilly system. Other decapod species are likely to be present in the sampled areas, but reference DNA sequence was not available and therefore these species could not be identified.

Background

Snowy Hydro is conducting an Environmental Impact Assessment (EIA) as part of the proposed expansion of its hydro-electric capacity (Snowy 2.0). Significant expansion and development of new infrastructure is proposed as part of Snowy 2.0, particularly the construction of an underground power station and tunnels connecting the existing Tantangara and Talbingo Reservoirs. The project will also include construction of new roads and upgrades to existing tracks to facilitate access. The proposed roads include the construction or upgrades of bridges and crossings at numerous creeks throughout the study area, which has the potential to impact on sensitive aquatic fauna.

Comprehensive aquatic surveys are being undertaken as part of the EIA, including macroinvertebrate and fish monitoring (Cardno 2018). Although standard electrofishing techniques are being used to assess fish and decapods throughout the project site, Snowy Hydro engaged EnviroDNA to use environmental DNA as an additional biodiversity assessment method. Environmental DNA (eDNA) is a non-invasive sampling technique that detects genetic material from a target species secreted into its surrounding environment (i.e. water). Quantitative comparisons with traditional sampling methods already indicate that eDNA methods can be superior in terms of sensitivity and cost efficiency, particularly for scarce, elusive or cryptic species (Biggs et al. 2015; Lugg et al. 2018; Smart et al. 2015; Weeks et al. 2015), enabling effective detection of species at low densities. Using metabarcoding approaches, it is now possible to identify entire taxonomic groups from environmental samples (Shaw et al. 2016; Thomsen and Willerslev 2015).

In this project we implemented eDNA metabarcoding techniques to investigate the presence of fish and decapod species throughout the Snowy 2.0 project area.

Methods

Survey sites were selected as part of the Aquatic Ecology Survey Plan (Cardno 2018). Sampling was conducted by EnviroDNA with staff from Snowy Hydro and EMM Consulting during February 2018 using standardised techniques (see below). Water samples from Tantangara Reservoir (Tar1-Tar9) and additional sites in Talbingo Reservoir (Tal11, Tal12) were collected as part of a separate project in October 2017 (Griffiths et al. 2017). See Figure 1 for sites where eDNA samples were collected for this project.

At each site, water samples were collected in triplicate and filtered *in situ* by passing up to 500 ml water (range 61-500 ml) through a 0.22 µm filter (Sterivex) using a sterile syringe. Care was taken to minimise contamination between sites by using clean equipment and gloves at each site and avoiding the transfer of water, soil, or organic matter. Filters were stored on ice for a maximum of 48 hrs before being

transported to the laboratory for processing. DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen DNeasy Blood and Tissue Kit).

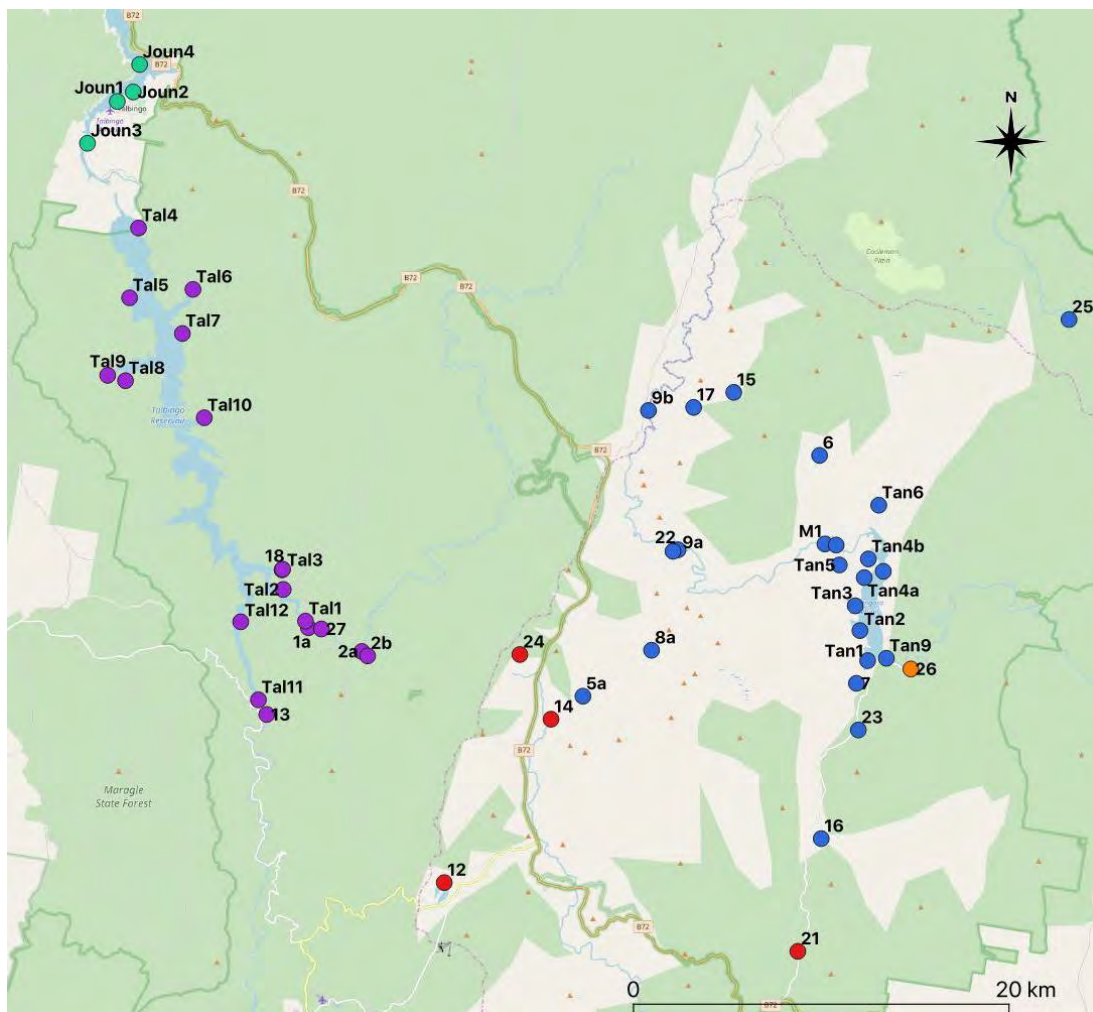


Figure 1. Map of water sampling locations. Catchments - Green closed circles (below Talingo), Purple closed circles (Talingo), Red closed circles (Eucumbene), Blue closed circles (Tantangara), Orange closed circles (below Tantangara). Site 28 (below Tantangara) is not shown on the map due to scale.

Biodiversity assessments were performed with two separate metabarcoding assays - a universal fish and universal decapod crustacean assay. For each assay, Polymerase Chain Reaction (PCR) was used to amplify a short, hypervariable marker region (located within the mitochondrial 12S ribosomal RNA gene) from the fish DNA or the decapod DNA present in each sample. This marker provides good resolution at lower taxonomic levels while having regions that are conserved at higher taxonomic levels. Four replicate reactions were performed per sample. The sequence of individual marker DNA molecules was then determined by Next Generation Sequencing (NGS). Following quality control filtering, DNA sequence reads were clustered into Operational Taxonomic Units (OTUs) on the basis of DNA sequence similarity (identity cut-off threshold = 98% fish, 99% decapods). The

proportion of reads corresponding to each OTU were calculated for each sample. Low abundance OTUs (less than 1% of the total sample reads) were filtered from the dataset on a sample-wise basis.

Each OTU was assigned a species identity by comparing with a custom fish/decapod reference sequence database containing data for native and introduced species occurring within south-eastern Australia (see Appendix 1 for available species in the study area). One representative DNA sequence was selected per OTU and searched against all DNA sequences in the reference database. Matches to the database were recorded using a step-wise process that was designed to maximise the accuracy of species assignment, while allowing for some within species variability. First, all OTUs sharing 100% DNA sequence identity with a database entry were assigned to that species. All unassigned OTUs were once again searched against the reference database, and those sharing $\geq 99\%$ DNA sequence identity with a database entry were assigned. This process was repeated sequentially for identity thresholds of 98% and 97%. A DNA sequence identity threshold of 97% is considered informally by the scientific community to be a rough benchmark for delineating different closely related species, although this will vary across different taxonomic groups and DNA marker regions. Data analysis was repeated with the OTU abundance threshold set at 0.1% of the total sample count, to check for the possibility of low-level detections. As the results were similar to those for the 1% threshold, they have been omitted.

Uncertainty in eDNA metabarcoding detections

There are two levels of uncertainty that can arise in eDNA metabarcoding studies; low level detections and confidence in species assignment (based on reference sequences). Low level detections can provide uncertainty as to the confidence in their origin. For instance, small amounts of DNA could be introduced to a water sample (site) by the movement of DNA from upstream, contamination from unknown sources (e.g. human or animal movement, non-sterile equipment etc) or introduced in the laboratory. Confidence in low level detections can be gained by increasing the number of samples at a site or sampling at the same site through time; however, this is not always feasible. For this study, we consider detections of 1% or less as low-level detections.

The second source of uncertainty within eDNA metabarcoding studies can arise when OTUs are not able to be assigned adequately to a species in the reference sequence database. This can be due to several reasons including a lack of reference sequences for a particular species (or group of species), a lack of reference sequences covering intraspecific diversity, or poor sequence resolution between closely related species. When this occurs, assignment at a higher taxonomic level is generally undertaken, and this is the approach we have adopted in this study.

eDNA persistence

The persistence of eDNA can also have an effect on the detection of different species. Generally, eDNA is considered to be a relatively real-time detection method, with eDNA persisting for a range of species from days to weeks, depending on environmental conditions (Lugg et al. 2018). The rate of degradation, however, will affect detectability for different species. Ideally sampling designs would incorporate information on degradation of eDNA for different species. In the absence of this information, sampling through time can provide a higher level of confidence in results (particularly absences at a site) as can be the case for other survey methods. Here we have undertaken a one-off survey event over a broad spatial scale, and therefore this should be taken into consideration when interpreting results.

Results and Discussion

The multi-species assays detected up to nine native fish species, eleven non-native fish species (Appendix 1, Table A1) and six decapod crustacean species (Appendix 1, Table A2) across the study area. Although similar numbers of native and non-native fish species were observed overall, it was clear that non-native fish dominate most habitats within the area, both in terms of number of species, their distribution, and presumably their biomass. Native fish species were generally detected at relatively few sites, with restricted localised distribution and low relative DNA concentrations. These results were not surprising given the dominance of introduced species, the extensive trout-stocking program carried out throughout the Snowy Mountains, and the well-known detrimental impacts that introduced species, particularly trout, can have on Australian native fish (Cadwallader 1996; Crowl et al. 1992; Lintermans 2007).

Fish biodiversity

Non-native fish

Invasive redfin perch (*Perca fluviatilis*) were detected at every site within Talbingo Reservoir and the downstream Jounama Pondage, as well as nearby sites upstream of Talbingo Reservoir within the Yarrangobilly River (Table 1). No redfin were identified within Tantangara Reservoir (agreeing with recent results from single-species assays undertaken by EnviroDNA; Griffiths et al. 2017), or its associated tributaries. Redfin were also not detected at other sites sampled during this study.

Salmonids were the most prevalent group of fish detected in this study (Appendix 1, Table A1). They were detected in every waterway and found at nearly every site sampled (46/50 sites). This dominance of salmonids was most pronounced at Tantangara Reservoir and its tributaries. The fish assay detected all three trout species known to be stocked within the study area (brown, rainbow and brook trout). Consistent with DPI fish stocking records (www.dpi.nsw.gov.au/fishing/recreational/resources/stocking - accessed 18th May 2018), both brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) were widely distributed throughout the study area, whereas brook trout (*Salvelinus fontinalis*) were restricted to Three Mile Creek, a tributary of Lake Eucumbene.

In addition to detecting DNA corresponding to the three trout species above, we also identified another salmonid genetic variant (haplotype) that shares 100% sequence identity with cutthroat trout reference DNA sequence. Cutthroat trout are not known to occur in Australia, and it is likely that the presence of this additional haplotype within the study area also corresponds to rainbow trout.

Cutthroat trout (*Oncorhynchus clarkii*) and rainbow trout (*O. mykiss*) are closely related sister species capable of interbreeding (Campton and Utter 1985; Young et al. 2001). It is therefore likely that the additional haplotype detected here is a shared haplotype between the species. This haplotype is therefore not included as a separate species in species counts (Appendix 1, Table A1).

Several other common invasive fish species were detected within the study area (Appendix 1, Table A1). Goldfish (*Carassius auratus*) and eastern mosquitofish (*Gambusia holbrooki*) had a mostly overlapping distribution; they were identified at many sites within Talbingo and Jounama and in the Murrumbidgee River at Cooma, downstream of Tantangara. Common carp (*Cyprinus carpio*) were also detected at the Murrumbidgee River at Cooma. Trace amounts of carp DNA (approx. 1% total site reads) was detected at one site within Talbingo Reservoir, at the entrance of Middle Creek. Given the invasive nature and high fecundity of carp, we would expect to detect the species at other sites if it was established in Talbingo Reservoir. This low-level detection may therefore be due to the transport of carp DNA via water or equipment (e.g. boats) from another location (as mentioned above in methods, uncertainty in low-level detections). Further sampling would be required through time to determine whether carp are indeed present within Talbingo.

There were also detections of additional non-native fish OTUs (up to 3 different OTUs) that could not be assigned adequately to species due to inadequate reference sequences. These all belonged to the Cypriniformes Order and were generally low-level detections (1%) at only a few sites and therefore must be regarded with a high level of uncertainty.

Native fish

There are several nationally threatened fish species that could potentially occur within the project area. Trout cod (*Maccullochella macquariensis*), Macquarie perch (*Macquaria australasica*) and Murray cod (*Maccullochella peelii*) are all known from the Murray-Darling system with historic records in the Murrumbidgee and Tumut drainages. More recently, stocking programs have also been undertaken at various locations in these drainages for each of these species. The recently described and critically endangered stocky galaxias (*Galaxias tantangara*) is also known from a single location in Tantangara Creek.

Neither trout cod nor Macquarie perch were detected by the fish assay. Murray cod were detected at a very low frequency in one sample from Jounama Pondage (Appendix 1, Table A1); they are known to have been stocked in Jounama by NSW DPI for the past five years at roughly equal numbers to the more widely detected brown and rainbow trout

(www.dpi.nsw.gov.au/fishing/recreational/resources/stocking - accessed 18th May 2018). These results are consistent with the limited distributions of these species.

Although once widespread within the Murrumbidgee catchment area, wild populations have declined severely due to overfishing, competition with introduced species and other anthropogenic effects, and are now patchily distributed (Allen et al. 2002; Lintermans 2002; Lintermans 2007). Golden perch (*Macquaria ambigua*), were detected at Jounama Pondage, where they are also stocked by NSW DPI, and also in a very low level detection in one sample from Talbingo reservoir. There is some uncertainty around these low level detections (at 1% or less, as discussed in methods). Additional survey work may be needed to confirm presence of species detected at this level or below.

The presence of several different galaxias species were identified within the study area (Appendix 1, Table A1), although results must be interpreted with caution given the close relationships of species within the *Galaxias* genus and lack of tissue samples from local populations to identify specific haplotypes. A haplotype that is common to several species in the mountain galaxias species complex was detected at Talbingo Reservoir. Based on the known distributions of the species within this complex, it is possible that the Talbingo individuals are either mountain galaxias (*Galaxias olidus*) and/or possibly obscure galaxias (*Galaxias oliros*) (Raadik 2014).

The climbing galaxias (*Galaxias brevipinnis*) was found within Gang Gang Creek and Wallaces Creek. Given the relatively high divergence of this species to other *Galaxias* species in the area within the selected marker region, we have high confidence that this haplotype represents the climbing galaxias. Gang Gang Creek represents part of the natural range of this species (Snowy River catchment), whilst it is possible that the results in Wallaces Creek are part of a translocated population, similar to those found in the Upper Murray River (Lintermans 2007).

A haplotype corresponding to the stocky galaxias (*Galaxias tantangara*) was detected within Tantangara Creek, Tantangara Reservoir, Kelly's Plain Creek and the Murrumbidgee River. This finding somewhat conflicts with the known distribution of the stocky galaxias, which has been described as being restricted to a small catchment (~4 km²) above Tantangara Creek Falls, approximately 4 km from the headwaters of the creek and 25 km upstream of Tantangara Reservoir (Raadik 2014; Unit 2017). The two Tantangara Creek sites included in the present study were approximately 5km and 10km downstream of this catchment area. The Kelly's Plain site and two of the three Murrumbidgee River sites that tested positive for the stocky galaxias haplotype were within the vicinity of Tantangara Creek/Reservoir, with the other positive Murrumbidgee site located approximately 100 km away, near Cooma. The observed haplotype may occur in other galaxias species (our reference database has haplotypes from five of the 15 known species within the mountain galaxias species complex to which the stocky galaxias belongs) and therefore we cannot be certain that these detections are indeed the stocky galaxias. Further work would be required to determine whether shared haplotypes exist within the mountain galaxias species (e.g. tissue samples and genetic/morphological identification). Similarly, some of the low detections may be due to the transport of DNA via water or other means (e.g. fish/bird predation and resulting faeces).

The two-spined blackfish (*Gadopsis bispinosus*), the flathead gudgeon (*Philypnodon grandiceps*) and the Australian smelt (*Retropinna semoni*) were all identified at a small number of sites at low DNA concentrations (Appendix 1, Table A1). The range of each of these species is known to include the study area (Allen et al. 2002; Lintermans 2007). The Australian smelt was also detected at 1% within Talbingo Reservoir, a very low level detection. Similarly, the Australian short-finned eel (*Anguilla australis*) was also detected at very low concentrations in Tantangara Reservoir and Talbingo Reservoir. This species is not considered native to the Murray-Darling basin, but there are occasional reports of it being present in the Upper Murrumbidgee (Lintermans 2007). Most occurrences are thought to be the result of translocations performed by anglers, but some may be due to rare natural dispersal events (Lintermans 2002; Lintermans 2007).

Decapod biodiversity

Three spiny crayfish taxa were identified within the study area (Appendix 1, Table A2). A haplotype representing Reik's crayfish (*Euastacus reiki*) was detected at a number of sites within the Tantangara Reservoir and Eucumbene catchments. The Murray crayfish (*Euastacus armatus*) was restricted to the Yarrangobilly subcatchment including sites in Wallaces Creek, Yarrangobilly River and Talbingo Reservoir at the inflow of the Yarrangobilly River. We detected an additional *Euastacus* haplotype at Wallace Creek that appears to be distinct from those corresponding to the Murray and the Reik's crayfish haplotype. Only three *Euastacus* species are known to occur within the study area (Morgan 1997; Shull et al. 2005),

and therefore it is possible that this haplotype corresponds to the Alpine spiny crayfish (*Euastacus crassus*). Unfortunately, this species is not represented in our reference DNA database. This species is also known to be closely related to Reik's crayfish (Shull et al. 2005), and therefore reference material is needed to confirm its presence, and to also confirm that it does not share a haplotype with Reik's crayfish.

The common yabby (*Cherax destructor*) was detected within both Talbingo and Tantangara reservoirs, Jounama Pondage and several creek/river sites (Appendix 1, Table A2). Although this species is widespread within the inland waterways of NSW, its natural range is generally restricted to low-mid altitudes. The presence of the yabby within the study area is presumably the result of one or more translocation events. Similar translocated yabby populations are known to exist in Lake Eucumbene and Lake Jindabyne. There is a risk these translocated populations may pose a threat to endemic crayfish species (Coughran and Daly 2012; Coughran et al. 2009; Hazlett et al. 2007).

The freshwater glass shrimp (*Paratya australiensis*) was found at Talbingo Reservoir, Jounama Pondage and within Middle Creek and the Murrumbidgee River at Cooma (Appendix 1, Table A2). We detected an additional shrimp from the family Palaemonidae within the Murrumbidgee, but were unable to identify it to species level. Our reference DNA sequence library does not currently contain data for all shrimp species that may occur within the study area. It is possible that there were other species present that we were unable to detect as a consequence. As more reference material/sequences become available, then more species may be detected in the current dataset from sites.

Conclusion

These results highlight the power of eDNA metabarcoding for identifying multiple species within taxonomic groups (fish and decapods) and provide a measure of biodiversity over a large spatial scale. The variability of the marker region used in this study was generally adequate for identifying a broad range of fish and decapod species. However, sequence variability within and between species can make it difficult to resolve closely related species such as found in the mountain galaxias species complex. Similarly, it is important that reference sequence data is available for correct taxonomic assignment. Species resolution is expected to increase in the future as more genetic data becomes available at the species and local haplotype level. The results from this project provide valuable baseline data on fish and decapod species within the Snowy Mountains region.

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Appendix 1. Fish and decapod eDNA metabarcoding results

Table A1. Summary of results from the fish multispecies assay, with species counts for each site (native and non-native), and site occupancy for each species. Numbers specify the relative abundance (%) of the DNA sequence reads for each species detected (averaged across site replicates). DNA abundance estimates for species are semi-quantitative only as estimates can be affected by life stage, shedding and DNA degradation rates, and environmental factors. *This Galaxias haplotype matched the Stocky galaxias haplotype in our reference database, but could be a shared haplotype with other species from the mountain galaxias species complex. **Cutthroat trout are likely to be rainbow trout (as discussed in main text) and have been treated as a single species in species counts. Catchments – TA (Tantangara), bTA (below Tantangara), EU (Eucumbene), TB (Talbingo), bTB (below Talbingo).

Site	Waterway	CATCHMENT	Australian shortfin eel	two-spined blackfish	Murray cod	golden perch	mountain galaxias species complex	climbing galaxias	Galaxias haplotype*	flathead muddeon	Australian smelt	goldfish	common carp	Cypriniforme OTU1	Cypriniforme OTU2	eastern mosquitofish	Cypriniforme OTU3	redfin	rainbow trout	cutthroat trout**	brown trout	brook trout	Total species	Native species	Non-native species
25	Goodradigbee Weir	TA																	33	67			1	0	1
6	Mufflers Creek	TA																	7	11	82		2	0	2
15	Ghost Gully Creek	TA																	16		84		2	0	2
17	Bally Creek	TA																	7	10	83		2	0	2
9b	Murrumbidgee River	TA																	11	13	77		2	0	2
5a	Gooandra Creek	TA													5					78	17		2	0	2
8a	Tantangara Creek	TA																	28	14	57		2	0	2
22	Tantangara Creek	TA							7										4	17	71		3	1	2
9a	Murrumbidgee River	TA							6						1				6	12	76		4	1	3
M1	Murrumbidgee River/ Tantangara	TA																	3	7	89		2	0	2
16	Nungar Creek	TA																	2	5	93		2	0	2
23	Kellys Plain Creek	TA																	14	80	6		3	0	3

Fish and decapod eDNA biodiversity surveys in the Snowy 2.0 project area

Site	Waterway	CATCHMENT	Australian shortfin eel	two-spined blackfish	Murray cod	golden perch	mountain galaxias species complex	climbing galaxias	Galaxias haplotype*	flathead audeon	Australian smelt	goldfish	common carp	Cypriniforme OTU1	Cypriniforme OTU2	eastern mosquitofish	Cypriniforme OTU3	redfin	rainbow trout	cutthroat trout**	brown trout	brook trout	Total species	Native species	Non-native species
7	Kellys Plain Creek	TA							3										13	12	72		3	1	2
Tan1	Tantangara Reservoir	TA																		13	87		1	0	1
Tan2	Tantangara Reservoir	TA																		21	73		1	0	1
Tan3	Tantangara Reservoir	TA																	12	8	80		2	0	2
Tan4a	Tantangara Reservoir	TA	<1																13	11	76		3	1	2
Tan4b	Tantangara Reservoir	TA																	4	13	82		2	0	2
Tan5	Tantangara Reservoir	TA							1										11	41	47		3	1	2
Tan6	Tantangara Reservoir	TA							1										4	4	88		3	1	2
Tan7	Tantangara Reservoir	TA							<1										22	33	45		3	1	2
Tan8	Tantangara Reservoir	TA																	19	23	58		2	0	2
Tan9	Tantangara Reservoir	TA							5										13	16	66		3	1	2
26	Murrumbidgee River	bTA							53										19	7	21		3	1	2
28	Murrumbidgee River	bTA							1			5	89			4			1				5	1	4
14	Eucumbene River	EU								4									3	1	91		3	1	2
24	Eucumbene River	EU																			97		1	0	1
12	Three Mile Creek	EU																	47	34	13	6	3	0	3

Fish and decapod eDNA biodiversity surveys in the Snowy 2.0 project area

Site	Waterway	CATCHMENT	Australian shortfin eel	two-spined blackfish	Murray cod	golden perch	mountain galaxias species complex	climbing galaxias	Galaxias haplotype*	flathead	Australian smelt	goldfish	common carp	Cypriniforme OTU1	Cypriniforme OTU2	eastern mosquitofish	Cypriniforme OTU3	redfin	rainbow trout	cutthroat trout**	brown trout	brook trout	Total species	Native species	Non-native species
21	Gang Gang Creek	EU						1		<1									6	72	11		4	2	2
2b	Wallaces Creek	TB						2											51	19	27		3	1	2
2a	Wallaces Creek	TB																	33	6	60		2	0	2
27	Yarrangibilly River	TB																20	40	9	31		3	0	3
1a	Yarrangibilly River	TB		3														6	45	8	38		4	1	3
Tal1	Talbingo Reservoir	TB		2								34				<1		11	31	7	15		6	1	5
Tal2	Talbingo Reservoir	TB										76				3		21			<1		4	0	4
Tal3	Talbingo Reservoir/ Middle Creek	TB										29				39		32					3	0	3
Tal4	Talbingo Reservoir	TB										<1				3		37	1		59		5	0	5
Tal5	Talbingo Reservoir	TB		5			10											86					3	2	1
Tal6	Talbingo Reservoir	TB		1		<1				<1		4	1			8		82			3		8	3	5
Tal7	Talbingo Reservoir	TB										4				1	1	91			4		5	0	5
Tal8	Talbingo Reservoir	TB								2		29		<1		8		61					5	1	4
Tal9	Talbingo Reservoir	TB										25				3		70	1				4	0	4
Tal10	Talbingo Reservoir	TB										63			<1	2		35					4	0	4
Tal11	Talbingo Reservoir	TB										30						70			<1		3	0	3
Tal12	Talbingo Reservoir	TB	<1				<1				1	30						67	1				6	3	3
13	O'Hares Creek	TB																	56	<1	43		2	0	2
Joun1	Jounama Pondage	bTB										26				<1		58	2	4	9		5	0	5
Joun2	Jounama Pondage	bTB		<1		1						26			<1	3		62	<1	<1	8		8	2	6
Joun3	Jounama Pondage	bTB				1						20				5		40	2		32		6	1	5
Joun4	Jounama Pondage	bTB			<1	1				1		9				34		43	2	2	8		8	3	5
	Occupancy (% sites detected)		4	10	2	8	4	4	18	1	2	32	4	2	8	28	2	36	76	70	84	2			

Table A2. Summary of results from the decapod multispecies assay, with species counts for each site, and site occupancy for each species. Numbers specify the relative abundance (%) of the DNA sequence reads for each species detected (averaged across site replicates). DNA abundance estimates for species are semi-quantitative only as estimates can be affected by life stage, shedding and DNA degradation rates, and environmental factors. At many sites, the sum of the abundance values is less than 100%, due to the presence of unassigned reads (not shown). Catchments – TA (Tantangara), bTA (below Tantangara), EU (Eucumbene), TB (Talbingo), bTB (below Talbingo).

Site	Waterway	CATCHMENT	freshwater glass shrimp	family Palaemonidae	common yabby	Murray crayfish	Reik's crayfish	unidentified spiny crayfish	Species
25	Goodradigbee Weir	TA					15		1
6	Mufflers Creek	TA					48		1
15	Ghost Gully Creek	TA					84		1
17	Bally Creek	TA					1		1
9b	Murrumbidgee River	TA					7		1
5a	Gooandra Creek	TA					13		1
8a	Tantangara Creek	TA					16		1
22	Tantangara Creek	TA					4		1
9a	Murrumbidgee River	TA					17		1
M1	Murrumbidgee River/ Tantangara Reservoir	TA			10		7		2
16	Nungar Creek	TA					1		1
23	Kellys Plain Creek	TA					2		1
7	Kellys Plain Creek	TA			20		18		2
Tan1	Tantangara Reservoir	TA			1				1
Tan2	Tantangara Reservoir	TA							0
Tan3	Tantangara Reservoir	TA			17				1
Tan4a	Tantangara Reservoir	TA			2				1
Tan4b	Tantangara Reservoir	TA			16				1
Tan5	Tantangara Reservoir	TA					1		1
Tan6	Tantangara Reservoir	TA			3		3		2
Tan7	Tantangara Reservoir	TA					1		1
Tan8	Tantangara Reservoir	TA			2				1
Tan9	Tantangara Reservoir	TA			3				1
26	Murrumbidgee River	bTA							0
28	Murrumbidgee River	bTA	29	4	22				3
14	Eucumbene River	EU					1		1
24	Eucumbene River	EU					24		1
12	Three Mile Creek	EU			25				1
21	Gang Gang Creek	EU					20		1
2b	Wallaces Creek	TB				5		4	2
2a	Wallaces Creek	TB				8			1
27	Yarrangibilly River	TB				3			1

Fish and decapod eDNA biodiversity surveys in the Snowy 2.0 project area

Site	Waterway	CATCHMENT	freshwater glass shrimp	family Palaemonidae	common yabby	Murray crayfish	Reik' s crayfish	unidentified spiny crayfish	Species
1a	Yarrangibilly River	TB				8			1
Tal1	Talbingo Reservoir	TB				1			1
Tal2	Talbingo Reservoir	TB							0
Tal3	Talbingo Reservoir/ Middle Creek	TB	1						1
Tal4	Talbingo Reservoir	TB	3		2				2
Tal5	Talbingo Reservoir	TB							0
Tal6	Talbingo Reservoir	TB	31		5				2
Tal7	Talbingo Reservoir	TB							0
Tal8	Talbingo Reservoir	TB	14		2				2
Tal9	Talbingo Reservoir	TB	20						1
Tal10	Talbingo Reservoir	TB							0
Tal11	Talbingo Reservoir	TB			3				1
Tal12	Talbingo Reservoir	TB	27						1
13	O' Hares Creek	TB							0
Joun1	Jounama Pondage	bTB	1		3				2
Joun2	Jounama Pondage	bTB							0
Joun3	Jounama Pondage	bTB							0
Joun4	Jounama Pondage	bTB			1				1
	Occupancy (% sites detected)		16	2	33	10	39	2	



Determining the presence of *Perca fluviatilis*, *Gambusia holbrooki*, *Galaxias brevipinnis* and *Macquaria australasica* across a range of locations within the Snowy Hydro region using environmental DNA

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Abbreviations

Abbreviations	Description
eDNA	environmental DNA
qPCR	quantitative polymerase chain reaction
EIS	Environmental Impact Statement

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Executive Summary

As part of environmental investigations for the Snowy 2.0 project, Snowy Hydro engaged EnviroDNA to undertake environmental DNA surveys for the presence of four species of fish at selected sites within the Snowy Mountains. The four species included two invasive species (*Perca fluviatilis* and *Gambusia holbrooki*) and two native species (*Macquaria australasica* and *Galaxias brevipinnis*). We developed and validated target single species quantitative PCR assays for *G. holbrooki*, *M. australasica* and *G. brevipinnis* and used a previously developed assay for *P. fluviatilis*. A total of 397 water samples were collected from 93 sites within the Main Works area, with 364 tested for the presence of both *P. fluviatilis* and *G. holbrooki* DNA, 33 tested for the presence of *M. australasica* DNA and 129 tested for the presence of *G. brevipinnis* DNA. Six sites were positive for *P. fluviatilis* DNA, 10 sites were positive for *G. holbrooki* DNA, two sites were positive for *G. brevipinnis* DNA and three sites were positive for *M. australasica* DNA.

Background

The Snowy 2.0 project involves the expansion of Snowy Hydro's hydro-electric storage capacity. The first stage of the project, Exploratory Works, has been approved and work has commenced. The approval process for Snowy 2.0 Main Works is now underway and involves a comprehensive Environmental Impact Statement (EIS). As part of this Snowy Hydro has sought to confirm the presence of a number of invasive and threatened fish species in seven reservoirs/dams and several river locations. Species for presence/absence assessment include:

- Redfin perch (*Perca fluviatilis*)
- Eastern mosquito fish (*Gambusia holbrooki*)
- Macquarie Perch (*Macquaria australasica*)
- Climbing galaxid (*Galaxias brevipinnis*)

Environmental DNA (eDNA) is a non-invasive sampling technique that detects traces of genetic material from a target species secreted into its surrounding environment (water). Quantitative comparisons with traditional sampling methods have demonstrated that eDNA methods can be superior in terms of sensitivity and cost efficiency, particularly for scarce, elusive or cryptic species (Biggs *et al.* 2015; Smart *et al.* 2015; Weeks *et al.* 2015), enabling effective detection of species at low densities and at all life stages. During this project, eDNA techniques were used to investigate the presence of the above four species. The following locations were surveyed:

- Lake Eucumbene
- Tumut Pond Reservoir
- T2 Dam
- Geehi Reservoir
- M2 Dam
- Jindabyne Reservoir
- Tantangara Reservoir
- Selected river sites between reservoirs
- Upper Murrumbidgee River below Tantangara Dam

Tantangara Reservoir was previously surveyed for *P. Fluviatilis* using eDNA in 2017, with all samples testing negative. The survey in 2019 provides further confidence surrounding assessment of the presence/absence of this species at this location.

Methods

Water sampling

Survey locations were advised by Snowy Hydro. Sampling was conducted by EnviroDNA with staff from Snowy Hydro between the 12th – 19th March 2019 using a boat (Lake Eucumbene, Jindabyne Reservoir, Tantangara Reservoir) or from the edge of waterways. Table 1 lists

sampling locations and the number of samples taken at each location for this project. Figure 1 shows each site where eDNA samples were collected for each location (see Appendix 1 for site names, locations and GPS coordinates of each sampling site).

At each sampling site, water samples were collected and filtered *in situ* by passing up to 500 ml water (range 40-500 ml) through a 0.22 µm filter (Sterivex) using a sterile syringe. Between 3 – 5 replicate samples were taken at any given site. Note: 6 samples were taken at Upper Murrumbidgee river sites, however only 3 at each site were analysed for this project.

Care was taken to minimise contamination between sites by using clean equipment and gloves at each site and avoiding the transfer of water, soil, or organic matter. Filters were frozen immediately in a portable car freezer before being transported to the laboratory for processing.

Table 1. Sampling locations, water sample numbers and species tested per samples.

Sampling location	# water samples	Species to be tested for at each location			
		<i>P. fluviatilis</i>	<i>G. holbrooki</i>	<i>G. brevipinnis</i>	<i>M. australasica</i>
Lake Eucumbene	99	yes	yes	Selected sites: LE01, LE02, LE03, LE06, LE07, LE12, LE20	no
Tumut Pond Reservoir	20	yes	yes	One site: TPR01	no
T2 Dam	15	yes	yes	One site: T2D01	no
Geehi Reservoir	18	yes	yes	One site: GR02	no
M2 Dam	15	yes	yes	no	no
Jindabyne Reservoir	84	yes	yes	One site: JR19	no
Tantangara	52	yes	yes	yes	no
9 river sites between reservoirs	28	yes	yes	no	no
Upper Murrumbidgee (11 sites)	33	yes	yes	yes	yes
Total samples	364				

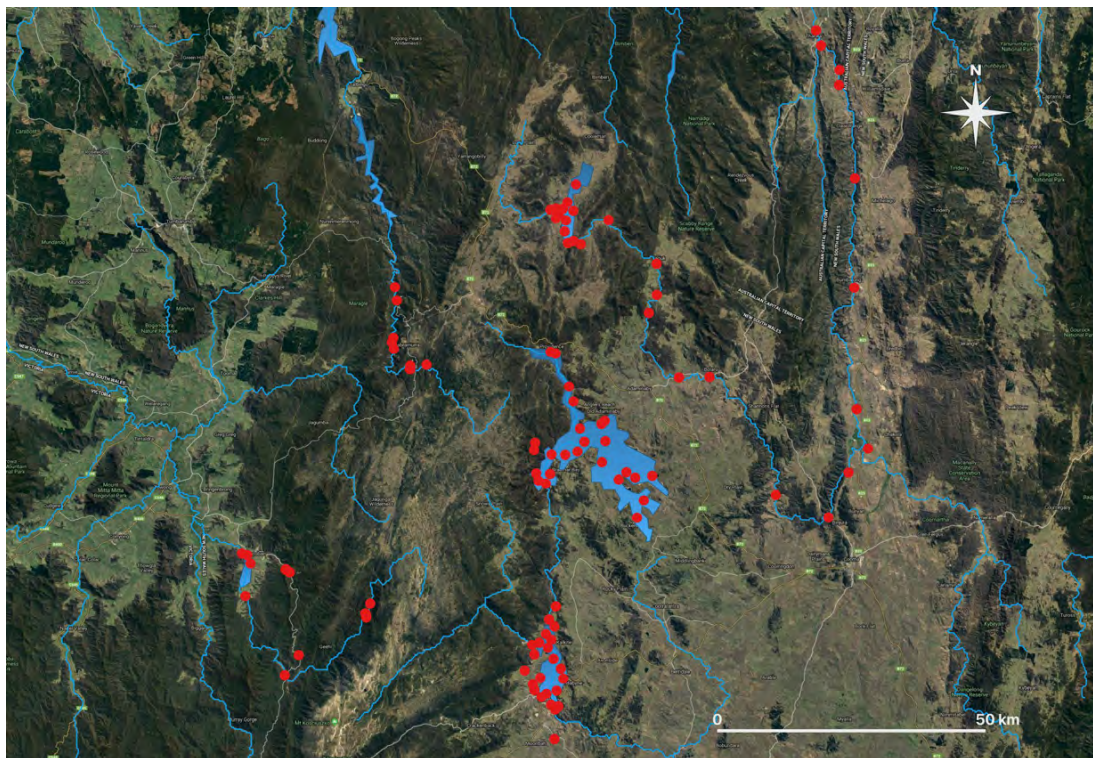


Figure 1. Location of sampling sites (see Appendix 1 for further site information).

EnviroDNA water sampling methodology for this project

EnviroDNA water sampling methodology undertaken for this Snowy Hydro project has been published by members of EnviroDNA's team (e.g. Lugg *et al.* 2018; Tingley *et al.* 2018), as well as others (e.g. Spens *et al.* 2016). Key aspects of this methodology include:

- **Water collection:** EnviroDNA filters water onsite, with no need to transport water, avoiding contamination risks and logistical challenges in remote areas. A Sterivex® filter cartridge is attached to a syringe that has been filled with sample water. The filter paper is enclosed within a plastic cartridge and cannot be opened or touched, avoiding the risk of contamination. Generally the maximum amount of water filtered per sample/cartridge in EnviroDNA's standard method is 500 ml. Given the proven sensitivity of this method, it was undertaken for this project. A previous project for Snowy Hydro used both EnviroDNA's standard water sampling approach in reservoirs, as well as a large 1 μ M filter which has greater throughput of water (between 10L-16L was collected for any one filter) due to the larger pore size. The results did not differ between filters, highlighting the sensitivity of EnviroDNA's standard water sampling approach.
- **Water filtration:** The filter paper pore size is 0.22 μ m, helping to yield the highest eDNA capture rate when compared to other filter paper pore sizes (Eichmiller *et al.* 2016; Spens *et al.* 2016; Li *et al.* 2018).

- *Water volume & number of samples:* A range of peer reviewed eDNA sensitivity studies using EnviroDNA's water collection and filtration method have been published for platypuses, mosquitoes, frogs, fish, newts and cane toads. Research has shown that the method is extremely sensitive.

Other key aspects to Snowy Hydro's eDNA water sampling regime include:

- *Where & when to sample:* This project targeted four different fish species (two invasives and two natives) on one eDNA sampling occasion at a number of different sites (rivers/streams and reservoirs) as requested by Snowy Hydro. Sampling was restricted to autumn 2019. EnviroDNA water sampling was targeted to relevant species ecology and behaviour where possible. For example, sampling sites within reservoirs were focused around edge habitat, tributary inlets and where suitable habitat is identified for *P. fluviatilis* and *G. holbrooki* (e.g. vegetation, rocks, snags). In river and stream locations, sampling focused on *G. brevipinnis* and *M. australasica* preferences where possible (e.g. clear fast-flowing shady streams with rocks, boulders and logs for *G. brevipinnis*. As well as clear and deep, rocky holes with lots of cover like vegetation, boulder and other debris for *M. australasica*).
- *How often to sample:* for this project, sampling was restricted to one sampling occasion. However, Tantangara reservoir has previously been sampled for *P. fluviatilis* using the same technique in 2017.

Target species assays

DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen DNeasy Blood and Tissue Kit). A species-specific marker and assay for *P. fluviatilis* had already been developed and validated in the laboratory and field during a previous Snowy Hydro project (Griffiths *et al.* 2017). For the remaining three species (*G. holbrooki*, *G. brevipinnis* and *M. australasica*), a species-specific marker targeting either the 12S, COI or control region of the mitochondrial genome of each species was developed by EnviroDNA using a combination of publicly available genetic data from NCBI GenBank and published literature (Waters *et al.* 2002; Ayres *et al.* 2012; Bylemans *et al.* 2017). The assays were validated in the laboratory, including specificity testing on a range of non-target fish species, which indicated the assays to be specific. For example, the *G. brevipinnis* assay was screened against tissue samples from a range of non-target species from the region; *Galaxias supremus* (Carruthers creek), *Galaxias tantangara* (Tantangara creek), and *Galaxias olidus* (Larrys Creek and Murrumbidgee River). This assay was also validated for specificity against a *G. brevipinnis* tissue sample sourced from Yarrangobilly River in the Snowy Mountains region.

Using a PrimeTime® probe assay (Integrated DNA Technologies), real-time quantitative polymerase chain reaction (qPCR) was carried out on samples to amplify the target DNA. All samples were screened in triplicate. Negative controls were included at both the extraction and qPCR stages so that laboratory contamination could be identified if present; no

contamination was found. For *G. brevipinnis*, we also screened samples from two sites in the project area that tested positive for this species in a previous eDNA metabarcoding study (see Robinson *et al.* 2019) as potential positive controls for this assay – discussed further in Findings.

For this project we consider three possible outcomes for testing based on single species target assays using qPCR across samples at the site level; 'negative' for the target species DNA (no qPCR technical replicates positive out of all samples tested), 'positive' for the target species DNA (3 or more qPCR positives per site) or an 'equivocal' result (2 or less qPCR positives per site). Having an 'equivocal' category can be considered somewhat conservative (see Hyatt *et al.* 2007) but can avoid the chance of false positives. As with many survey methods (e.g. aural detections, point-count surveys), there is potential for one or more species to be detected at an unoccupied site (i.e. false positive detection) with eDNA sampling. In the field setting, false positives can enter the detection process via several pathways; sample contamination (e.g. introduction of species DNA from another site caused by humans or another animal), eDNA transport (e.g. natural transport of eDNA downstream in lotic systems), and prolonged eDNA persistence after the extirpation of a species at a site (e.g. a species' DNA in the environment could be sourced from a live or dead organism). Resampling the area can increase the level of confidence in results if this was required. With laboratory quality control protocols followed the risk of false positives in the lab is generally minimal. However, background amplification of non-target DNA can sometimes occur very late in a qPCR (see Rees *et al.* 2014), which can give rise to a false positive. Our equivocal category is used for this reason.

Findings

Perca fluviatilis

We screened a total of 364 samples for the presence of *P. fluviatilis*. All samples from Tantangara Reservoir ($n = 52$), Lake Eucumbene ($n = 99$) and Jindabyne Reservoir ($n = 84$) tested negative for the presence of *P. fluviatilis* DNA. Similarly, all samples from Geehi Reservoir ($n = 18$), Tumut Pond Reservoir ($n = 20$), T2 Dam ($n = 15$), M2 Dam ($n = 15$), Mowamba Weir Pool ($n = 4$), and Bogong Creek ($n = 3$) also tested negative for *P. fluviatilis* DNA. One site each out of two at Tumut River (TR01) and Swampy Plains River (SPC02) tested positive for *P. fluviatilis*, with all replicates ($n = 3$) from these sites being positive. All three sites at Khancoban Reservoir also tested positive in all replicates taken at these sites. One site in the Murrumbidgee River tested positive (MR12) and two sites (MR15 and MR16) returned equivocal results out of the 11 sites tested in this system.

The negative results for Tantangara Reservoir were consistent with previous eDNA results for this reservoir in Griffiths *et al.* (2017).

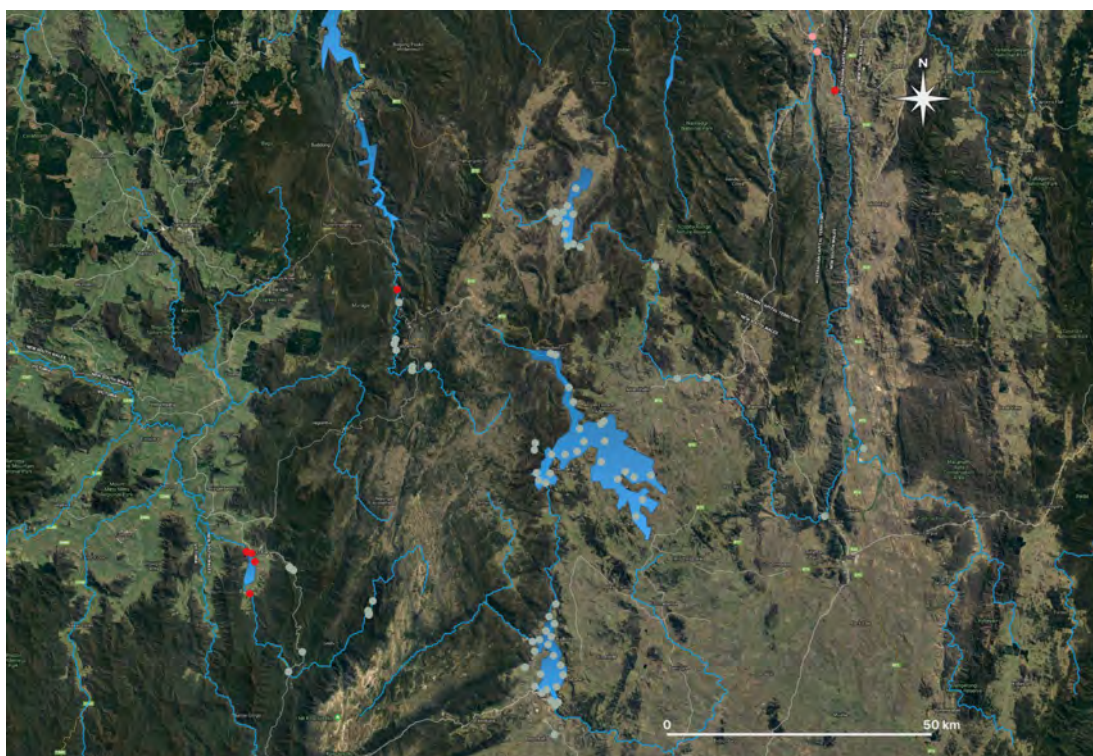


Figure 2. Site locations of eDNA testing and detections for *P. fluviatilis* from March 2019 sampling. Red circles indicate positive results, closed grey circles indicate negative results, and closed pink circles indicate equivocal results.

Gambusia holbrooki

We screened the same 364 samples for the presence of *G. holbrooki* DNA, with a total of 10 sites testing positive, 1 site returning an equivocal result and all other sites testing negative. Positive sites were largely from the Murrumbidgee River (9 out of 11 sites positive), with only 1 site in Lake Eucumbene (LE01) testing positive. The positive site at Lake Eucumbene was a weak level positive (only 3 replicates out of 12 technical replicates from four samples), indicating a likely low abundance of *G. holbrooki* at this site and more generally in Lake Eucumbene. One site from Jindabyne Reservoir (JR05) returned an equivocal result (2 out of 12 technical replicates were positive) indicating either a very low level detection at this site or a false positive. Ideally this site would be subject to follow up testing at a different time point to confirm the presence or absence of *G. holbrooki* from this site. The positive sites found in the Murrumbidgee River is consistent with the previous results from Robinson *et al.* (2019) who also found *G. holbrooki* in the Murrumbidgee River with an eDNA metabarcoding survey.

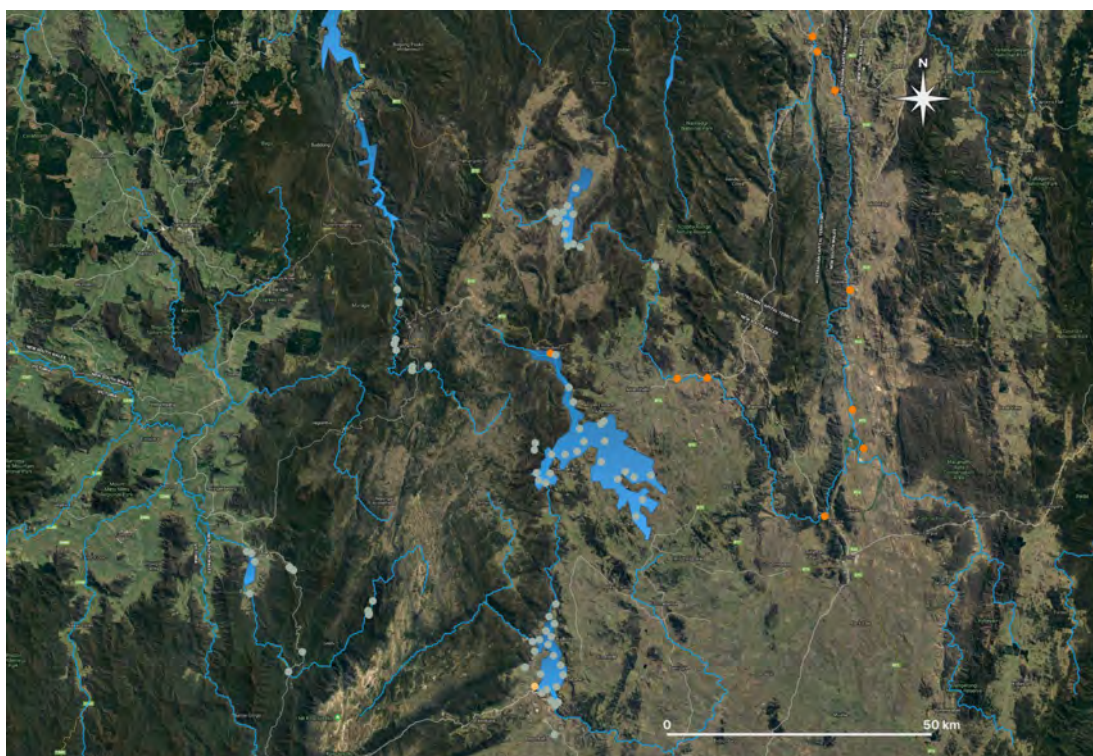


Figure 3. Site locations of eDNA testing and detections for *G. Holbrooki* from March 2019 sampling. Orange circles indicate positive results, closed grey circles indicate negative results, and yellow circles indicate equivocal results.

Macquaria australasica

We tested for *M. australasica* in samples from the Murrumbidgee River (11 sites, 33 samples). Three sites were found to be positive for *M. australasica* DNA (MR06, MR08, MR11), one site was equivocal (MR06a, sampled close to the positive MR06 site) and seven sites were negative. This species is known to be present in the Murrumbidgee River, with historical records, and more recently stocking programs undertaken in this system. The highest concentration of DNA was found at site MR08 (with 7 out of 9 technical replicates from the 3 samples testing positive), indicating a relatively higher amount of *M. australasica* DNA at this site relative to other positive sites. Over all positive sites, however, detections were relatively low in terms of DNA concentration and technical replicates returning positive results. All detections were in a somewhat similar stretch of the Murrumbidgee River (the two remaining sites MR09a and MR10, tested negative).

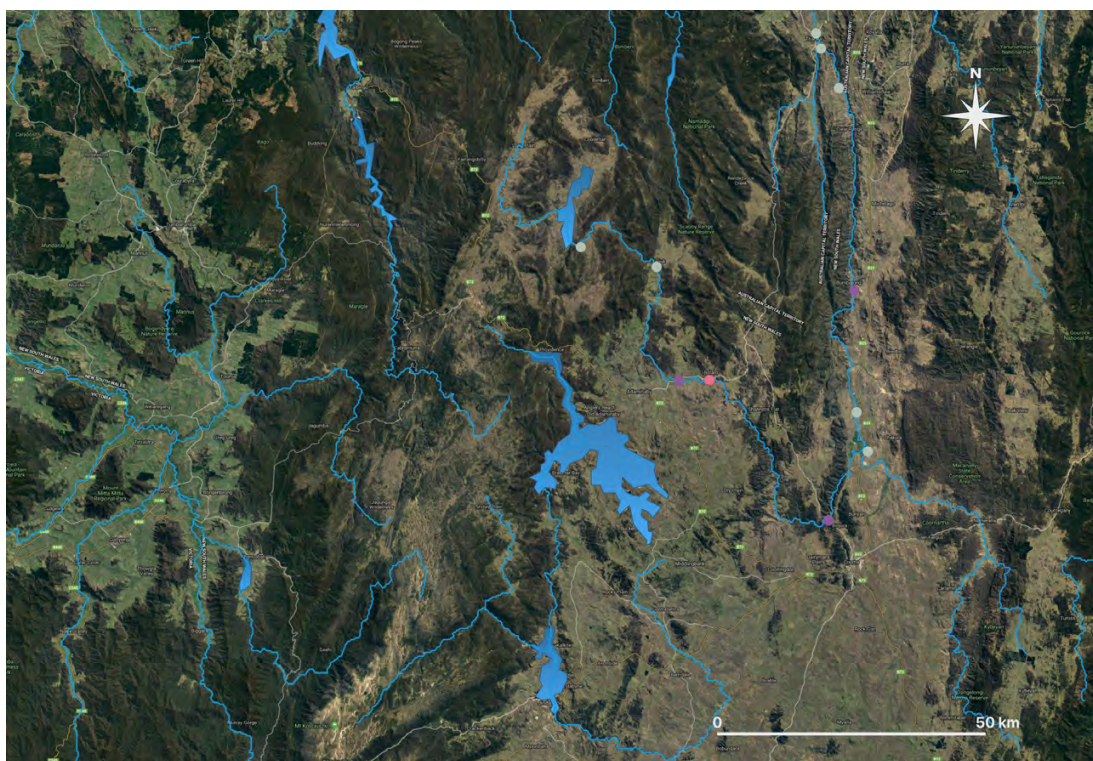


Figure 4. Site locations of eDNA testing and detections for *M. australasica* from March 2019 sampling. Purple circles indicate positive results, grey indicate negative results, and pink circles indicate equivocal results.

Galaxias brevipinnis

We tested samples from 35 sites ($n = 129$ samples) for the presence of *G. brevipinnis* DNA with our assay. Only two sites tested positive; one in Jindabyne Reservoir (JR19, 5 out of 12 replicates from 4 samples) and one in the Geehi Reservoir (GR02, 7 out of 9 replicates from 3 samples). Two sites returned equivocal results; both in Lake Eucumbene (LE01 and LE12; 1/12 and 2/9 respectively for replicate qPCRs). All other sites (31 sites) tested negative, including 5 sites ($n = 20$ samples) from Lake Eucumbene, all 13 sites ($n = 52$ samples) from Tantangara Reservoir, and sites tested from Tumut Pond Reservoir (1 site), T2 Dam (1 site) and the Murrumbidgee River (11 sites).

We also tested samples collected in February 2018 from two sites from a previous study in this system that had returned positive results for *G. brevipinnis* using eDNA metabarcoding (sites 21 and 2b; Robinson *et al.* 2019). For the eDNA metabarcoding study, *G. brevipinnis* was detected in all 3 samples at site 21 with an average frequency of 11%, whereas for site 2b, *G. brevipinnis* was only detected in 1 sample at an average frequency of 2%. Using our single species qPCR assay, site 21 (Gang Gang Creek) returned a positive result for *G. brevipinnis* DNA (8/9 qPCRs from three samples), while site 2b (Wallaces Creek) returned a negative result (0/9 qPCRs from three samples). Site 2b does not present a surprising result given the time period from sampling, the freeze thawing of the extracted DNA from the previous study and the likely further degradation of DNA in these samples.

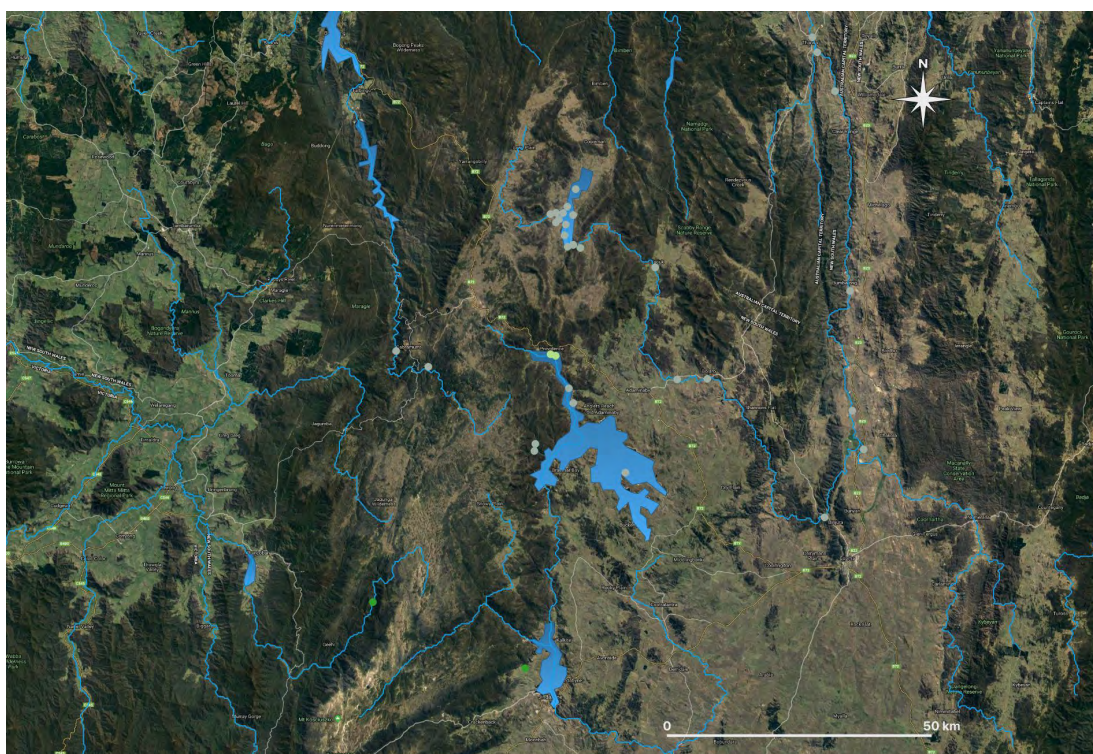


Figure 5. Site locations of eDNA testing and detections for *G. brevipinnis* from March 2019 sampling. Dark green circles indicate positive results, closed grey circles indicate negative results, and light green circles indicate equivocal results.

Conclusions

In this project we used targeted single species qPCR assays and eDNA water sampling at a range of sites in the Snowy Hydro catchment area to determine the presence/absence of DNA from four species; *P. fluviatilis*, *G. holbrooki*, *M. australasica* and *G. brevipinnis*. We developed and validated assays for the latter three species and used the same assay as in Furlan and Gleeson (2016) for *P. fluviatilis*. A total of 397 water samples from 93 sites were collected and DNA extracted, with 364 tested for the presence of the invasive species *P. fluviatilis* and *G. holbrooki*, 33 tested for the presence of the native *M. australasica*, and 129 tested for the presence of the native *G. brevipinnis*. The assays detected each species, although each was detected at only a limited number of sites within the Snowy Hydro project area.

Acknowledgments

We thank Elizabeth Pope (Snowy Hydro) for project support, Mic Clayton (Snowy Hydro) for help with water sampling field work, and Tarmo Raadik (Arthur Rylah Institute) for providing tissue samples to assist with validation of the *Galaxias brevipinnis* probe/assay.

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Appendix 1. Site/sampling details and target species detection results

Site	Waterway	Latitude	Longitude	Collection date	# samples	<i>Perca fluviatilis</i>	<i>Gambusia holbrooki</i>	<i>Galaxias brevipinnis</i>	<i>Macquaria australasia</i>
LE01	Lake Eucumbene	-35.9460225	148.6253995	15/3/19	4	negative	positive (3/12)	equivocal (1/12)	not tested
LE02	Lake Eucumbene	-35.9929378	148.6564595	12/3/19	4	negative	negative	negative	not tested
LE03	Lake Eucumbene	-36.0131322	148.663967	12/3/19	4	negative	negative	negative	not tested
LE04	Lake Eucumbene	-36.0499293	148.6747942	12/3/19	4	negative	negative	not tested	not tested
LE05	Lake Eucumbene	-36.0436375	148.7107443	13/3/19	4	negative	negative	not tested	not tested
LE06	Lake Eucumbene	-36.0693897	148.5994971	13/3/19	4	negative	negative	negative	not tested
LE07	Lake Eucumbene	-36.0791713	148.5978233	13/3/19	4	negative	negative	negative	not tested
LE08	Lake Eucumbene	-36.0849981	148.6271774	13/3/19	4	negative	negative	not tested	not tested
LE09	Lake Eucumbene	-36.1105906	148.601378	13/3/19	4	negative	negative	not tested	not tested
LE10	Lake Eucumbene	-36.1207136	148.6052404	13/3/19	4	negative	negative	not tested	not tested
LE11	Lake Eucumbene	-36.1244575	148.6174713	13/3/19	4	negative	negative	not tested	not tested
LE12	Lake Eucumbene	-35.9481765	148.6339846	15/3/19	3	negative	negative	equivocal (2/9)	not tested
LE13	Lake Eucumbene	-36.1114655	148.6246455	13/3/19	4	negative	negative	not tested	not tested
LE14	Lake Eucumbene	-36.0854531	148.6500635	13/3/19	4	negative	negative	not tested	not tested
LE15	Lake Eucumbene	-36.0811176	148.670625	13/3/19	4	negative	negative	not tested	not tested
LE16	Lake Eucumbene	-36.0677632	148.6821693	13/3/19	4	negative	negative	not tested	not tested

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Site	Waterway	Latitude	Longitude	Collection date	# samples	<i>Perca fluviatilis</i>	<i>Gambusia holbrooki</i>	<i>Galaxias brevipinnis</i>	<i>Macquaria australasia</i>
LE17	Lake Eucumbene	-36.0669654	148.7174027	13/3/19	4	negative	negative	not tested	not tested
LE18	Lake Eucumbene	-36.1165796	148.7673276	13/3/19	4	negative	negative	not tested	not tested
LE20	Lake Eucumbene	-36.1089526	148.7525648	13/3/19	4	negative	negative	Negative	not tested
LE21	Lake Eucumbene	-36.1472899	148.7817686	13/3/19	4	negative	negative	not tested	not tested
LE22	Lake Eucumbene	-36.1140319	148.7959523	13/3/19	4	negative	negative	not tested	not tested
LE23	Lake Eucumbene	-36.1706122	148.7704679	13/3/19	4	negative	negative	not tested	not tested
LE26	Lake Eucumbene	-36.1190948	148.7397213	13/3/19	4	negative	negative	not tested	not tested
LE29	Lake Eucumbene	-36.0955299	148.7116361	13/3/19	4	negative	negative	not tested	not tested
LE30	Lake Eucumbene	-36.0394711	148.7163141	13/3/19	4	negative	negative	not tested	not tested
JR01	Jindabyne Reservoir	-36.4298784	148.6331805	16/3/19	4	negative	negative	not tested	not tested
JR02	Jindabyne Reservoir	-36.4233777	148.6264515	16/3/19	4	negative	negative	not tested	not tested
JR03	Jindabyne Reservoir	-36.4254368	148.6385429	16/3/19	4	negative	negative	not tested	not tested
JR04	Jindabyne Reservoir	-36.4129182	148.6104226	16/3/19	4	negative	negative	not tested	not tested
JR05	Jindabyne Reservoir	-36.4036457	148.5985779	16/3/19	4	negative	equivocal (2/12)	not tested	not tested
JR06	Jindabyne Reservoir	-36.3879886	148.6467968	18/3/19	4	negative	negative	not tested	not tested
JR07	Jindabyne Reservoir	-36.3615032	148.6305534	16/3/19	4	negative	negative	not tested	not tested

eDNA study for four fish species in the Snowy Mountains.

Site	Waterway	Latitude	Longitude	Collection date	# samples	<i>Perca fluviatilis</i>	<i>Gambusia holbrooki</i>	<i>Galaxias brevipinnis</i>	<i>Macquaria australasia</i>
JR08	Jindabyne Reservoir	-36.3423454	148.5945043	16/3/19	4	negative	negative	not tested	not tested
JR09	Jindabyne Reservoir	-36.3397181	148.6065207	16/3/19	4	negative	negative	not tested	not tested
JR10	Jindabyne Reservoir	-36.35548	148.5983668	16/3/19	4	negative	negative	not tested	not tested
JR11	Jindabyne Reservoir	-36.2906169	148.6345015	16/3/19	4	negative	negative	not tested	not tested
JR12	Jindabyne Reservoir	-36.3081179	148.6229144	16/3/19	4	negative	negative	not tested	not tested
JR13	Jindabyne Reservoir	-36.3177314	148.6320124	16/3/19	4	negative	negative	not tested	not tested
JR14	Jindabyne Reservoir	-36.3357179	148.6267123	16/3/19	4	negative	negative	not tested	not tested
JR15	Jindabyne Reservoir	-36.3464448	148.6210134	16/3/19	4	negative	negative	not tested	not tested
JR16	Jindabyne Reservoir	-36.3278614	148.6177722	16/3/19	4	negative	negative	not tested	not tested
JR17	Jindabyne Reservoir	-36.4043637	148.6355946	18/3/19	4	negative	negative	not tested	not tested
JR18	Jindabyne Reservoir	-36.3869706	148.6084292	16/3/19	4	negative	negative	not tested	not tested
JR19	Jindabyne Reservoir	-36.3773182	148.5821025	16/3/19	4	negative	negative	positive (5/12)	not tested
JR20	Jindabyne Reservoir	-36.3746948	148.6427302	16/3/19	4	negative	negative	not tested	not tested
JR22	Jindabyne Reservoir	-36.4083638	148.6187477	16/3/19	4	negative	negative	not tested	not tested
TAN01	Tantangara Reservoir	-35.7528551	148.6250717	15/3/19	4	negative	negative	negative	not tested
TAN02	Tantangara Reservoir	-35.7510451	148.6344784	15/3/19	4	negative	negative	negative	not tested

eDNA study for four fish species in the Snowy Mountains.

Site	Waterway	Latitude	Longitude	Collection date	# samples	<i>Perca fluviatilis</i>	<i>Gambusia holbrooki</i>	<i>Galaxias brevipinnis</i>	<i>Macquaria australasia</i>
TAN03	Tantangara Reservoir	-35.7643834	148.6345213	15/3/19	4	negative	negative	negative	not tested
TAN04	Tantangara Reservoir	-35.7602045	148.6394565	15/3/19	4	negative	negative	negative	not tested
TAN05	Tantangara Reservoir	-35.7513933	148.6465805	15/3/19	4	negative	negative	negative	not tested
TAN06	Tantangara Reservoir	-35.7184149	148.6681382	15/3/19	4	negative	negative	negative	not tested
TAN07	Tantangara Reservoir	-35.7425225	148.6537616	15/3/19	4	negative	negative	negative	not tested
TAN08	Tantangara Reservoir	-35.7541498	148.6637343	15/3/19	4	negative	negative	negative	not tested
TAN09	Tantangara Reservoir	-35.7673193	148.6513154	15/3/19	4	negative	negative	negative	not tested
TAN10	Tantangara Reservoir	-35.7822156	148.6491859	15/3/19	4	negative	negative	negative	not tested
TAN11	Tantangara Reservoir	-35.7978809	148.6543358	15/3/19	4	negative	negative	negative	not tested
TAN12	Tantangara Reservoir	-35.795279	148.6638844	15/3/19	4	negative	negative	negative	not tested
TAN13	Tantangara Reservoir	-35.7952667	148.6640747	15/3/19	4	negative	negative	negative	not tested
GR01	Geehi Reservoir	-36.2998328	148.3150779	19/3/19	5	negative	negative	not tested	not tested
GR02	Geehi Reservoir	-36.2866873	148.323123	19/3/19	3	negative	negative	positive (7/9)	not tested
GR03	Geehi Reservoir	-36.3028577	148.3166637	19/3/19	5	negative	negative	not tested	not tested
GR04	Geehi Reservoir	-36.3056806	148.3164083	19/3/19	5	negative	negative	not tested	not tested

eDNA study for four fish species in the Snowy Mountains.

Site	Waterway	Latitude	Longitude	Collection date	# samples	<i>Perca fluviatilis</i>	<i>Gambusia holbrooki</i>	<i>Galaxias brevipinnis</i>	<i>Macquaria australasia</i>
TPR01	Tumut Pond Reservoir	-35.9633044	148.4175638	14/3/19	5	negative	negative	negative	not tested
TPR02	Tumut Pond Reservoir	-35.9643006	148.3902986	14/3/19	5	negative	negative	not tested	not tested
TPR03	Tumut Pond Reservoir	-35.9691183	148.3894416	14/3/19	5	negative	negative	not tested	not tested
TPR04	Tumut Pond Reservoir	-35.9696395	148.3903106	14/3/19	5	negative	negative	not tested	not tested
T2D01	T2 Dam	-35.941682	148.3627583	14/3/19	5	negative	negative	negative	not tested
T2D02	T2 Dam	-35.9328331	148.3594648	14/3/19	5	negative	negative	not tested	not tested
T2D03	T2 Dam	-35.926791	148.3620138	14/3/19	5	negative	negative	not tested	not tested
M2D01	M2 Dam	-36.2406574	148.1810986	19/3/19	5	negative	negative	not tested	not tested
M2D02	M2 Dam	-36.2401635	148.1817754	19/3/19	5	negative	negative	not tested	not tested
M2D03	M2 Dam	-36.2444258	148.1882815	19/3/19	5	negative	negative	not tested	not tested
MWP01	Mowamba Weir Pool	-36.4696426	148.6320142	16/3/19	4	negative	negative	not tested	not tested
TR01	Tumut Rv	-35.858041	148.3646348	14/3/19	3	positive (9/9)	negative	not tested	not tested
TR02	Tumut Rv	-35.8760598	148.3677123	14/3/19	3	negative	negative	not tested	not tested
SPC01	Swampy Plains River	-36.3836169	148.1798058	18/3/19	3	negative	negative	not tested	not tested

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Site	Waterway	Latitude	Longitude	Collection date	# samples	<i>Perca fluviatilis</i>	<i>Gambusia holbrooki</i>	<i>Galaxias brevipinnis</i>	<i>Macquaria australasia</i>
SPC02	Swampy Plains River	-36.2766112	148.1137745	18/3/19	3	positive (9/9)	negative	not tested	not tested
BC01	Bogong Ck	-36.3564096	148.2032809	18/3/19	3	negative	negative	not tested	not tested
KR01	Khancoban Res	-36.2212364	148.1170634	18/3/19	3	positive (9/9)	negative	not tested	not tested
KR02	Khancoban Res	-36.2326606	148.1225351	18/3/19	3	positive (9/9)	negative	not tested	not tested
KR03	Khancoban Res	-36.2189513	148.1081434	19/3/19	3	positive (9/9)	negative	not tested	not tested
MR01	Murrumbidgee Rv	-35.7995679	148.6761574	15/3/19	3	negative	negative	negative	negative
MR03	Murrumbidgee Rv	-35.8264099	148.8033125	3/12/19	3	negative	negative	negative	negative
MR06	Murrumbidgee Rv	-35.9807865	148.8406696	3/12/19	3	negative	positive (9/9)	negative	positive (5/9)
MR06a	Murrumbidgee Rv	-35.9799522	148.8919818	3/12/19	3	negative	positive (9/9)	negative	equivocal (1/9)
MR08	Murrumbidgee Rv	-36.1703549	149.091261	3/12/19	3	negative	positive (8/9)	negative	positive (7/9)
MR10	Numeralla Rv	-36.0769241	149.1576672	3/12/19	3	negative	positive (5/9)	negative	negative
MR09a	Murrumbidgee Rv	-36.0237344	149.1385972	3/12/19	3	negative	positive (9/9)	negative	negative
MR11	Murrumbidgee Rv	-35.8589112	149.1346161	3/12/19	3	negative	positive (5/9)	negative	positive (3/9)
MR12	Murrumbidgee Rv	-35.5830926	149.1090326	3/12/19	3	positive (7/9)	positive (4/9)	negative	negative
MR15	Murrumbidgee Rv	-35.5291508	149.0789003	12/3/19	3	equivocal (1/9)	positive (6/9)	negative	negative
MR16	Murrumbidgee Rv	-35.5083977	149.0706053	12/3/19	3	equivocal (1/9)	positive (6/9)	negative	negative

Appendix 2. Larger maps of sampling sites and detections

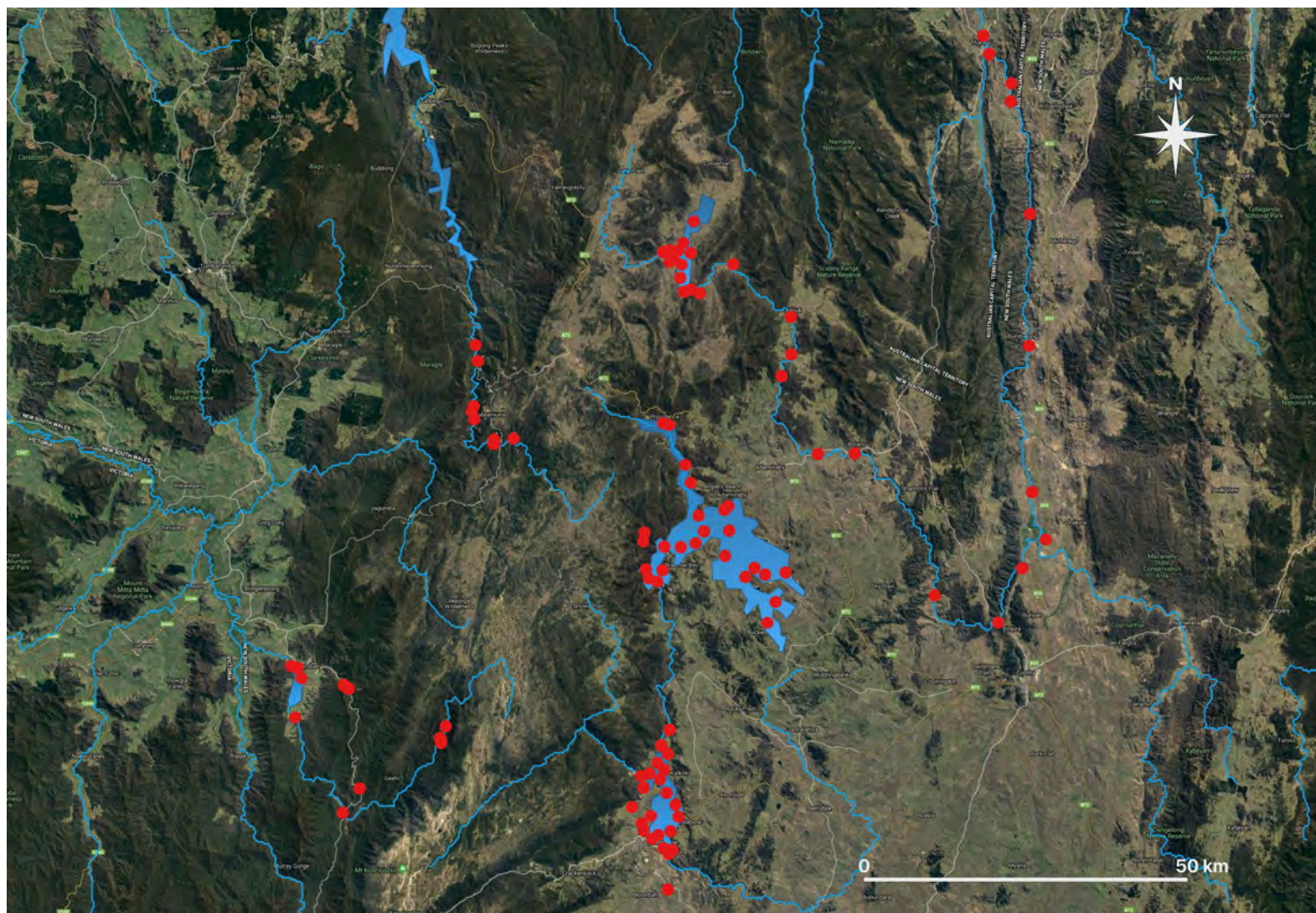


Figure 1. Location of sampling sites (see Appendix 1 for further site information).

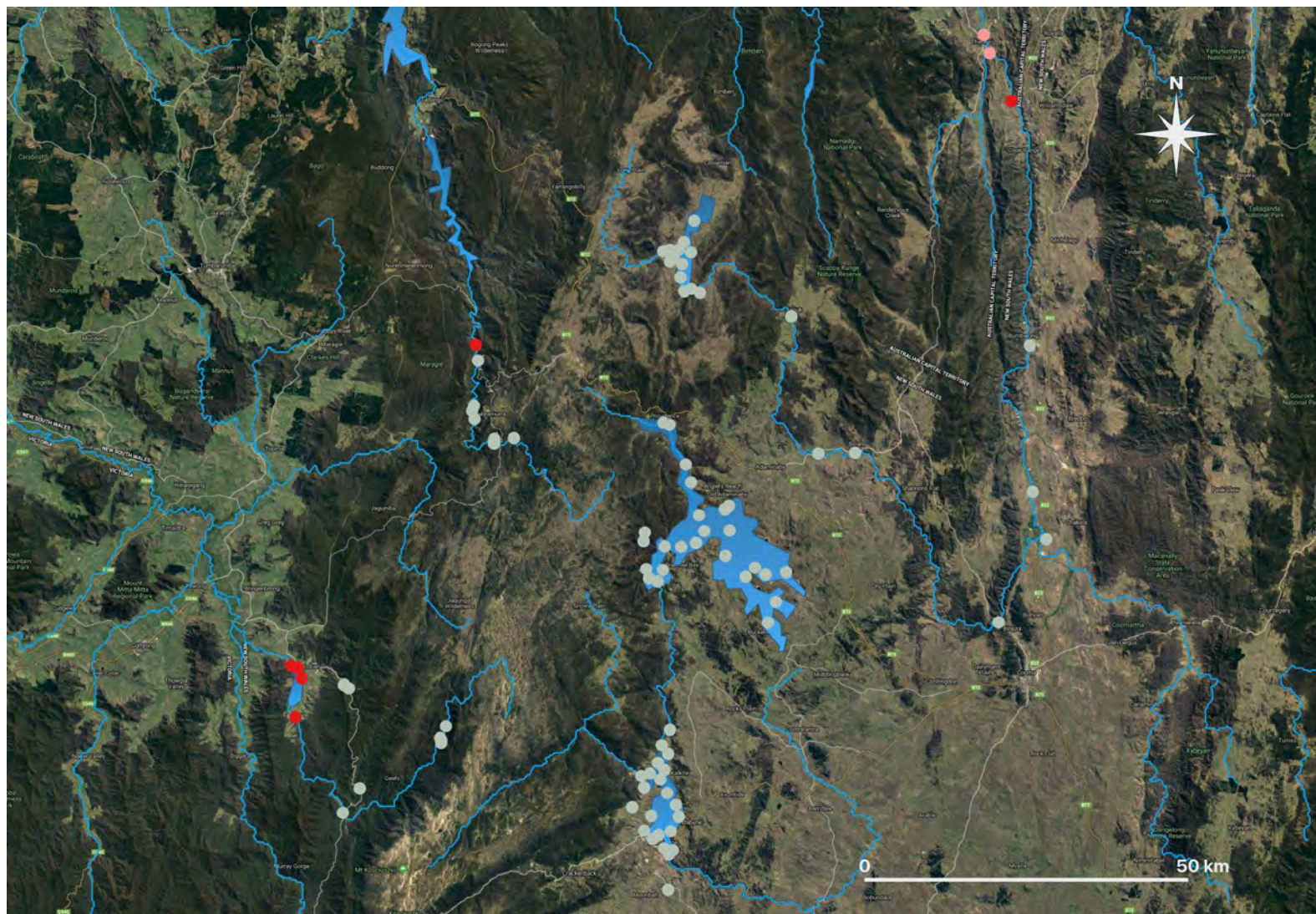


Figure 2. Site locations of eDNA testing and detections for *P. fluviatilis* from March 2019 sampling. Red circles indicate positive results, closed grey circles indicate negative results, and closed pink circles indicate equivocal results.

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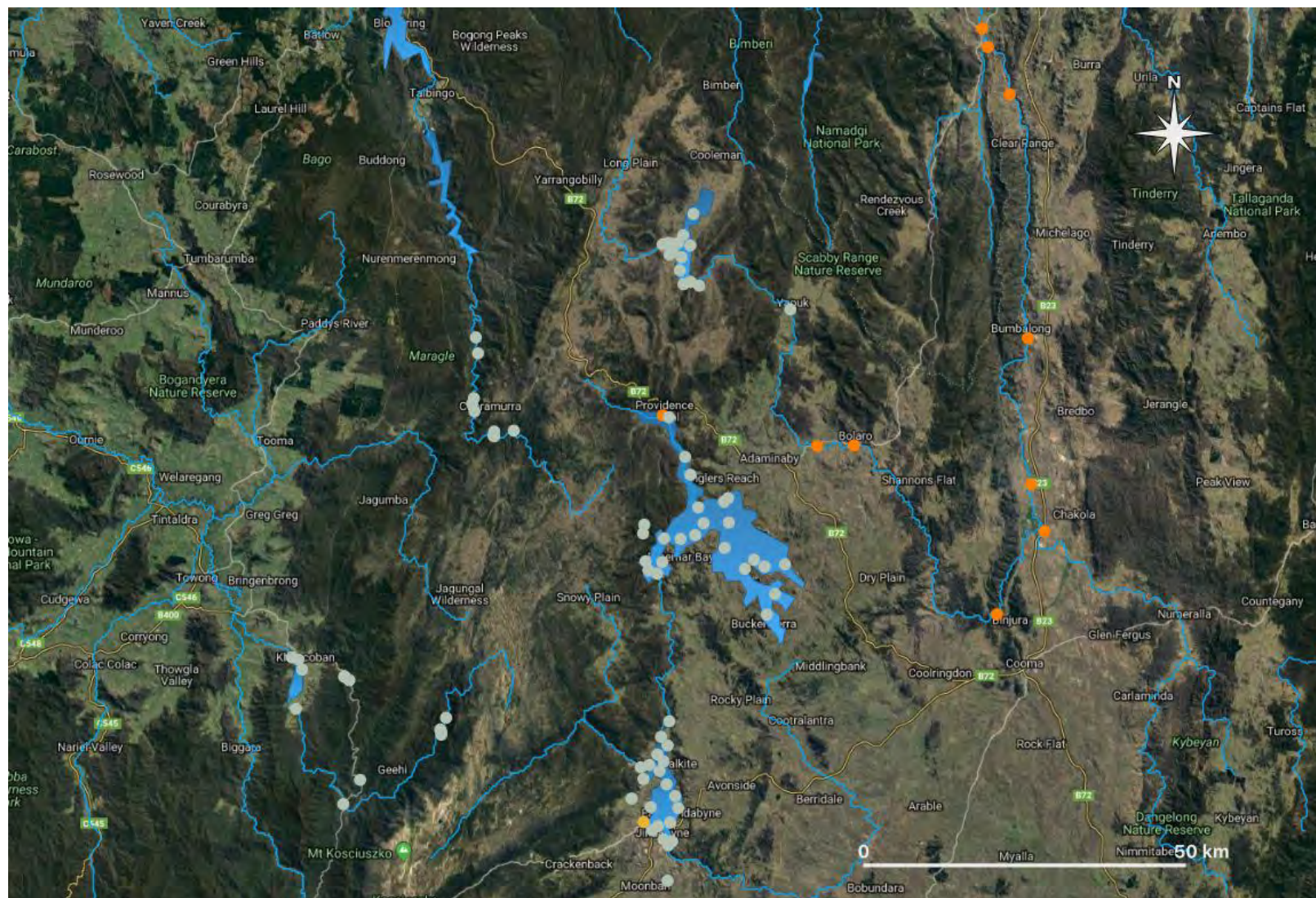


Figure 3. Site locations of eDNA testing and detections for *G. Holbrooki* from March 2019 sampling. Orange circles indicate positive results, closed grey circles indicate negative results, and yellow circles indicate

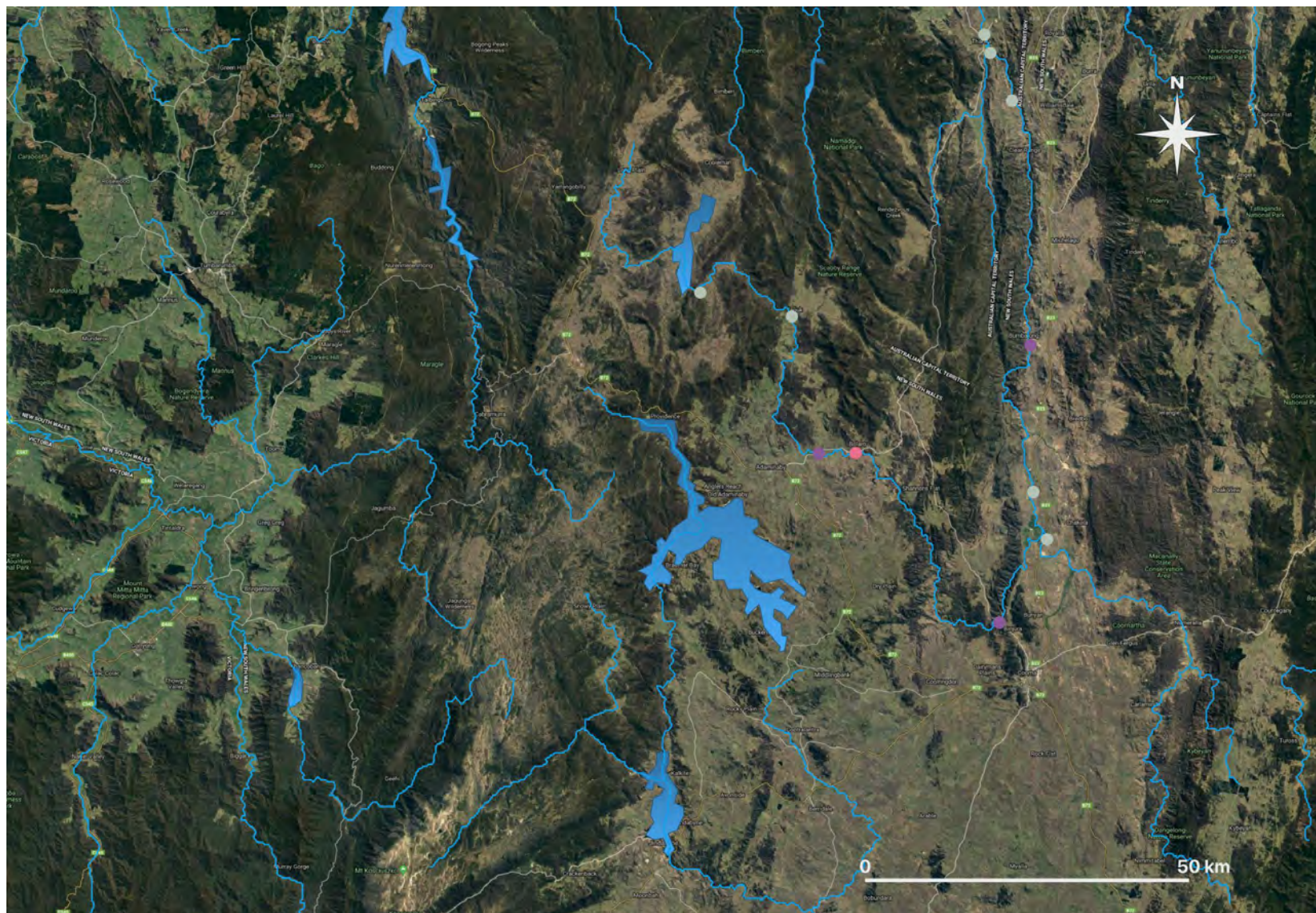


Figure 4. Site locations of eDNA testing and detections for *M. australasica* from March 2019 sampling. Purple circles indicate positive results, grey indicate negative results, and pink circles indicate equivocal results.

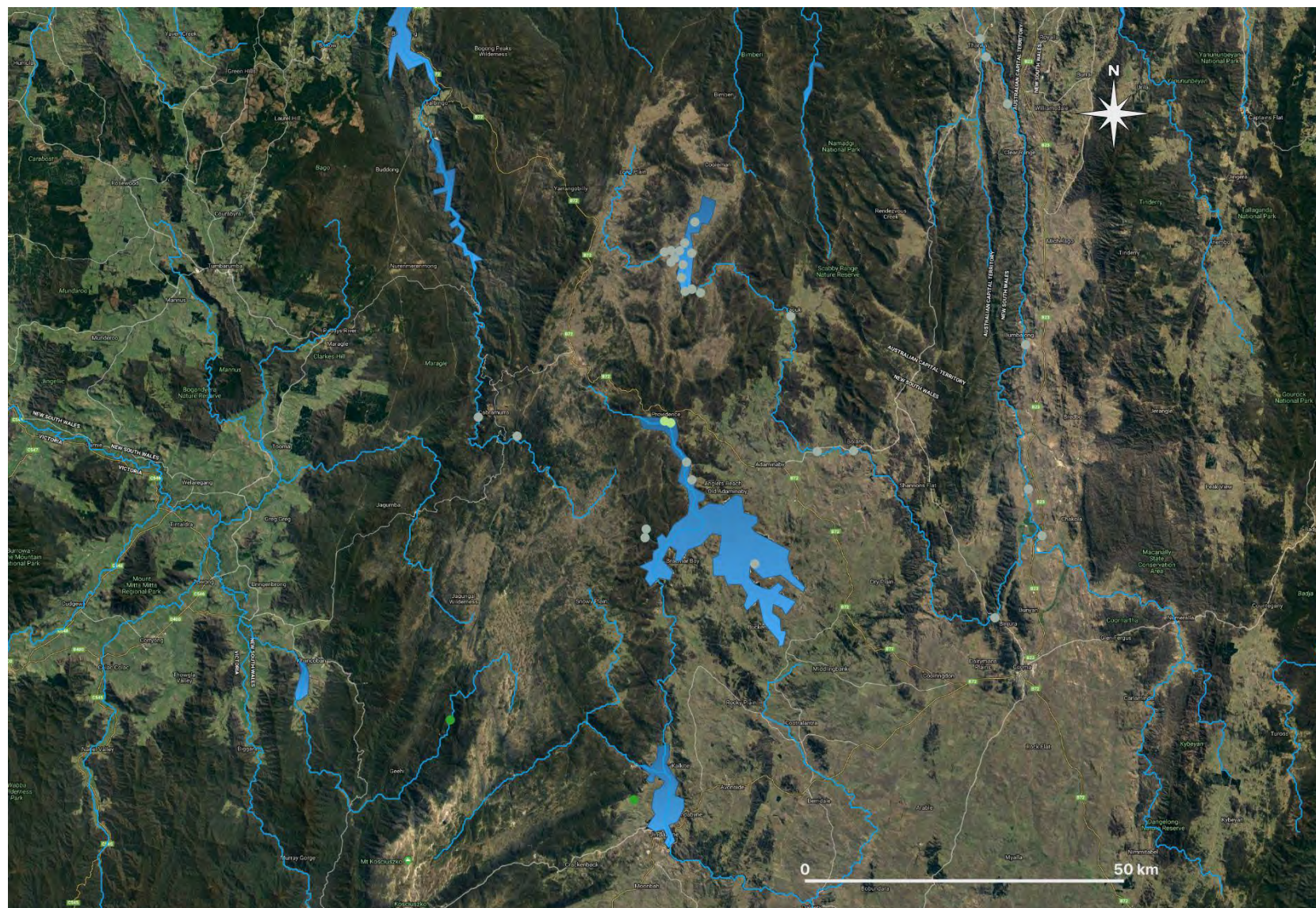


Figure 5. Site locations of eDNA testing and detections for *G. brevipinnis* from March 2019 sampling. Dark green circles indicate positive results, closed grey circles indicate negative results, and light green circles indicate equivocal results.

Final Report

Assessment of the potential for increased distribution of *Epizootic haematopoietic necrosis virus* (EHNV) associated with Snowy 2.0

Consultation to EMM Consulting Pty Ltd for Snowy Hydro Limited

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Executive Summary

Snowy 2.0 will create a direct hydrodynamic connection between Talbingo and Tantangara reservoirs via a 27km tunnel with high water exchange. One consideration for Snowy 2.0 is the potential to increase the range of *Epizootic haematopoietic necrosis virus* (EHN). This is a viral pathogen of fish that is of international concern and is listed by The World Organization for Animal Health (OIE). EHN has been restricted to south-eastern mainland Australia where it has caused sporadic outbreaks of high mortality disease in redfin perch (*Perca fluviatilis*) since the 1980s. It has also caused a less spectacular, low mortality disease in farmed rainbow trout (*Oncorhynchus mykiss*). Experimental studies indicate that several native Australian fish species including the endangered Macquarie perch (*Macquaria australasica*) are susceptible to EHN although natural disease events caused by EHN have never been detected in species other than redfin perch and rainbow trout. EHN is considered to be endemic in waters where it has previously occurred. This presence is maintained as persistent subclinical infection of redfin perch, low grade endemic infection cycles that do not induce high mortality outbreaks and by virtue of EHN being able to remain infectious for periods of many months in the environment outside of a host. The severity of EHN disease has been moderated after the first outbreaks in an area by the reduced population of naïve fish. The geographic range of EHN can increase by movement of live fish with subclinical infection. There is a much higher probability of EHN spreading to new locations during a disease outbreak because of the large quantity of virus produced and potential dispersion via contamination of human recreational equipment, spread by birds and other vectors.

The low efficacy of passive surveillance for this disease reduces the reporting of disease events. Further, there are insufficiently detailed epidemiological investigations of the detected outbreaks which leaves uncertainty about environmental risk factors that might contribute to the sporadic high mortality outbreaks.

A key consideration in preventing the spread of EHN is to avoid establishment of the amplifying host (redfin perch) in waters that can enable spread to less susceptible species located proximately. Of particular concern is the high mortality when a previously uninfected population of redfin perch is first infected. This results in a large disease outbreak that amplifies the virus to a quantity that can be transmitted large distances, including free in water, and over long time periods because EHN can remain infectious for survive several months outside of a host. Restricting live fish movement is also important as EHN can spread with movement of apparently healthy fish including rainbow trout and redfin perch that have a subclinical infection. Even with low and moderate probability of transmission of EHN to a new location the risk is rated as high where the impact includes the possibility of mortality in an endangered species, for example, a breeding population of Macquarie perch in the upper Murrumbidgee River.

Background

Snowy 2.0 is a project designed to increase the capacity of the Snowy Mountains Hydroelectricity Scheme (Snowy Hydro, 2018). Specifically, a 27 km underground tunnel network that incorporates a power station is proposed to connect the Talbingo and Tantangara Reservoirs. At times when energy is abundant, water will be pumped to the elevated Tantangara Reservoir. Release of water back to Talbingo can generate electricity at times of peak demand and when other sources of electricity are not productive. The reservoirs and rivers referred to in this document, which have different fish populations and disease risks for Epizootic haematopoietic necrosis virus (EHN) are shown in Figure 1.

Tantangara Dam on the Murrumbidgee River upstream of Adaminaby creates the Tantangara Reservoir. The volume of impounded water fluctuates based on inflow and management of the Snowy Scheme and environmental flows. The capacity is 254 gigalitres (GL), the surface area is 2100 hectares and the catchment area is 460 square kilometres within the Kosciuszko National Park (Dams Australia, 2010). Outflow to the Murrumbidgee River is regulated at the dam and the reservoir is connected by a 16.6 km tunnel to Lake Eucumbene. Lake Eucumbene is the largest reservoir in the Snowy Scheme and is connected to Lake Jindabyne by the Eucumbene River. Water from Lake Eucumbene is distributed throughout the Scheme via gravity fed tunnels to Geehi Reservoir in the Upper Murray catchment and Tumut Pond Reservoir on the Tumut River upstream of Talbingo Reservoir. Brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) are present in Tantangara Reservoir and have value as a recreational fishery. Fishery management does not include a stocking program as the intention is to allow

the average size of fish and the population to fluctuate according to natural recruitment and lake productivity (NSW Department of Primary Industries, 2018). An endangered fish species, stocky galaxias (*Galaxias tantangara*), is known only from one locality in the headwaters of Tintangara Creek, upstream of the Tintangara Reservoir (NSW Department of Primary Industries, 2016).

Talbingo Dam on the Tumut River upstream of Talbingo, creates the Talbingo Reservoir which is an important component of the Snowy Mountains Hydroelectricity scheme. At full capacity (921GL) it holds 3.6 times more water than Tintangara reservoir with a similar surface area of approximately 2,000 hectares and a catchment area is 1,093 square kilometres (Dams Australia, 2010). This reservoir is more accessible compared to Tintangara for recreational activities including boating and fishing for species including non-native brown trout, rainbow trout and redfin perch (*Perca fluviatilis*, also referred to as European perch). Other pest fish species present include goldfish (*Carassius auratus*) and eastern gambusia (*Gambusia holbrooki*). Endangered native species including trout cod (*Maccullochella macquariensis*) and Macquarie perch (*Macquaria australasica*) have been stocked but not recently detected (EMM Consulting, 2018).

Epizootic haematopoietic necrosis virus (EHN) is a viral pathogen that has been of international concern since its emergence in the early 1980s and is listed by The World Organization for Animal Health (OIE, 2015b). The OIE lists 12 diseases of fish requiring notification to ensure the safe trade of aquatic animals and their products to protect aquatic resources worldwide (OIE, 2018). EHN has caused epidemic mortality events in redfin perch and rainbow trout. Infection with the virus is systemic and causes Epizootic hematopoietic necrosis (EHN), a disease characterised by extensive visceral tissue damage leading to mortality (Hick et al., 2017a). Originally detected in Victoria in 1984, EHN spread within the subsequent decade with EHN epidemics documented across south-eastern Australia including South Australia, New South Wales and the Australian Capital Territory. Currently, the frequency of outbreaks has diminished and EHN remains restricted to parts of south-eastern Australia. The reasons for this have not been fully elucidated, but it may reflect host adaptation (Becker et al 2016), rather than evolution of the virus (Hick et al 2017). The most recent reported outbreaks in Victoria occurred in 2012, The Australian Capital Territory in 2011, New South Wales in 2009 and South Australia in 1992 (Animal Health Australia, 2018). *European catfish virus* (ECV) is a related virus that has been reported in Europe (Hick et al., 2016). Both EHN and ECV can infect several species of fish, but the host range of these virus species is restricted to fish and other classes of animals are not infected. Laboratory studies have demonstrated that EHN is

capable of causing fatal disease in several native fish species including Macquarie perch (*Macquaria australasica*), although natural disease outbreaks have not been recorded (Becker et al., 2013).

Taxonomically, EHN is placed in the genus *Ranavirus* within the Family *Iridoviridae* (Chinchar et al., 2017). EHN is a large double stranded DNA virus that can be released from a host cell by lysis (non-enveloped virion) or budding (enveloped virion) (Williams et al., 2005). Transmission of EHN between susceptible hosts is possible via water or ingestion of tissues from infected fish (Becker et al., 2013; Langdon, 1989; Whittington and Reddacliff, 1995). EHN infects fish through the skin, gills or mouth. Infection results in multifocal necrosis of the haematopoietic tissue found in the spleen, liver and kidney (Langdon and Humphrey, 1987; Langdon et al., 1988; Reddacliff and Whittington, 1996; Whittington et al., 1994). Most infected fish quickly succumb and die within a few weeks, but disease expression is highly dependent on water temperature (Whittington and Reddacliff, 1995).

EHN is presumed to be endemic in the Blowering Reservoir (Whittington et al., 2010; Whittington et al., 2011), which is downstream of the Talbingo Reservoir on the Tumut River, where it has caused multiple outbreaks of disease in redfin perch. Unfortunately there was not a full epidemiological investigation of these disease events to determine the full extent of the disease and the risk factors for its occurrence. The status of EHN in Talbingo is unknown with no reports of disease. A new connection between Talbingo and Tantangara Reservoir which has direct hydrodynamic connection to Lake Eucumbene and the Upper Murrumbidgee River potentially expands the range of EHN. This includes the potential exposure of a critical breeding population of Macquarie perch in the Murrumbidgee River between Tantangara Dam and Cooma (Faulks et al., 2010), susceptible rainbow trout in Tantangara and Eucumbene Reservoirs and the endangered stocky galaxias which has unknown susceptibility.

The objective of this desktop review was to assess the potential for Snowy 2.0 to lead to the transfer of EHN through the hydraulic connection of Talbingo and Tantangara reservoirs, and consider the risk that EHN poses to endangered native species of fish and the recreational trout fishery, if transfer was to occur. Based on synthesis of what is known about the epidemiology of EHN, the risk posed of creating a hydrodynamic connection between Talbingo and Tantangara Reservoirs will be assessed under different scenarios for distribution of host fish species and application of disease mitigation measures on transmission via the tunnel system. There is limited information about the role of environmental and host risk factors that lead to outbreaks of EHN. Therefore, assessments of the risk posed by EHN can only be qualitative and contain an inherent uncertainty.

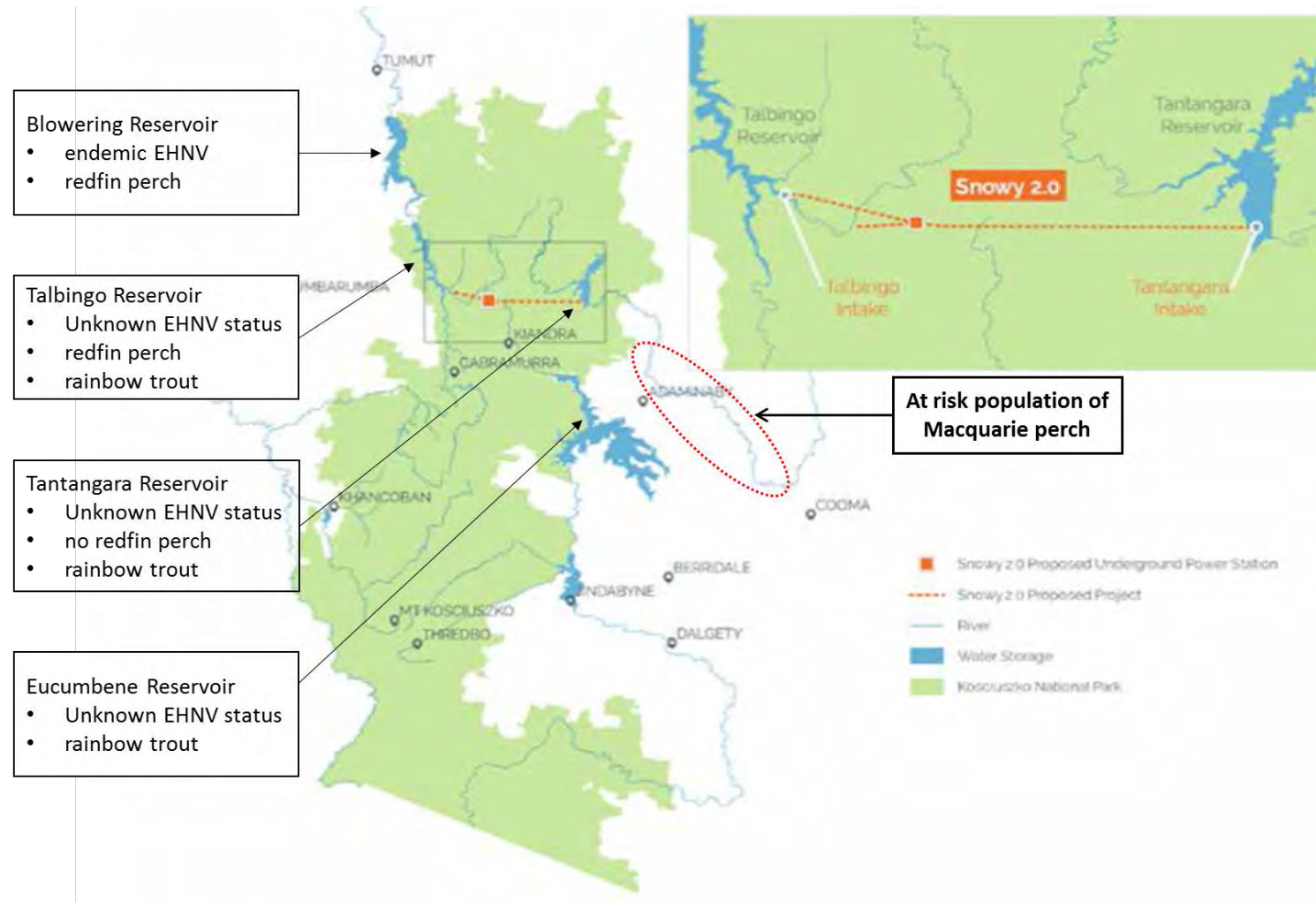


Figure 1. Map showing the proposed tunnel connecting Talbingo and Tantangara Reservoirs. Blowering Reservoir is on the Tumut River downstream of Talbingo. Tantangara Reservoir discharges into the Murrumbidgee River at Tantangara Dam and is connected by an existing tunnel to Eucumbene Reservoir. Known populations of redfin perch, rainbow trout and Macquarie perch (between Cooma and Yass) with the known distribution of EHN within Blowering Reservoir. Image adapted from Snowy Hydro, (2018). Snowy 2.0 Pumped Hydro Project Update October 2018.

1. Spatiotemporal distribution of EHN

- Historical distribution of EHN and patterns of EHN disease outbreaks in Australia, and specifically the Murrumbidgee catchment.

The first epidemics caused by EHN occurred in freshwater impoundments in central Victoria including Lakes Mokoan and Nillahcootie in the Broken River catchment. Several populations of wild redfin perch (*Perca fluviatilis*) were impacted in the period 1984 - 1986 (Langdon and Humphrey, 1987; Langdon et al., 1986). These first epidemics often resulted in mortality of tens of thousands of juvenile redfin perch, and in some outbreaks, adults were affected. From 1986 to the mid-1990s, outbreaks of EHN in redfin perch and rainbow trout were reported across the Murray Darling Basin in south-eastern Australia, including Lake Hume, Tumut River below Blowering Dam, Burrinjuck Reservoir, Blowering Reservoir, Lake Burley Griffin and Googong Reservoir (Whittington et al., 2010). Mortality rates in initial EHN epidemics in Victoria and NSW were estimated to be > 90% and in some smaller water bodies 100% mortality in redfin perch was reported (Langdon and Humphrey, 1987; Langdon et al., 1986; Whittington et al., 2010). The disease impacted recreational fishing. There has been limited field epidemiology undertaken in the case of these EHN disease and the full geographic extent and any environmental risk factors for disease have not been determined. From these outbreaks, there was evidence of both downstream and upstream spread of EHN as well as an instance outside of the Murray Darling Basin catchments (Whittington et al., 2010). A summary of the geographic distribution of EHN in rainbow trout and redfin perch populations is provided (Figure 2 and 3, respectively). The spread of EHN was considered to be in an upstream direction (Whittington et al. 2010). Additionally, it was translocated outside the Murray Darling Basin with the trade in live rainbow trout fingerlings to the upper Shoalhaven River catchment (Whittington et al., 1999). There are 2 rainbow trout farms in the Murrumbidgee catchment in which EHN has been reported, described as Farm A and Farm C by Whittington et al., (1999), and a farm was affected east of

Adaminaby on the Murrumbidgee river in 1986 (R. Whittington pers.com). Rainbow trout farms were affected from the use of river water contaminated with EHN from outbreaks in redfin perch (Whittington et al., 1994) as well as by movement of live fish (Whittington et al., 1999).

The occurrence of EHN has been discontinuous over time and space with discrete disease events occurring within the endemic regions without a long-term pattern of recurrence. After spreading to new locations the incidence of disease has been infrequent (Hick et al., 2017b). Incidences of EHN that have been reported are limited to scenarios in which government fisheries officers have identified fish kills and submitted suitable diagnostic samples to a laboratory. Generally passive surveillance has been poor and it is possible that low grade EHN occurs without notification. There have been no reports of EHN since an outbreak limited to redfin perch within Lake Ginninderra in the Australian Capital Territory (ACT) in 2011 and in Victoria in 2012. Mortality in the ACT outbreaks were limited to redfin perch in reservoirs (Lake Burley, Googong Dam and Lake Ginninderra) with no reports of disease in upstream locations. High mortality outbreaks of EHN are most likely limited to situations where a large population of naïve redfin perch is exposed, as would occur with a change in the distribution of the virus or a long period of time without low grade endemic disease transmission. Comprehensive surveillance of the Murray-Darling Basin reported in 2011 found EHN is still endemic in the upper Murrumbidgee River catchment in the Murray-Darling Basin (Whittington et al., 2011). However, not all parts of the upper Murrumbidgee catchment were surveyed and some, for example Cotter Reservoir were not infected. There was evidence of freedom from EHN in other parts of the Murray Darling Basin.

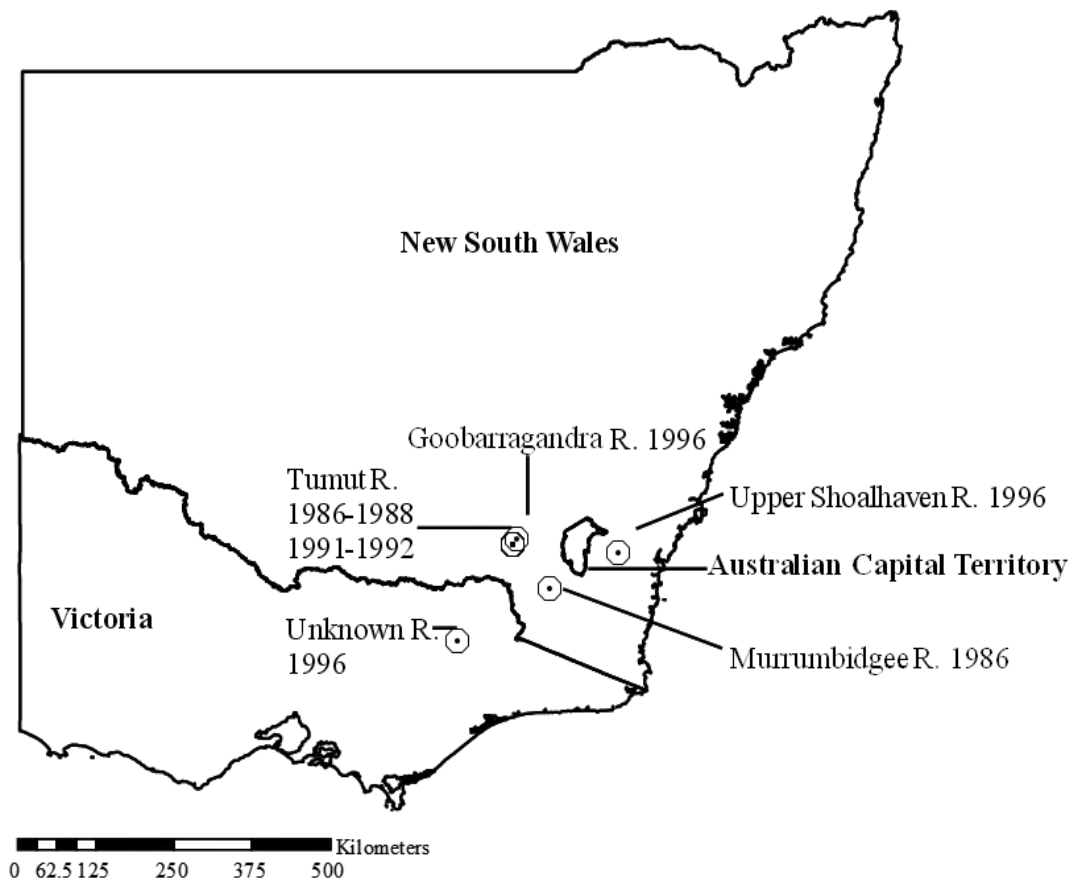


Figure 2. The geographic distribution of EHN outbreaks (year) at rainbow trout farms on various rivers (R.) in south-eastern Australia (Langdon et al., 1988, Whittington et al., 1990). (Adapted from Whittington et al., 2010).

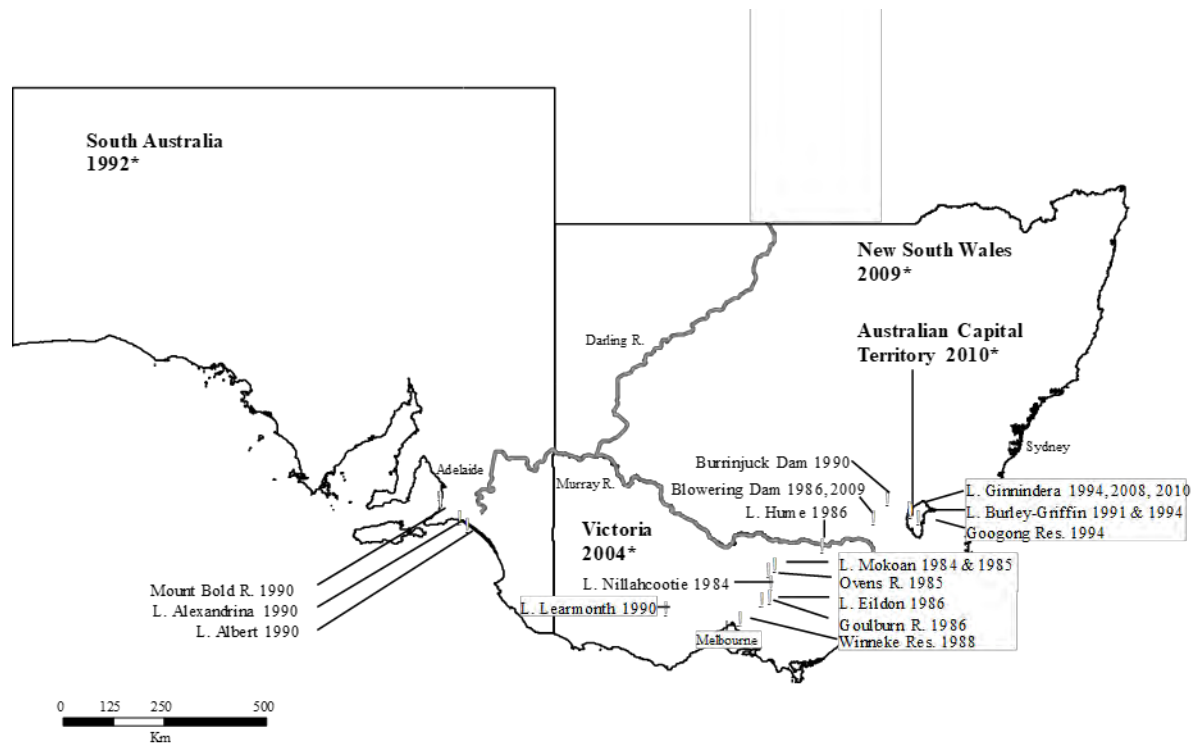


Figure 3. Geographic distribution of outbreaks of EHN in redfin perch populations in lakes (L.), reservoirs (R.) and rivers (R.) in south-eastern Australia (Langdon and Humphrey 1987, Whittington et al., 1996, 2010, 2011). (Adapted from Whittington et al., 2010)

2. Susceptible hosts and environmental conditions required for disease

- Describe the known susceptible hosts and environmental conditions required for disease, and mechanisms for spread of EHN.

There are dramatic species differences in susceptibility with redfin perch being highly susceptible to EHN compared to rainbow trout. Infection with EHN at rainbow trout farms has been characterized as being subclinical at the population level with low prevalence (e.g. <8%) not always presenting as obvious disease (Whittington et al., 1999). Further, the minimum infective dose for redfin perch was <0.1 50% tissue culture infective dose per ml (TCID₅₀/ml) for infection by bath challenge compared to rainbow trout which required at least 10³ TCID₅₀/mL (Whittington and Reddacliff, 1995). Redfin perch can develop an EHN carrier state with persistent subclinical infection (Becker et al., 2016), which was not observed in earlier studies by Langdon (1989) and Whittington and Reddacliff (1995). Age of fish influences the likelihood of developing disease and dying, with juvenile fish being more susceptible and succumbing more rapidly to disease. From experimental challenges, juvenile redfin perch died 3-6 days after exposure compared to adults at around 10-11 days, when held at similar water temperatures (Whittington and Reddacliff, 1995). The infection trials indicate that fish are susceptible to bath exposure, such that EHN can be infectious when free as would occur during a disease outbreak. EHN associated with debris from dead fish can be carried by vectors and on fomites due to the considerable resilience of the virions to loss of infectivity. Less is known about the amount of contact or the conditions that will enable transmission from fish with subclinical infection. Testing populations for freedom from infection with EHN is good practice when restocking water bodies and is practiced for rainbow trout according to guidelines provided by the OIE.

Redfin perch and rainbow trout can survive infection with EHN and go on to develop an immune response including antibodies specific for EHN (Whittington et al., 1994; Whittington and Reddacliff, 1995). From experimental challenges, adult redfin perch can have anti-EHN antibody in serum as well as live virus in kidney, spleen and liver (Whittington et al., 2011). In addition, antibody

responses can develop following exposure to EHN by a natural route of infection and may be quite persistent, demonstrated in some cases to last for at least 18 months (Whittington et al., 2011; Whittington et al., 1994). Therefore, following exposure to EHN, some individual fish will succumb to the virus and die within 4 weeks of exposure; other fish may become infected and remain carriers of the virus; a third group become infected and then recover; finally, some fish do not become infected (Becker et al., 2013; Langdon, 1989; Whittington et al., 2011). Whether an individual fish dies, becomes a carrier or recovers with a specific immune response may depend on the dose of EHN that it was exposed to, inter-current stress, genotype and a wide range of environmental factors. The prevalence of infection in rainbow trout during disease outbreaks was 60 – 80% whilst only 0 – 4% of in-contact fish were positive for EHN when tested by virus isolation and no detection in survivors of the disease (Whittington et al., 1994; Whittington et al., 1999). Early studies found EHN by virus isolation in apparently healthy redfin perch in disease affected populations, but the duration of the infection was not known (Langdon and Humphrey, 1987).

It was shown from experimental studies that EHN can kill several native freshwater fish species of high conservation value including: Macquarie perch, Murray cod (*Maccullochella peelii peelii*), freshwater catfish (*Tandanus tandanus*), Murray Darling rainbowfish (*Melanotaenia fluviatilis*) and silver perch (*Bidyanus bidyanus*) (Becker et al., 2013; Langdon, 1989). There is no record of disease or EHN infection in any of these species from natural disease outbreaks. Additional species considered to satisfy the OIE criteria for susceptibility to EHN are mountain galaxias (*Galaxias olidus*), and internationally, black bullhead (*Ameiurus melas*) northern pike (*Esox lucius*), pike-perch (*Sander lucioperca*) were shown to be susceptible (OIE, 2015b). Notably the mosquito fish (*Gambusia holbrooki*) are susceptible and are one of the most abundant fish in the Murrumbidgee catchment (Gilligan, 2005). Other species were confirmed to be resistant to infection with EHN under the conditions of the studies, for example trout cod (*Maccullochella macquariensis*). For other species such as climbing galaxias (*Galaxias brevipinnis*), there

has been no assessment of susceptibility. There is no experimental data assessing the susceptibility of brown trout. This species has also been present in waters where EHN disease has occurred without any reports of mortality suggesting brown trout are not highly susceptible to disease. A species from one family may be susceptible while another species from the same or related families within the same order is not. For example, within the order *Atheriniformes*, the Murray–Darling Rainbowfish (family *Melanotaeniidae*) and the Unspecked Hardyhead (family *Atherinidae*) differed in susceptibility following bath challenge with the virus (Becker et al., 2013). Closely related species within a genus differed in susceptibility depending on the challenge model used. For the genus *Macquaria*, only the Macquarie Perch was susceptible following bath challenge, while Australian Bass and Golden Perch were susceptible only after an intraperitoneal injection challenge (Langdon 1989). Based on these observations, identifying potential hosts for EHN infection should not rely solely on taxonomic relatedness as a predictor of susceptibility (Becker et al., 2013). Consequently, the susceptibility of the critically endangered stocky galaxias cannot be predicted.

As fish are ectothermic, water temperature plays a pivotal role in determining if fish exposed to a pathogen are infected and if this culminates in progression to disease. The thermal range for disease expression (resulting in mortality) from an infection with EHN is between 12°C and 26°C. The optimal range of 19-21°C identified by Whittington and Reddacliff (1995) was confirmed by international studies using European stocks of redfin perch and rainbow trout (Ariel and Jensen, 2009; Borzym and Maj-Paluch, 2015). The thermal range for disease expression can be used to identify the risk periods for transmission of EHN and disease outbreaks for a specific waterbody. In the case of Tantangara Reservoir there are periods of 6 months each summer when disease is possible, with peak risk periods when the temperature is optimal for disease expression spanning approximately 4 months. A response of fish to disease is to seek a thermal environment that suits recovery (Boltana et al., 2018). In the case of redfin perch, early studies recognised that fish in thermally stratified water bodies may have sought avoidance

of disease in cooler water (Whittington and Reddacliff, 1995). It is possible that thermal gradients in deep reservoirs may influence the risk period for EHN infection and the population level disease outcome. Whilst poor water quality, for example low dissolved oxygen is a potential risk factor, there is no data in the scientific literature which identifies other risks for EHN disease outbreaks.

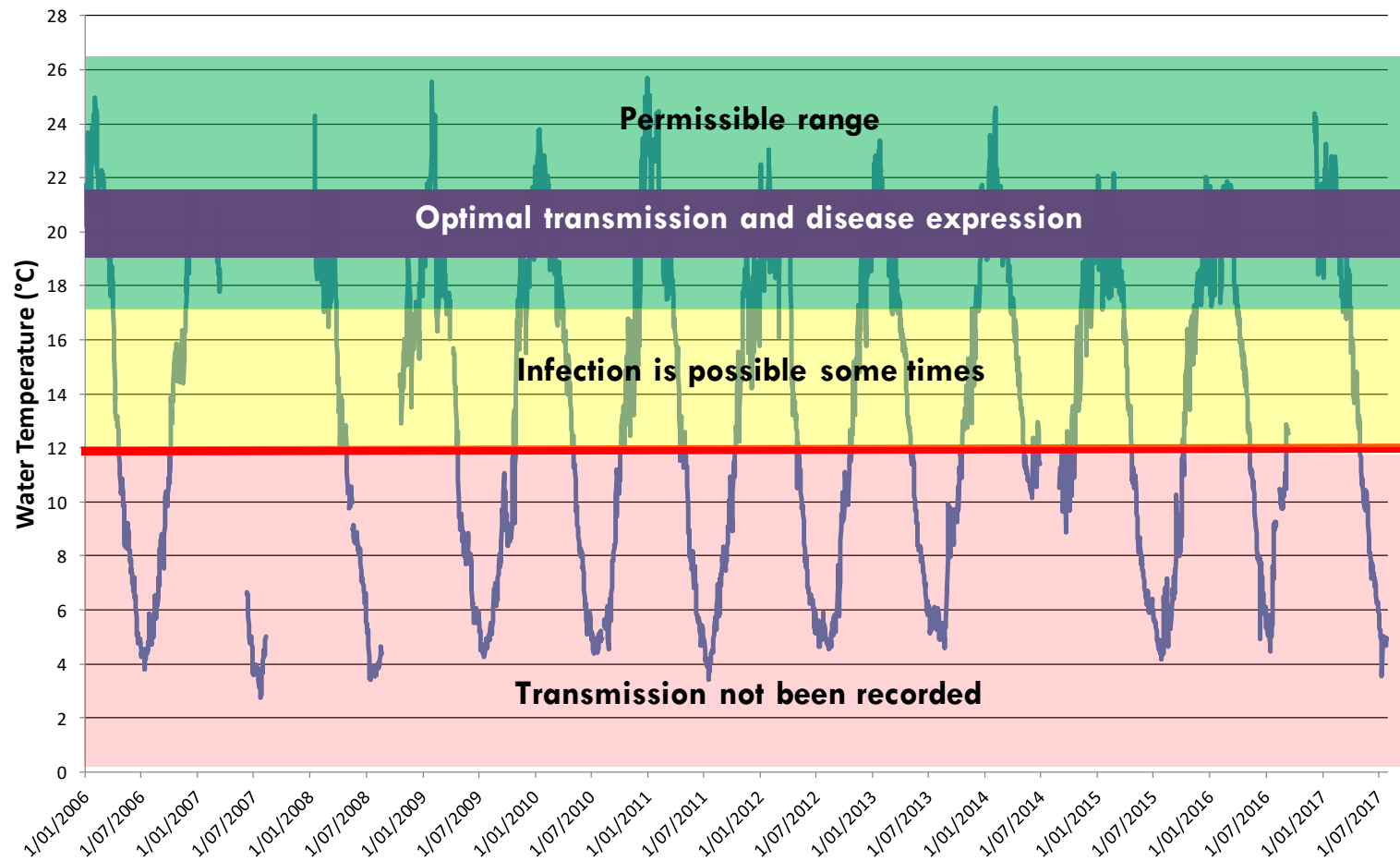


Figure 4. The average daily water temperatures of the top 2m of Tantangara Reservoir from 2006 to 2017. Temperature data were supplied by Snowy Hydro. Overlaid is the transmission profile associated with water temperature for infection and disease expression for EHN described by Whittington and Reddacliff (1995).

3. Presence or absence of EHN in Talbingo and Tantangara Reservoirs

To date, there have been no reports of disease caused by EHN in Talbingo or Tantangara Reservoirs. There has been no active surveillance and passive surveillance would have low sensitivity for detection of moderate disease outbreaks (Whittington et al., 2011). Details of a recent surveillance project commissioned by Snowy Hydro (Song et al., 2018) in which EHN was not detected were provided.

A convenience sample of 50 redfin perch was obtained by electro fishing from nine sites in Talbingo Reservoir in February 2018. From Tantangara Reservoir, 50 trout were sampled (24 brown trout and 26 rainbow trout). The susceptibility of brown trout to EHN has not been evaluated, so the effective sample size from a known susceptible species was 26. These samples were reported to be tested by qPCR using a method described by Pallister et al., (2007) with an unusually high false positive rate for the type of assay. Samples subsequently tested negative using conventional PCR described in the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE, 2015b). Interpretation of the results of qPCR, conventional PCR and sequencing of qPCR amplicons in series provided the interpretation that all samples were negative for EHN. However, to use these test results to interpret the survey requires knowledge of the expected prevalence of EHN and the diagnostic sensitivity and specificity of the assays to interpret the likelihood of freedom from infection. There are no published validation data for the Pallister assay. A different qPCR assay for EHN has been evaluated according to the OIE validation pathway including standardisation of tissue preparation and nucleic acid purification using virus isolation in cell culture as a reference assay (Jaramillo et al., 2012). The diagnostic sensitivity was 94.3 – 98.3 % and the diagnostic specificity was 91.3 – 99.6%. A minimum expected prevalence of 2% is frequently considered in an endemic disease scenario (OIE, 2015). Thus, the present survey would require a sample size of 530 or 131 fish per population to demonstrate freedom with 95% confidence for an expected prevalence of 2% or 5%, respectively. These calculations were undertaken using the FreeCalc algorithm (AusVet epitools,

<http://epitools.ausvet.com.au/content.php?page=FreeCalc2>) which is based on Cameron and Baldock (1998) with the parameters: population size (100,000); test sensitivity (98%); test specific (99%); design prevalence (2% – 5%) and the modified hypergeometric exact calculation method. As the population size is unknown the calculation was repeated for 10-fold difference in population size between 10^3 and 10^6 for the 2% prevalence resulting in a change in the required sample size from 453 to a maximum of 531 as the population increased. For 5% prevalence the sample size was 126 at a population size of 10^3 and did not exceed 131 as the population size was increased. Alternatively, the present survey can be interpreted as estimating the true prevalence of EHN in each water body as 0 – 6.7% (95% Wilson confidence limits) according to the AusVet epitool calculator for true prevalence (<http://epitools.ausvet.com.au/content.php?page=TruePrevalence>) which is based on the method of Rogan and Gladen (1978). These interpretations are dependent on random sampling, which was not undertaken.

The survey also considered qPCR tests on environmental samples with negative results obtained. The diagnostic sensitivity and specificity of eDNA methods for detecting EHN in environmental water samples is not known. Techniques for sampling environmental water have been demonstrated for Cyprinid herpes virus 3 (Minamoto et al., 2009), for example. In the case of EHN, free virus in the environment is likely to exhibit extreme spatiotemporal clustering outside of a disease outbreak scenario so the predictive value of this methodology is expected to be low.

Options for further surveillance

There is no known active surveillance for EHN in NSW or the ACT that is currently ongoing. Greater knowledge of the presence or absence of EHN in Talbingo and Tantangara Reservoirs would enable the effective evaluation and management of disease risks. A risk-based surveillance program that provides an efficient approach to detecting EHN in new locations is detailed in Section 6.1. Such a program would be ongoing and include assessments of the populations at risk and the environmental conditions to identify changes in disease risk and targeted tests for EHN.

Preceding this surveillance program is the need to further evaluate the presumption of freedom from infection in Talbingo and Tantangara Reservoirs. These activities could include:

- Testing an increased sample size to accommodate the low minimum expected prevalence and clustering for endemic EHN. The principles of risk-based sampling to target the samples with the highest probability of EHN infection can be used to maximise the negative predictive value the survey (Section 6.1).
- Use of diagnostic tests with estimates of diagnostic sensitivity and specificity based on the most comprehensive available validation data. These should be undertaken in a laboratory with ISO 17025 accreditation for the test.
- A sero-survey for EHN-specific antibodies can provide additional information about the exposure history of fish in each location because the antibody response can last longer than persistent infection in surviving fish (Whittington et al., 1994). An enzyme linked immunosorbant assay (ELISA) for detection of EHN antibodies assay can be conducted in the OIE reference laboratory for EHN according to the methods described by (Whittington et al., 1999). This assay is not recognised by the OIE as a standard diagnostic test due to uncertainty about the duration and variability in the immune response of fish (OIE 2015). It has been used to evaluate the EHN

exposure status of native Australian species (Whittington et al., 2011). The prevalence of antibodies in grow-out rainbow trout after an outbreak of EHN was 0.02 – 3.7% compared with below the limit of detection for agent detection tests for EHN (Whittington et al., 1999). Tests for EHN antibodies in blood serum samples provide a method of identifying prior exposure of a population and can be useful for surveillance of populations in areas where EHN may be endemic outside of times when there is a disease outbreak.

4. Evaluate transmission pathways for EHN

- Evaluate EHN transmission pathways and potential for persistence of EHN in waterways under a scenario where the Snowy 2.0 project proceeds. Specifically, if EHN was present at some time in the future in Talbingo identify the pathways from Talbingo to Tantangara, from Tantangara to the Upper Murrumbidgee River and from Tantangara Reservoir to Eucumbene Reservoir via the Murrumbidgee-Eucumbene Tunnel.

Transmission of EHN

EHN has been detected in three different scenarios:

(i) disease epidemic with high prevalence, high viral load and high mortality in redfin perch. Generally the period of disease is short (months), the virus is considered endemic in the water body but recurrence of disease is infrequent.

(ii) Subclinical infection of redfin perch with EHN. The virus was isolated from apparently healthy redfin perch that survived a natural disease outbreak suggesting a possible reservoir, although the duration of infection is not known (Langdon and Humphrey, 1987).

(iii) subclinical or low mortality disease in farmed rainbow trout.

Laboratory studies suggest that a disease outbreak and subclinical infection may be possible in other species (Section 2). None of the species evaluated so far appear to be susceptible to disease that produces the same high viral load and high mortality as in redfin perch. This species is therefore considered the

amplification host for the pathogen. Spread of EHN between water bodies has been frequently associated with active disease in redfin perch. Transmission has occurred in an upstream as well as downstream direction (Whittington et al., 2010), has been mediated by birds through regurgitation after feeding on EHN-infected fish carcasses (Whittington et al., 1996), live fish movements (Langdon et al., 1988) and by anthropogenic activities including on fomites such as boats and fishing equipment, illegal relocation of redfin perch and use of frozen redfin perch as bait (Whittington et al., 2010). Transmission through water was demonstrated by the occurrence of EHN disease in rainbow trout has been linked pumping of river water from a diseased area into aquaculture facilities independent of the movement of fish (Whittington et al., 1999).

Horizontal transmission from fish with subclinical infections is a source for EHN spread. EHN was spread by movement of infected rainbow trout fingerlings between farms (Langdon et al., 1988, Whittington et al., 1994, Whittington et al., 1999). However, without the movement of live fish, the risk of disease spread from subclinical infections is much lower because the viral load and prevalence are low. There is no evidence to suggest EHN can be transmitted vertically from adults to eggs (Whittington et al., 2010).

Transmission of EHN is influenced by the infectious dose of EHN required to establish infection, which is extremely different between redfin perch and rainbow trout host species (Section 2). Recent studies indicate that the infectious dose is also variable within a host species due to differences between host populations and the nature of the challenge for redfin perch and rainbow trout (Becker et al., 2013; Borzym and Maj-Paluch, 2015). Effective contact is a combination of the amount of EHN, the susceptibility of the individual fish and the mode of contact. Cohabitation of infected fish provides the highest chance of effective contact. Retention of EHN in tissues of a size likely to be ingested increases the chance of infection compared to immersion exposure in free EHN, where a relatively large quantity of virus is required to obtain an effective concentration.

The stability and long term survival of EHN outside of a host creates multiple potential transmission pathways independent of live fish. EHN and similar ranaviruses are extremely stable in the environment under certain conditions. EHN retained its infective titre for 97 days at 15°C and 300 days at 4°C (Langdon, 1989). Further, experiments have shown the virus to be resistant to desiccation, retaining infectivity after drying for 113 days at 15°C. EHN has also been shown to remain infective in frozen fish for at least 2 years (Whittington et al., 1996). Infectivity of a related ranavirus (*Bohle iridovirus*) declined at 44°C and further at 52°C but treatment at 58°C for 30 minutes was required for complete loss of infectivity (La Fauce et al., 2012).

Thus, spread of EHN via fomites is a relevant transmission pathway and waterborne transmission has the potential to occur over a long period of time. There are limited studies of the efficacy of some chemical disinfectants for EHN and related ranaviruses under a low soiling load. These suggested that sodium hypochlorite at 200 mg.L⁻¹; 70% ethanol; 150 mg.L⁻¹ chlorhexidine as 0.75% Nolvasan® for 1 minute; 200 mg.L⁻¹ potassium peroxymonosulfate were effective (Bryan et al., 2009; La Fauce et al., 2012; Langdon, 1989). Higher soiling loads will reduce the efficacy of disinfection protocols.

Specific scenarios in which probability of EHN transmission pathways is to be considered:

The present risk assessment is qualitative and reflects the opinion of the authors based on available data summarised in this report, and conducted according to the method reviewed by Thrush et al. (2011). A qualitative rating scale was applied for the probability of transmission of EHN to new locations (negligible, low, moderate and high). The consequence of the spread of EHN was assessed qualitatively for disease impact on fish populations based on the following definitions: *insignificant*, subclinical infection that is not persistent; *minimal*, EHN disease resulting in short-term disturbance of feral pest fish species only (including short term increase in transmission risk to new areas); *major*, was a long term change in the ecosystem due to the impact of EHN disease on fish populations or endemic disease is

established and able to transmit to a new location; *massive*, high mortality disease of an endangered fish species. The risk was determined as a combination of probability and consequence according to the following matrix:

		Consequence of EHN disease			
		Insignificant	Minimal	Major	Massive
Probability of EHN transmission	Negligible	Low	Low	Low	Moderate
	Low	Low	Low	Moderate	Moderate
	Moderate	Low	Moderate	Moderate	High
	High	Low	Moderate	High	High

Current risk in the absence of Snowy 2.0

There are existing risks from natural and anthropogenic spread of EHN on vectors including, recreational anglers and from birds and the possibility of emergence from an unknown reservoir host with changes in the environment (Whittington et al., 2010). For Tantangara, Lake Eucumbene and the Upper Murrumbidgee River, the baseline risks were considered low, low and moderate, respectively.

4.1 No fish are transferred to Tantangara

The baseline risk is defined by the probability of the at-risk population of fish in Tantangara being effectively exposed to EHN. An effective exposure depends on the state of the at-risk population, the environment as well as the nature of any EHN entry to the water body. The resident population of rainbow trout provides a moderately susceptible host species. The probability of this population becoming infected with EHN will vary periodically. The risk is significantly increased at times of strong recruitment, with an abundance of young of the year fish increasing the proportion of the most susceptible age class. The seasonal risk will vary with water temperature with a peak in the period October until May of each year.

The exposure to EHN can occur without transfer of fish or direct contact and it is unlikely that darkness, turbulence or pressure in the tunnel would impact the infectivity of EHN. Waterborne transmission of EHN requires a dose in the order of 1000 TCID₅₀/ml to infect rainbow trout. Establishment of the disease in the population is also unlikely for an isolated single infection event. Establishment of an infection cycle within the reservoir is more likely if a minimum prevalence threshold is exceeded by a pathway that exposes multiple fish to infection from Talbingo rather than an isolated single event. An effective dose and number of initial effective contact transmissions is much more likely to be reached if there is a high mortality outbreak of disease in redfin perch in Talbingo Reservoir, otherwise the EHN risk for Talbingo reservoir is low. It is unlikely that EHN will be uniformly distributed in the water column under circumstances of a disease outbreak. Rather, virus will cluster with small particles derived from necrotic fish tissue, mucous and attachment to particles in the water an unpredictable distribution. These small boluses of EHN will be stable for more than a week and provide opportunity for rainbow trout to be exposed to infection by contact or ingestion. There are precedents whereby rainbow trout in farms have been infected by EHN via pumping of water from a location where redfin perch are experiencing disease (Whittington et al., 1994). A key to effective biosecurity will be a sensitive surveillance strategy to identify times when EHN is occurring in Talbingo and when EHN transmission risk is therefore exponentially higher.

If an effective transmission of EHN to rainbow trout in Tantangara occurs, it is likely that a low grade disease expression will occur which might not be readily apparent. Therefore active surveillance would be required to obtain a timely detection and enable a disease control response. It is also likely that EHN will become endemic in the waterway with the potential for seasonal recurrence and exacerbation of mortality when the population structure changes and environmental risk factors occur. In the event that an EHN outbreak occurs in Talbingo and this is subsequently transmitted to the population of rainbow

trout in Tantangara, the long term potential for transmission to fish in water bodies with a hydrodynamic connection is high.

4.2 No live Redfin are transferred to Tantangara

- No live Redfin are transferred to Tantangara (but dead fish may be delivered to Tantangara)

The scenario in which whole dead redfin perch (or other fish species) might be transferred to from Talbingo to Tantangara is a similar but slightly higher risk scenario compared to that described in Section 4.1. A probability of an infectious dose making effective contact with a susceptible rainbow trout from a whole fish is only marginally different to that in which particles are transferred. The risk factors for establishment of EHN in Talbingo and further spread are the same as Scenario 4.1. The risk associated with this pathway would be high if active EHN disease expression occurs in Talbingo and low at other times.

4.3 Live Redfin only colonise Tantangara Reservoir and further expansion is prevented via fish exclusion devices

A scenario in which live redfin perch colonise Tantangara creates a high risk for spread of EHN. The highly susceptible redfin perch in an environment that will be conducive to EHN disease expression substantially increases the probability of an effective contact being made between Talbingo (or indirectly from another proximate waterbody) resulting in EHN infection and widespread disease expression in Tantangara. The probability of EHN effectively transmitting to Tantangara is considerably higher in the presence of redfin perch by virtue of the susceptibility to some fish to as little as 0.1 TCID₅₀/ml, a 10,000-fold lower dose for immersion exposure compared to rainbow trout. Therefore the long term risk is high. This reflects a high risk of EHN transmission in the presence of an outbreak in Talbingo and a moderate risk of transmission and the major impact of endemic EHN in redfin perch in Tantangara. The risk period for EHN transmission varies with the water temperature profile of both reservoirs as in Section 4.1. The first

exposure of naïve redfin perch would likely result in an explosive outbreak, as has been seen for the first occurrence in other waterbodies.

The potential consequences of EHN reaching Tantangara are much higher in the presence of redfin because of the higher likelihood and more rapid spread to new populations and locations due to the presence of an amplification host for EHN. Under this scenario it is expected that EHN would become endemic in the reservoir and the long term probability of continued spread would be high. The expression of disease might recur seasonally and will be much greater compared to rainbow trout, generating high loads of EHN associated with a high mortality outbreak. It is likely that rainbow trout in Tantangara Reservoir would become infected and may create another reservoir for endemic infection and a risk pathway for spread due to movement of recreational fish. This facilitates the full range of transmission opportunities that would put fish in connecting water bodies at moderately high risk of infection. The severity of the outbreak will depend on the water temperature and other environmental conditions at the time. It will also depend on the abundance and age structure of the redfin perch population. An understanding of the probable population dynamics and productivity for Tantangara for this species would further inform the disease risk. A delay between transfer of fish and EHN transmission would potentially exacerbate the risk due to an increased size of the redfin perch population. Further, a newly established population of redfin is likely to have a population structured skewed towards an abundance of naïve young fish which will increase the magnitude of the outbreak. The higher the EHN load inputting into spread pathways, the higher the likelihood of effective contact with additional host populations. During a disease outbreak with abundant dead fish, the risk of water borne, inadvertent anthropogenic and bird mediated transmission is high. Under a disease outbreak scenario, fish exclusion devices at the dam and tunnel will not substantially reduce the high risk of transmission to susceptible host populations in water bodies with a hydrodynamic connection. This includes rainbow trout in Lake Eucumbene and Macquarie perch in the Murrumbidgee River upstream of Cooma. Upstream spread is possible in the

presence of a susceptible host, so exposure of the endangered population of galaxid species in Tantangara Creek may occur, although the susceptibility of this species is not known. However, if the disease occurs in Talbingo and if Redfin are transferred to Tantangara and the disease becomes endemic, during periods where EHN is endemic without active disease in redfin the risk for spread will be much lower. During these times, fish exclusion at the dam and tunnel will further reduce the risk of EHN spread via redfin perch or rainbow trout with subclinical infection.

An established population of Redfin perch in Tantangara provides a stepping stone to increase the distribution of EHN to new populations and locations. By acting as an amplifying host, redfin perch can increase the probability of generating a transmission pathway and EHN load that will infect species with lower susceptibility (resident rainbow trout) and geographically more separated (Macquarie perch).

4.4 Live Redfin colonise Tantangara and secondary locations of the Upper Murrumbidgee River and Eucumbene Reservoir

The scenario in which redfin perch colonise Eucumbene Reservoir and the Upper Murrumbidgee River in addition to Tantangara Reservoir provides the highest risk of spread of EHN and impacts on new host populations. The scenario is influenced by the same factors described in Section 4.3. The increased risk is generated by the increased probability of transferring EHN from Tantangara to these waters. The relevant risks are the lower infectious dose required by redfin perch to establish infection in the new location and the higher disease expression. The scenario in which an outbreak of EHN occurs in direct contact with the at risk populations provides the highest likelihood of establishing infection in the less susceptible species. Opportunities to implement eradication are not feasible when there are wild susceptible hosts.

5. EHN to Macquarie Perch in the Murrumbidgee River

- Evaluate the likelihood of transmission of EHN to the Macquarie Perch population in the Murrumbidgee River at Cooma under all 3 scenarios listed in Section 4.

Water outflows from Tantangara Reservoir at the dam enter the upper Murrumbidgee River where an important population of the endangered Macquarie perch are present between Tantangara dam and Goorudee River, 53 km upstream of Cooma. There is contemporary documentation of Macquarie perch spawning activity in this area (Bylemans et al., 2017). Snowy 2.0 creates a plausible transmission pathway between Talbingo and this Macquarie perch population. The important factors in this pathway were described in different scenarios under Section 4. Specific considerations in assessing the risk of this pathway are:

- The presence of EHN in Talbingo Reservoir. There have been no recorded instances of EHN outbreaks in Talbingo. At present it is not known if a low prevalence of endemic infection is present in one or more species, particularly redfin perch, rainbow trout and gambusia. The presence of a large population of redfin perch in the absence of prior exposure provides the highest risk of transmission of EHN to connected waters in the event that an outbreak occurs. However, if EHN is absent from Talbingo, it would be of value to seek to identify the natural barrier to spread from Blowering, one of the most active sites for EHN. Determining the likelihood of spread of EHN from Blowering Reservoir to Talbingo using disease modelling supported by additional EHN surveillance is key to evaluating the risk pathway.
- An EHN disease epidemic in Talbingo Reservoir would create a period of high risk. The risk can be reduced by rapid identification and implementation of higher level disease control for the high risk period based on an effective surveillance system.
- Endemic disease in Talbingo Reservoir creates a low-moderate risk that would be somewhat mitigated by preventing live fish transfers.

- Establishment of EHN in Tantangara Reservoir has a variable risk that depends on the nature of resident fish populations and the effective transmission of EHN. There is a suitable water temperature profile and the present rainbow trout population is sufficient to create an endemic infection if spread occurs. Effective prevention of live fish movements out of Tantangara Reservoir would reduce the risk to hydrodynamically connected waterways in this scenario. Exclusion of redfin perch from this water body would provide a substantial barrier to the initial spread and establishment in this water body and also reduce the infection pressure that will be placed on connected waters during a disease epidemic.
- Finally, the risk to Macquarie perch is reduced if redfin perch are not present in the Murrumbidgee River between Tantangara Dam and Cooma. This species would provide an amplification host that could increase the infection pressure and probability of effective contact.
- The risk of transmission of EHN is increased by stepping stone populations of susceptible hosts and particularly the amplification host, redfin perch, at each step. Rainbow trout provide a much lower risk compared to redfin perch which are more easily infected and amplify the virus more effectively. The stability of EHN in the environment is sufficient to enable it to remain viable for the duration required to move from Talbingo to Tantangara and to the Macquarie perch population in the upper Murrumbidgee River if water is released from the reservoir during an active disease outbreak. The probability of an effective dose of EHN contacting Macquarie perch is low in the absence of a large disease epidemic occurring within Tantangara. There are no reports of transmission to species other than redfin perch or rainbow trout within the current range of EHN.

Overall, there is a moderate increase in the risk of EHN transmission between Talbingo Reservoir and the population of endangered Macquarie perch in the Murrumbidgee River upstream of Cooma due to a hydrodynamic connection between Talbingo and Tantangara reservoirs. The risk can be reduced with implementation of measures to mitigate disease and EHN transmission. The key risk of EHN exposure of Macquarie perch in the Upper Murrumbidgee River without Snowy 2.0 depends on upstream spread from the endemic waters in the ACT with some risk of spread with recreational fishing. At present these are low risk transmission pathways and effective contact is reduced without a proximate population of amplification hosts.

6. Disease mitigation measures to minimise risk of spreading EHN

- Identify steps/measures/operating procedures that could be implemented to minimise the risk of transmission of EHN to Tantangara Reservoir and beyond if an outbreak occurs in Talbingo

6.1 Surveillance for EHN in Talbingo and Tantangara

EHN is listed in Australia's National List of Reportable Diseases of Aquatic Animals (Animal Health Committee, 2018) and is a notifiable disease in the New South Wales Biosecurity Regulation 2017. At present the NSW DPI implements a passive surveillance program whereby investigation of notified fish kill events includes testing for EHN when it is considered to be a differential diagnosis. The efficiency of this surveillance system depends on visible high mortality disease, willingness of the public or government staff to make notifications, and timely submission of suitable diagnostic samples. There is no known active surveillance for EHN, although hatchery produced rainbow trout are screened by laboratory testing to confirm freedom from EHN before stocking (NSW DPI, 2005). A monitoring program for EHN disease risk would provide the necessary knowledge for an evidence-based and adaptable disease mitigation strategy aimed at reducing the risk of spread of EHN. Active EHN disease events provide a much higher risk for spread of EHN to new locations compared to endemic subclinical situation. Therefore prompt identification of a disease outbreak would enable options to provide enhanced biosecurity that would

minimise the risk of iatrogenic spread. According to the OIE definition of a surveillance system, several components are integrated to generate information about the distribution and incidence of disease caused by a pathogen (OIE, 2015a). For the risk associated with Snowy 2.0, this would incorporate surveys in Talbingo and Tantangara Reservoirs for EHN. Active sampling is required because of the recognised weaknesses of passive surveillance for disease in wild fish which includes poor detection of diseased animals and failure to obtain suitable diagnostic specimens under time delay. Further, EHN is present with a low prevalence of subclinical infection and low viral load in an endemic subclinical infection scenario. The ongoing activity would lead to progressive increases in the confidence of the infection status of the monitored areas. Additional information to be monitored could include: fish populations (susceptible species presence or absence; density; age structure); and environmental risk factors for disease (water temperature; outbreaks of EHN in other waters; changes to biosecurity such as stocking practices).

A risk-based approach for detection of EHN provides the most efficient method of surveillance under a scenario where the virus has not been previously detected in the waterways under consideration. Risk-based surveillance for aquatic animal disease is applicable to minimise the cost whilst maximising the confidence of detecting changes in the incidence and distribution of disease (reviewed by Oidtmann et al., 2013). The method requires targeted sampling that is informed by known risk factors whereby samples are preferentially tested based on the highest likelihood of detecting EHN. In the case of infection with EHN, risk factors are: health status (sick and recently dead > healthy); species (redfin perch > rainbow trout > other fish species susceptible in experimental settings); age of fish (juvenile > adults); water temperature (19 – 21°C > between 22°C and 26°C > between 12°C and 18°C); proximity, or other epidemiological connection to known EHN infected fish (epidemiological trace forward of suspect or confirmed incidence of EHN).

6.1.1. Objective and definitions

The objective of a surveillance program would be detection of EHN as rapidly as possible in areas that are presumed free from infection. Under this scenario the confidence of freedom from infection increases as negative surveillance results accumulate. A detection of EHN under the risk-based protocol does not measure the prevalence or describe the distribution of EHN. If EHN was determined to be present in a waterway, it would be appropriate to change the surveillance strategy to measure the prevalence and spatiotemporal distribution in fish populations stratified by species and age/size class with an appropriately designed survey. The 4 relevant epidemiological units of interest are all fish of susceptible species in: Talbingo Reservoir; Tantangara Reservoir; Lake Eucumbene; and the Murrumbidgee River between Tantangara Dam and Cooma. Infection with EHN is defined by the diagnostic test.

6.1.2 Targeted sample collection

Point in time surveys for EHN can be integrated into an ongoing monitoring programme. The majority of samples would be collected according to an active sample collection strategy. The timing of sample collection is informed by risk factors that increase disease risk: water temperature in the optimal range, (19 – 21°C); preferential sampling of young redfin perch, or the next most susceptible group in areas where redfin are absent (i.e. young rainbow trout). The design prevalence should consider that EHN can be present at low prevalence and the sample size for wild rainbow trout and apparently healthy redfin perch should be calculated for a minimum expected prevalence of 2% and the sample size should be adjusted for clustering.

Additional samples should be tested when risk factors are identified. Most particularly, sampling should be responsive to changes in the risk factors described in Section 6.3. Reports of fish disease in the project area should trigger rapid deployment of a properly equipped sample collection team who will preferentially seek moribund fish or conspecifics from the location of the reported disease. Changes in

risk should trigger additional surveillance. For example, redfin perch entering a new water body or a substantial change in the age structure of redfin perch or rainbow trout with high recruitment or productivity changes of the waters.

In the absence of a well characterised system to collect an appropriate sample, environmental samples are not recommended for EHN surveillance. A validation study would need to be undertaken to identify the best protocol for sample collection with consideration of the impact of processing techniques on analytical and diagnostic characteristics. This is needed as the negative predictive value of existing test results is unknown. An environmental sampling strategy requires a risk-based approach that accounts for spatiotemporal clustering of EHN outside of a host.

The advantages of sero-surveys to increase the sensitivity by identifying previous history of EHN infection justify their incorporation into the surveillance system. The higher prevalence of EHN antibodies in surviving fish and the potential to collect blood samples without sacrificing fish can increase the effective sample size. In an endemic disease scenario the prevalence of antibodies will inform the disease risk as a higher prevalence might indicate low susceptibility to infection at population level. It should be noted that the use of serological tests requires validation to estimate the sensitivity and specificity of detecting EHN specific antibodies in different fish species.

Samples should be collected in a manner that maximises the utility of diagnostic tests by ensuring false positive results are not caused by cross contamination and false negative results are not caused by inappropriate sample preservation, preparation or inhibition. Blood samples for sero-surveys require trained personnel to ensure survival of fish not intended for sacrificial testing.

6.1.3 Diagnostic tests for EHN

The assay, with the most comprehensive validation data should be used according to internationally agreed protocols and in a laboratory with ISO 17025 accreditation for the test, rather than accreditation only for other tests, i.e. qPCR according to Jaramillo et al., (2012). Confirmatory tests including sequence analysis and virus isolation should be undertaken to resolve equivocal results. This requires samples to be transported to the laboratory on ice within 36 hours in preference to preservation in 80% molecular biology grade ethanol if logistically possible. Notification of suspected emergency disease and positive test results for EHN should be undertaken according to legislative requirements.

There are standard operating procedures for indirect enzyme linked immunosorbent assay for antibodies to EHN (EHN Ab ELISA) for serum samples from all surveyed fish and virus isolation confirmation of positive qPCR samples (Whittington et al., 2011). Histopathology of correctly preserved diseased fish tissues in the case of a disease outbreak is important for understanding if EHN is causative through histopathological examination with immunohistochemistry to attribute the presence of EHN (if detected) as the cause of disease. The diseased fish which are a high priority sample for EHN testing require abdominal contents fixed in 10% neutral buffered formalin in addition to unpreserved tissue samples.

6.1.4 Additional options for the surveillance system

A mechanism to collate passive surveillance on fish disease, changes in fish population structure and environmental data would feed into decision making for risk-based surveillance. It is noted that recreational anglers will provide the best opportunity for passive disease detection and providing them with a clearly communicated, informed and incentivised notification pathway would be desirable.

Infection pressure. An outbreak of EHN disease in a neighbouring waterway will significantly increase the risk of EHN infection in the target water bodies. Detection of EHN through passive surveillance should trigger increased surveillance efforts and precautionary disease mitigation. The sensitivity of this pathway might be increased by offering incentives to recreational fishing groups to report suspicions of disease. Trace forward epidemiological analyses of any reports of EHN should rigorously consider a change in risk to the four areas under consideration and trigger additional surveillance if a potential link is found.

Water temperature. Water temperature monitoring broadly informs the risk periods for EHN transmission risk and the highest risk periods for disease outbreaks. The timing of routine surveillance is informed by long term temperature data.

Fish populations. The presence of redfin perch in target water bodies other than Talbingo Reservoir, increases in the population density of all susceptible species and reduction of the age profile of susceptible species are risk factors for EHN transmission. Data from direct and indirect (e.g. environmental sampling, eDNA for fish species assessment) methods of fish monitoring in the relevant waterways could be used.

6.2 Options for reducing infective EHN spread with Snowy 2.0

- Discuss potential measures to reduce the risk of EHN transmission at Tantangara Dam and the entrance of the Murrumbidgee-Eucumbene Tunnel to prevent the transmission of EHN beyond Tantangara Dam.

The physical separation between Talbingo and Tantangara and the differential disease risk between these two waterbodies provides the most efficient place for EHN prevention in Tantangara and beyond. Preventing an increase in the geographical distribution of redfin perch would be the most effective way to reduce the likelihood of EHN spread to Tantangara Reservoir and beyond. Using an ongoing active surveillance system to detect EHN disease would enable adaptive increases in biosecurity at these times of substantially increased risk.

6.2.1 Restrict the range and size of the amplifying host fish population

The risk of spread of EHN to Tantangara Reservoir and beyond is greatly reduced by ensuring redfin perch do not establish outside of Talbingo Reservoir. The establishment of Redfin in Tantangara would increase the probability of effective transmission of EHN into the reservoir and increase the magnitude of disease outbreaks in the system and thus the infection pressure in neighbouring waterbodies. Surveillance for detection and quantification of redfin populations in Tantangara is required to detect a breach in this system or entry by another means. Detection could trigger an eradication or population control strategy.

6.2.2 Reduce the burden of EHN in water conduits

There is a confirmed potential for transmission of EHN to rainbow trout when rainbow trout aquaculture facilities pumped water at low volume over a short distance from a source in which EHN disease was active in redfin perch (Whittington et al., 2010). The details of the distance and volume of the pumped water in this example are not recorded, but over a much lower volume and distance compared to Snowy 2.0. Regardless, the environmental resilience of EHN enables it to remain infectious for a period of 3 months at 15°C, thus probably able to be transferred in high volume, through a tunnel of 27 km. Exclusion of the movement of live fish substantially reduces the risk of transmission of EHN when disease is subclinical, but is not sufficient to reduce the disease risk when disease is active. EHN associated with dead fish, mucous and necrotic tissues are a high risk for transmission and a high soiling load provided by such biological materials reduces the efficacy of chemical and physical disinfection procedures (Department of Agriculture, Fisheries and Forestry, 2008). Disinfection data for EHN is available to enable feasibility of water disinfection treatments at high volume to be evaluated. In the absence of a suitable chemical or physical disinfection measure, periods of higher risk for EHN transmission identified by surveillance for EHN disease can be identified and measures adopted that would seek to limit the areas exposed to the virus. There is no evidence to inform the use of release of water preferentially from different depths as a

method of reducing EHN spread with water conduits. Active disease surveillance would help identify such periods of heightened risk due to EHN disease.

6.2.3 Apply biosecurity to reduce other EHN transmission pathways

Transmission independent of a hydrodynamic connection is possible with a high risk during a disease epidemic. Particular risks are birds that feed on dead and dying fish and inadvertent anthropogenic spread on fomites, particularly recreational anglers. Surveillance for disease events to rapidly detect an epidemic might allow timely control measures to be implemented to reduce bird activity such as collection and destruction of dead and diseased fish and dispersal of birds. Community awareness and activities to enforce biosecurity targeted to the most relevant groups such as recreational fishers and campers will reduce the risk of spread on fomites. Stocking of fish for recreational fishing and conservation requires consideration of virus transfer with subclinical infection, particularly with exposure to multiple infection points in a hatchery environment. This risk can be reduced by batch testing juvenile fish for freedom from infection and using effective hatchery biosecurity that includes considerations about the source of water and broodstock.

Conclusions

Spread of EHN into a new location with a naïve host fish population will result in a high mortality disease epidemic before an endemic infection cycle is established. A direct water connection between Talbingo and Tantangara Reservoirs increases the risk for expanding the range of EHN in the event that EHN is found to be present in Talbingo or occurs at some point in the future. Without any disease mitigation, there is a high risk of EHN accessing new locations and impacting susceptible fish populations including a population of an endangered species. The risk of spread could be reduced if appropriate disease mitigation strategies are implemented. The following key additional information would be valuable to inform appropriate management, specifically: the present EHN status of Talbingo; investigations of any

natural barriers that have prevented spread of EHN from Blowering to Talbingo Reservoir; and UV efficacy for disinfection of EHN contaminated reservoir water. Disease risk varies with the structure of the at-risk host populations, the environment conditions and is much greater during a disease event. An adaptive biosecurity response can be implemented if it is informed by a high quality EHN surveillance system.

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A review of fish information from the Upper Murrumbidgee and Upper Tumut catchments.

Mark Lintermans



Report to EMM Consulting Pty Ltd.

28 February 2019

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Cover photo: Tantangara dam wall. (Photo Mark Lintermans).

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Introduction

Snowy Hydro Limited (Snowy Hydro), the operator of the Snowy Mountains Hydro-electric Scheme (Snowy Scheme), is proposing to build and operate Snowy 2.0. Snowy 2.0 is a project that will increase the pumped hydro-electric capacity within the existing Snowy Scheme by linking the Tantangara and Talbingo reservoirs with tunnels feeding a new underground power station. The project will involve tunnelling and excavation works between the two reservoirs to depths of up to 1 kilometre (km).

Snowy 2.0 will provide large-scale storage of energy that will be available as quick-start electricity generation at critical times of peak demand. When operational, Snowy 2.0 will function primarily as an energy storage facility; pumping water out of Talbingo Reservoir (the lower reservoir) to Tantangara Reservoir (the upper reservoir) in the storage mode and allowing the water to flow from Tantangara Reservoir into Talbingo Reservoir in the generating mode. Decisions concerning the operational mode, flow rates and flow duration would be made remotely by Snowy Hydro on the basis of the state of the national electricity market (NEM) with due regard given to operational and licensing constraints, including the need to maintain downstream supply and environmental flows.

Snowy 2.0 has been declared Critical State Significant Infrastructure (CSSI) in accordance with the provisions of the New South Wales (NSW) *Environmental Planning and Assessment Act 1979* (EP&A Act) with the declaration coming into effect on 9 March 2018. As a result, Snowy 2.0 may be carried out without obtaining development consent under Part 4 of the EP&A Act. However, Snowy 2.0 is subject to Division 5.2 of the EP&A Act, which requires the preparation of an environmental impact statement (EIS) and the approval of the NSW Minister for Planning.

Water transfers between river basins is one of the known pathways for the transfer of fish species (Lintermans 2004) with the Snowy scheme considered the means by which the coastal *Galaxias brevipinnis* was introduced to the upper Murray (Waters et al. 2002). Introduction of alien species is a major threat to the conservation of native fish communities worldwide, as well as in Australia (Dudgeon et al. 2006; Vorosmarty et al. 2010; Lintermans 2013a). Prevention of such transfers is usually far more cost effective than attempts to later control or eradicate introduced species (Wittenberg and Cock 2005; Simberloff et al. 2013; Rytwinski et al. 2018).

To inform the development of Snowy 2.0, information on the fish fauna of both the source catchment (upper Tumut) and receiving (upper Murrumbidgee) catchments is required. This report assembles existing information—largely unpublished—collected by or known to the author. It does not attempt a comprehensive synthesis of all fish knowledge from the catchments of interest, and has little to contribute to knowledge of the fish fauna of the

Tumut impoundments (Talbingo Reservoir, Jounama Pondage, Blowering Reservoir) as I have not sampled these reservoir locations. However, this report seeks to augment existing fish studies that are being conducted as part of Snowy 2.0 to enable a more-rounded understanding of the existing fish assets and potential threats that may need to be addressed.

This report and associated dataset provides:

1. recent and historic (since ~1950s) freshwater fish records from my personal knowledge in the catchments of the upper Tumut (from upstream of Talbingo to around Blowering reservoirs) and the upper Murrumbidgee (from around Cooma upstream to above Tantangara Reservoir).
2. a professional assessment on likely abundance/status of significant species (i.e. threatened or invasive species).
3. a professional assessment for nationally or state-listed threatened species, as to whether the species is likely to be currently present in the area of record.
4. a brief assessment of potential threats to freshwater fish populations from the operation of Snowy 2.0.

Results

Compilation of recent and historic fish records

Known fish data from my personal observations and sampling was combined with other relevant data for the two focal areas. The fish data has been collected using a variety of sampling methods and intensities and is not standardised. Therefore, catch-per-unit-effort data cannot be meaningfully assembled or compared. Consequently, the data is simply presented as presence/absence of individual fish species. Where samples have been collected over multiple years from the same site, a range of results from multiple years is presented to allow an appreciation of the potential variability in detected species presence. The data are supplied as an excel spreadsheet, and also attached to this report as an appendix (Appendix 1). Data fields included are:

- Date of sampling/observation
- Site location
- Easting and northing (decimal degrees) (approximate only for angler records).
- Nearest town
- Data source and/or relevant publication
- List of fish species recorded at the site.

A total of 18 fish species are recorded from a total of 84 sampling locations or sampling events (Appendix 1). There were 10 native species and 8 alien species (including 1 translocated native). Fourteen of the 84 records were from historical angler reports, two were from eDNA sampling, and the remainder are records from scientific sampling involving the capture of fish.

Six species listed as threatened on either state or national listings were recorded from the data trawl (Table 1).

Table 1. Listed threatened species recorded (and conservation status under each listing). Vu= vulnerable, Crit En = critically endangered, En = endangered.

Species	State listed	Nationally listed
<i>Gadopsis bispinosus</i>	ACT (Vu)	-
<i>Galaxias tantangara</i>	NSW (Crit En)	*(Crit En) #
<i>Maccullochella macquariensis</i>	NSW (En), Vic (En), ACT (En)	En
<i>Macquaria australasica</i>	NSW (En), Vic (En), ACT (En)	En
<i>Maccullochella peeli</i>	-	Vu
<i>Euastacus armatus</i>	NSW (Vu), ACT (Vu)	-

listed as critically endangered by the Australian Society for Fish Biology (Lintermans 2016)

*= currently being assessed under the EPBC Act

Three alien/invasive species of particular concern were also recorded: Redfin Perch *Perca fluviatilis*, Climbing galaxias *Galaxias brevipinnis*, and eastern Gambusia *Gambusia holbrooki*. Published literature identifies another three native fish species known from the upper Tumut catchment (Golden perch *Macquaria ambigua*, Australian smelt *Retropinna semoni*; Flatheaded gudgeon *Philypnodon grandiceps*). No additional species are known to occur in the upper Murrumbidgee catchment above Cooma from the published literature. The additional fish species identified from the upper Tumut catchment are not considered further in this report.

The 6 listed threatened species and the three alien/invasive species are discussed further below.

Professional assessment on likely abundance of significant species (i.e. threatened or invasive species).

Two-spined blackfish *Gadopsis bispinosus*

Two-spined blackfish is a small-medium sized fish generally confined to upland, rocky-bottomed streams (Sanger 1994; Lintermans 2007). It is relatively widespread and abundant on the eastern side of the upper Tumut catchment, occurring in a number of tributaries of the Goobarragandra and Tumut rivers. It is interesting to note that the DPI catch record data presented in the Snowy 2.0 Exploratory Works Environmental Impact Statement (EMM Consulting 2018) reports that Northern river blackfish has been recorded from Jounama Creek. To my knowledge these are the first records of this species from this water body, and I would have thought this locality is more suited to Two-spined blackfish. I have sampled

Jounama Creek on multiple occasions and have only captured Two-spined blackfish (Lintermans 1998, unpubl data). I have sampled Northern river blackfish from more lowland habitats in the Tumut or adjacent catchments such as Gilmore Creek and Adjungbilly Creek. I suggest that the DPI catch reports of Northern river blackfish in the Snowy 2.0 Exploratory Works Environmental Impact Statement are incorrect, and that in fact they are/were Two-spined blackfish.

By contrast, Two-spined blackfish is exceedingly rare in the upper Murrumbidgee catchment upstream of the ACT (Lintermans 2002). It has only been recorded at two sites upstream of Cooma, each on only single occasions. The record from the Murrumbidgee River at Killarney in 1991 has never been able to be replicated in the two subsequent sampling events in 1998 and 1999 at this site, despite suitable sampling techniques being deployed (Fyke nets). The single record from the Murrumbidgee River at Platypus Lodge in 1998 detected small numbers of this species by both boat electrofishing (2 individuals) and fyke nets (2 individuals). The habitat at this site was noted to be heavily impacted by fine sediment which is a known threat to this species, which lays demersal adhesive eggs on clean boulder and cobble substrates (Lintermans 1998; 2007; 2012). This sedimentation is thought to be a result of the lack of flushing flows downstream of Tantangara Reservoir (which almost never spills). The species has been extirpated below another impoundment in the upper Murrumbidgee catchment (Cotter Dam, ACT) as a result of lack of flushing flows and infilling of the substrate through flow deprivation (Lintermans 2002, 2012).

Stocky galaxias *Galaxias tantangara*

Stocky galaxias is a newly described species (Raadik 2014) with an extremely limited distribution. Like most other Australian members of the Galaxiidae, it is a small-bodied fish with a maximum length of ~ 100 mm (H Allan unpublished data). Only known from a small section of Tantangara Creek, it is now confined to only 3 km of stream above a waterfall which excludes predatory trout. The species is thought to have lost > 90% of its former range in the Tantangara Creek sub-catchment (NSW FSC 2016) and wider sampling in the subcatchment has failed to locate any individuals of this species (Raadik 2014; H. Allan unpublished data). Sampling immediately downstream of the barrier waterfall in 2016/17 (Allan & Lintermans unpubl. data) has captured abundant Rainbow Trout and Brown Trout and no galaxias, reinforcing the conclusion about the susceptibility of *G. tantangara* to salmonid predation and extirpation. The species is not known from the Tumut catchment, or anywhere else in NSW/Australia.

Trout cod *Maccullochella macquariensis*

Trout cod is a large-bodied fish that was first listed as nationally threatened in 1980 (Burbidge and Jenkins 1984). It has been the subject of national recovery program for 3 decades, with hatchery-breeding and stocking the mainstay of the recovery effort (Koehn et al 2013; Lintermans et al. 2018). It has been stocked in the upper Murrumbidgee catchment below Tantangara since 1988 with almost 335,000 individuals stocked in this sub-catchment

between 1988 and 2009 (Koehn et al. 2013). There have been several stocking sites around or upstream of Cooma, with the most recent stockings occurring in 2008 (NSW Fisheries unpubl data). Irregular monitoring of the success of these stockings in the upper Murrumbidgee has continued, and there is some evidence since the late 2000s of wild recruitment. However, there have also been some anecdotal observations of potential hybrid offspring from Trout cod/Murray cod crosses, with genetic work confirming that hybrid larvae are present in the upper Murrumbidgee and that F1 hybrids are fertile (Couch et al. 2016). Trout cod were formerly present between Cooma and Tantangara Dam as recently as the 1960s/early 1970s but were effectively extinct in the upper Murrumbidgee by the mid-late 1970s (Berra 1974, Lintermans et al. 1988).

‘Cod’ were also historically known from the Tumut River system upstream of Blowering, but it is not certain whether these were Murray cod, Trout cod, or both (Trueman 2012). Stocking of Blowering Reservoir and Jounama Pondage with Murray cod for recreational purposes has occurred in recent years, and stocking of Talbingo Reservoir with Trout cod for recreational purposes occurred in 1996 and more recently in 2014–2016 and 2018. To my knowledge there has been no evidence of wild recruitment in the upper Tumut from these stockings.

Macquarie perch *Macquaria australasica*

Macquarie perch were historically widespread in the upper Murrumbidgee catchment, but suffered precipitous declines since the mid 1980s (Lintermans 2002). There are anecdotal reports of them as high up the system as Tantangara reservoir, where they were relatively abundant after the dam filled in the late 1960s (Trueman 2012). They now exist as a series of scattered populations in the upper Murrumbidgee, usually in gorge country with relatively intact vegetation cover. The Tantangara Reservoir population is presumed to be extinct, but there is a reliable angler report of the species from immediately below the dam wall in 1991. The uppermost population extends from around Cooma to just downstream of Yaouk bridge (Killarney), and this population demonstrates successful recruitment to young-of-year life-stage (YOY) every year (Lintermans 2016, unpubl data). Adults and subadults are much less common than YOY (Lintermans 2016).

In the Tumut drainage, Macquarie perch were historically present at least until the early 1960s (Trueman 2012) but are now extremely uncommon. A stocking of Macquarie perch into Talbingo Reservoir occurred in 1995/96, and there is an angler report from the upper Tumut in 1998/99.

Murray cod *Maccullochella peelii*

The largest freshwater fish in Australia, reaching 1.8 m in length and 113.6 kg (Lintermans 2007), Murray cod is now an extremely rare fish in the upper Murrumbidgee catchment at or above Cooma. Lintermans (2002) reported that Murray cod did not occur upstream of the ACT, but vague reports of ‘cod’ (of unknown species) are scattered through old newspapers. Most (if not all) of these anecdotal reports are presumed to be Trout cod (Trueman 2012).

However this situation has changed since 2008 when NSW Fisheries commenced stocking Murray cod downstream of Cooma (Couch et al. 2016), and it is also likely that some private stocking of farm dams has also occurred since hatchery breeding became commonplace in the 1980s (Rowland 1983, 1988). Approximately 4000 Murray cod were stocked between 2008 and 2011. The anecdotal records of hybrid cod and the genetic verification of hybrid cod larva all post-date this stocking program.

In the Tumut system, there have been large releases of hatchery-bred Murray cod for many years, particularly in Blowering Reservoir and also in Jounama Pondage. Murray cod are known to breed in Blowering Reservoir, but the Murray cod fishery in many impoundments is sustained predominantly by stocking (Forbes et al. 2016).

Murray crayfish *Euastacus armatus*

Murray crayfish is the second-largest freshwater crayfish in the world, growing to 3.5 kg and living for up to 30-50 years (Lintermans 2002; Gilligan et al. 2007). The species has suffered significant declines across its range over the last 50 years, and is listed as threatened in the ACT and NSW. Populations in lowland rivers have suffered badly in the last decade with the blackwater events that followed the ending of the Millennium Drought (1997-2010), causing high mortality (McCarthy et al. 2014) and recovery taking many years (Whiterod et al. 2018). Upland populations have fared a little better, with no blackwater events occurring in these reaches, but severe floods have had localised impacts on populations in the upper Murrumbidgee (Noble and Fulton 2017). The fish sampling methodologies largely employed in the datasets that have generated this report are not well suited to sampling Murray crayfish, and so there are relatively few records contained within this report.

Murray crayfish are much more abundant in the upper Tumut system, particularly in impoundments, than they are in the upper Murrumbidgee catchment. The Snowy 2.0 Exploratory Work Environmental Impact Statement (EMM Consulting 2018) did not record the species in Talbingo Reservoir, and concluded that the likelihood of the species occurring in Talbingo was low. This is incorrect. Murray crayfish occur in Blowering Reservoir downstream, and are known to occur in Talbingo Reservoir.

Murray crayfish are not known from the sole impoundment within the focal area of this report in the upper Murrumbidgee (Tantangara Reservoir) and to my knowledge have not been reported in riverine habitats upstream of Cooma. They are present in riverine habitats and impoundments (e.g. Burrinjuck) in the upper Murrumbidgee catchment downstream of Cooma (Lintermans and Rutzou 1991; Lintermans 2002; Fulton et al. 2012).

Redfin perch *Perca fluviatilis*

Despite >30 years of active fish sampling in the upper Murrumbidgee by the author, there are no confirmed records of Redfin perch upstream of the southern ACT border (Lintermans 2002; unpublished data). The NSW Fisheries freshwater database contained a single record from October 1990 of this species from the Murrumbidgee river at Mittagang Crossing (see

Lintermans 2007), but recent investigation has revealed this to be a data entry error: the field datasheet contains no mention of Redfin perch (L. Pearce pers. comm. 2018). This means that the Murrumbidgee catchment upstream of the southern ACT Border is considered to be Redfin-free. Redfin are present up to the Tharwa area of the Murrumbidgee River in the ACT, but have not been recorded upstream of Gigerline Gorge which appears to be a barrier to the upstream movement of several fish species (Golden perch *Macquaria ambigua*, Murray cod and Redfin perch) (Lintermans 2002) during 'normal' flows since the construction of Tantangara Dam. That this barrier can be breached (or most likely bypassed) is demonstrated by the presence of Carp, and Oriental weatherloach upstream (likely the result of illegal transfer of bait fish) and the presence of Murray cod upstream since stocking in the Numeralla catchment by NSW DPI (Couch et al. 2016). Redfin are common in the upper Tumut catchment, particularly in Blowering Reservoir where they are a sought-after recreational target. The species is also present in Talbingo Reservoir, the Tumut River upstream of Talbingo, Jounama Creek and several tributaries of Blowering Reservoir. I assume they are present in Jounama Pondage.

Climbing Galaxias *Galaxias brevipinnis*

The Climbing galaxias is a fish that is native to coastal drainages in southeastern Australia. It normally deposits its eggs in flooded riparian vegetation with the larvae then being swept out to sea to develop before returning to freshwaters. However, the species has been likely transferred to inland catchments in the Murray-Darling Basin via the Snowy Mountains Hydroelectric Scheme, where individuals from the Snowy River drainage have been diverted into the Upper Murray drainage (Waters et al 2002; Lintermans 1998). In these inland, landlocked populations, large impoundments fulfil the role of the marine environment for larval development. 1993 was the first record of this species from the Murrumbidgee catchment, when the species was recorded in Morris Creek, a small tributary of Blowering Reservoir (Lintermans unpublished data) with the species again recorded in this location in 2002 (Raadik 2003; Lintermans 2007). The recent Snowy 2.0 Exploratory Works Environmental Impact Statement also recorded this species in Wallaces Creek, a tributary of the Yarrangobilly River (EMM Consulting 2018). The records of this species in Wallaces Creek suggests that there is a high likelihood that the species is or will soon become established in the Tumut drainage. This is the first record of this species since the 1993 and 2002 captures from Morris Creek and is a significant record both for the Murrumbidgee catchment as a whole, and for Snowy 2.0.

There are no records of Climbing galaxias in the Murrumbidgee system, other than those around Blowering and Talbingo. It is confidently considered that they are absent from the upper Murrumbidgee catchment upstream of Burrinjuck Dam.

Eastern gambusia *Gambusia holbrooki*

Eastern gambusia is a small-bodied highly invasive species that along with its close relative Western mosquitofish, is globally distributed. Gambusia are listed in the 100 worst invasive species worldwide (Lowe et al. 2000). Eastern gambusia is widely distributed within the

Murray-Darling Basin and elsewhere in Australia, occurring in all states and territories (Lintermans 2007; Rowe et al. 2008). It is known to be present in Talbingo Reservoir. *Gambusia* are highly aggressive towards other fish, causing fin damage, subsequent bacterial and fungal infection, as well as competing for food and space, and preying on small juveniles (Pyke 2005, 2008; Macdonald & Tonkin 2008; Rowe et al. 2008). The species is present in the Upper Murrumbidgee from the bottom of the study reach (Cooma) to at least as far upstream as Adaminaby, but are not known to be present in Tantangara Reservoir.

Professional assessment for nationally or state-listed threatened species, as to whether the species is likely to be currently present in the area of record.

Two-spined blackfish *Gadopsis bispinosus*

This species is known to be present in the upper Tumut system (as noted in the Environmental Impact Statement prepared for the Exploratory Works for Snowy 2.0).

Stocky galaxias *Galaxias tantangara*

This species is definitely present in the Tantangara Creek sub-catchment of the upper Murrumbidgee (NSW FSC 2016; Allan and Lintermans 2018; Driscoll et al. 2018). I currently have a Masters student describing the ecology of this species.

Trout cod *Maccullochella macquariensis*

Trout cod have been stocked into the Tumut catchment (Talbingo Reservoir) in recent years and are proposed to be stocked into Blowering Reservoir and Jounama Pondage (NSW DPI 2012) as part of a plan to develop a recreational fishery around this species. To date there is nothing to suggest that they will reproduce in the wild in this environment. It is essentially a put and take fishery. There is similarly no evidence to suggest that the stocking of Trout cod into the Talbingo Reservoir has resulted in any natural recruitment.

In the upper Murrumbidgee there are clear indications that Trout cod are breeding, but whether there are pure Trout cod offspring is unknown. The genetic studies of Couch et al. (2016) lower down in the Upper Murrumbidgee only found hybrid Trout cod x Murray cod larvae, or pure Murray cod larvae.

Macquarie perch *Macquaria australasica*

Macquarie perch have established self-sustaining populations in the upper Murrumbidgee as far upstream as Killarney (3.5 river km downstream of Yaouk bridge and ~30.5 Km downstream of Tantangara Dam). The self-sustaining population may extend upstream of Yaouk but the typically very low flow released from Tantangara Reservoir in conjunction with the rocky gorge topography commonly found along this reach suggest that natural instream barriers may limit Macquarie perch dispersal upstream. Science-based fish sampling records upstream of Yaouk are extremely sparse, as are angling reports. It must be noted that should adequate environmental water releases be made from Tantangara Reservoir in the future (i.e. releases of > 5000 ML/d) or if instream barriers are identified and

remediated, Macquarie perch would be expected to recolonise the area upstream to the Tantangara dam wall.

Similarly, there has been sparse scientific sampling in the main stem of the Murrumbidgee upstream of Tantangara or in Tantangara Reservoir itself. It seems unlikely that Macquarie Perch are currently present in or above Tantangara Reservoir (but the species was present in these locations in the 1950s (Trueman 2012)).

There does not seem to be any evidence that there is a self-sustaining population of Macquarie perch in the Tumut River upstream of Blowering Reservoir, including within Talbingo Reservoir.

Murray cod *Maccullochella peelii*

Murray cod are not present as a self-sustaining population in the upper Murrumbidgee above Cooma. There is some reproduction of Murray cod occurring in Blowering Reservoir, but the future for such natural recruitment appears in doubt, as the closed season to protect spawning fish in this impoundment has recently been repealed. There is a substantial stocking effort for Murray cod in the upper Tumut (Blowering and Jounama).

Murray crayfish *Euastacus armatus*

There is no population of Murray crayfish upstream of Cooma including in Tantangara Reservoir. There are numerous angler reports of populations of this species in Blowering and Talbingo reservoirs and Jounama Pondage.

Fish sampling issues worthy of note

One of the issues associated with gaining a representative sample of the fish fauna in a particular location is the need to use multiple sampling techniques, and potentially sampling periods (i.e. seasons). Different species have differing susceptibility to gear types, and differing reproduction and activity patterns and these considerations can greatly influence the overall catch and how the resulting data is interpreted.

Sampling effort also needs to be sufficient, and there are many studies that show that increased sampling effort increases the number of taxa recorded at a site, and it is usually the rare species that are missed (Lyons 1992; Cao et al. 2004; Ebner et al. 2008).

Sampling techniques need to be tailored to target species. For example, if sampling for Macquarie perch the most efficient sampling method to reduce the likelihood of false negatives is the use of fyke nets (Lintermans 2013b, 2016). The use of fyke nets significantly reduced false negatives in studies in the Queanbeyan River (Lintermans 2013) and the upper Murrumbidgee (Lintermans 2016). If for example, only backpack electrofishing or boat electrofishing had been used, the detection rate at sites where Macquarie perch were known to occur would have been < 35% compared to fyke nets which had detection rates of 92-100% (Lintermans 2016). Similarly, different life-stages of fish are susceptible to different

gear types. Again, for Macquarie perch, fyke nets captured mainly Young-of-Year and age 1+ fish whereas boat electrofishing or gill nets capture predominantly adult fish (Lintermans 2013, 2016). Therefore, if the intent is to gain information about recent recruitment, then fyke nets would be more suitable.

The sampling employed as part of the Snowy 2.0 Exploratory Work Environmental Impact Statement was restricted to a single technique (electrofishing) with backpack electrofishing effort in streams restricted to 4 x 2 minute shots per site. This is half the standard effort routinely employed in fish surveys such as the Sustainable Rivers Audit (Davies et al. 2012). The boat electrofishing effort deployed in reservoirs as part of the Snowy 2.0 Exploratory Work Environmental Impact Statement is not specified (either the length of the shot, or the number of shots). Boat electrofishing is not a suitable sampling method for detecting Murray crayfish in deep reservoirs, and it is not surprising that it failed to capture this species in Talbingo Reservoir. The issues of sampling effort and methods and their usefulness in detecting specific target taxa are of critical importance for the Snowy 2.0 Main Works EIS.

Issues of concern from the operation of Snowy 2.0

The major issues of concern from the operation of Snowy 2.0 are:

- Redfin and/or EHN virus transfer from Talbingo Reservoir to Tantangara Reservoir.
- Transfer of Climbing galaxias from Talbingo Reservoir to Tantangara Reservoir
- Transfer of Oriental weatherloach from Tantangara Reservoir to Talbingo Reservoir
- Potential transfer of Eastern gambusia from Talbingo Reservoir to Tantangara Reservoir

Redfin perch and/or EHN virus transfer from Talbingo Reservoir to Tantangara Reservoir.

Redfin perch are a significant predator of small-bodied native fish and also juveniles of larger-bodied species (Lintermans 2007; Morgan et al. 2002; Rowe et al. 2008; Wedderburn and Barnes 2016) but the major potential impact to native fish in the Snowy 2.0 area is the role of redfin perch as a vector and reservoir of Epizootic Haematopoietic Necrosis Virus (EHNV). EHNV is considered endemic to the Murrumbidgee catchment and was first recorded in NSW from Blowering Reservoir (Whittington et al. 1996, 2011). EHNV has not been reported from natural environments upstream of Cooma.

A major potential impact of EHNV is on Macquarie perch populations in the upper Murrumbidgee. Laboratory studies have demonstrated that Macquarie perch were extremely susceptible to EHNV, with 100% mortality reported with 6-11 days of bath exposure or 4-6 days of intraperitoneal injection of EHNV (Langdon 1989). If Redfin perch are transferred from Talbingo Reservoir to Tantangara Reservoir and establish a

viable population, and these fish become infected with EHN virus at some point in the future, this would provide a means for the EHN virus to infect Macquarie perch and a dispersal source for infected Redfin to spread downstream of Tantangara.

Mountain galaxias was also shown in laboratory trials to be susceptible to EHN virus with all exposed fish dying in 5-10 days (Langdon 1989). As stocky galaxias were part of the Mountain galaxias complex until described as a new species in 2014, it is possible that this species would also be susceptible if exposed to the virus.

Transfer of Climbing galaxias from Talbingo Reservoir to Tantangara Reservoir

The species is not known, and is considered to not occur in the Upper Murrumbidgee catchment (Lintermans 2002). As their name suggests, Climbing galaxias are excellent climbers, able to ascend moist vertical surfaces such as waterfalls tens of metres high (McDowall and Fulton 1996). This is of extreme concern for the conservation of Stocky galaxias (*Galaxias tantangara*), which are currently confined to a single small stream above a waterfall. The 6 m waterfall which excludes trout from the upper reaches of Tantangara Creek is considered highly unlikely to provide protection against invasion from Climbing Galaxias (Lintermans pers observation; McDowall and Fulton 1996). The species is thought to have been transferred from its native coastal catchments to the upper Murray catchment via transfer through the Snowy Mountains Hydroelectric Scheme (Waters et al. 2002), where it has expanded to occupy a broad diversity of stream types and sizes (Lintermans 2007). If transferred to Tantangara Reservoir, the species would be expected to rapidly invade upstream, and its documented climbing ability means that existing waterfall barriers are unlikely to prevent the species invading the entire current range of *G. tantangara*. The existence of a large reservoir (Tantangara) to satisfy the lacustrine/marine larval life-stage requirements of this species indicates that if introduced, it would most likely establish. Ecological impacts are largely unknown, but it is predicted that it would likely compete with other galaxiids for food, and space (Lintermans 2007). As the existing habitat of *G. tantangara* is extremely limited (only 3 km of a small (av. 1m width and 0.1 m depth) stream, there is little opportunity for habitat or niche partitioning between the two species. The precise impacts of invading *G. brevipinnis* on *G. tantangara* are unknown, but the entire known distribution of *G. tantangara* is susceptible to such invasion and consequent impacts unless suitable barriers able to prevent passage of *G. brevipinnis* can be constructed. Invasion of Lake Pedder, Tasmania by Brown Trout (*Salmo trutta*) and *G. brevipinnis*, resulted in the extirpation of native *G. pedderensis* from this waterbody (Hardie et al. 2006). Similarly in New Zealand, it has been hypothesized that *G. brevipinnis* has displaced small non-migratory *G. vulgaris* in streams above impoundments (McDowall and Allibone, 1994).

Transfer of Oriental Weatherloach from Tantangara to Talbingo

Oriental weatherloach is a small-bodied very hardy fish that once established in a region (discarded aquarium fish initially) is then subsequently spread in southern Australia by anglers using it as live bait (Lintermans 2004). Once established in a new location, the species then spreads downstream by flooding, or passive downstream transport. The initial establishment of weatherloach in the Canberra region was presumably from aquarium fish discarded in Ginninderra Creek in urban Canberra. Subsequent spread occurred when these fish were then collected by people who used them as live bait when travelling outside Canberra. Its popularity as live bait is a result of its ease of collection (it reaches high densities, can be easily collected with a dipnet); its hardiness (individuals can be transported without water as the species uses aerial respiration); it is extremely muscular so individuals stay on a hook and wriggle; and when discarded, it can rapidly establish a new population.

Since weatherloach established in the ACT in the mid 1980s (Lintermans et al. 1990a,b) the species has spread to numerous nearby waterways (Lintermans 1993a,b) and then subsequently downstream. Weatherloach became established in Lake Eucumbene by the early 1990s, presumably through use as bait fish by Canberra-based anglers (Swales 1992). Transfer of fish between Lake Eucumbene and Tantangara Reservoir is already thought to occur (Shortfinned eel *Anguilla australis* are reported to move between the two water bodies either through the connecting tunnel when water transfer from Tantangara Reservoir to Lake Eucumbene is occurring; or through overland dispersal across the very low divide between the two catchments)(Lintermans 2002). The movement of anglers between Lake Eucumbene and Tantangara Reservoir is also common, and so the transfer of bait fish, such as weatherloach, would also be expected to occur, although this practise is illegal. It seems inevitable that weatherloach will establish in Tantangara Reservoir (if they are not already there: fish sampling for small-bodied fish in Tantangara Reservoir is essentially non-existent). Therefore, if weatherloach are able to establish in Tantangara Reservoir, as a hardy, small-bodied fish, it is likely they will be transferred downstream via water transfers from Snowy 2.0. This is just to highlight that all the issues with transfer of fish or pathogens is not unidirectional.

Potential transfer of Eastern Gambusia from Talbingo to Tantangara

The absence of this species in Tantangara Reservoir needs to be confirmed. The habitat preferences of this species (shallow margins) means that conventional fish sampling techniques do not capture this species well (electrofishing, nets, etc), but they are usually highly visible if looked for. If the species is confirmed to be present in Tantangara Reservoir, then Snowy 2.0 poses no additional threat from this species. If absent from Tantangara Reservoir, then the potential transfer of this species via the operation of Snowy 2.0 is cause for concern. This species can form self-sustaining populations in montane lakes, with the

species known to occur in Three Mile Dam in the adjacent Snowy River catchment (Lintermans 1998). The establishment of a population of this species towards the top of the catchment is highly undesirable, both for the impacts on native fish, frogs and invertebrates in Tantangara Reservoir, and as a source for downstream dispersal to currently uninfected areas.

Conclusion

There are several native and non-native species that are present in either the upper Murrumbidgee or Upper Tumut catchments, but not common to both. The risk of transferring such species via the Snowy 2.0 scheme's operation are, therefore, of real concern. There are five species in the upper Tumut system that could be transferred and establish in Tantangara Reservoir (Australian smelt, Flatheaded gudgeon, Climbing galaxias, Redfin perch and potentially Eastern gambusia). There are two nationally threatened fish species in the upper Murrumbidgee that are at real risk from such inter-basin fish and pathogen transfers: Macquarie perch and Stocky galaxias. The major risks are the transfer of Redfin perch and the EHN virus, and Climbing galaxias from the Tumut system to the upper Murrumbidgee.

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Appendix 1. Locations, years and species recorded.

see separate Excel data file



research for a sustainable future



Predicting invasive fish survival through the Snowy 2.0 pumped hydro scheme

Research commissioned by Snowy Hydro
Report No. 123

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Executive summary

Snowy Hydro Limited is proposing to develop Snowy 2.0 — a pumped hydropower facility with the capacity to generate 2000 MW of electricity. The facility will connect the existing Talbingo (lower) and Tantangara (upper) reservoirs within the Snowy Scheme via underground tunnels and a pumped hydro-electric power station. During the pumping phase, Snowy 2.0 will transfer water from Talbingo Reservoir and discharge it into Tantangara Reservoir, while the direction of water transfer will be reversed during the generation phase.

The facility promises to provide a large-scale source of reliable renewable energy for Australia but there is a risk that aquatic biota could be inadvertently transferred between Talbingo and Tantangara reservoirs during the pumping phase. In particular, there is concern that invasive fish including Redfin perch (*Perca fluviatilis* Linnaeus, 1758; 'redfin') and Eastern gambusia (*Gambusia holbrooki* Girard, 1859; 'gambusia'), as well as the native Climbing galaxias (*Galaxias brevipinnis* Günther, 1866) could be transferred from Talbingo Reservoir to Tantangara Reservoir, where they are currently considered to be absent. Tantangara Reservoir supports a recreational trout fishery. It is also connected via tunnel to Lake Eucumbene on the Eucumbene River, a tributary of the upper Snowy River. Releases are made from Tantangara Reservoir to the upper Murrumbidgee River system, which contains a population of Macquarie perch (*Macquaria australasica* Cuvier, 1830). Translocation of redfin, and gambusia could therefore have unintended adverse impacts on both the trout fishery and native species in Tantangara Reservoir and connected waterways. Thus, it is important to understand the likelihood that these fish species could survive transport through Snowy 2.0.

During the early stages of the project, it was identified as important to determine (a) the likelihood of fish entrainment and (b) whether redfin and gambusia would survive passage through the Snowy 2.0 facility during its pumping phase. During this phase, fish could be exposed to potentially harmful compression, shear stress and mechanical blade strike, all of which are known to have negative impacts on fish. Snowy Hydro Limited commissioned a research team to experimentally quantify the expected survival rates of redfin and gambusia through the proposed pumped hydro facility.

The Snowy 2.0 facility will be among the largest pumped hydro systems in the world and there are currently no global data upon which a baseline level of survival could be assessed for the target fish species. Therefore, a series of experimental facilities were constructed to simulate the hydraulic conditions within the proposed turbine system. A laboratory-based shear chamber and hyperbaric chamber were constructed to define the critical tolerances of each species. Different life stages were then exposed to extreme pressure changes and shear forces; determined by modelling the expected passage through the Snowy 2.0 facility. The ranges tested were based on design parameters and operational conditions provided by Snowy Hydro Limited at the time of the experiments, and assume that no mitigation measures are in place.

Entrainment likelihood

Survival through the pumped hydro facility is immaterial if there is no chance that fish will be entrained into the intake. A qualitative assessment was performed to ascertain if there was a credible likelihood that (a) redfin and gambusia would be present in the proposed intake area; (b) the intake dimensions suit entrainment; (c) the hydraulics of the intake will entrain fish; (d) habitat around the intake would be suitable; and (e) fish are present at the entrainment depth. Results from the qualitative entrainment review indicated that — in the absence of any controls — there is a possible-to-likely likelihood of redfin and gambusia being entrained within the Snowy 2.0 intake.

Shear tolerance

Shear strain was modelled upon expected shear events within the Snowy 2.0 facility related to CFD-modelled data. Five different shear scenarios were simulated, including a 'no shear' control. The shear strain rates needed to be scaled to account for the size of target life history stages. For instance, an egg will experience fluid shear on a much finer scale (mm) than an adult fish (cm). So shear strain needed to be standardised and scaled to the experimental

subject. Shear ranges tested were 0 – 6177 1/s for eggs; 0 – 3706 1/s for both larval stages; and 0 – 1853 1/s for juvenile redfin and adult gambusia. Adult redfin were not tested as they were too large for the shear chamber.

Based on results from immediate recovery of all life stages from the shear chamber, redfin eggs survived all but the highest two strain rates tested (5623 and 6177 1/s). Under those conditions eggs were completely destroyed. However, below 4203 1/s strain rates, the majority of eggs survived and subsequently hatched. Redfin larvae (12-18 days post hatch) survived strain rates less than the highest strain rate (3706 1/s). Older redfin larvae (28-30 days post hatch) did not survive shear strain rates of 3706 1/s, but there was significant survival below 3374 1/s. Juvenile redfin survived all shear levels. Adult gambusia also survived all shear levels.

Blade strike tolerance

Blade strike effects were assessed using both stochastic and deterministic modelling approaches. In the absence of actual data for these species, it was assumed that a strike event would lead to mortality. Stochastic modelling indicated that the Snowy 2.0 strike survival rate was approximately 100% for redfin eggs, 98% for redfin larvae, 83% for juvenile redfin, 71% for adult redfin, and 94% for adult gambusia. Similarly, the deterministic model predictions of fish survival rates in response to the risk of blade strike within the Snowy 2.0 facility ranged from more than 99% for redfin eggs to 95% for gambusia, and approximately 75% for redfin adults, with larger fish being more likely to be struck than the smaller fish. These values are likely to have underestimated survival rates in response to blade strike within the Snowy 2.0 facility, since the model predictions were based on the assumption that all blade strikes would lead to mortality. However, some fish might survive a strike event.

Pressure tolerance

A world-first hyperbaric pressure chamber was constructed to simulate pressure changes through the proposed Snowy 2.0 facility and define the critical tolerances of each species. Adult, juvenile, larval and egg life stages were exposed to extreme pressure changes determined by modelling the expected passage through the system. The ranges tested were based on design parameters and operational conditions provided by Snowy Hydro Limited at the time of the experiments, and assumed that no mitigation measures were in place.

Two pressure scenarios were simulated. One where all six pump-turbines (T_6) would be operating in pump mode – determined to represent the most extreme of the pumping scenarios. During this simulation, passage through the entire system would take approximately two hours and expose the fish to pressures up to 76 bar. The other scenario simulated three pump-turbines operating (T_3) in pump mode where passage would take approximately 3.8 hours. Fish would be exposed to pressures in excess of 75 bar. Survival was assessed immediately after each experiment, and then again after 24 hours and 5 days from exposure.

The pressure tolerance experiments demonstrated high survivorship for both species for both the T_6 and T_3 scenarios. For T_6 , there was high immediate survivorship of all life stages of redfin, including eggs (55%), 12-18 day old larvae (96%), and 28-30 day old larvae (76%), juveniles (100%) and adults (100%). All adult gambusia (100%) survived the T_6 exposure. Juvenile and adult redfin, and adult gambusia all continued to survive for up to five days post exposure, whereas larval redfin survivorship decreased slightly after 5 days.

For the life stages subjected to the T_3 scenario, there was also high immediate survivorship in adult redfin (50%), juveniles (100%), and 12-18 day old larvae (85%). Adult redfin survivorship remained the same for the first five days post exposure, but juvenile and larval redfin survivorship declined to 61% and 45%, respectively.

Survival in response to the combined effects of shear stress, blade strike and pressure changes







Following the independent assessments of shear, blade strike and pressure impacts on redfin and gambusia, their combined impacts were calculated to give an overall survival estimate. This involved applying the principles of multiplicative probability and assumed fish would be first subjected to shear stress as they moved through the draft tube, then blade strike as they passed through the turbine, and finally a rapid increase in pressure. Two scenarios were tested

– one based on the experimental results for the shear ranges tested, and the other adjusted for the likelihood of exposure to the different shear ranges tested (using CFD modelled data). The combined stressor analysis for both scenarios indicated that all life stages of redfin and adult gambusia would have survival rates, across all life history stages, ranging between a minimum of 15% (redfin eggs) and a maximum of 94% (gambusia adults) (see Table 1).

Conclusions

In the absence of control measures, the experiments undertaken in this study indicate that redfin and gambusia are likely to survive passage through the Snowy 2.0 facility because (1) there are actively recruiting populations of redfin and gambusia already present in Talbingo Reservoir; (2) there is a possible-to-likely likelihood of redfin and gambusia being entrained into the Snowy 2.0 intake; and (3) the results indicated it is likely that a proportion of any redfin or gambusia entrained at the intake in Talbingo Reservoir would survive the shear, blade strike and pressure impacts expected to occur within the Snowy 2.0 facility. Identified knowledge gaps for further research are discussed, including quantifying entrainment risk, investigations of other species at risk of transfer through the system and empirical studies addressing cumulative shear, blade strike and pressure impacts.

Table 1. Combined stressor survival estimations with shear, blade strike and pressure effects (for the six-turbine (T_6) simulation) for all life stages of redbfin and adult gambusia. Scenario 1 relates to survival based on the experimental survival rates for the shear ranges tested in experiments; and Scenario 2 relates to survival based on the experimental shear survival rates after they had been adjusted for the actual frequency distribution of shear ranges expected to occur within the Snowy 2.0 facility. All estimates have been adjusted for shear and pressure control group mortalities.

Combined stressor survival estimation			
Test group	Stressor	Scenario 1: Survival based on experimental data (%)	Scenario 2: Survival based on simulated probability of exposure (%)
Adult redbfin 	Shear	100*	90*
	Blade strike	71	71
	Pressure	100	100
Combined survival range		71	63
Juvenile redbfin 	Shear	100	90
	Blade strike	83	83
	Pressure	100	100
Combined survival range		83	75
Redfin larvae (28 – 30 DPH) 	Shear	42	71
	Blade strike	98	98
	Pressure	82	82
Combined survival range		44	66
Redfin larvae (12-18 DPH) 	Shear	46	69
	Blade strike	99	99
	Pressure	99	99
Combined survival range		34	58
Redfin eggs (with eyes formed) 	Shear	22	51
	Blade strike	100	100
	Pressure	70	70
Combined survival range		15	36
Adult gambusia 	Shear	100	91
	Blade strike	94	94
	Pressure	100	100
Combined survival range		94	86

*Likelihood of survival of adult redbfin to shear strain has been based on juvenile results since we did not expose adults to shear strain because they did not fit into the shear chamber delivery tube. Shear injuries generally inversely scale with size, so if juveniles survived, we can be confident that adults will.

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The map illustrates the Snowy Mountains Water Supply Scheme, showing the route from Tumut to Canberra. The route is marked by an orange line, which is the Snowy Mountains Highway. Key locations and features include:

- Regions:** Northern Snowy-Tumut and Southern Snowy-Murray.
- Power Stations:** Blowering Power Station, Snowy Mountains Hydro Power Station, Tumut 1 Power Station, Tumut 2 UG Power Station, Tumut 3 UG Power Station, Khancoban Power Station, Murrumbidgee Power Station, Gungahlin Power Station, Jindabyne Pumping Station, and Jindabyne Mini Hydro Power Station.
- Reservoirs and Dams:** Blowering Reservoir, Talbingo Reservoir, Tantangara Reservoir, Toompa Dam, and Lake Eucumbene.
- Locations:** Tumut, Tumbarumba, Kiandra, Cabramurra, Adaminaby, Cooma, Jindabyne, Thredbo, and Dalgety.
- Legend:**
 - Blue circle: Reservoir
 - Red circle: Dam
 - Orange line: Snowy Mountains Highway
 - Black box: Power Station
 - Blue line: Snowy Mountains Water Supply Scheme

Charles Sturt University and NSW Department of Primary Industries

Issues related to redfin and gambusia and the Snowy 2.0 facility

Redfin are a medium-sized, freshwater fish that are naturally distributed throughout Europe and parts of Asia (McDowall 1996). Redfin were translocated to Australia by acclimatisation societies from Britain in 1861, and are now found in all states apart from Queensland and the Northern Territory (Hammer and Walker 2004). The potential translocation of redfin into Tantangara Reservoir via Snowy 2.0 has possible implications for both the trout fishery and native species in Tantangara Reservoir and connected waterways. The threat associated with redfin is due to a number of traits that they possess; namely, they are a notifiable pest in NSW, are highly invasive and fecund, and are difficult to eradicate once introduced. The unintended transfer of gambusia to Tantangara Reservoir may also present a number of ecological risks to that reservoir and/or the other waterways connected to it.

The likelihood of redfin and gambusia surviving passage through the Snowy 2.0 facility

The likelihood of Snowy 2.0 facilitating the transfer of redfin and gambusia to Tantangara Reservoir during pumping would depend on three factors: 1) the likelihood that these fish will be entrained at the intake in Talbingo Reservoir, 2) the likelihood that fish would survive the hydraulic stresses to which they are exposed during passage through the turbine and distribution tunnels, and 3) the ability of these fish to survive and breed within Tantangara Reservoir. With respect to likely survival during passage through the turbine and tunnels, fish tolerance to three main stresses needs to be considered: both acute and sustained pressure change, elevated fluid shear and possible strike from the pump-turbine blades.

At conventional hydropower facilities where fish pass during the generation phase, fish initially experience a slow increase in pressure as they are entrained and approach the turbine blades, at which time they then experience extremely rapid decompression (typically experiencing negative pressures) as they pass the turbine blades (Brown et al. 2014). Since the velocity of the water passing through the turbine often surpasses the maximum swimming speed of the fish, the fish lose their ability to control their orientation, leaving them vulnerable to physical strike on the turbine blades (Coutant and Whitney 2000, Brown et al. 2014). The fish then enter the river downstream and are often exposed to shear stress as a result of discharged water interacting with other moving bodies and objects (Deng et al. 2005). Such stresses have the potential to cause significant injury to fish (Neitzel et al. 2004, Brown et al. 2009, Schweizer et al. 2012, Boys et al. 2016).

The three stresses of pressure, shear and blade strike also have the potential to occur during pumping at pumped hydropower facilities like Snowy 2.0. However, the way in which fish are exposed to these stresses will differ to that during passage through conventional hydropower facilities during generation. During the pumping phase, fish may be exposed to a slow compression as they are drawn down towards the turbine/pump, and then high water velocities and associated shear stresses near the turbine. As the fish pass from the lower to the upper side of the turbine blades, they are exposed to blade strike impacts, and an extremely rapid (instantaneous) increase in pressure that is generated by the head of hydrostatic pressure exerted by the height of the headwater reservoir. They are also likely to move from a rapid to slower flowing environment and experience elevated shear as they pass the turbine blades. Unlike traditional turbine passage, very few published studies have considered the survival of fish following passage through a pumped hydro plant (but see a few exceptions like Nestler et al. (1999) and Yang and Jackson (2011)), and the impact of extreme compression on fish has only been studied on a small number of species, and not to the degree expected at Snowy 2.0.

Aims of this report

The purpose of the current report was to quantify the likely survival of redfin and gambusia during passage through the Snowy 2.0 facility during the pumping phase (Figure 2). This was achieved by:

1. conducting a qualitative desktop review of the likelihood of entrainment for redfin and gambusia into the Snowy 2.0 intake (Chapter 2) (Figure 2).
2. experimentally examining (within a laboratory shear chamber and hyperbaric chamber) the tolerances of each species and varying life stages to the shear forces and extreme pressure changes that may be expected within the Snowy 2.0 facility (Chapter 3). The ranges tested were based on estimates of the design and operational conditions provided by Snowy Hydro Limited at the time of the experiments, and assume that no mitigation measures are in place.
3. modelling the impacts of turbine blade strike on redfin and gambusia, which are likely to occur within the proposed Snowy 2.0 facility (Chapter 3).
4. estimating the overall survival rate for each life stage and species (taking into account all three sources of mortality — shear, blade strike and pressure) (Chapter 4).
5. discussing the implications of the findings (Chapter 4), and highlighting knowledge gaps (Chapter 4).

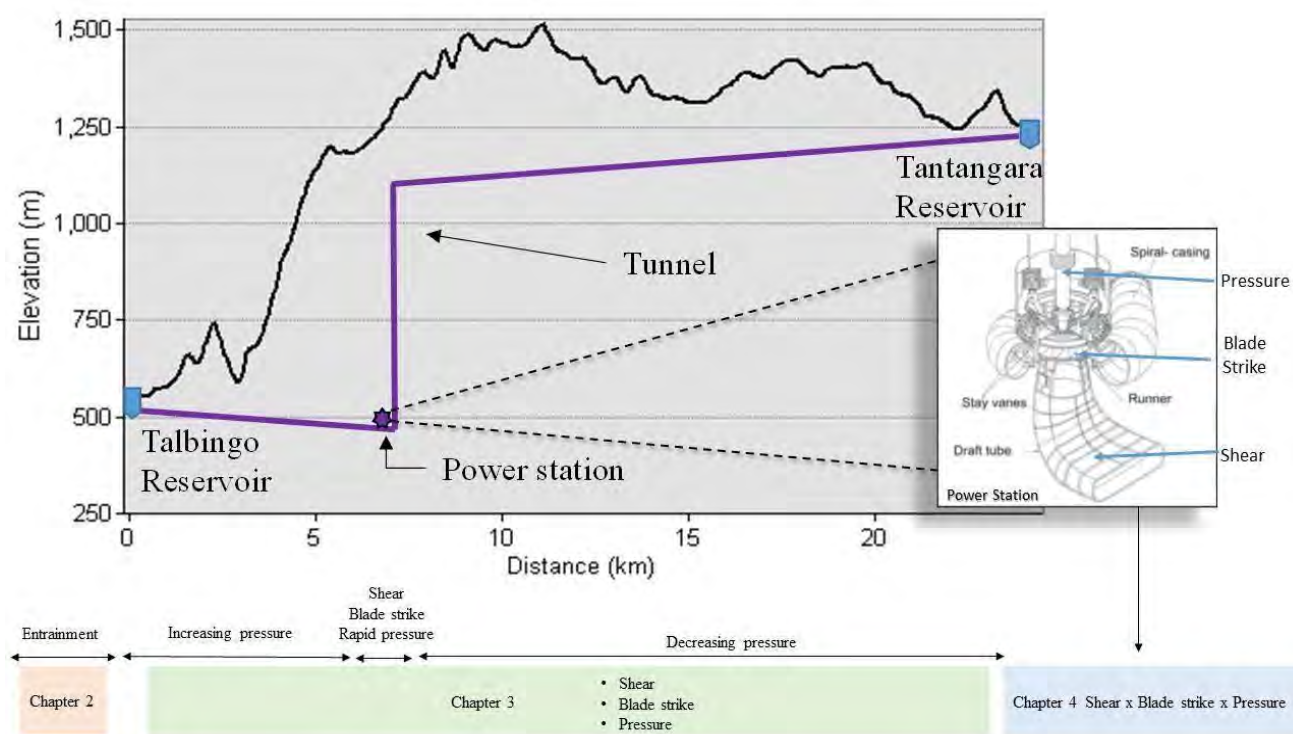


Figure 2. Schematic of the Snowy Hydro 2.0 facility showing the proposed tunnel between Talbingo and Tantangara reservoirs, along with the factors influencing the risk of fish transfer within each region of the facility. The relationship between each chapter has been linked logically to the sequence that fish will be exposed to during passage through the system.

Chapter 2: Fish entrainment likelihood associated with the Snowy 2.0 facility

Chapter overview

The probability of invasive fish successfully passing through a pumped hydropower facility is dependent on their likelihood of being entrained within the water intake. There are few studies of entrainment from pumped hydropower stations globally (but see a few examples like Northfield Mountain Pumped Storage Project (No. 2485) and Turners Falls Hydroelectric Project (No. 1889) 2016). There are however examples of fish entrainment and mortality in the draft tubes of conventional hydropower facilities that can be related to the pumping stage scenarios of pumped hydropower facilities (Andrade et al. 2012; Silva et al. 2019). Fish entrainment and the factors influencing entrainment in these facilities during pumping can also be adopted from cooling water intakes for coal and nuclear power generating facilities. The literature can also offer qualitative suggestions for approaches to assess entrainment impacts and/or risks for fish. Considering all of these factors can provide a mechanism to qualitatively assess whether redfin and gambusia may be entrained within the Snowy 2.0 intake.

Fish entrainment

Entrainment refers to the process of fish being involuntarily transferred with water flow through an intake or channel from one location to another (Barnthouse 2013, Mussen et al. 2013). It occurs when 'approach' velocities – of the water being drawn into the intake – exceed the ability for fish to escape, or where fish are unable to detect sensory cues that would alert them to the danger. Where trashracks or small-mesh screens are installed, entrainment can also be associated with impingement, with the latter occurring when the fish become trapped against a physical structure during the process of being drawn along with the flow of water (Barnthouse 2013). Fish entrainment and/or impingement are global issues and have been investigated within a range of settings, including irrigation channels (Post et al. 2006, Carlson and Rahel 2007, Boys et al. 2013a, Boys et al. 2013b), water diversions (Grimaldo et al. 2009, Mussen et al. 2013), and intakes (Dadswell et al. 1986, Nestler et al. 1992) or turbine draft tubes (Andrade et al. 2012; Suzuki et al. 2017) for hydroelectric facilities and other power generating (e.g. coal and nuclear) facilities (Jensen et al. 1982, Jensen 1982, British Energy Estuarine and Marine Studies 2010, Barnthouse 2013). However, the majority of studies undertaken thus far currently remain in the grey literature, or are protected under commercial disclosure arrangements; and the approaches, methods and objectivity of quantifiable conclusions from these studies all greatly vary (Post et al. 2006). In addition, most of the focus has gone into assessing the impacts of entrainment on fish populations and/or communities (Jensen et al. 1982, Jensen 1982, Lorda et al. 2000, Barnthouse 2013), rather than the likelihood of specific species and/or life stages being entrained (but see a few exceptions like Mussen et al. (2013) and Mussen et al. (2014).

Factors influencing the likelihood of fish entrainment in turbines within hydropower facilities

The likelihood of fish being entrained into hydropower facilities is influenced by both the attributes of the intake and surrounds, and the ecological characteristics of the species under consideration (Figure 3) (Coutant and Whitney 2000, Grimaldo et al. 2009, Rytwinski et al. 2017). Significant factors associated with the intake include the hydraulic conditions, depth, and surface area; the turbine type, size and power output (Rytwinski et al. 2017); as well as the surrounding habitat conditions influencing the species' persistence (e.g. the thermal conditions, and the presence of spawning substrate) (Grimaldo et al. 2009). Key ecological characteristics include the species' biology (e.g. the obstacle-sensory capacity and swimming performance of each life stage (Rytwinski et al. 2017)), population dynamics (e.g. seasonal changes in the abundance of particular life stages (Grimaldo et al. 2009)), and habitat requirements (e.g. the species' life stage-specific habitat/temperature/depth preferences) (Coutant and Whitney 2000,

Grimaldo et al. 2009). The spatial and temporal dynamics of each of these intake- and species-related factors play a key role in shaping the influence of each of these components, because fish may be more vulnerable to the effects of entrainment during particular life stages; and/or in certain habitats or at specific times of the year (Coutant and Whitney 2000, Grimaldo et al. 2009, Barnthouse 2013).

Fish can also be entrained into the draft tubes of conventional hydropower facilities in different operational scenarios, particularly during flow reduction to stop the turbine for maintenance (Andrade et al. 2012). In this scenario, turbine flow rates seem to play a significant role in fish attraction towards the draft tube outlet and make a particular area more susceptible to fish entrainment (Suzuki et al. 2017). This has been observed in Neotropical systems and demonstrates fish entrainment in different civil structures at various hydropower plants (Silva et al. 2019).

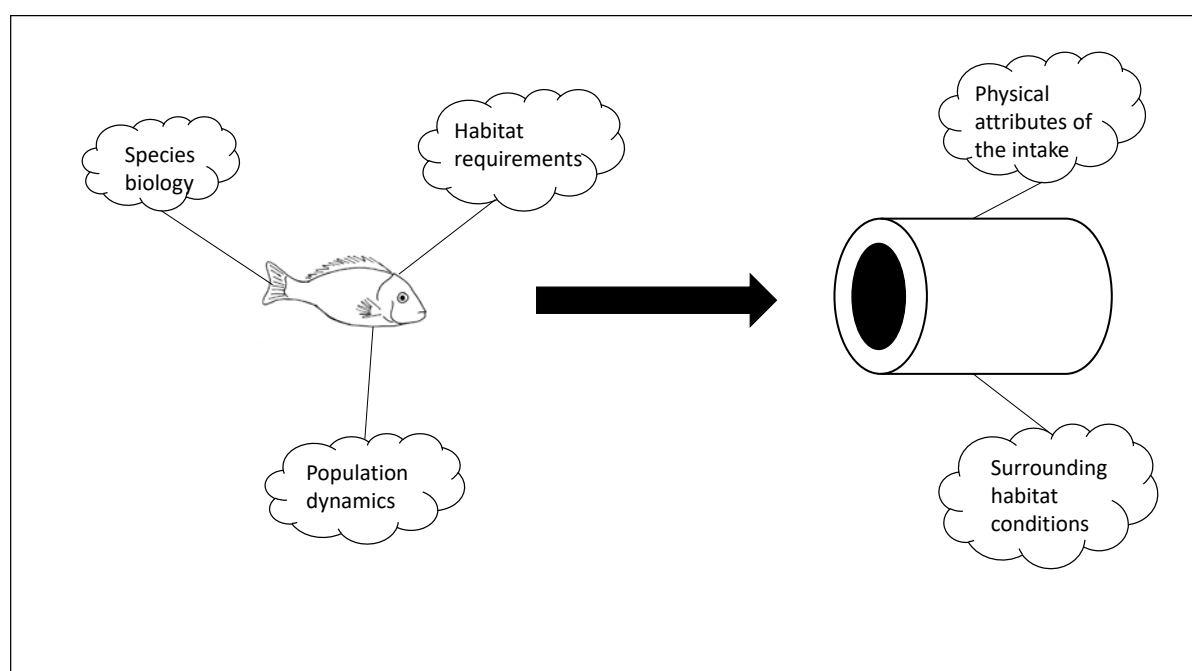


Figure 3. Key factors influencing the likelihood of fish entrainment in hydropower turbine intakes.

Hydraulic conditions created by turbine intakes

Pumped hydropower intake facilities result in a range of velocity and turbulence conditions, which are influenced by physical factors such as the intake surface area and depth; as well as turbine type, size, and power output (Rytwinski et al. 2017). The velocity conditions associated with turbine intakes are largely determined by the magnitude of the approach velocities (i.e. the velocities generated perpendicular to the intake surface) relative to the sweeping velocities (i.e. the velocities occurring parallel to the intake) (Swanson et al. 2004). The likelihood of entrainment is minimised when approach velocities are reduced relative to sweeping velocities, by increasing the surface area of the intake, or angling the intake to increase cross flow (Swanson et al. 2004). Snowy 2.0 has an operational framework where there are multiple possible pumping volumes depending on the number and type of turbines (i.e. variable or fixed speed) that are operating. This will influence the volume of water being pumped at any one time and the consequent velocities experienced in front of the intake. Information provided by Snowy Hydro indicates that peak velocities will be 2 m/s at the intake entrance during pumping at full capacity. Velocities will be lower when pumping at reduced capacities and will decrease with distance from the intake. Detailed modelling indicating how velocity will change with distance from the intake is not currently available. Furthermore, fish availability will vary seasonally, with eggs and larvae only occurring in certain months for some species. Therefore, there will be a strong temporal aspect to the hydraulic conditions at the pump intake which will influence the likelihood of entrainment.

The response of fish to the hydraulic conditions created by turbine intakes

Coutant and Whitney (2000) argue that the response of fish to the hydraulic conditions created by intakes depends on various morphological, physiological and behavioural attributes of the species and life stage in question, including its:

- ability to recognise and avoid obstacles
- swimming performance
- buoyancy and stability
- behaviour in turbulent flow
- pre-existing stress.

These factors, either singly or in-combination, could influence the ability for fish of various sizes to be entrained into the Talbingo intake.

Ability to recognise and avoid obstacles

Alterations to water flow patterns near the intake (e.g. rapid acceleration) may be perceived by fish as 'obstacles' to try to avoid (Coutant and Whitney 2000). Indeed, many fish are thought to be able to use their lateral line sensory system to respond to such obstacles, since that organ is dedicated to detecting fluid movement (Popper and Platt 1993). However, these sensory systems would need to be capable of responding very efficiently given the rapid acceleration of flows and/or high level of turbulence often associated with a turbine intake. For example, it has been suggested that juvenile fish may be particularly susceptible to entrainment, partly owing to their sub-optimally developed sensory capabilities, in addition to their typically high abundance and inclination to disperse (Coutant and Whitney 2000). Alternatively, fish which are positively rheotactic and move downstream may see water flowing into a pump turbine as a 'cue' for dispersal and, therefore, be motivated to move through. It appears that no-to-very little empirical data have been collected thus far regarding redbfin or gambusia rheotaxis, so this remains a major knowledge gap.

Swimming performance

Even if fish are able to efficiently detect alterations to flow patterns near an intake using their lateral line system or some other sensory system, they may still be entrained unless they possess strong sustained swimming or burst swimming abilities to overcome the altered flows (Boysen and Hoover 2009). The swimming abilities of fish can vary greatly among species and life stages, and are influenced by the prevailing environmental conditions (Swanson et al. 2004, Boysen and Hoover 2009, Srean et al. 2017). For instance, there is some suggestion that fish may be more vulnerable to entrainment during periods of extreme cold, since such conditions can potentially place them in a state of cold torpor and allow them to passively drift into intakes (Coutant and Whitney 2000). Adults also possess a far greater swimming ability than larvae. So there is a distinct difference in entrainment likelihood among life history stages based on swimming ability alone (Swanson et al. 2004, Boysen and Hoover 2009). Eggs (if free-floating) and larvae will always be more susceptible to entrainment based on their reduced ability to escape strong approach velocities.

Buoyancy and stability

The buoyancy and stability of fish also play an important role in determining their ability to maintain their body orientation and water column-positioning in turbulent flows associated with turbine intakes (Coutant and Whitney 2000, Webb 2002). Fish that are either unable to retain their vertical position (quick changes in buoyancy), and/or to orient themselves into the flow (i.e. do not exhibit positive rheotaxis) are more likely to be entrained within turbine intakes (Coutant and Whitney 2000, Boysen and Hoover 2009). Pelagic fish lacking swim bladders (e.g. paddle fish) are particularly vulnerable to entrainment because they are reliant upon the motion of constantly swimming to remain buoyant. If they lose hydrodynamic control (i.e. the capacity to maintain their orientation and lift due to hydraulic forces (e.g. in the turbine intake)), they sink (Coutant and Whitney 2000). Redfin eggs do not possess any air and are negatively buoyant (i.e. sink to the bottom). Thus, any eggs within the intake region at the start of pumping will be entrained if velocities at the bottom of the intake are high enough. Redfin larvae also have

poorly developed swim bladders. Observations from larval rearing (this study; Chapter 3) suggested that a large majority of redfin larvae are positively buoyant (floating) up until six days post-hatch (unpublished data, authors). Thus, these fish would be susceptible to any entrainment where water is collected near the surface. This is not the case with the Talbingo intake which will always be at least 8 m below the surface of the water. Nonetheless, observations from the wild would be required to validate this assertion.

Behaviour in turbulent flow

There is currently little known about the behaviour of fish in turbulent conditions (but see Pavlov et al. (2000) and Odeh et al. (2002)). Pavlov et al. (2000) examined the effect of turbulence on the behaviour and distribution of fish; while Odeh et al. (2002) investigated the effects of both shear stress and turbulence on fish, and showed that turbulence can influence the startle response of fish. The only other knowledge of the behaviour of fish in turbulent conditions comes from studies that have considered high speed water jets where shear forces cause tissue injuries (Groves 1972). While the latter research is not directly relating to turbulence impacts, it would be useful to determine whether fish in highly turbulent intake regions can modify their trajectories by changing their behaviour (Coutant and Whitney 2000). For instance, it has been shown that juvenile salmon can use aspects of turbulent flow to hasten their downstream migrations (Coutant 1998). This behaviour suggests that they may have a sensory ability to detect these flow characteristics, and that they can modify their body orientation to take advantage of the flow characteristics (Coutant 1998). There will likely be a variety of different turbulence profiles associated with different pumping operations and reservoir levels at the Talbingo Snowy 2.0 intake, and each may have different physiological effects on fish depending on the respective hydraulic conditions.

Pre-existing stress

Fish suffering from pre-existing stress may have a lesser ability to deal with the hydraulic conditions created by turbine intakes than fish that are not as initially stressed (Coutant and Whitney 2000). This is because stressed fish may respond abnormally to many of the physiological and behavioural features outlined in the preceding sections (i.e. ability to recognise and avoid obstacles, swimming performance, buoyancy and stability and behaviour in turbulent flow) (Jones 1971, Coutant and Whitney 2000). For example, the responsiveness of fish to obstacles associated with intakes may be dramatically reduced in individuals that are infected with disease (Coutant and Whitney 2000). Likewise, stress caused by low dissolved oxygen (DO) concentrations or pollutants could affect the swimming performance of fish in turbine intakes (Jones 1971).

Entrainment studies on redfin and gambusia

No studies could be identified that have considered the likelihood of redfin or gambusia entrainment within power (nuclear, coal or hydropower) facility intakes. Most entrainment studies on perch (Perciformes) and other fish have primarily been concerned with identifying and quantifying the impacts to the source population from the loss of individuals through an intake rather than assessing the likelihood of entrainment. Jensen (1982) quantified the effects of entrainment and impingement at the Monroe coal-fired power plant in Michigan (USA) on the Yellow perch (*Perca flavescens* Mitchell, 1814) population in the western basin of Lake Erie. Using fishery assessment models, he concluded that entrainment and impingement would cause only a 2–3% impact on the biomass of the Yellow perch stocks at that location (Jensen 1982). Jensen et al. (1982) applied the same types of models to investigate the effects of entrainment and impingement at 15 nuclear power plants on rainbow smelt (*Osmerus mordax* Steindachner, 1870), alewife (*Alosa pseudoharengus* Wilson, 1811) and Yellow perch stocks in Lake Michigan. The authors again found that entrainment and impingement only had a 0.28% impact on the Yellow perch population, and not much more of an impact on the rainbow smelt (0.76%) and alewife (2.86%) populations (Jensen et al. 1982). These suggest that entrainment impacts are variable. However, it is important that entrainment estimates are kept in context. If zero entrainment is the target, these figures may still be unacceptably high.

The only entrainment studies on fish in the genus *Gambusia* relate to mortal impacts. Čada et al. (1980) investigated the mortality of juvenile *Gambusia affinis* to entrainment mortality caused by a nuclear power plant simulator, by exposing the fish to different combinations of pump speed and water temperatures in the simulator. They compared the sensitivity of *Gambusia affinis* with that of larval bluegill sunfish (*Lepomis macrochirus*, Rafinesque, 1810), channel catfish (*Ictalurus punctatus* Rafinesque, 1818), European carp (*Cyprinus carpio*, Linnaeus, 1758), largemouth bass (*Micropterus salmoides* Lacepède, 1802), and smallmouth bass (*Micropterus dolomieu* Lacepède, 1802); in addition to juvenile bluegill (Čada et al. 1980). The species showed high variation in their sensitivity to pipe and condenser passage. While carp and largemouth bass larvae exhibited high mortalities after passing through the condenser in the power plant simulator, *Gambusia affinis* (along with channel catfish and smallmouth bass larvae and juvenile bluegill) suffered relatively low mortalities, even when combined with thermal shocks of 10°C above ambient temperatures (Čada et al. 1980). Boys et al. (2013b) used a field-based approach to examine the suitability of different screen design characteristics for the protection of a lowland Australian river fish community at an experimental irrigation pump (Boys et al. 2013b). They found that *Gambusia holbrooki* (n=1) were only occasionally entrained in low abundances in comparison to other species like Carp gudgeon (*Hypseleotris* spp.) (n = 138) and Australian smelt (*Retropinna semoni*) (n = 29).

The likelihood of redfin and gambusia entrainment into the Snowy 2.0 intake

The following section investigates the risk of redfin and gambusia entrainment into the Snowy 2.0 facility by reviewing the characteristics of the proposed Snowy 2.0 intake and assessing how well they match with the ecological characteristics of each fish species.

Physical characteristics and likely habitat conditions of the Snowy 2.0 intake

- The Snowy 2.0 intake would be located in Yarrangobilly River arm at the south-eastern end of the reservoir (Snowy Hydro 2017).

Depth and surface area of the Talbingo intake:

- The Snowy 2.0 intake is a bellmouth structure transitioning to the 9.8 m diameter tunnel. The structure consists of four openings of 10 m high by 4.75m wide, corresponding to an approximate gross surface area of 190 m², and the top of the intake would be 9–18 m below the surface of Talbingo depending on the reservoir level (Snowy Hydro 2018).
- An approach channel will be constructed in front of the Talbingo intake to enable the smooth intake and exit of water during operation. All potential fish habitat such as timber or rocky outcrops would be expected to be removed during construction (Snowy Hydro, pers. comm.).

Hydraulic conditions around the Snowy 2.0 intake:

- The ability for a fish to escape or avoid entrainment is strongly influenced by the magnitude of velocities generated perpendicular to the turbine intake (approach velocities) relative to that along the intake (sweeping velocities) (Swanson et al. 2004).
- Reducing approach velocities relative to sweeping velocities minimises entrainment risk (Swanson et al. 2004). Owing to its location in a large reservoir, it is unlikely that any sweeping velocity would be present in the vicinity of the proposed Snowy 2.0 intake, except during times of very high flow in the Yarrangobilly River.
- Velocity at the trash racks will not exceed 2 m.s⁻¹ during full station pumping (i.e. when all turbines are in operation) and will reduce if less turbines are in operation (Snowy Hydro, pers. comm.). Velocity will decrease quickly with distance from the intake. If there is no pumping or generation, there will be no velocity and fish could enter the intake.
- In the absence of further controls, it is assumed that any fish which enters the area around the trash racks and is unable to swim against the flow into the intake during pumping, could be entrained if they are small enough to pass through the trash racks.
- Ultimately the hydraulic conditions around the intake will continually vary in association with changes between pumping mode and generation mode. Water flow velocities will

(1) be directed towards the intake during pumping mode, (2) be directed away from the intake during generation mode, and (3) be negligible or low at any stage when neither mode is being implemented or when transitioning between the two. Thus, fish will be provided with a broad range of water velocities (both magnitudes and directions) around the intake (including slow/no-flow conditions), suggesting that the hydraulic conditions will be suitable for the entrainment of particular species and/or life stages at some stage.

Redfin perch ecological characteristics and their match with the Snowy 2.0 intake characteristics

Habitat and food preferences

- Redfin can live in a wide variety of habitats, including various streams and rivers, lakes and even estuarine lagoons (Freyhof and Kottelat 2008).
- Their use of habitat varies among life stages due to changes in resource partitioning and feeding behaviours (Coles 1981, Persson 1988, Eklöv 1997).
- During the larval stage, they tend to occupy open water habitats, where they consume pelagic organisms like zooplankton, and are partially dispersed by currents (Coles 1981, Freyhof and Kottelat 2008).
- They move into the littoral zone and start feeding on larger invertebrates and fish during their first year of development (Coles 1981, Persson 1988, Eklöv 1997), before eventually occupying both littoral and pelagic habitats once they become large enough to feed mainly on fish (Persson 1988, Eklöv 1997).
- The observed high densities of juveniles observed in Talbingo, suggest that conditions in Talbingo are suitable for recruitment and development of all life stages of redfin.

Depth preferences

- An acoustic tagging survey of the redfin community in Suma Park Reservoir (Australia) found that all fish occurred in the top 5–10 metres of the water column, potentially due to anoxic conditions below this depth (L. Faulks pers. obs.).
- Redfin appeared to prefer the areas of the dam with deeper water (i.e. the front of the dam) even though the full extent of the water column was not utilised (reservoir depths at the front of the dam reached 25 m) (L. Faulks pers. obs.).
- Čech et al. (2005) investigated the diel vertical migration patterns of redfin fry (L_T 11–35 mm) in Slapy Reservoir (Czech Republic), and found that they migrated to depths of 13–14 m during the noon period in May.
- Likewise, Vejřík et al. (2016) found that juvenile redfin were using deep (12–15 m) hypoxic waters as a refuge from large predators in Vír Reservoir in Moravia, Czech Republic.
- Furthermore, redfin spawning has also been observed at depths ranging from 0.2 m to 12 m (Gillet and Dubois 1995, Smith et al. 2001).
- According to Craig (1977), redfin depth usage changes diurnally and seasonally.
- For example, Jellyman (1980) noted redfin foraging during daylight in the shallows (0.5–2.0 m) of Lake Pounui, Wairarapa, before moving into deeper areas (1.5–3.0 m) during the early evening to feed on small fish around macrophyte beds.
- Depth usage also varies seasonally, with redfin tending to utilise deeper sections during winter (Craig 1977).
- Jellyman (1980) observed redfin near macrophytes at depths of 2.0–3.5 m over the autumn/winter period in Lake Pounui (Wairarapa), although they exhibited similar depth usage patterns around spawning in spring.

Suitability of Snowy 2.0 intake characteristics

- The proposed Snowy 2.0 intake (9–18 m) occurs within the depth range that adult (5–10 m depth; juvenile (12–15 m; Vejřík et al. 2016) and larval (13–14 m; Čech et al. 2005) redfin have been observed to occupy in reservoirs — when Talbingo Reservoir is low.
- Redfin spawning has also been observed at depths ranging from 0.2 m to 12 m (Gillet and Dubois 1995, Smith et al. 2001) — a range that will not overlap with the base of the intake at any reservoir level (i.e. the base of the intake will always be a minimum of 19 m below the surface).

Swimming ability

- Swimming respirometer studies have shown that redfin are capable of critical swimming speeds of 6.3 (178 mm fish swimming at 1.13 m.s⁻¹) and 7.9 (101 mm fish swimming at 0.80 m.s⁻¹) body lengths per second (BL.s⁻¹) (Tudorache et al. 2008); and maximum burst swimming speeds of between 12.6 BL.s⁻¹ (115 mm fish swimming at 1.45 m.s⁻¹) and 20 BL.s⁻¹ (250 mm fish swimming at 0.5 m.s⁻¹) (however the duration over which the maximum burst swimming speeds were maintained was not reported, although the standard convention assumes < 20 s) (Clough et al. 2004).
- Davies (2000) investigated the swimming performance of redfin and compared it with the swimming performance of four other species: *Galaxias truttaceus* Valenciennes, 1846, *Galaxias maculatus* Jenyns, 1842, *Salmo trutta* and *Pseudaphritis urvillii* Valenciennes, 1831.
- He found that redfin was the weakest swimmer of the five species tested, in terms of both burst swimming (mean = 0.32 ms⁻¹) and maximum sustained swimming (mean = 0.15 ms⁻¹) (median fork length = 103.5 mm, range = 82–221 mm) (Davies 2000).
- Pavlov et al. (1972) and Wolter and Arlinghaus (2003) reported redfin U_{crit} values ranging between 0.56 and 0.60 m.s⁻¹, and a U_{max} of 1.26 m.s⁻¹ for fish ranging from 46–64 mm in length.
- The approach velocity at the Snowy 2.0 intake trash racks will be <2 m.s⁻¹ during full station pumping (i.e. when all turbines are in operation) and will reduce if less turbines are in operation. It will also transition down to zero when pumping ceases. In situations where the approach velocity is at the upper level and redfin are within the area immediately influenced by this current velocity, the fish would not be able to swim against it to escape.
- The area over which adults may be involuntarily entrained into the Snowy 2.0 intake will be smaller than the area over which larvae and eggs could be involuntarily entrained, owing to the adults having a greater swimming ability.
- Boys et al. (2012) investigated the criteria for screening water diversions to minimise the entrainment or impingement of a range of Murray-Darling Basin fishes, and noticed that increases in approach velocity from 0.1 to 0.5 ms⁻¹ resulted in a significant increase in the rate of screen contact for fish smaller than 150 mm. Consequently, they recommended that approach velocities for intakes not exceed 0.1 ms⁻¹ as a precautionary approach (Boys et al. 2012).
- To date, very few published studies have considered the ability of redfin to orient themselves into the flow. One study, Pavlov et al. (2011), reported that 93% of the redfin (SL 52–90 mm) in an experiment oriented themselves away from the flow, and actively moved down stream, with their heads forward. Further studies are needed to better understand redfin orientation and behaviour with respect to flow.

Suitability of Snowy 2.0 intake characteristics

- CFD modelling would be necessary to determine the exact area over which critical swimming velocities would be likely to be exceeded at the intake region.
- The data above suggests that at least some redfin may not be able to avoid being entrained within the intake once they are in the region influenced by the intake's

approach velocities, particularly during full station pumping operation when velocities are expected to be highest.

- The area of involuntary entrainment would be expected to be quite small for adult fish with developed swimming abilities, and larger for larvae with poor swimming abilities.

Breeding

- Redfin are oviparous and typically become sexually mature during the period from autumn through winter (Hokanson 1977), with males capable of reaching sexual maturity in the first year and females in the second year (Morgan et al. 2002).
- Spawning generally occurs once annually in spring in slow moving water (Nunn et al. 2007) at depths ranging from 0.2 m (Smith et al. 2001) to between 4 and 12 m (Gillet and Dubois 1995).
- Strings of eggs are quickly fertilised after being released, and the fertilised egg strands are entangled around substrate, such as submerged macrophytes or other vegetation (Smith et al. 2001).
- The eggs typically develop and hatch within 1–3 weeks, with the larvae forming large schools to avoid predation (McDowall 1996).

Suitability of Snowy 2.0 intake characteristics

- The base of the intake will always be a minimum of 19 m below the surface so will be outside the expected limit of redfin spawning depth (0.2 m to 12 m (Gillet and Dubois 1995, Smith et al. 2001)).
- Redfin spawned on the PVC standpipe of the broodstock tank during our experiments, even though we had added natural (timber) and artificial (lobster spawning mops) substrate to that tank (L. Baumgartner pers. obs.) indicating that the species does not necessarily need any natural substrate features to spawn.
- If eggs floated into the intake area, it is possible they could be entrained through the intake as they are not adhesive.

Current distribution of redfin in Talbingo

- Talbingo currently supports a large self-sustaining population of redfin (Cardno 2018; NSW Fisheries database).
- Redfin were already prevalent throughout the Murrumbidgee and Tumut rivers at the time of the construction of Talbingo Reservoir in 1971, and thus it is likely that they became trapped in the reservoir after the impoundment was completed (R. Farragher and R. Tilzey pers. comm.).
- Surveys have also detected redfin in the Yarrangobilly River (Cardno 2018).
- "Numerous" (>200) redfin (mainly juveniles ≤ 5 cm) were caught amongst macrophytes in Talbingo between February and March, 2018 (Cardno 2018).

Suitability of Snowy 2.0 intake characteristics

- High numbers of redfin have been observed in Talbingo in late summer including in the Yarrangobilly arm in the vicinity of the proposed intake (Cardno 2018).
- There are currently no data available on redfin depth usage patterns in Talbingo. Based on existing literature, the depth of the proposed intake would be inside the commonly expected depth range of the species whilst reservoir levels were low since the depth to the top of the intake (9–18 m) occurs within the depth range that adult (5–10 m depth; L. Faulks pers. obs.), juvenile (12–15 m; Vejřík et al. 2016) and larval (13–14 m; Čech et al. 2005) redfin have been observed to occupy in reservoirs.
- Therefore, overall, it is likely that juvenile and adult redfin could be found in the vicinity of the proposed intake during low reservoir levels, even if the area would not be considered preferred habitat

Gambusia ecological characteristics and their match with the Snowy 2.0 intake characteristics

Habitat and food preferences

- Like redfin, gambusia are able to utilise a wide variety of habitats, including rivers, creeks, lakes, wetlands and channels (Specziár 2004, Pyke 2005, Lintermans 2007).
- They prefer still- or slow-water habitats, mostly around the littoral margins, and particularly where there is aquatic vegetation (Pyke 2005, Lintermans 2007).
- The habitat preferences of gambusia can vary depending on their life stage and sex (Specziár 2004).
- For example, Specziar (2004) found that during the breeding season in Lake Heviz (Hungary), females preferred the sheltered habitats of the lake's mudholes, whereas males and juveniles occurred in all macrophyte-covered habitats.
- In terms of feeding, gambusia are regarded as adaptable generalist predators that are capable of altering their diet according to available prey (McDowall 1996).
- Juveniles tend to consume copepod nauplii, small cladocerans and diatoms; while adults tend to consume a variety of aquatic macroinvertebrates, terrestrial insects and spiders, and the early life stages of fish and frogs (Stoffels and Humphries 2003, Pyke 2005).
- Cannibalism of young has also been documented (Macdonald and Tonkin 2008).
- Gambusia usually forage near the water surface and they often feed on items at the water surface itself (Pyke 2005).

Suitability of Snowy 2.0 intake characteristics

- The Yarrangobilly arm currently has suitable nearshore littoral zone habitats for adult and juvenile gambusia, and gambusia have been observed near macrophyte beds within the reservoir (see the current distribution section P. 27 – Cardno 2018), suggesting there are adequate food resources to support a population of the species.
- However, the Snowy 2.0 intake area within the Yarrangobilly arm will consist of a deep rock and concrete lined artificial channel with no timber. It is likely that there will be few (if any) natural habitat features (e.g. macrophytes) in the area directly in front of the intake (Snowy Hydro, pers. comm.).
- Given the apparent preference of gambusia for shallow, slow-flowing areas with abundant cover, the area directly in front of the intake would probably not contain desirable conditions for them.
- Nonetheless, gambusia have been observed in nearby regions of the reservoir, indicating that these nearby regions contain suitable habitat.

Depth preferences

- Gambusia prefer habitats that are relatively shallow (often < 15–20 cm) (Pyke 2005), but the literature regarding their maximum depth preferences is limited.

Suitability of Snowy 2.0 intake characteristics

- The top of the proposed Snowy 2.0 intake will be located at a depth of between 9 and 18 m, depending on the level of Talbingo.
- Based on current data available from other locations and the surface-feeding preference of gambusia, this depth range seems to be far deeper than that utilised by the species.
- Gambusia may be even less likely to be distributed near the intake if the intake occurs at hypoxic depths.

Swimming ability

- Gambusia are regarded as poor swimmers (Rowe et al. 2008).
- Srean et al. (2017) found *G. holbrooki* to have a lower critical swimming speed (mean $U_{crit} < 0.15 \text{ ms}^{-1}$ at 25 °C) than many other similar-sized species, including those within the same family or genus.
- Critical swimming speed varied according to fish size and sex, with males of the same body mass having much higher critical swimming speeds (Srean et al. 2017).
- In situations where the Snowy 2.0 intake approach velocity is at its upper level and gambusia are within the area immediately influenced by this current velocity, the fish would not be able to swim against it to escape.
- The area over which adult gambusia may be involuntarily entrained into the Snowy 2.0 intake will be smaller than the area over which juvenile gambusia could be involuntarily entrained, owing to the adult gambusia having a greater swimming ability.
- No published studies have considered the ability of gambusia to orient themselves into the flow.
- One study observed some individuals of the poeciliid species, *Poecilia reticulata* (i.e. guppies) to undertake positive rheotaxis in an artificial stream, but the nature and magnitude of the rheotactic response varied among populations from different habitats (Mohammed et al. 2012).

Suitability of Snowy 2.0 intake characteristics

- Gambusia appear to be relatively poor swimmers in comparison to many other small-bodied species (Srean et al. 2017).
- Based on the projected maximum velocity at the intake of $<2 \text{ m.s}^{-1}$, and the ability of gambusia to orient themselves into the flow, it should be assumed that gambusia will not be able to avoid being entrained within the intake if they were to occur in the region influenced by the intake's approach velocities (as has been recommended for redfin).

Breeding

- The breeding season for gambusia in most locations lasts from about mid-spring through to mid-autumn, and peaks in summer (Pyke 2005).
- Female gambusia are internally fertilised, and can reach sexual maturity within 1–2 months of being born (Pyke 2005).
- Spermatozoa are released from the testis for essentially the entire breeding season (Pyke 2005).
- Ovarian maturation in mature females begins in mid-spring and oogenesis occurs throughout the period between mid-spring and mid-autumn (Pyke 2005).
- The gestation period is usually around 2–3 weeks, with young developing within the mother until they are born as free-swimming small fish (Pyke 2005).

Suitability of Snowy 2.0 intake characteristics

- Based on current data available from other locations and the surface-feeding preference of gambusia, the depth range of the Snowy 2.0 intake seems to be far deeper than that utilised by the species.

Current distribution of gambusia in Talbingo

- 'Numerous' gambusia were caught amongst macrophytes in Talbingo between February and March, 2018 (Cardno 2018).
- However, there are presently no other data available on their depth preferences or seasonal dynamics.

Suitability of Snowy 2.0 intake characteristics

- Gambusia have been observed in Talbingo in late summer (Cardno 2018), but data are scant.
- There are currently no data available on gambusia depth usage patterns in Talbingo.

Entrainment likelihood assessment for redfin and gambusia

The entrainment likelihood data collated for redfin and gambusia were analysed (Table's 2 - 4), in conjunction with a conceptual model of fish entrainment likelihood adapted from Boysen and Hoover (2009) (Figure 4). This involved assessing the likelihood of each risk factor, using standard likelihood categories (Table 2). Specifically, entrainment likelihood was considered by assessing the suitability of Talbingo's habitat conditions for the species/life stage (which encompassed its habitat, food, water quality and thermal requirements), and by assessing the species/life stage's depth usage patterns and hydraulic requirements (particularly its ability to orient itself towards flow (i.e. positive rheotaxis), and its escape swimming ability).

Table 2. Definitions of the likelihood ratings used to determine entrainment risk.

Likelihood	Definition
Almost certain	Very likely to occur
Likely	Likely to occur
Possible	May occur about half of the time
Unlikely	Unlikely to occur
Rare	Very unlikely to occur

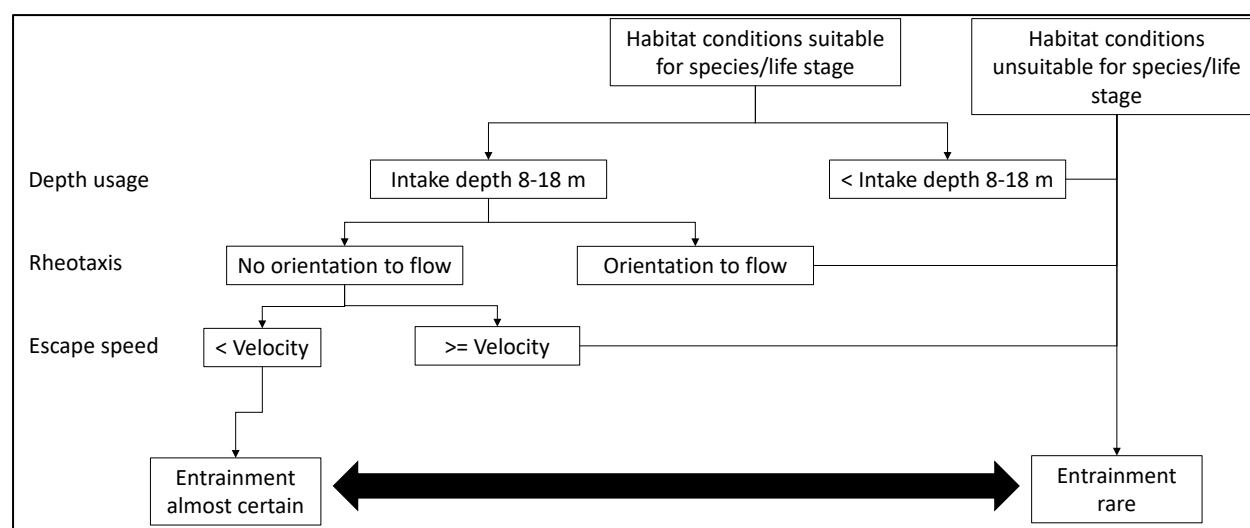


Figure 4. Conceptual model of fish entrainment likelihood adapted from Boysen and Hoover (2009). Entrainment likelihood was considered in the current study by assessing the species/life stage's depth usage patterns and hydraulic requirements (particularly its ability to orient itself towards flow (i.e. positive rheotaxis), and its escape swimming ability).

Table 3. Matrix outlining the key risk factors for redfin entrainment within the proposed Snowy 2.0 intake in Talbingo Reservoir – specifically in the region near the Yarrangobilly arm. The likelihood levels for each risk factor are for a scenario in which no mitigation measures are in place.

Risk factor	Likelihood
Risk that the Snowy 2.0 intake area (in the Yarrangobilly arm) contains suitable habitat conditions for all redfin life stages: The Snowy 2.0 intake area within the Yarrangobilly arm will consist of a deep rock and concrete lined artificial channel with no timber (Snowy Hydro, pers. comm.). Thus, the intake area itself may not contain preferable habitat conditions, but redfin have been observed in nearby regions of the reservoir, indicating that these regions contain suitable habitat.	Likely
Risk that the depth and surface area of the proposed Snowy 2.0 intake are suitable for redfin entrainment: Intake will fall within the depth range adult, juvenile and larval redfin have been observed to occupy in reservoirs when reservoir levels are low. Spawning has been observed at depths ranging from 0.2 m to 12 m (Gillet and Dubois 1995, Smith et al. 2001). Given the base of the intake will always be at least 19 m below the surface, it is unlikely that the area in front of the intake would be used for spawning. Nonetheless, this likelihood rating could be upgraded if data reveals that egg ribbons are deposited in the depth range of the intake.	Likely (larvae, juveniles and adults) Unlikely (eggs)
Risk that the hydraulic conditions associated with the proposed Snowy 2.0 intake are suitable for redfin entrainment: Observations of redfin swimming ability have been variable, and there are currently few data regarding the velocity conditions associated with the proposed Snowy 2.0 intake or the ability of redfin to orient themselves into the flow. It should be assumed that redfin will not be able to avoid being entrained within the intake if they are present in the region influenced by the intake's approach velocities. Additional CFD modelling would be required to confirm this.	Likely
Risk that the known spatial and temporal patterns for redfin in the Yarrangobilly arm are suitable for entrainment: High numbers of juvenile redfin have been observed in Talbingo in late summer (Cardno 2018), but data are scant. There are no data on redfin depth usage patterns in Talbingo, but results from the international literature suggest that redfin can occur within the depth range for the Snowy 2.0 intake.	Possible

Table 4. Matrix outlining the key risk factors for gambusia entrainment within the proposed Snowy 2.0 intake in Talbingo Reservoir. The likelihood levels for each risk factor are for a scenario in which no mitigation measures are in place.

Risk factor	Likelihood
Risk that the Snowy 2.0 intake area (in the Yarrangobilly arm) contains suitable habitat conditions for all gambusia life stages: The Snowy 2.0 intake area within the Yarrangobilly arm will consist of a deep rock and concrete lined artificial channel with no timber (Snowy Hydro pers. comm.). Therefore, the intake area itself may not contain preferable habitat conditions, however gambusia have been observed in nearby regions of the reservoir, indicating that these regions contain suitable habitat.	Possible
Risk that the depth and surface area of the proposed Snowy 2.0 intake are suitable for gambusia entrainment: Current data suggests that gambusia prefer shallow areas (often < 15-20 cm) (Pyke 2005), and therefore, that they are unlikely to be distributed near the depth of the top of the intake (9-19 m). However, there are currently a lack of data regarding the maximum depth limits of gambusia, so it is not possible to discount this risk.	Unlikely
Risk that the hydraulic conditions associated with the proposed Snowy 2.0 intake are suitable for gambusia entrainment: Gambusia are relatively weak swimmers compared to many other small-bodied fish (Rowe et al. 2008), but there are currently no data pertaining to the velocity conditions associated with the proposed Snowy 2.0 intake or the ability of gambusia to orient themselves into the flow. Thus, it should be assumed that gambusia will not be able to avoid being entrained within the intake if they were to occur in the region influenced by the intake's approach velocities. In any case, the slow transition between pumping and generation mode would create periods where suitable hydraulic conditions exist for fish to enter the intake.	Likely
Risk that the known spatial and temporal patterns for gambusia in the Yarrangobilly arm are suitable for entrainment: Gambusia have been observed in Talbingo in late summer (Cardno 2018), but data are scant. There are no data on gambusia depth usage patterns in Talbingo.	Possible

Likelihood assessment results for redfin and gambusia

- Overall, based on the known features of the proposed intake and the life history characteristics of the species, entrainment of redfin into the intake is considered to be 'Likely', whereas entrainment of gambusia is considered to be 'Possible'. The primary driver for the difference in likelihood is related to the depth preferences of each species.

Knowledge gaps for assessing the likelihood of entrainment into the Snowy 2.0 intake

Our review has identified the following knowledge gaps which, if addressed, could enable a more complete understanding of the likelihood of redfin and gambusia being entrained into the Snowy 2.0 intake:

Velocity conditions for the Snowy 2.0 intake

- There are limited empirical data regarding the velocity conditions that are likely to occur around the proposed Snowy 2.0 intake, and consequently, the swimming abilities and rheotactic behaviours (i.e. orientation with respect to flow) of redfin and gambusia cannot be assessed within the context of the hydraulic conditions associated with the intake.
- Once CFD modelling is complete, data on velocity around the intake could be compared to burst swimming speeds of the fish from the literature or tested experimentally.

Spatial and temporal distribution of redfin and gambusia in Talbingo Reservoir

- Spatio-temporal distribution data are scant for redfin and gambusia in Australian lakes and reservoirs.
- Few studies have surveyed the fish community in Talbingo Reservoir (but see Cardno (2018); and none of these have been done at the spatial scale needed to understand the spatial distribution of redfin and gambusia in the reservoir (e.g. their depth preferences), or taken into account the temporal dynamics of their various life stages.
- This data could be collected by undertaking targeted field studies using acoustic tracking, underwater sonar and/or baited remote underwater video (BRUV) stations.

Chapter 3: Investigations to determine the probability of fish survival through the Snowy 2.0 facility

Introduction

The likelihood of fish successfully passing through hydroelectric facilities is critically dependent upon their ability to survive passage (Čada 2001). There are three main stressors that influence fish welfare through a hydropower facility: shear stress, blade strike and pressure changes (Coutant and Whitney 2000). The effects of shear stress, blade strike and pressure changes on fish during conventional hydropower generation have been well documented (Čada 2001, Brown et al. 2009, Rytwinski et al. 2017), but empirical data regarding the influence of these stressors during the pumping phase of pumped hydropower facilities are currently scarce (Baumgartner et al. 2018).

Shear stress

Shear stresses occur naturally in rivers and streams when two intersecting water masses move at different velocities and/or in different directions to one another (Boys et al. 2014). Fish are often subjected to shear stresses in rivers and streams, but usually at levels that are too low to cause any harm (Čada et al. 2007). However, when the shear stress levels exceed the tolerance threshold of a fish, they can cause serious injuries or even mortality (Neitzel et al. 2004, Deng et al. 2005). High shear stress levels are likely to also occur in pumped hydropower facilities, but few studies have effectively quantified expected values for such facilities.

Blade strike

Blade strike is responsible for many instances of fish injury and mortality in hydropower facilities (Coutant and Whitney 2000) and is particularly common (Deng et al. 2007). The likelihood of fish being injured or killed from blade strike depends on a multitude of factors (Coutant and Whitney 2000, Deng et al. 2007). These include the specifications of the turbine and intake, the hydraulic conditions within the hydropower facility, and the morphology and swimming ability of the fish (Coutant and Whitney 2000, Deng et al. 2007). Computational fluid dynamics (CFD) modelling is often applied to develop design specifications for turbines that are less likely to cause blade strike injuries to fish (Coutant and Whitney 2000). However, this requires knowledge of how fish move into and through turbines, and the underlying geometry and operating modes.

Pressure changes

During conventional power generation, fish are subjected to an increase in pressure as they initially approach the deepest part of the forebay, and then a rapid decompression as they enter the draft tube or swim under the spillway gate (Brown et al. 2014). The rapid decompression has been shown to cause injury (and in some cases mortality) through the overexpansion of gas-filled organs like the swim bladder, and/or the creation of gas bubbles (referred to as emphysema) in vasculature or organs (Brown et al. 2012b, Boys et al. 2014). The pumping phase exposes fish to a rapid compression, with the range of pressure change dependent on the overall operating head. Few empirical studies have investigated the tolerance thresholds of fish to compression, but the data available suggests that the effects of compression vary considerably among species and life stages (Belaud and Barthelemy 1973, Sebert and Macdonald 1993).

The proposed Snowy 2.0 facility

The design of the proposed Snowy 2.0 scheme incorporates pressure profiles that are currently at the higher end of global hydropower schemes. Modelling for the proposed scheme suggests that fish may be subjected to rapid increases in pressure, to levels greater than 68 bar in less than a second (Snowy Hydro Limited). It could be expected that these rapid pressure changes would influence fish welfare (Baumgartner et al. 2018). Nevertheless, there is currently a lack of empirical data on the tolerance levels of redfin and gambausia to rapid compression and shear stress; nor is there any empirical data regarding their tolerance levels to blade strike impacts; these are crucial to assess survival.

The current chapter investigates the tolerance levels of redbfin and gambusia to the shear-, blade strike- and pressure-related impacts expected to occur in association with the proposed Snowy 2.0 facility.

Methods

Fish collection, production and handling

Juvenile and adult redbfin

Juvenile and adult redbfin (Figure 5) were collected from Bethungra Dam, New South Wales (NSW) (34° 45' 46.89" S, 147° 54' 14.42" E) (Figure 6) between late August to September 2018. Boat mounted electrofishing (7.5 GPP Smith-Root) was mostly used to catch juveniles and gill nets (1.8 m drop, 25 m long and mesh size 60 and 80 mm) used to catch adults. Bethungra Dam was selected as the source of redbfin as it was known to possess a sufficiently large population of redbfin and was within reasonable proximity to the Charles Sturt University Fish Laboratory (CSUFL) in Thurgoona where the experiments were conducted. Expeditions to Talbingo Reservoir yielded few adult fish, so a decision to change the source population was made in agreement with SHL. The CSUFL was a purpose-built facility exclusively designed to house fish for use in hydropower simulation experiments.

Redfin were transported to CSUFL in a specialised 600-L aerated fish transporter fitted with oxygen tanks and aerators, and filled with dam water. On arrival, juvenile and adult redbfin were placed in separate quarantine 1000 L tanks and monitored for a week prior to release into other holding tanks. All fish were observed daily to monitor their health and condition. After quarantine, juveniles and adults were placed in separate 1000 L tanks, in accordance with best practice optimal stocking densities of 20 kg/m³ (Rougeot and Toner 2008) (Figure 7). Tap water was added to the holding tanks and treated with Safe™ (1g / 1000 L) to remove chlorine. Water was recirculated and filtered in each tank with a biological filter (AST Endurance Polygeyser Bead Filter Model 4000). To minimise the risk of stress-related disease following transport, all fish were given a prophylactic salt treatment that involved raising the salinity of the holding system to 5 ppt for one week, after which was maintained at approximately 2.5 ppt throughout the study. All tanks were maintained at a stocking density of < 2.5 kg / 1000 L.

Fish were housed at CSUFL for at least 5 days prior to experimentation to allow for acclimation to hatchery conditions and to be assessed for any sign of disease. Water quality parameters (temperature, pH, dissolved oxygen (%), mg/L) conductivity (ms/cm⁻¹), ammonia, nitrate and nitrite) were measured daily. Throughout the study, the mean (± SE) temperature was 11.12 ± 0.016°C (range of 6.73-16.22); pH was 7.6 ± 0.001 (range of 7.02-7.86); conductivity was 6.4 ± 0.016 ms / cm⁻¹ (range of 0.07 – 11.4); turbidity was always 0 NTU, dissolved oxygen was 10.15 ± 0.008 mg L⁻¹ (range of 5.91 to 13.05); total dissolved gas saturation was 97.09 ± 0.074% (range of 56.8 to 119); ammonia was 2.01 ± 0.010 (range of 0 – 4), nitrite was 0.05 ± 0.001 (range of 0 – 2) and nitrate was 0.18 ± 0.007 (range of 0 – 5).

A 25% water change was carried out once per week, which was adequate to maintain water quality and remove any uneaten food. Adult, juvenile and broodstock redbfin were fed twice daily on a mix of frozen blood worms, live earthworms and yabbies (*Cherax destructor*) and a commercial pellet (Skretting floating pellets, 3 mm for juveniles and 5 mm for adults).



Figure 5. Top: Juvenile redfin. Bottom: Adult redfin.

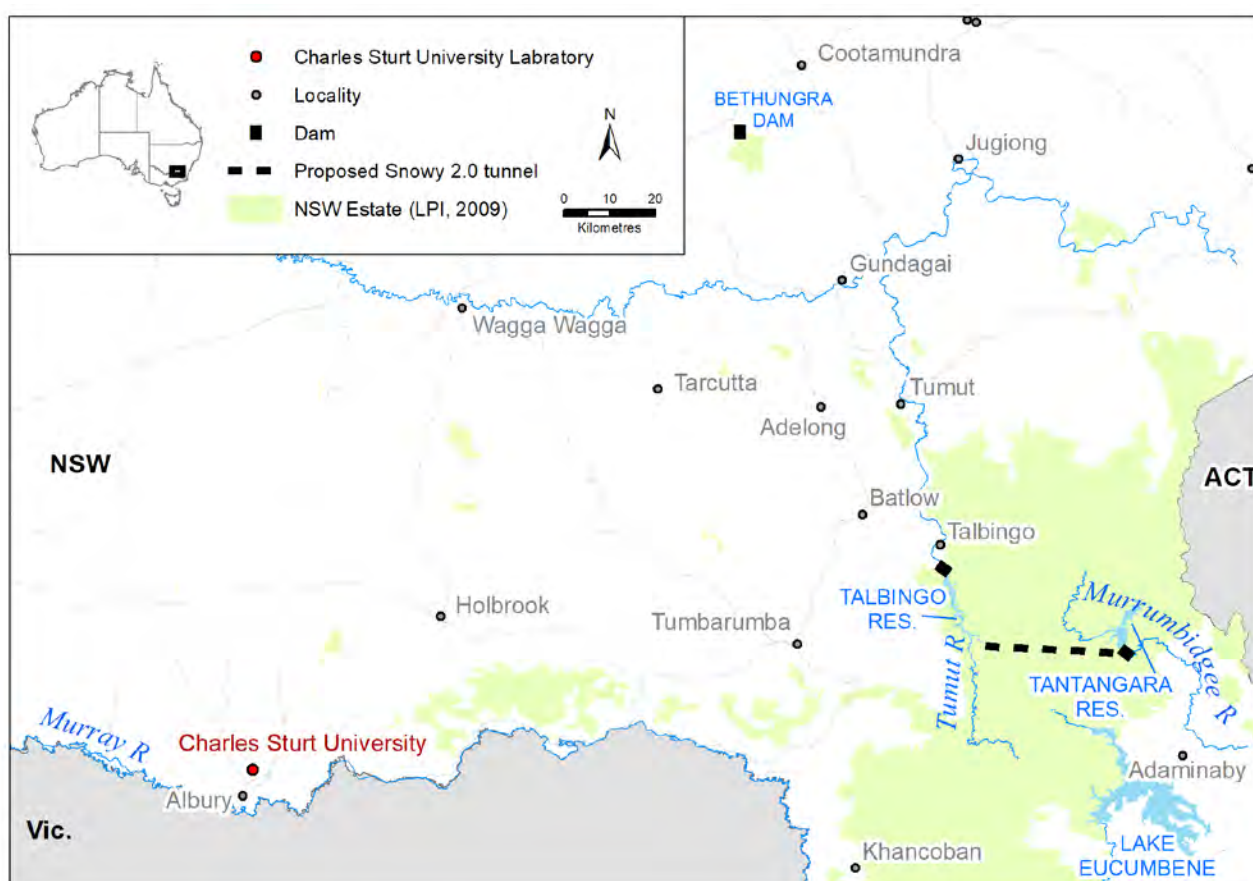


Figure 6. Map showing the (1) site where redfin were sourced from, Bethungra Dam; (2) site where the experiments were undertaken, Charles Sturt University – Albury campus; and (3) the location of the proposed Snowy 2.0 facility between Talbingo and Tantangara reservoirs.



Figure 7. The 1000-L polyethylene fish holding tanks at CSUFL.

Spawning

Each adult redbfin was assessed for fecundity, with fecund females identified as those with a swollen red papilla, and mature males identified as those that expelled milt on handling. These fish were separated and placed into a broodstock holding tank. Broodstock fish were not measured to minimise handling. Housing of the adult redbfin coincided with their natural reproductive season in the southern hemisphere (September to October). Fortuitously, adult broodfish naturally spawned in the holding tanks without the need for artificial induction. Artificial spawning substrates (lobster spawning mops, and the internal stand pipe of the tank) were used as spawning sites and to aid egg collection (Figure 8).

Throughout the study, the mean (\pm SE) temperature was 12.28 ± 0.06 °C (range of 9.28 – 16.03); pH was 7.61 ± 0.004 (range of 7.21 – 7.76); conductivity was 4.80 ± 0.035 ms / cm⁻¹ (range of 2.54 – 5.51); turbidity was 0.14 ± 0.026 NTU (range of 0 – 3.7), dissolved oxygen was 9.63 ± 0.025 mg L⁻¹ (range of 6.67 – 10.57); total dissolved gas saturation was 94.39 ± 0.266 % (range of 65 – 104.8); ammonia was 2.19 ± 0.050 (range of 0.25 - 4), nitrite was 0.03 ± 0.004 (range of 0 – 0.5) and nitrate was always 0.

Adults typically spawned overnight once spring temperatures reached approximately 12 °C. Within 12 hours of spawning, egg ribbons (Figure 9) were harvested and placed into mesh baskets in their source tank for two days. Ribbons were then transferred to aerated 20-L buckets until they were either utilised for egg experiments or subsequently hatched for larval experiments. Broodstock redbfin were held at a ratio of 1 female: 2 males.



Figure 8. Lobster spawning mop (circled, RHS of image) in 1000-L holding tank, used as artificial spawning substrate for redfin.



Figure 9. A single redfin egg ribbon held in a mesh basket.

Eggs and larvae

Egg development was monitored daily under a dissecting microscope (Olympus Model Number SZ2-ILST). Eggs (mean (± 1 SD) diameter = 1.4 ± 0.032 mm; range = 1.34-1.44 mm) for experimentation were kept until they had reached a development stage where eyes were visible and then used in experiments (Figure 10). Once hatched, redfin larvae were kept prior to experimentation in aerated glass aquaria (450 mm (L) x 290 mm (W) x 350 mm (D)) containing a sponge filter (Aqua One Filter Air 60 Breeder Sponge Filter) maintained at the lowest air setting. Larvae were fed with newly hatched *Artemia* nauplii and egg yolk 5 times daily at the rate of approximately five feeds per day until experimentation. Larvae were experimented on during two distinct phases 12 - 18 days (Figure 11) and 28 - 30 days post hatch (DPH).

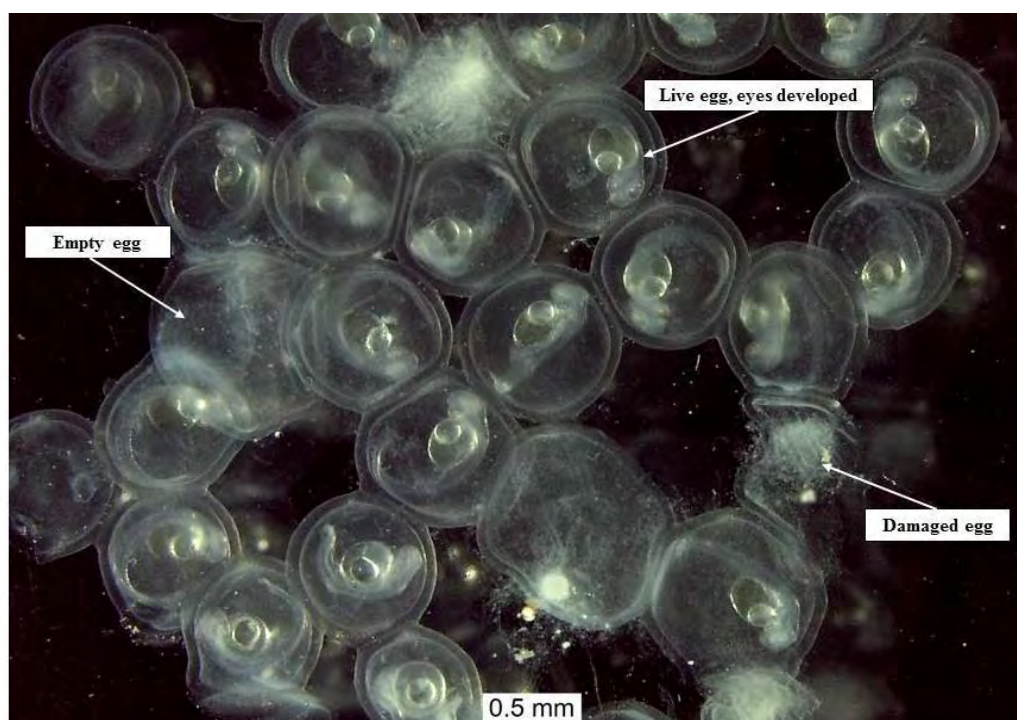


Figure 10. Redfin egg ribbon fragment containing live eggs with developed eyes, damaged eggs and empty eggs.



Figure 11. Photo of larval redfin aged between 12 - 18 DPH.

Adult gambusia

Adult gambusia were collected from storage dams at Charles Sturt University, Albury campus, (NSW) (36° 2' 19.88" S, 146° 59' 15.66" E) (Figure 6) between September and October 2018, using a 4mm mesh seine net and unbaited traps. Fish were transported to the laboratory in 100 L 'nelly' bins with dam water. To minimise the risk of stress-related disease following transport, the fish were given a prophylactic salt treatment (5 ppt for one week) and maintained at approximately 2.5 ppt throughout the study. The mean (\pm SE) temperature was 9.76 ± 0.091 °C (range of 7.68 – 12.07); pH was 8.04 ± 0.006 (range of 7.89 – 8.14); conductivity was 0.33 ± 0.003 ms / cm⁻¹ (range of 0.29 – 0.46); turbidity was always 0 NTU, dissolved oxygen was 9.73 ± 0.071 mg L⁻¹ (range of 7.46 – 11.33); total dissolved gas saturation was 89.90 ± 0.77 % (range of 68.4 – 110.1); ammonia was 0.5 ± 0.021 (range of 0.25 - 1), nitrite was 0.02 ± 0.005 (range of 0 - 0.25) and nitrate was always 0. In the laboratory, gambusia were held for 5-7 days in 1000 L tanks and fed *Artemia* nauplii before being used for the experiments.

Shear experiments

Fish production and handling

Redfin eggs, larvae, juveniles and adults and adult gambusia were collected, bred, harvested and housed at the CSUFL as per the methods described under the 'Fish collection, production and handling' section aforementioned.

Shear chamber

Shear strain experiments were conducted using a cylindrical plexiglass chamber (1.95 m (L) x 0.44 m diameter) connected to a submerged jet at one end, and to a fibreglass reservoir tank (2.10 m (L) x 2.10 m (W) x 0.9 m (D) at the other end (Figure 12). Attached to the chamber is a clear polycarbonate deployment tube fixed above the submerged jet at an angle of ~30° (Figure 12). This tube was used to introduce eggs, larvae and juvenile redfin and adult gambusia to the point in the chamber where the flow velocity was known.

A 3-phase electric pump (Grundfos® NBG 125), capable of achieving volumes of 153 m³ h⁻¹, circulated water through the chamber via a 15cm diameter PVC pipe. On entry to the chamber, a conical plastic nozzle reduced the diameter of flow from 15 cm to 5 cm over a distance of 26 cm effectively accelerating the flow and creating a submerged jet into the chamber, similarly to the approach described by Neitzel et al. (2004) (Figure 12).

Within the range of flows obtained in the chamber, maximum velocities of up to 18.5 m s⁻¹ were possible in the centre of the nozzle. A butterfly valve was used to control the flow rate within the chamber and, therefore, allow for the production of different water velocities within the jet. An inline flow meter (Wollman Silver Turbo Water Meter, ARAD Waterworks®) was used to calculate the flow rate for the different butterfly valve openings. Once the flow rate was established, a total tube was inserted into the chamber to determine the jet velocities and allow for further calculation of the shear strain rates.

Characterisation of the shear environment

Mean jet velocities for the different flow rates applied were obtained using a total tube connected to a pressure gauge ranging from 0 to 250 kPa with 5 kPa increments (Figure 13). The total tube was positioned 90 mm distant from the nozzle and measured velocity in the centre of the jet. The pressure differential for the different jet velocities was obtained and jet velocities calculated using Bernoulli's equation:

$$H = \frac{v^2}{2g}$$

where H is the total head (m), v is the velocity (m s⁻¹) and g the gravitational constant (m² s⁻¹).

Once mean jet velocities were obtained the shear strain rates were calculated using the equation suggested by Neitzel et al. (2004) and based on the original method described by Groves (1972):

$$e = \frac{\partial v}{\partial y}$$

where v is the mean water velocity and y is the distance perpendicular to the force. Distance was defined at different values for each life stage and species tested to provide a fine scale measurement of shear strain rate at the width of the fish (Neitzel et al. 2004). Therefore, y values for redfin were 0.3 cm for eggs (egg yolk diameter), 0.5 cm for larvae (both 12 - 18 and 28 - 30 DPH – larvae width) and 1 cm for juvenile (body width) (Table 5). For adult gambusia the y value was also defined at 1 cm (body width) (Table 5).

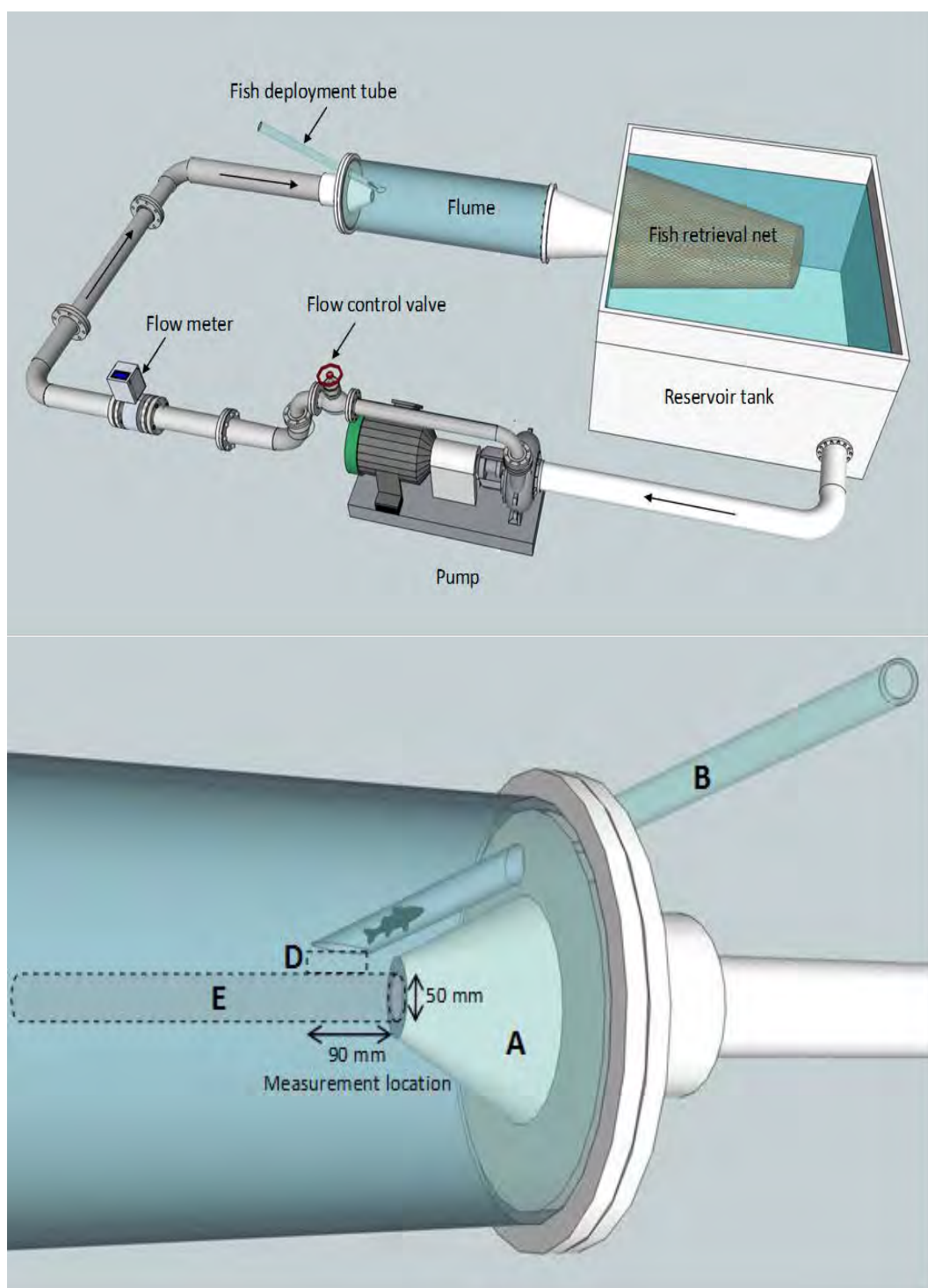


Figure 12. Shear chamber facilities that were used to simulate Snowy 2.0 conditions on redfin and gambusia. Fish are released in the deployment tube, exposed to a jet in the chamber then collected in the retrieval net before being transferred to holding facilities (adapted from Boys et al. 2014).

Table 5. Chamber flow rates, mean jet velocities and shear strain rates calculated for each species and life stage tested.

Chamber flow rate (Ls ⁻¹)	Mean jet velocity at the nozzle (m s ⁻¹)	Shear strain rate (1/s)	Species / life stage
0	0	0	Redfin and gambusia / all life stages
12	5.05	1683	Redfin / eggs
25	12.61	4203	
34	16.87	5623	
40	18.53	6177	
12	5.05	1010	Redfin / larvae 12 - 18 and 28 - 30 DPH
25	12.61	2522	
34	16.87	3374	
40	18.53	3706	
12	5.05	505	Redfin / juveniles and gambusia / adults
25	12.61	1261	
34	16.87	1687	
40	18.53	1853	



Figure 13. Calibration of the shear chamber using a total tube with a pressure gauge attached. Calibration was conducted to determine the flow velocity within the jet in front of the nozzle in the chamber and provide for the calculation of the shear strain rates to which fish were exposed.

Species, life stage shear experiments

Shear strain experiments were conducted on redfin eggs, larvae and juveniles and adult gambusia (Table 6). All life stages and species were inserted in the chamber in front of the nozzle and 25 mm above the jet centreline using a delivery tube (Figure 12).

Egg experiments

Five replicate test groups of redfin eggs (mean 24.4 eggs \pm 4.4 SD, range 19 – 34 eggs) were randomly exposed to five strain rate treatments (Table 6). At the beginning of each experiment, an egg ribbon was collected using an aquarium net from a bucket containing aerated water and cut with sharp scissors into fragments (about 30 mm x 30 mm that contained approximately 20 eggs). An egg ribbon was collected using an aquarium net from a bucket containing aerated water and cut with sharp scissors into fragments (about 30 mm x 30 mm that contained approximately 20 eggs). Egg ribbon fragments were placed on a petri dish and individual eggs were counted using a dissecting microscope (Olympus SZ2 -ILST). Eggs were included in the count if they 1) had visible eyes, and 2) the developing larvae showed signs of movement within 20 seconds. Eggs with eyes and movement indicated that the egg survived exposure and may hatch. Eggs that were damaged, empty, had a fungus or had no eyes or no movement were not counted but were left attached to the egg ribbon fragment, although not used in the experiments. It was originally intended to allocate the same number of eggs to each capsule (i.e. 20), but the actual number of eggs varied to minimise disturbance from multiple cuts to the egg ribbon. Once counted, each egg fragment was transferred through the deployment tube into the shear flume. A gentle flow of water was passed down the deployment tube to ensure that all eggs were flushed into the chamber and exposed to a shear event. Following exposure to the shear event, the eggs were collected from the fish retrieval net and placed into aerated 700-mL plastic jars for each replicate, and held for 24 hours to assess survival.

Eggs were considered to have survived if they had hatched and the emerging larvae were free swimming larvae or were moving in the egg following examination under the dissecting microscope. All other eggs, including dead larvae, broken eggs, fungal-affected eggs or those not moving inside were considered to not have survived. Only survival was recorded to eliminate inaccuracy of counting of hatched eggs or broken eggs (which had numerous protein fragments). The survival counts were conducted at 0 h and 24 h to calculate the total survival rate as a percentage for each test group replicate.

Larval experiments

Five replicate test groups of 15 larval redfin 12 - 18 DPH (larvae at this life stage were not measured) and the same for an additional group of 5 larval redfin 28 - 30 DPH (mean length 10.1 mm (FL) \pm 1.5 SD, range 6 – 14 mm) were exposed to the same five strain rate treatments (Table 6). Larvae for each test group were pipetted from the glass holding aquaria and transferred via the deployment tube into the shear chamber.

Juvenile redfin and gambusia experiments

The absolute numbers of fish assessed per replicate were dependent on the physical characteristics of the deployment tube. Juvenile redfin were assessed using ten replicates, consisting of one fish per replicate because only a single fish could fit into the tube at once. Adult gambusia are much smaller and were assessed using five replicate test groups of 10 individuals each. Juvenile redfin and adult gambusia were each subjected to the same five strain rate treatments (Table 5) and protocols used in the redfin larvae experiments. Individuals of each species were dip-netted from holding tanks and transferred via the deployment tube into the shear chamber. Survival following shear experiments was examined using the same assessment protocols as that applied in the pressure experiments. The 0 cm s⁻¹ cm⁻¹ shear strain rate control was applied to consider any potential handling effects, and involved introducing the eggs via a duplicated deployment tube that was directed into the capture net (Figure 14). The unit to represent shear strain rates (cm s⁻¹ cm⁻¹) is simplified to 1/s hereafter.

Data analysis

Data obtained for the shear experiments were analysed independently for each species and life stage tested. For redfin eggs and both larval stages (12-18 and 28-30 DPH), survival probability was calculated based on the number of eggs or larvae retrieved after shear strain exposure. This approach provided for a conservative estimation of survival because sometimes eggs and larvae were not recaptured, but this did not influence further replicates. For redfin juvenile and adult gambusia, survival was estimated for each fish since they were individually exposed to the shear strain and all retrieved after exposure. Survival probability was obtained instantaneously (immediately after the experiment), 24 hours and five days after exposure for each replicate and shear strain rate.

Mean survival probability was then calculated for each experimental group (control and shear strain rates) for instantaneous, 24 hours and five days post-exposure. Comparisons between treatments were conducted using a non-parametric analysis of variance (Kruskal-Wallis test). Then, a pair-wise comparison of means (Scheffe test) was used to identify groups that had significantly different estimations of survival. Tests were performed using Statistica, and considered significant at $P < 0.05$.



Figure 14. Deployment tube attached to the shear chamber and a duplicated deployment tube used as a control that was directed into the capture net (LHS).

Table 6. Summary of life stage, experimental treatments and fish size for shear experiments. Eggs were not measured[#] and the table below provides the mean number of eggs (with standard deviation) used in each treatment.

Life stage	Treatment (shear strain 1/s)	Replicates	No. per replicate	Fish Size (FL, mm)	Size range (mm)
<i>Redfin</i>					
Egg	0	5	1 – 2 fragments	24.4 ± 4.4 (No. of eggs)	19 – 34 (No. of eggs)
	1683	5			
	4203	5			
	5623	5			
	6177	5			
Larvae 12 - 18 DPH	0	5	15	No measurement	No measurement
	1010	5			
	2522	5			
	3374	5			
	3706	5			
Larvae 28 - 30 DPH	0	5	10	10.1 ± 1.5 *	6 – 14
	1010	5			
	2522	5			
	3374	5			
	3706	5			
Juvenile	0	10	1	115.6 ± 6.1	98 - 127
	505	10			
	1261	10			
	1687	10			
	1853	10			
<i>Gambusia</i>					
Adult	0	5	10	23.6 ± 5.1 *	12 – 47
	505	5			
	1261	5			
	1687	5			
	1853	5			

[#]A separate sample of eggs was measured for the blade strike model to determine average size range of 1.34 – 1.44 mm. Eggs used in experiments were not measured for experiments to avoid handling.

*Adult gambusia and redfin larvae 12 – 18 DPH were measured by their total length (TL).

Blade strike modelling

Francis Turbine blade strike model

Blade strike modelling is an important and cost-effective approach to evaluate the biological performance of turbine design and operations. Von Raben (1957) proposed the first deterministic blade strike model based on the assumption that fish must pass through a turbine runner leading edge plane after the sweep of one blade and before the sweep of the next one to avoid strike by a runner blade. Turnpenny et al. (2000) defined the 'water length' as the distance between two successive blades along the flow line and derived the blade strike model for Kaplan turbines. Fish aligned with the flow lines and longer than the water length would be struck by the runner blades.

Strike probability was given by:

$$P_{\text{Blade Strike}} = \frac{\text{Fish Length}}{\text{Water Length}}$$

Since fish can enter the turbine in random orientations rather than aligning with flow lines, which can shorten the apparent fish length, this model would maximize the blade strike risk.

Deng et al. (2007) defined 'critical passage time' as the time between sweeps of two successive blades. Considering the time a fish needs to pass safely through the plane of the leading edges of the runner blades, Deng et al. (2007) derived the same blade strike model for Kaplan turbines as Turnpenny et al. (2000) and introduced a stochastic blade strike model and evaluated the validity of using blade strike modelling as an estimate of the biological performance of several large Kaplan turbines.

We derived the blade strike model for Francis turbines, following Deng et al. (2007), to approximate strike probabilities for Snowy 2.0. We first calculated the surface of the imaginary cylinder of a Francis turbine wicket gate exit (Figure 15):

$$A_{wgc} = 2\pi R_{wgc} h_{wg} \quad (1)$$

where R_{wgc} is the radius of the imaginary cylinder of the wicket gate exit and h_{wg} is the height of the wicket gate.

For the proposed Snowy 2.0 pump-storage turbine, the turbine runner leading edge cylinder is adjacent to the imaginary cylinder of the wicket gate exit due to its high head (> 600 m) and low specific speed, so we get $R_{wgc} = R_1$, where R_1 is the radius of the turbine runner leading edge.

The radial velocity at the wicket exit is:

$$V_r = \frac{Q}{A_{wgc}} = \frac{Q}{2\pi R_{wgc} h_{wg}} = \frac{Q}{2\pi R_1 h_{wg}} \quad (2)$$

Assuming that the angle of a fish at the wicket exit is the same as the wicket gate opening angle θ , then the time a fish needs to safely pass through the imaginary cylinder of the turbine runner leading edges is:

$$t = \frac{l \cdot \sin \theta}{V_r} \quad (3)$$

where l is the fish length.

The 'critical passage time' t_{cr} , which is the time between sweeps of two successive blades is:

$$t_{cr} = \frac{1}{n \cdot (\frac{N}{60})} \quad (4)$$

where n is the number of runner blades and N is the runner speed in revolutions per minutes (RPM).

A fish will experience a blade strike if it does not pass through the imaginary cylinder of the turbine runner leading edges within time t_{cr} , so the probability of blade strike can be expressed as:

$$P = \frac{t}{t_{cr}} = \frac{l \cdot \sin \theta \cdot n \cdot (\frac{N}{60})}{V_r} \quad (5)$$

Von Raben (1957) observed that his blade strike model always produced an estimate of blade strike that was higher than the proportion of live fish he observed to be injured during passage through the turbine he was modelling. To account for the observation that not all fish struck by a turbine blade were injured, Von Raben introduced the idea of a mutilation ratio (MR). The mutilation ratio was simply the ratio between the proportion of fish he estimated to be struck by a turbine blade and the proportion he observed to be injured. To deal with the same issue in his experiments, Turnpenny et al. (2000) empirically developed a regression equation of MR for different fish lengths:

$$MR = 0.15533 \ln(l) + 0.0125 \quad (6)$$

where MR is mutilation ratio, \ln is natural logarithm, and l is fish length (in centimetres).

For Snowy 2.0, because of the high rotation speed of the Francis turbine and lack of experimental data on blade strike injury for redbfin and gambusia, the approach taken was to consider the worst scenario and assumed that all the fish struck by a blade will be mortally injured (i.e. the MR is assumed to be 100% regardless of the fish length). Therefore, the modelling output would likely overestimate fish strike injuries, especially for small fish or eggs, which have relative large surface area to mass ratio that are likely being pulled around the blade by the drag force of the flowing water rather than colliding with turbine blades (Turnpenny 1998).

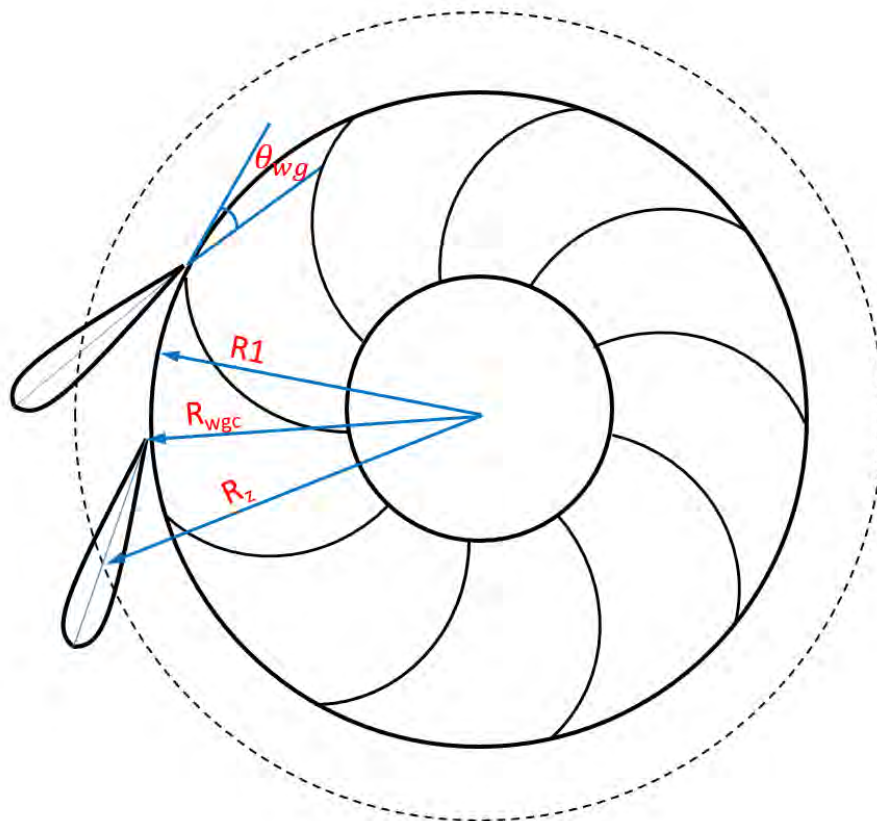


Figure 15. Schematic showing the wicket gate angle and radius of the imaginary cylinder of the wicket gate exit.

Pressure experiments

Hyperbaric chamber design

A pressure chamber (fast transient hyperbaric chamber) capable of generating extremely fast pressure transients (to the millisecond) was designed and manufactured specific to this project requirement. The chamber was designed to simulate the pressure profile experienced by a fish as it would travel through the Snowy 2.0 facility while maintaining a stable environment (oxygen and temperature levels). The chamber was manufactured to have enough bandwidth to meet the 'spike' imposed by the turbine stage of the simulation. This 'spike' had to be accurate and repeatable within a very tight window of error, further challenges were in creating a well-oxygenated environment under these high-pressure conditions. These challenges were met within the following specifications:

Specifications

- User programmable test profiles with up to 10 hours test duration controlled by a user-friendly LabVIEW based software. 5 KHz measurement bandwidth.
- Pressure rate of change better than 3 Bar/milli second with less than 2% overshoot.
- Dissolved oxygen measurement via an optical luminescent DO probe with a 0.1mg/l accuracy (0-20mg/l range)
- Water temperature measurement via a K-type Thermo-couple 1 °C accuracy
- Pressure measurement with 0.5% measurement accuracy, 1 millisecond response time.

Hardware description

The system was built around a 100l pressure vessel with ASME B16.1 Class 600 6-inch flanges on both ends, one end having a viewing port (composed of a thick acrylic) and the other a removable flange connected to a hydraulic ram for the water displacement (Figure 16 and Figure 17). The flange/ram is suspended on a rail system for easy removal during the loading of the test specimens. The chamber has a 100 Bar bleed off and water circulation system for measurement and oxygenation of the water at ambient pressures. The system is powered by a 2.2Kw hydraulic fluid pump and cooling systems. A 230 Bar 20L hydrogen charged accumulator was used to store the large amount of energy required for the rapid pressure changes within the system (Figure 17). Charging time would take around 1min at 2.2Kw. The rapid pressure changes were implemented by making use of a high-end embedded Moog motion controller moving a high bandwidth Moog hydraulic ram/valve combination to accurately and rapidly displace water in the test chamber. Measurement of all signals were achieved using Beckhoff digitisation hardware. Communication between the PC, motion controller and Beckhoff PLC was implemented over a standard TCP/IP local network.

Calibration procedure

Thermocouple temperature channels were calibrated by using a Fluke 725 process calibrator to simulate the sensor characteristics. The dissolved oxygen sensor was calibrated with the calibration tool and simulation solution provided by the supplier. The pressure sensor came with calibration values for zero point and span from the supplier and was calibrated as per these values.

Fish Pressure Chamber procedure

At the beginning of each experiment, the flange with the hydraulic ram end of the pressure chamber was unbolted and removed (Figure 16). Test specimens (eggs, larvae and fish) were inserted into two cylindrical Perspex capsules (350 mm (L), diameter 100mm, hereafter the Fish Pressure Chamber – FPC capsules) via an opening on the top side of each capsule (275mm (L) x 50mm (W)) and filled with the same water from the fish holding tanks. This opening was then covered with a velcro-attached mesh (1.5mm x 1.5mm mesh size) window to prevent the fish from escaping but still allowed for the exchange of oxygenated water between the capsule and inside the pressure chamber (Figure 18).

The two FPC capsules were then inserted into the pressure chamber from the hydraulic ram end, and the flange was bolted back into place. Water was delivered to the pressure chamber using a submersible pump (Ozito Model: PSDW-750 Dirty Water Submersible Pump) being drawn from a sump containing water from the same tanks in which the fish were held prior to experimentation. Once the pressure chamber was full, the chamber was switched to purge mode to remove any air bubbles from the system. After purging, the chamber was set to auto mode to initiate pressure changes according to the desired experimentation scenario.

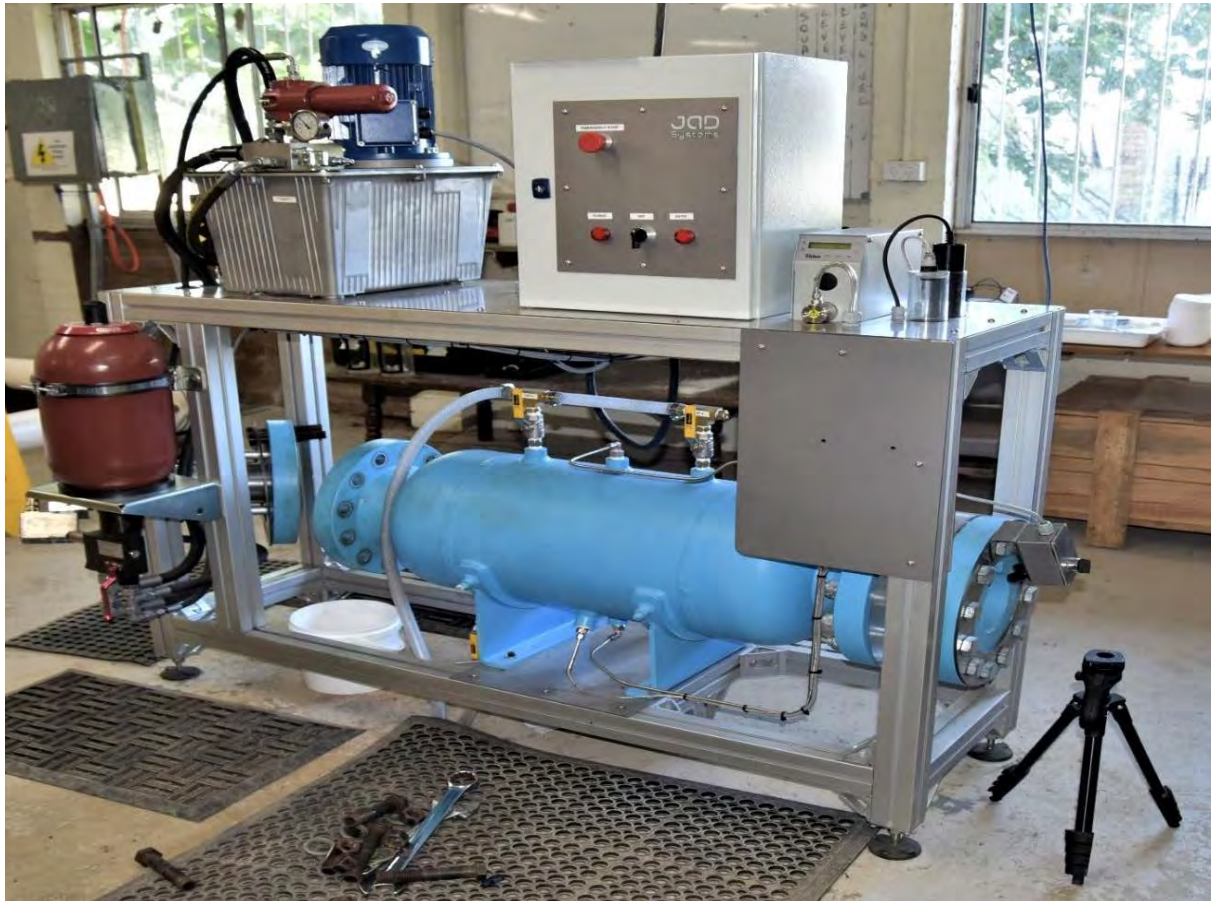


Figure 16. Photo of hyperbaric chamber showing the hydraulic ram end of the chamber unbolted (on the left side of the chamber from this view).

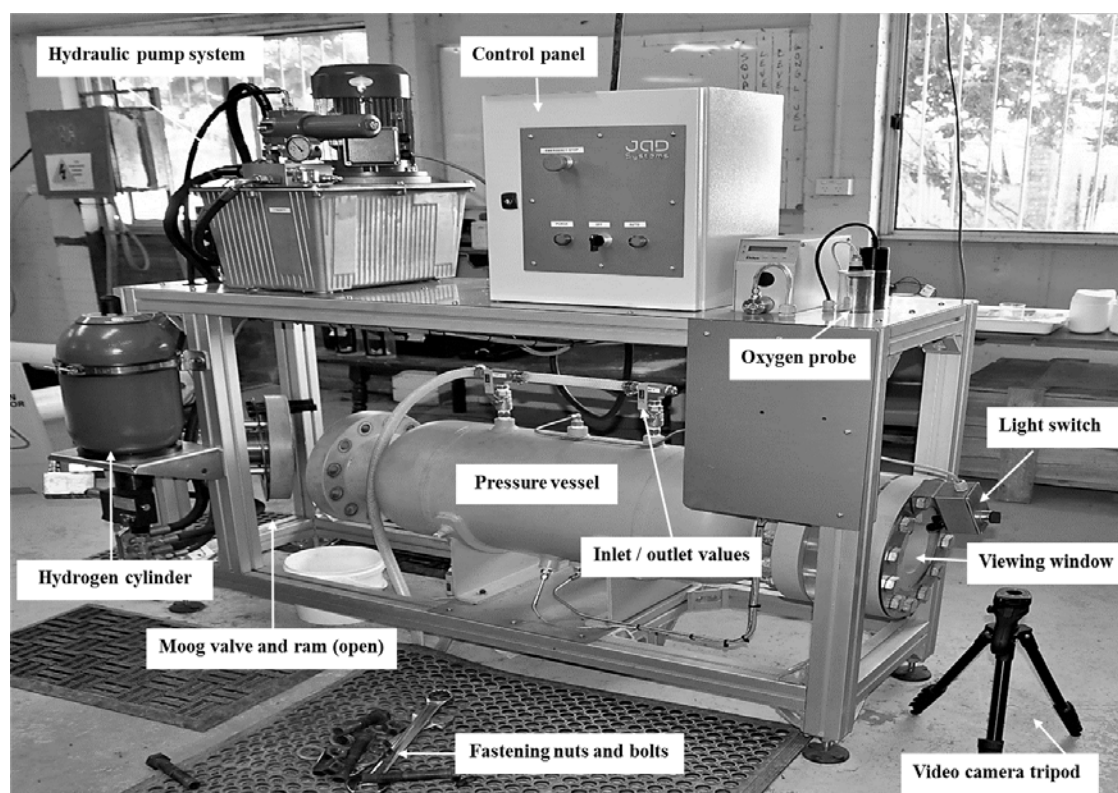


Figure 17. The hyperbaric chamber with various operating components labelled.

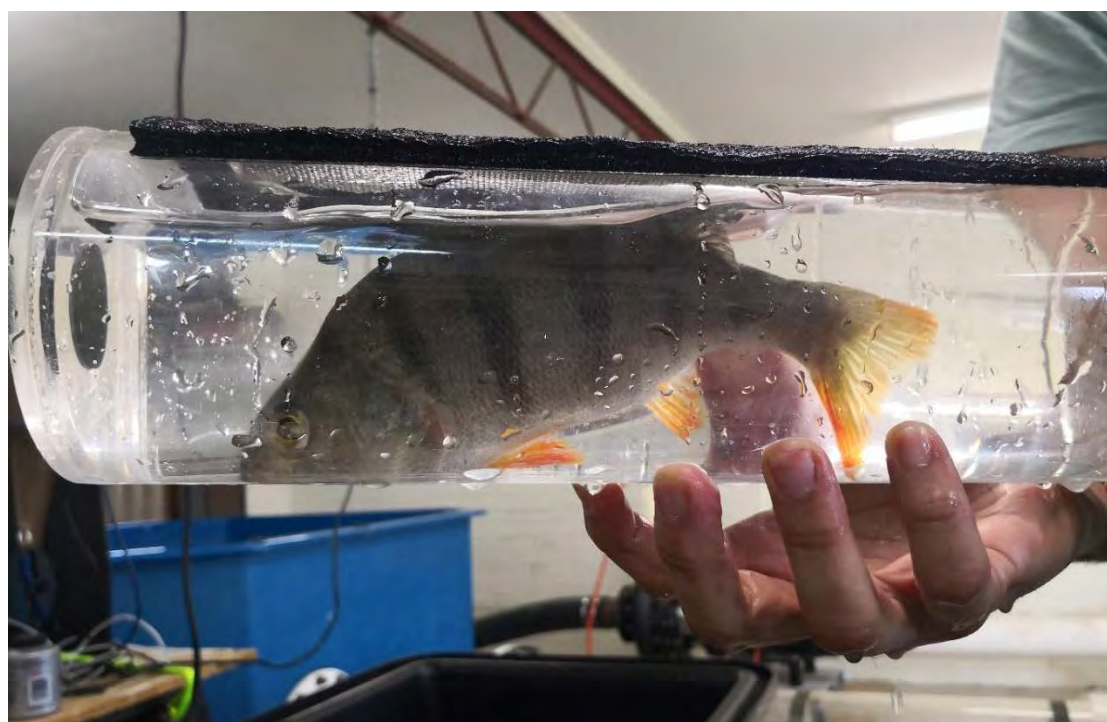


Figure 18. Photo of adult redfin in the Fish Pressure Chamber capsule prior to being added into the pressure chamber.

The hydraulic ram was controlled by a computer program with graphical user interface (GUI; LabView) and generated pressure changes in accordance to a pre-loaded profile. The computer program provided for the entry of up to 13 different pressure values and the correspondent time lag for the chamber to operate within that value. Two different pressure profiles simulating operational scenarios with three (T_3) and six (T_6) turbines operating at maximum flow were loaded and saved in the computer program (see next section). After loading the desired pressure profile in the system the user initiated the pressure simulation in the chamber. Throughout the experiment, fish were viewed through a viewing window until the replicate concluded.

At the end of the pressure exposure, water from the pressure chamber was drained back into an intermediary water holding tank using an in line pump (Ozito Model: TRP-650 Transfer Water Pump). The hydraulic ram end of the chamber was again unbolted and the capsules were retrieved from inside the chamber. The fish were collected from the FPC capsules and placed into holding tanks that contained baskets labelled based on each experimental replicate. All pressure profile experiments were also coupled with a control that consisted of the identical protocol but without imposing fish to pressure exposure. Following the completion of each pressure test, the computer program saved data for the pressure profile achieved, temperature and dissolved oxygen throughout the entire experiment. Saved data could be accessed using the GUI system or exported as a csv file for viewing in alternative software (e.g. Microsoft Excel). The csv file was used to confirm the pressure profile fish were exposed to and to verify oxygen and temperature stability in the chamber throughout the experiment.

Pressure profiles tested

Two user-defined pressure profiles were simulated in the hyperbaric chamber. These were considered characteristic of what would be experienced by a fish entrained at the Talbingo intake and passing through the Snowy 2.0 facility during the pumping phase (Table 7; Figure 19). The profile is based on the 'reference design' of the station as the final design was not known at the timing of the experiments. Some changes have subsequently been made to the design of the station however the source of primary compression however remains unchanged as this occurs when transported through the runner/impeller of the unit itself. For the layout, the primary change has been the adoption of a single inclined pressure shaft which results in a more gradual reduction in pressure (moving towards Tantangara away from pressure source - pump) and the movement of the cavern closer to Talbingo, which results in greater pressure being available at the suction of the pump. This is slightly offset due to a smaller tunnel of 9.8 m versus 10 m which will result in greater head loss.

In both scenarios, the highest simulated pressure achieved would be up to 76 Bar at the turbines and the lowest 1 Bar when reaching Tantangara Reservoir. What differed between the two scenarios was the transient times between different sections of the facility and the total passage time:

T_6 – Full pumping capacity with all six turbines operating simultaneously at full speed. Resulting in a total passage time of 2 hours (~7,133 seconds).

T_3 - Three pump-turbines in pump mode operating at full speed. Resulting in a total passage time of 3.8 hours (~13,652 seconds) (Figure 20).

Together these two simulated scenarios account for a reasonable proportion of expected operating conditions once Snowy 2.0 is completed. A single pump-turbine operating scenario was not simulated in this study, because the travel time for a fish exposed to that scenario is expected to be greater than 18 hours, and thus it was impractical to run replicate pressure trials for that duration in the time available to complete the project. Likewise, simulating any other pressure scenarios involving the operation of fewer than three pump-turbines or between three and six pump-turbines was beyond available resources.

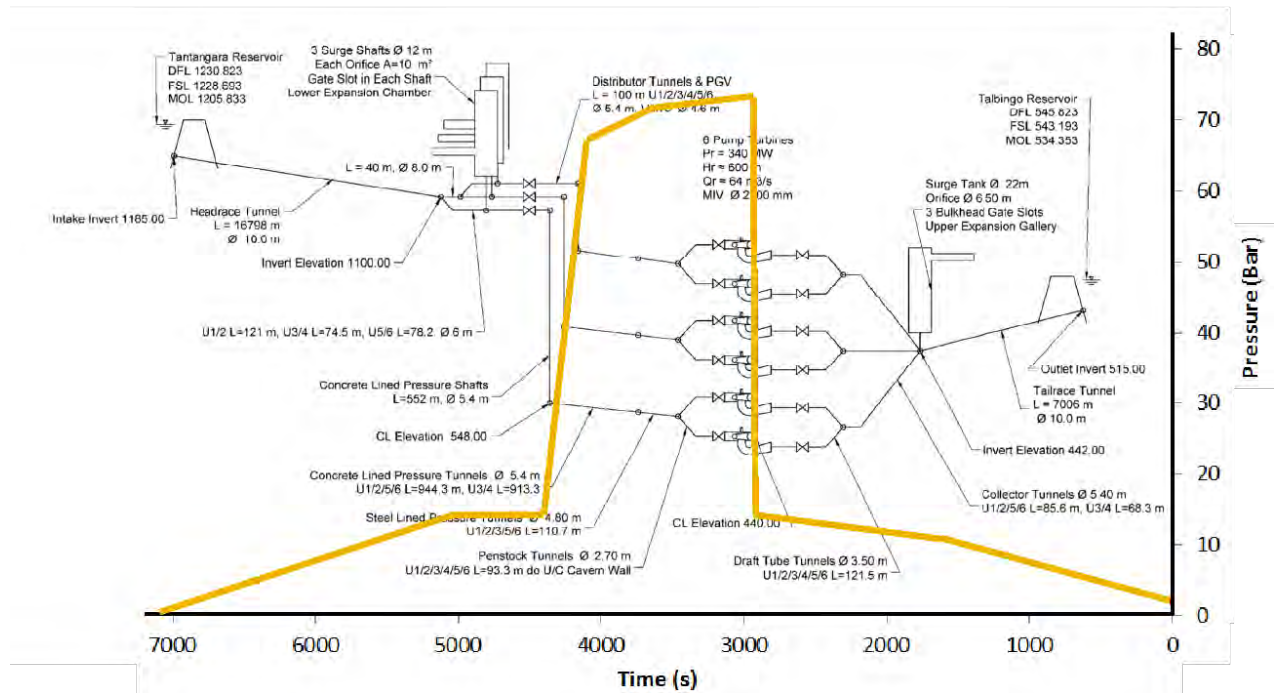
Pressure changes and travel time were simulated for both scenarios considering the water level assuming Talbingo at Full Supply Level (FSL) and Tantangara at Minimum Operating Level

(MOL). For this condition of water level in the reservoirs, fish would be exposed to slightly lower pressure changes along the system when compared to the opposite scenario where Tantangara is at FSL and Talbingo is at MOL (Table 8). Therefore, the tested scenario would be considered the least harmful for fish in regards to pressure exposure, and survival likelihood would be expected to be higher than for other scenarios. Nonetheless, a constantly full supply level at Tantangara and a minimum supply level at Talbingo would be a rare scenario. It is more likely that the levels of each reservoir would be fluctuating in-between. Although the profile tested here was the least harsh (with respect to peak pressures) of all scenarios, it was necessary to test this scenario to provide a conservative assessment of the risk of redfin and/or gambusia being successfully transferred from Talbingo Reservoir to Tantangara Reservoir and beyond. If 100% mortality was experienced at the lowest end of severity, there would be no need to test more severe scenarios.

Species and life stage fish pressure chamber experiments

Pressure experiments were undertaken on five life stages of redfin (egg, larvae 12 - 18 DPH, larvae 28 - 30 DPH, juvenile and adult) and adult gambusia (see Table 9 for the number of replicates for each life stage). Due to constraints in the availability of fish eggs and 28 - 30 DPH larvae, these were not tested at three turbines (T₃) scenario. For each pressure exposure treatment a similar number of control replicates were performed where fish were placed in the hyperbaric chamber for a similar 'passage' time, but were kept at atmospheric pressure.

(a)



(b)

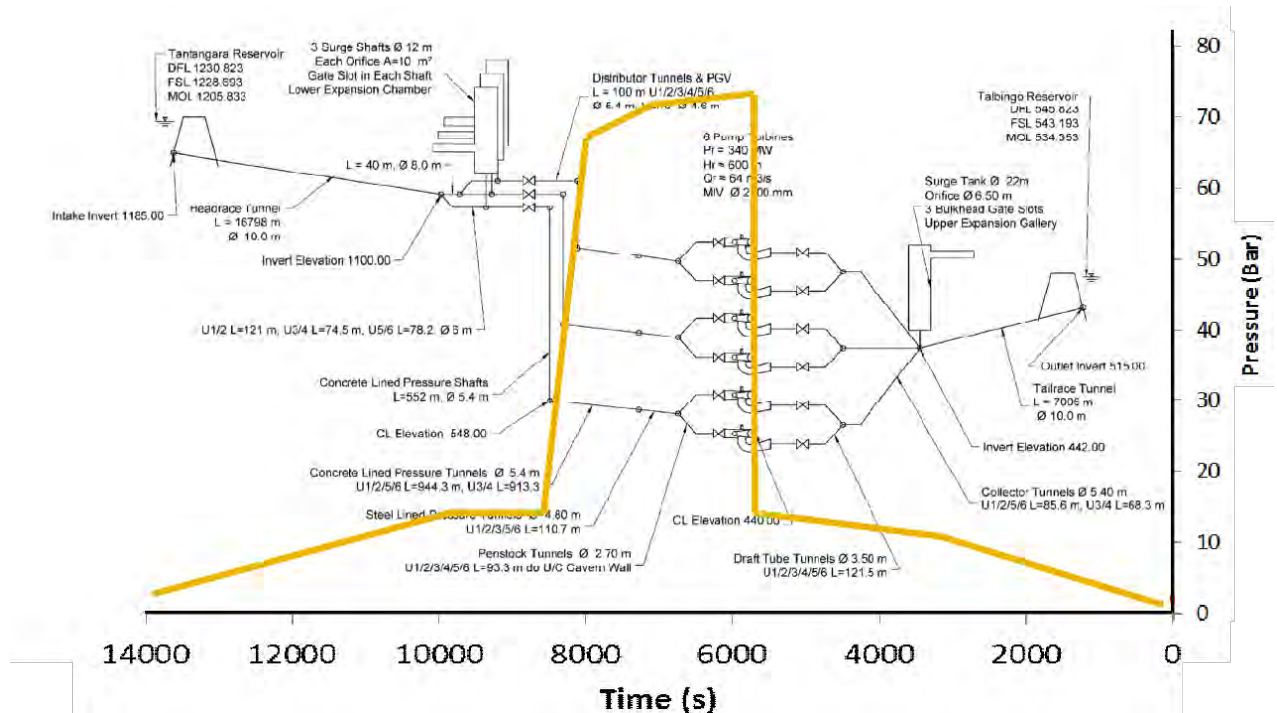


Figure 19. (a) T₆ (2 hour) and (b) T₃ (3.8 hour) pressure profiles applied during the experiments. The profiles have been overlayed onto the schematic of the Snowy 2.0 facility to provide an indication of the proposed pressure change at each of the operating locations.

Table 7. Summary table of pressure changes and travel times expected to occur across the Snowy 2.0 scheme for two different operating scenarios for Talbingo reservoir operating at full supply level (FSL) and Tantangara at minimum operating level (MOL).

Location in the Snowy 2.0 scheme	Full pumping mode (all turbines operating – maximum flow; T ₆)			Intermediate pumping mode (three turbines operating; T ₃)		
	Gauge Pressure (Bar)	Duration (s)	Elapsed time (s)	Gauge Pressure (Bar)	Duration (s)	Elapsed time (s)
Intake (I)	2	1	1	2	1	1
Intake to ST (ST)	10	1,951	1,952	10	3,744	3,745
ST to Bifurcate (B1)	11	21	1,973	11	40	3,785
Bifurcate to Draft tube (DT)	11	24	1,997	11	24	3,809
Draft tube to Turbine (T)	76	0.019	1,997	75	0.019	3,809
Turbine to MIV (MIV)	76	1	1,998	74	12	3,821
MIV to Bifurcate (B2)	75	11	2,009	74	40	3,861
Bifurcate to Reducer (R)	75	21	2,030	65	441	4,302
Reducer to Lower Bend (LB)	66	230	2,260	10	258	4,560
Lower bend to Upper bend (UB). UB to ST and to bifurcate	11	194	2,454	10	117	4,677
3 (B3)						
Bifurcate to Outlet (O)	1	4,678	7,132	1	8,975	13,652

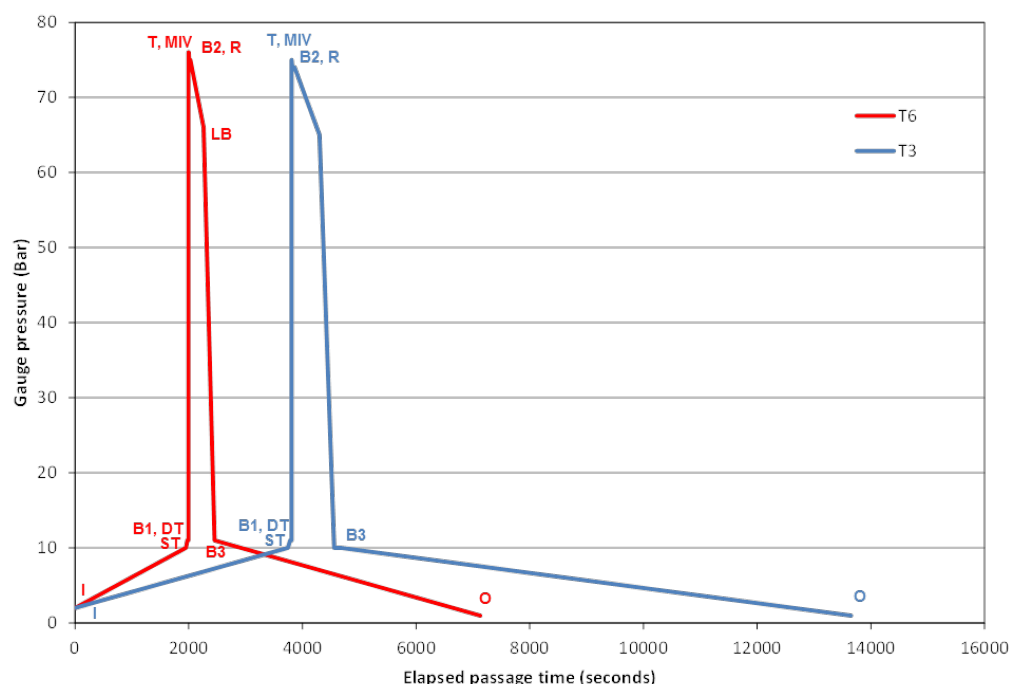


Figure 20. The two pressure profiles simulated in the hyperbaric chamber: T₆ (full pumping capacity – all six turbines) and T₃ (intermediate pumping capacity – three turbines). See Table 9 for an explanation of location labels and full description of pressures and transient times for both scenarios.

Table 8. A comparison of the expected pressure changes in the most severe scenario with Tantangara at FSL and Talbingo at MOL (scenario B) and that currently tested (scenario A). Pressure changes occurring in scenario B would be 1 Bar lower in the section from Talbingo Reservoir to turbine draft tube, 2 Bar higher from the draft tube to the upper bifurcate and 3 Bar higher from the upper bifurcate to Tantangara Reservoir.

Pressure on each location (BAR)		
Scenario A – Tantangara at MOL and Talbingo at FSL	Scenario B – Tantangara at FSL and Talbingo at MOL	Location in the Snowy 2.0 scheme
2	1	Intake
10	9	Intake to Surge Tank (ST)
11	9	ST to Bifurcate
11	10	Bifurcate to Draft tube
76	78	Draft tube to Turbine
76	78	Turbine to Main Inlet Valve (MIV)
75	77	MIV to Bifurcate
75	77	Bifurcate to Reducer
66	68	Reducer to Lower bend
11	13	Lower bend to Upper bend
11	13	Upper bend to ST and to bifurcate
1	4	Bifurcate to Tantangara

Redfin egg experiments

Five replicate test groups of approximately 20 redfin eggs (mean 25.9 eggs \pm 4.7 SD, range 20 – 35 eggs) were exposed to the T₆ scenario, and the same number of replicate test groups of eggs (n=5) were treated as controls. Egg ribbon fragments were counted using the same approach used for the shear experiment. Once counted, each egg fragment was transferred to the FPC capsule (two capsules per treatment) containing water from the same source. Eggs were inserted into the capsule, loaded into the pressure chamber and exposed to the defined pressure profile. At the end of the pressure exposure, both egg ribbon fragments were collected from the two FPC capsules, instantaneously assessed for survival, placed into aerated 700-mL plastic jars for each replicate, and held for 24 hours to reassess their survival (Figure 21). This 24 hour viability assessment period ensured that all eggs were given an adequate duration to hatch, since viable redfin eggs are known to hatch within 14 days of being produced/fertilised at 13 °C (Toner and Rougeot 2008), and the eggs in this experiment had been produced approximately 12 days prior to experimentation. Following exposure to the pressure event, the eggs were collected from the fish retrieval net and placed into aerated 700-mL plastic jars for each replicate, and held for 24 hours to assess survival following the same procedure as the shear experiments.

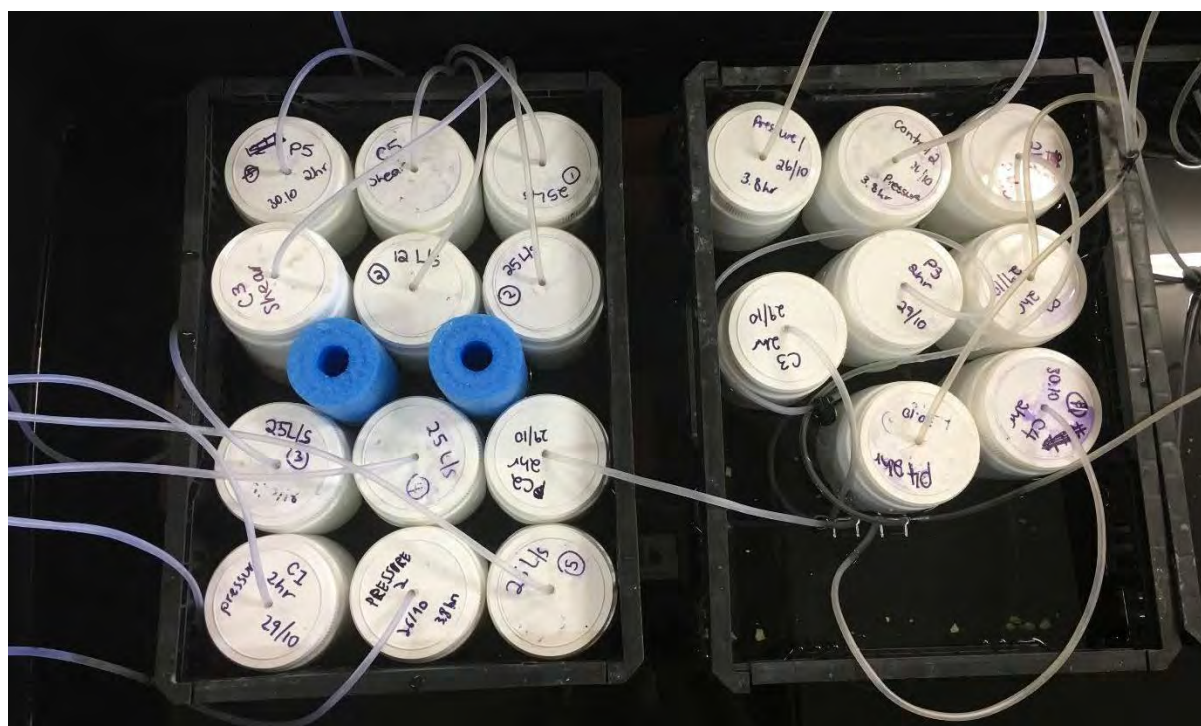


Figure 21. Photo of aerated 700-mL plastic jars used to hold egg and larval life stages of redfin for each experimental replicate.

Redfin larvae experiments

Larval redbfin were tested at two age classes including those between 12–18 and 28–30 days old.

Larvae 12 - 18 DPH

Five replicate test groups of 15 larval redfin (mean length 5.8mm (TL) \pm 0.6 SD, ranging from 4.1 – 7.3 mm, Figure 22) per capsule (total 30 per replicate) were subjected to the same protocols used for the egg pressure profile experiments under the T₆ scenario. Moreover, three replicate tests groups of 15 larval redfin (mean length 7.0 mm (TL) \pm 1.1 SD, ranging from 4.5 – 9.5 mm) per capsule (total 30 per treatment) were subjected to the same protocols used for the egg pressure profile experiments under the T₆ scenario, although using a different pressure profile programmed for the T₃ scenario. The same number of replicate test groups of 12 - 18 DPH were treated as controls for each pressure profile.

Larvae were pipetted from their glass aquaria to the FPC capsules in equal numbers (i.e. 15 larvae per capsule), and the pressure profiles were applied. At the end of the pressure exposure, the test group of larvae were transferred from both FPC capsules into aerated 700-mL plastic jars for 5 days to assess for survival. Larvae were placed individually on a Sedgewick Rafter Counting Cell and measured under a dissecting microscope (Olympus SZ2 -ILST) once they had died (checked immediately following the experiment and again at 24 hours and 5 days after exposure) or following the completion of the experiment to avoid handling during the 5 day holding period.

Some larvae were smaller than the capsule mesh size and during experiments escaped throughout the pressure chamber. A proportion of survival of the larvae that remained in the capsules was used in this instance for further calculation of survival probability for each replicate.



Figure 22. Photo of larval redfin aged between 12 - 18 DPH.

Larvae 28 - 30 DPH

Five replicate test groups of 5 larval redfin (mean length 9.7 mm (FL) \pm 1.1 SD, range 7 – 13 mm) per FPC capsule (total 10 per replicate, Table 9) were subjected to the same protocols used for the egg pressure profile experiments under the T_6 scenario. Larvae were captured from their glass aquaria using an aquarium net (100mm wide) and transferred to the FPC capsules. Equal numbers of test and control groups were exposed to each pressure profile. At the end of the pressure exposure, the test group were transferred from both capsules into aerated 700-mL plastic jars and held for 5 days to assess survival. Larvae were placed individually on a Sedgewick Rafter Counting Cell and measured under a dissecting microscope (Olympus SZ2 - ILST) once they had died (checked immediately following the experiment and again at 24 hours and 5 days) or following the completion of the experiment to avoid handling during the 5 day holding period.

Redfin juvenile and adult experiments

Juvenile and adult redfin were each subjected to the same protocols used for the egg and larvae experiments. Five replicate test groups of three juveniles (mean length 114.1 mm (FL) \pm 6.5 SD, range 96 – 131 mm) per capsule (6 in total per replicate, Figure 23) were assessed for the T_6 scenario and three replicate tests groups of three juveniles (mean length 113.1 mm (FL) \pm 7.3 SD, range 100 – 135 mm) per capsule (6 in total per replicate) were assessed for the T_3 scenario (Table 9). The same number of replicate test groups of juveniles were treated as controls for each pressure profile.

Five replicate test groups of a single adult redfin (mean length 189.5 mm (FL) \pm 19.1 SD, range 164 – 221 mm) per capsule (2 in total per replicate) were assessed for the T_6 scenario and three replicate tests groups of a single adult redfin (mean length 189.5 mm (FL) \pm 13.1 SD, range 164 – 209 mm) per capsule (2 in total per replicate) were assessed for the T_3 scenario (Table 9). The same number of replicate test groups of adults were treated as controls for each pressure profile.

After pressure exposure, juvenile and adult redfin were collected from the capsules and placed into holding tanks that contained baskets for each experimental replicate (Figure 24). The number of surviving juveniles or adults in each test group was assessed immediately following each pressure trial and then again after 24 hours and 5 days, and total survival rate was established as a proportion of individuals surviving for each test group replicate. All fish still alive after 5 days were euthanised in 100 mg L⁻¹ benzocaine.



Figure 23. Photo of juvenile redfin in the Fish Pressure Chamber capsule ready to be added to the pressure chamber.



Figure 24. Photo of holding basket for juvenile and adult redfin for each experimental replicate.

Gambusia experiments

Gambusia were subjected to the same protocols used for the redfin experiments. Five replicate test groups of ten adult gambusia (mean length 30.5 mm (TL) \pm 6.0 SD, range 20 – 47 mm) per capsule (20 per replicate) were exposed to the T₆ scenario. The same number of replicate test groups of adult gambusia were treated as controls for each pressure profile. Gambusia were collected from the FPC capsules and placed into a holding tank that contained baskets based on each experimental replicate (Figure 27).

The number of surviving gambusia in each test group was assessed immediately following each pressure trial and then again after 24 hours and 5 days, and total survival rate was established as a proportion of individuals surviving for each test group replicate. All fish still alive after 5 days were euthanised in 100 mg L⁻¹ benzocaine.



Figure 25. Holding baskets for gambusia for placement of experimental fish after pressure exposure.

Data analysis

Survival of larvae, juvenile and adult fish was quantified instantaneously (i.e. immediately after retrieval from the capsules), and at 24 hours and 5 days after pressure exposure for all species and life stages tested. Egg survival was only quantified for instantaneous and 24 hours post-exposure by identifying eggs that had hatched or where the embryo was clearly seen to be alive and wriggling within the egg.

Results were expressed as survival percentages, and means were plotted for each species and life stage for both the pressure treatments (T₆ and T₃) and their associated control for instant survival, 24 hours and 5 days. The mean percentage of fish surviving was compared between a treatment and its paired control using a two-tailed independent T-test, with the significance level set at 0.05. All T-tests were performed in the package, Real Statistics for Excel.

Table 9. Summary table of fish species and life stages examined during the pressure experiments. No. eggs refers to the average number of eggs per replicate. T₆ refers to 6 turbine/pump operation; T₃ to 3 turbine/pump operation.

Life stage	Treatment [#]	Mean Fish Size (FL, mm)	Size range (mm)	No. of Replicates	No. of Control	No. fish per replicate
<u>Redfin</u>						
Egg	T ₆	25.9 ± 4.7* (No. eggs)	20 – 35* (No. eggs)	5	5	Max 30
Larvae 12 - 18 DPH	T ₆	5.8 ± 0.6 [^] (TL)	4.1 – 7.3	5	5	30
	T ₃	7.0 ± 1.1 [^] (TL)	4.5 – 9.5	3	3	30
Larvae 28 - 30 DPH	T ₆	9.7 ± 1.1	7 – 13	5	5	10
Juvenile	T ₆	114.1 ± 6.5	96 – 131	5	5	6
	T ₃	113.1 ± 7.3	100 – 135	3	4	6
Adult	T ₆	189.5 ± 19.1	164 – 221	5	5	2
	T ₃	189.5 ± 13.1	164 – 209	3	3	2
<u>Gambusia</u>						
Adult	T ₆	30.5 ± 6.0 [^] (TL)	20 – 47	5	5	20

*Each egg fragment was cut from the egg ribbon and consisted of between 20 – 35 eggs. [#]Treatment consists of either T₆ (full pumping capacity - 2 hour pressure profile) or T₃ (half pumping capacity - 3.8 hour pressure profile). [^]Gambusia and 12 – 18 DPH larvae were measured as total length (TL).

Results

Shear results (survivorship comparisons between experimental groups)

Redfin eggs

Mean survival of eggs immediately after being exposed to shear strain rates between 1683 1/s and 6177 1/s ranged from 28 % to 0 %, respectively (Figure 26). Egg survival significantly decreased at higher shear strain rates (> 5623 1/s). Comparisons among treatments showed a significant difference in survival (Kruskal-Wallis test; $H = 18.9$, $n = 5$, $P < 0.01$), with the two highest shear strain rates (5623 1/s and 6177 1/s) significantly more harmful to fish than the controls (Table 10). No eggs were recovered alive after exposure to these two shear strain rates.

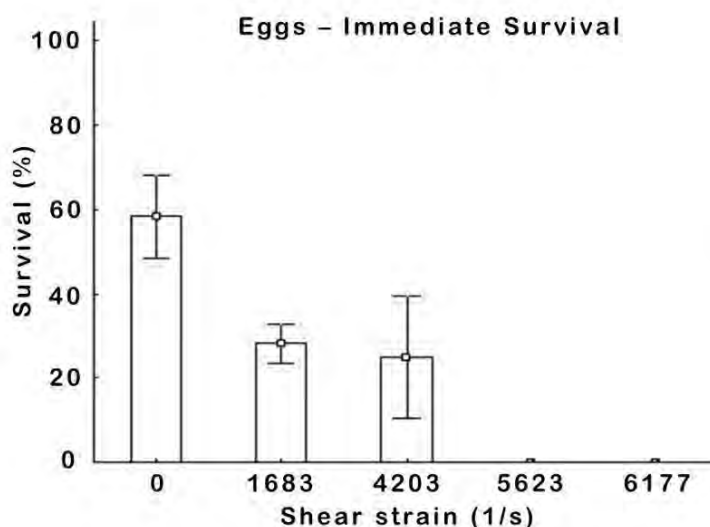


Figure 26. Mean (\pm SE) survival (%) for redfin eggs within the first 24 hours after exposure to shear strain rates ranging from 0 (1/s; control) to 6177 (1/s).

Redfin larvae

Mean instantaneous survival of redfin larvae 12- 18 DPH ranged from 78 % to 24 % when exposed to 1010 1/s to 3706 1/s, respectively (Figure 27). Larval survival significantly decreased with increasing shear strain (Kruskal-Wallis test; $H = 21.6$, $n = 5$, $P < 0.01$), with the most notable difference between the two highest shear strain rates (3374 1/s ($P < 0.01$) and 3706 1/s ($P < 0.01$); Kruskal-Wallis test) when compared to the controls (Table 10).

After 24 hours post shear exposure, survival rates significantly decreased across all experimental groups with mean survival ranging from 64 % (1010 1/s) to 1 % (3706 1/s) (Kruskal-Wallis test; $H = 21.5$, $n=5$, $P < 0.01$) (Figure 27). Significantly lower survival was identified for the two highest shear strain rates (3374 1/s ($P < 0.05$) and 3706 1/s ($P < 0.01$)) (Table 10). Survival was significantly higher in controls and low shear treatments. Survival rates decreased further after five days, with mean survival ranging from 50 % (1010 1/s) to no survival observed for the two highest shear strain rates (3374 1/s and 3706 1/s) (Figure 27).

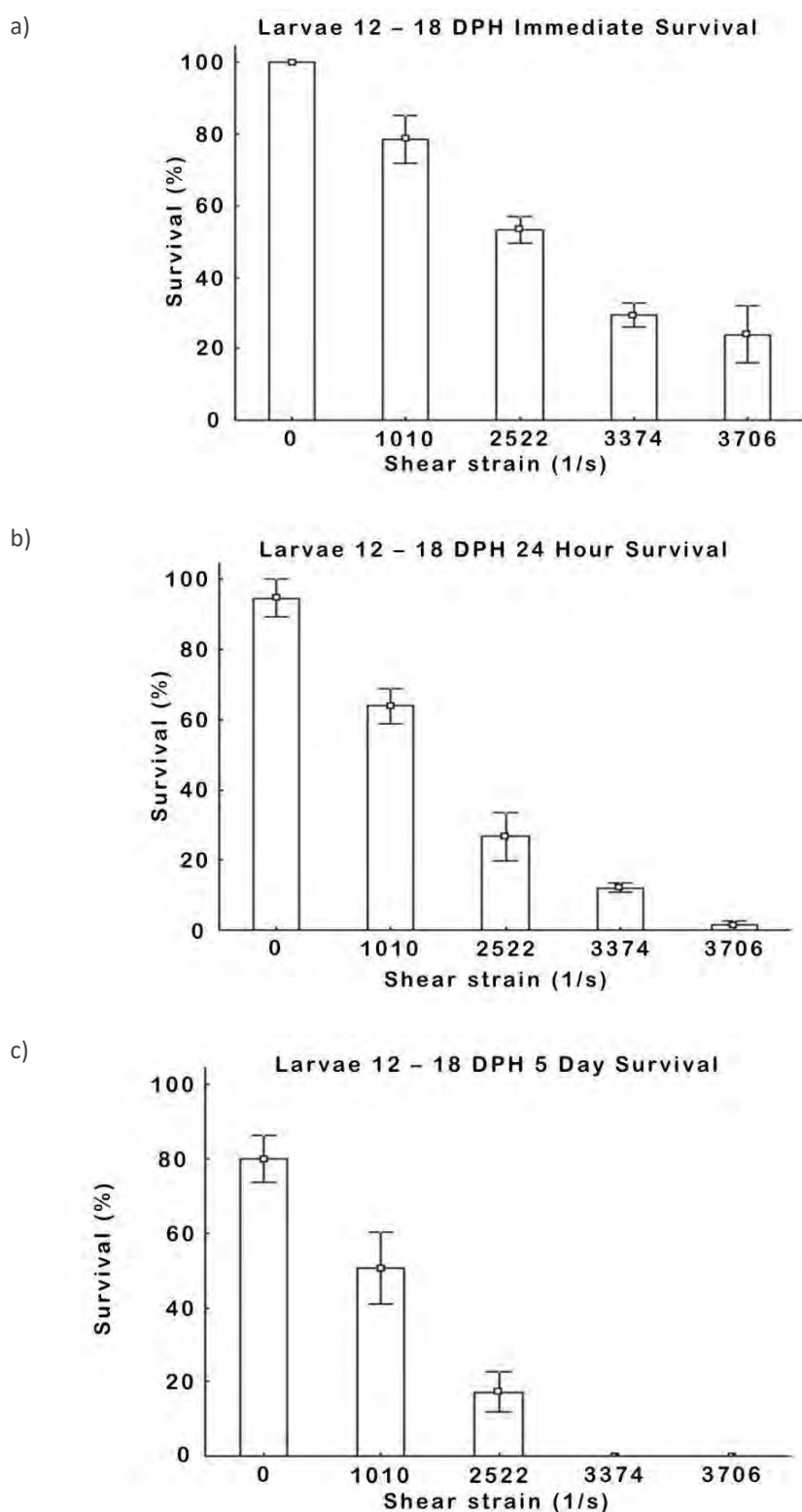


Figure 27. Mean (\pm SE) survival (%) for redfin larvae 12 – 18 DPH a) immediately after, b) 24 hours after and c) 5 days after exposure to different shear strain rates ranging from 0 (1/s; control) to 3706 (1/s).

Instantaneous redfin larvae (28-30 DPH) survival ranged from 80 % to 0 % at 1010 1/s and 3706 1/s respectively (Figure 28). Larval survival significantly decreased with increasing shear strain (Kruskal-Wallis test; $H = 20.0$, $n = 5$, $P < 0.01$) (Table 10, Figure 28). Elevated water temperatures, from an unexpected overnight loss of mains power, contributed to high mortality in the control group following shear experiments so 24 hour or 5 day survival could not be calculated for this life stage.

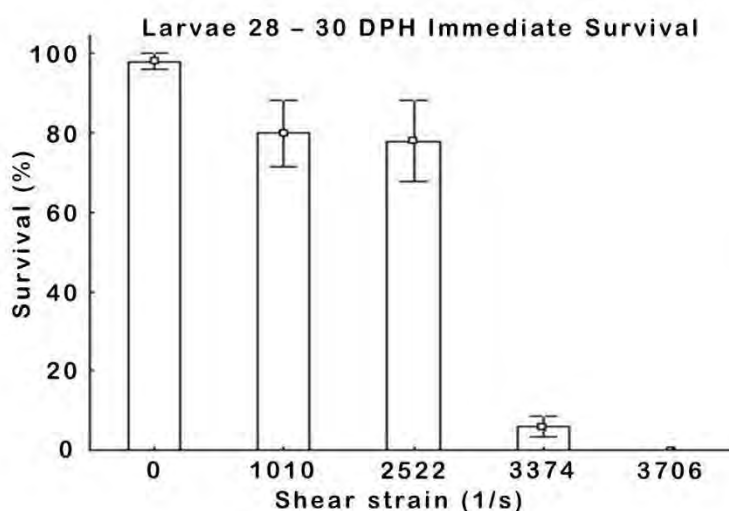


Figure 28. Mean (\pm SE) survival (%) for redfin larvae 28 – 30 DPH immediately after exposure to different shear strain rates ranging from 0 (1/s; control) to 3706 (1/s).

Redfin juveniles

All redfin juveniles survived immediately after exposure to shear strain rates between 505 1/s and 1853 1/s. But survival decreased to 70 % for the highest shear strain rate (1853 1/s) after 24 hours (Figure 29). These patterns did not change for the first 5 days after exposure (Kruskal-Wallis test; $H = 12.51$, $n = 10$, $P < 0.05$) (Table 10).

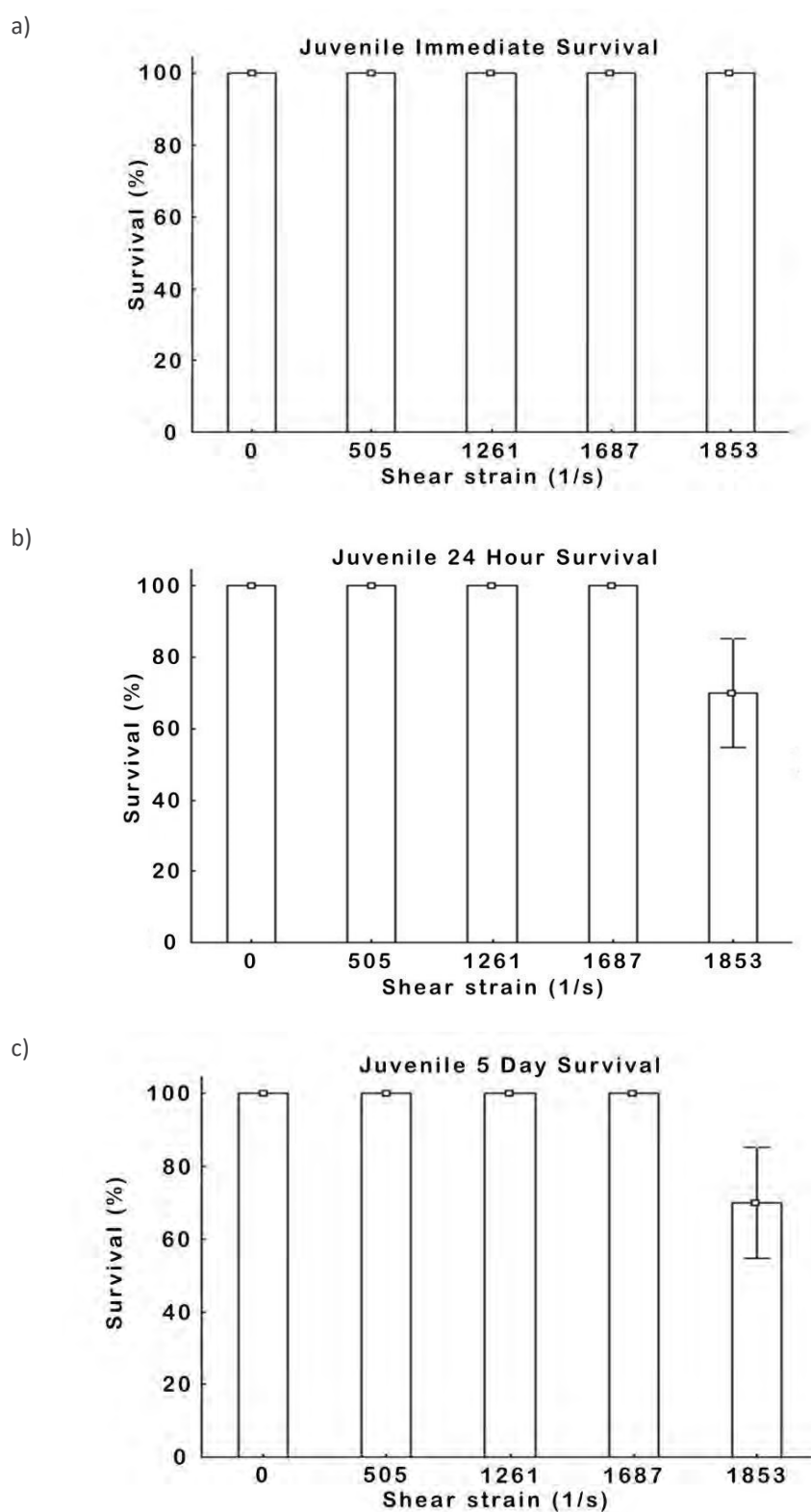


Figure 29. Mean (\pm SE) survival (%) for redfin juveniles a) immediately after, b) 24 hours after and c) 5 days after exposure to different shear strain rates ranging from 0 (1/s; control) to 1853 (1/s).

Gambusia

All gambusia exposed to shear strain rates between 505 1/s and 1853 1/s had 100% survival immediately post experiment (Figure 30). Although there was some mortality in the four treatments 24 hours after exposure to shear strain, there was no significant difference compared to the lower strain rates (Kruskal-Wallis test; $H = 0.00$, $n = 5$, $P > 0.05$) (Table 10). After 5 days however, there was a significant difference in survival (Kruskal-Wallis test; $H = 14.36$, $n = 5$, $P < 0.01$), mainly due to the 1687 1/s treatment group experiencing high mortality. This higher mortality was possibly due to a fungal infection observed in fish within this replicate (Figure 30c).

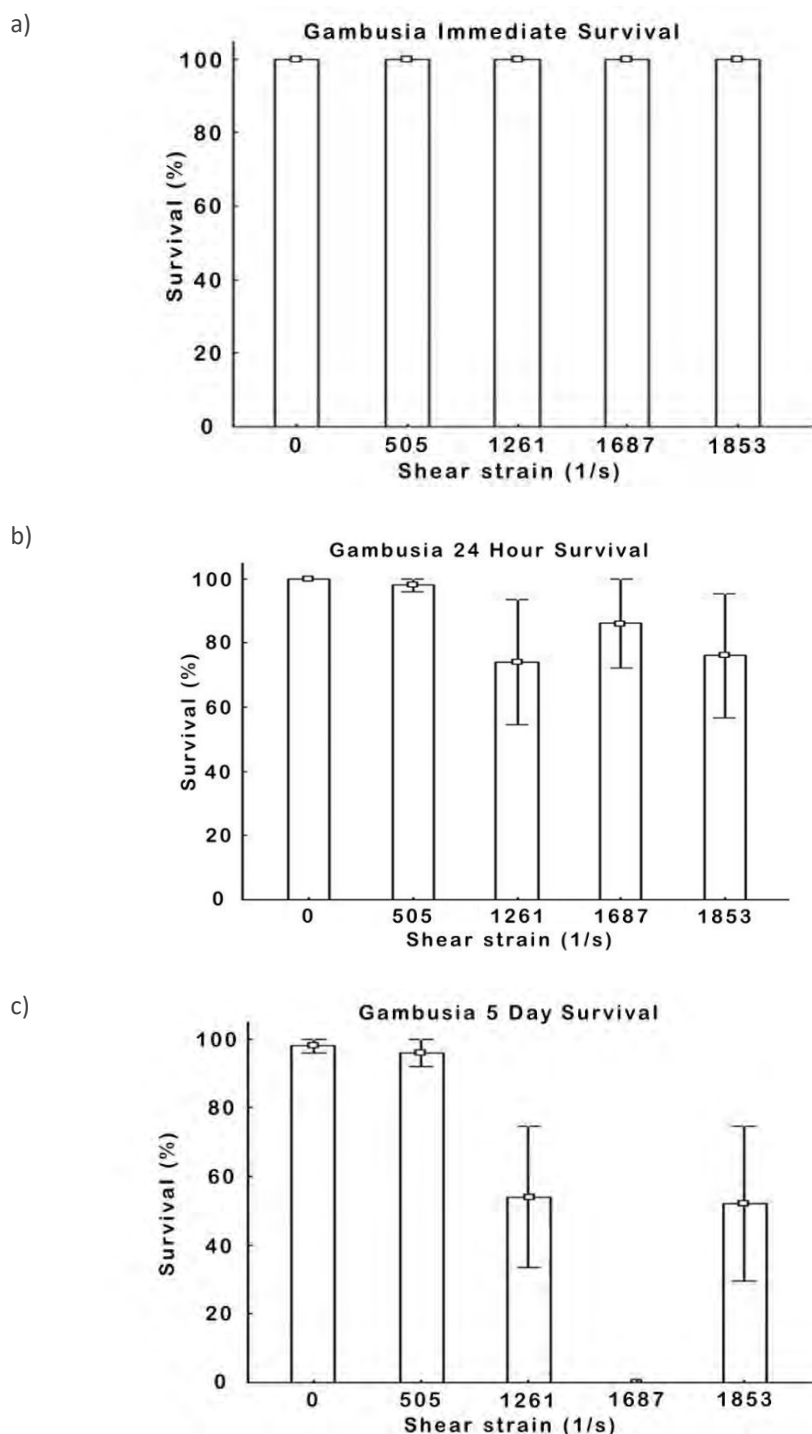


Figure 30. Mean (\pm SE) survival (%) for gambusia a) immediately after, b) 24 hours after and c) 5 days after exposure to different shear strain rates ranging from 0 (1/s; control) to 1853 (1/s).

Table 10. Main Kruskal-Wallis test and pairwise comparison results for redfin and gambusia survival after exposure to different shear strain rates.

Species and life stage	Time post exposure	Main test H statistic	Main test P value	Pairwise treatment comparison tests and P values
Redfin eggs	0-24 h*	18.899	0.008	0-5263 (0.010), 0-6177 (0.010)
	5 d			Not assessed
Redfin 12-18 DPH	0 h	21.596	0.002	0-3374 (0.002), 0-3706 (0.001)
	24 h	21.514	0.003	0-3374 (0.030), 0-3706 (0.000), 1010-3706 (0.018)
	5 d	21.485	0.003	0-3374 (0.004), 0-3706 (0.004)
	0 h	20.004	0.005	0-3374 (0.025), 0-3706 (0.002)
Redfin 28-30 DPH	24 h	11.655	0.02	No pairwise comparisons significant
	5 d	9.186	0.057	Main test not significant
	0 h	0.000	1	Main test not significant
Redfin juveniles	24 h	12.511	0.014	No pairwise comparisons significant
	5 d	12.511	0.014	No pairwise comparisons significant
	0 h			
Redfin adults	24 h			Not assessed
	5 d			
	0 h	0.000	1	Main test not significant
Gambusia adults	24 h	3.267	0.514	Main test not significant
	5 d	14.358	0.006	0-1687 (0.023), 505 - 1687 (0.028)
	0 h			

Blade strike modelling results

Deterministic model predictions

We applied a deterministic model to the Snowy 2.0 Hydro pump-storage turbine (Table 11) to each species and life stage at three given discharges: minimum flow (31.05 m³/s), mid-point flow (44.01 m³/s), and max flow (49.02 m³/s). The corresponding wicket gate opening angles were 13°, 20°, and 21°, respectively. A deterministic model lacks probability, that is, it predicts a single unique estimate for each combination of input values. Since fish orientation relative to the flow direction ranges from 0° to 90°, we used the arithmetic mean 45° as the fish relative orientation to the flow direction and the fish apparent length is calculated as Fish Length*cos(45°). In this deterministic model, blade strike predictions are a function of fish apparent length and radial injection location, runner geometry, runner rotation rate, and axial flow. Without applying a mutilation ratio, the result that predictions of blade strike probability are likely overestimated the fish strike injuries. The blade strike probabilities ranged from 0.2% to 28.9%, with larger fish being more likely to be struck than smaller fish (Table 12). Although the probabilities for redfin eggs and larvae are already small (< 2%), their large surface area to mass ratio may help them to be pulled around the blade rather than colliding with the blade, which may even lower their blade strike probabilities.

Table 11. Snowy 2.0 pumped hydro turbine parameters.

No. of blades	9
No. of wicket gates	20
Wicket gate spacing pitch diameter (m)	4.914
Wicket gate height (m)	0.242
RPM	500
Runner diameter (m)	4.166
Hub diameter (m)	1.83

Table 12. Predictions of blade strike probabilities using the deterministic model.

Species	Treatment	Min Flow			Mid Flow			Max Flow		
		Min Length	Mean Length	Max Length	Min Length	Mean Length	Max Length	Min Length	Mean Length	Max Length
Redfin Eggs	T6	0.23%	0.24%	0.25%	0.25%	0.26%	0.27%	0.23%	0.24%	0.25%
Larvae 12-18 DPH	T6	0.5%	0.7%	0.9%	0.5%	0.8%	1.0%	0.5%	0.7%	0.9%
Larvae 12-18 DPH	T3	0.5%	0.9%	1.2%	0.6%	0.9%	1.2%	0.6%	0.9%	1.2%
Larvae 28 - 30 DPH	T6	0.9%	1.2%	1.6%	0.9%	1.3%	1.7%	0.9%	1.2%	1.6%
Juvenile Redfin	T6	11.7%	13.9%	15.9%	12.5%	14.9%	17.1%	11.7%	14.0%	16.0%
Juvenile Redfin	T3	12.2%	13.8%	16.4%	13.1%	14.8%	17.6%	12.2%	13.8%	16.5%
Adult Redfin	T6	20.0%	23.1%	26.9%	21.4%	24.7%	28.8%	20.1%	23.2%	27.0%
Adult Redfin	T3	20.0%	23.1%	25.4%	21.4%	24.7%	27.3%	20.1%	23.2%	25.6%
Adult Gambusia	T6	2.4%	3.7%	5.7%	2.6%	4.0%	6.1%	2.4%	3.7%	5.7%

Stochastic model predictions

The stochastic version of the model was implemented using @RISK (the Palisade Corporation, Ithaca, New York). The software allows users to define distributions for any or all independent variables so that the variation in variable values is propagated through calculations and is reflected in model predictions. The Monte Carlo simulation method and 10,000 realisations were used for all analyses.

In this analysis, three variables were assigned distributions of possible values in simulations: wicket gate angle, fish size, and fish orientation relative to flow direction. Wicket gate angle was assigned a uniform distribution ranging from the minimum flow angle (13°) to the maximum flow angle (21°). The discharge was linear interpolated using given flow information (Figure 31): when the wicket gate opening is in-between 13° and 20°, the corresponding discharge was linear interpolated using the minimum flow discharge 31.05 m³/s and mid-point flow 44.01 m³/s; when the wicket gate opening is in-between 20° and 21°, the corresponding discharge was linear interpolated using the mid-point flow discharge 31.05 m³/s and maximum flow 44.01 m³/s. Fish size was assigned a normal distribution. Fish orientation relative to flow direction was assigned a uniform distribution from 0° to 90°.

In addition, sensitivity and scenario analysis reports were performed using the @RISK software, which identifies the input distributions most critical to the predicted results. The higher the regression coefficient between the input the output, the more significant the input is in determining the value of the output. Except for fish eggs, the probability of blade strike depended most significantly on fish length (Figure 32–35).

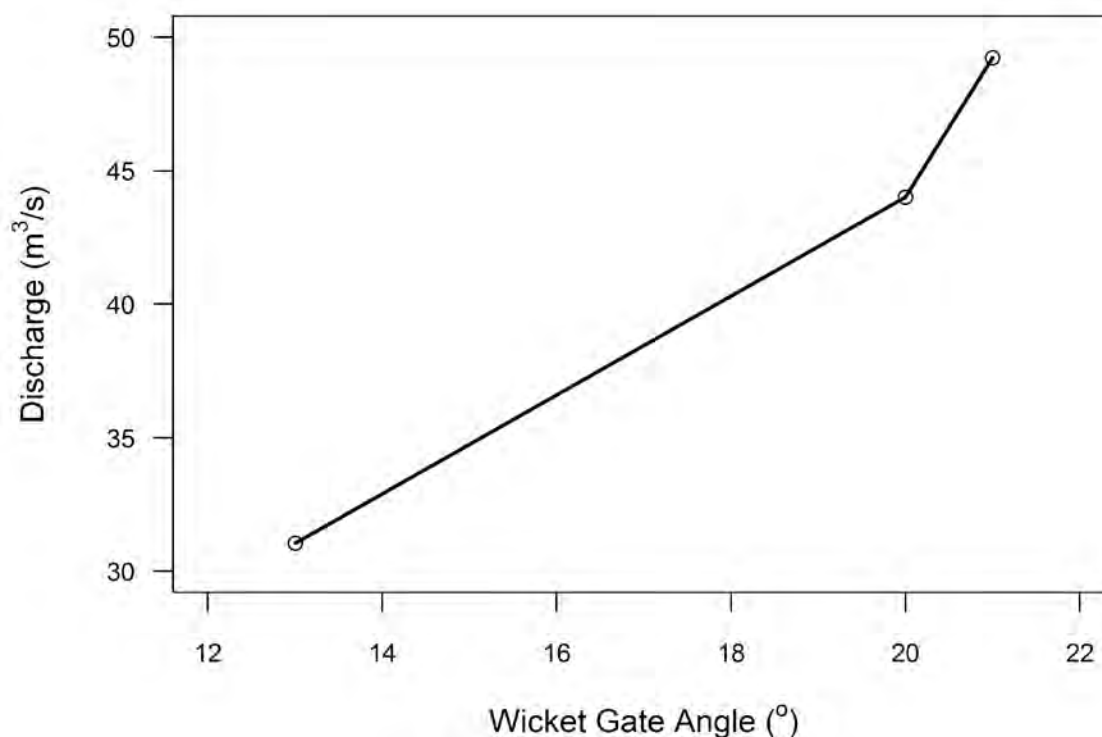


Figure 31. Relationship between Snowy 2.0 Wicket Gate Angle and Discharge.

Redfin eggs

Redfin eggs ranged in diameter from 1.34–1.44 mm, with a mean of 1.4 mm and standard deviation of 0.032 mm (Figure 32). The probability of blade strike for redfin eggs ranged from 0.23% to 0.27%, with a mean value at 0.25% (Table 12). The standardised regression coefficients for egg size and for wicket gate opening angle were 0.66 and 0.52 (Figure 32), respectively, indicating that both egg size and wicket gate angle contributed significantly to the probability of blade strike.

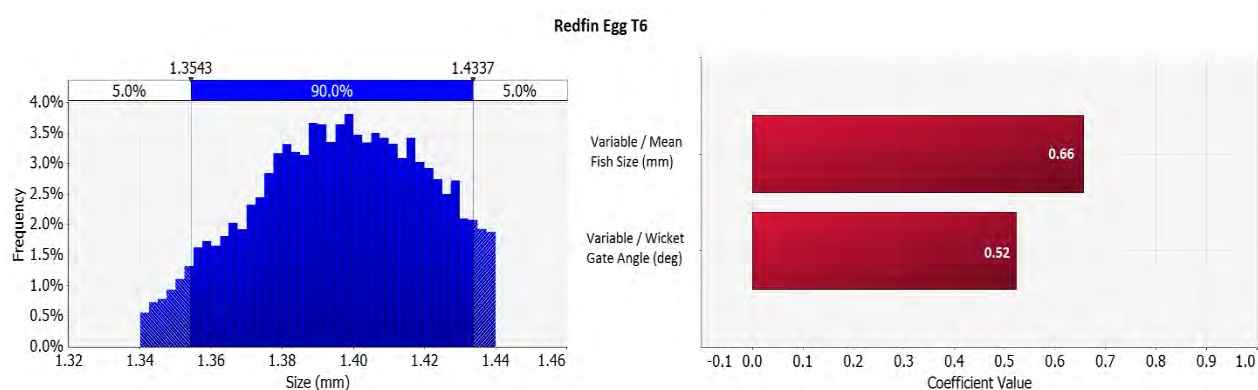


Figure 32. Input Distributions for Redfin Egg Size (Left) and Standardised Regression Coefficients (Right) indicating the Sensitivity of Blade strike Predictions to Size and Wicket Gate Opening Angle.

Redfin larvae

Redfin 12-18 DPH larvae subjected to the T6 experiments ranged in TL size from 4.1–7.3 mm (mean \pm SD 5.8 ± 0.6 mm), while redfin 12-18 DPH larvae subjected to the T3 experiments ranged in TL size from 4.5–9.5 mm (mean \pm SD 7.0 ± 1.1 mm) (Figure 33). Redfin 28-30 DPH larvae subjected to the T6 experiments ranged in TL size from 7–13 mm (mean \pm SD 9.7 ± 1.1 mm). The probability of blade strike for redfin larvae ranged from 0.5% to 1.7%, with mean values at 0.8%, 0.9%, and 1.3% for three distributions (Table 12). The standardised regression coefficient for fish size was 1.00 (Figure 33), indicating that fish size contributed more significantly to the probability of blade strike.

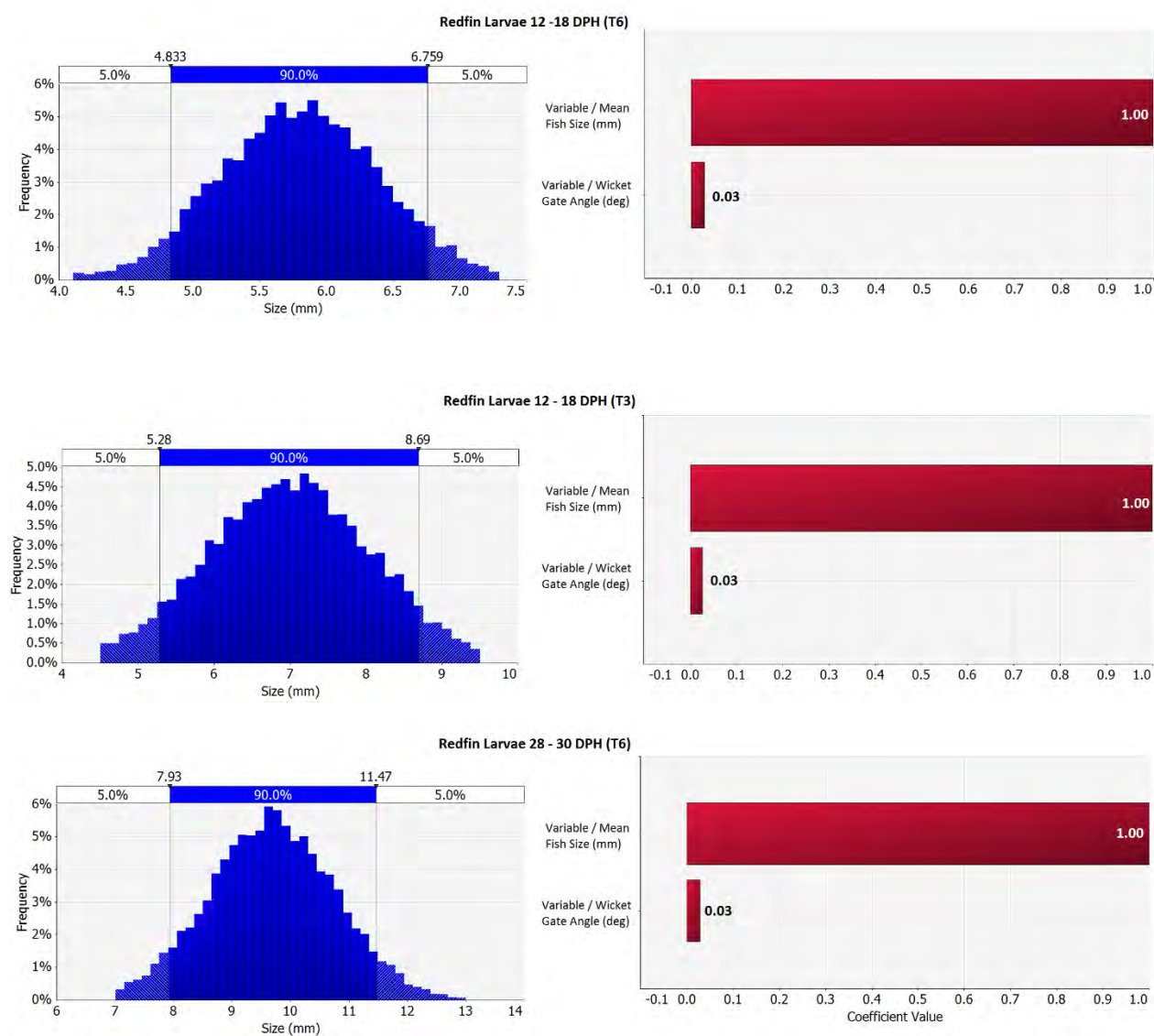


Figure 33. Input Distributions for Redfin Larvae at 12-18 DPH (T_6 and T_3) and 28-30 DPH (T_3) treatments (Left) and Standardised Regression Coefficients (Right) indicating the Sensitivity of Blade strike Predictions to Size and Wicket Gate Opening Angle.

Redfin juveniles

Redfin juveniles subjected to the T6 experiments ranged in FL size from 96–131 mm (mean \pm SD 114.1 \pm 6.5 mm), while redfin juveniles subjected to the T3 experiments ranged in FL size from 100–135 mm (mean \pm SD 113.1 \pm 7.3 mm) (Figure 34). The probability of blade strike for redfin juveniles ranged from 11.7% to 17.6%, with the mean value at about 15% for both distributions (Table 12). The standardised regression coefficients for fish size was 1.00 (Figure 34), respectively, indicating that fish size contributed more significantly to the probability of blade strike.

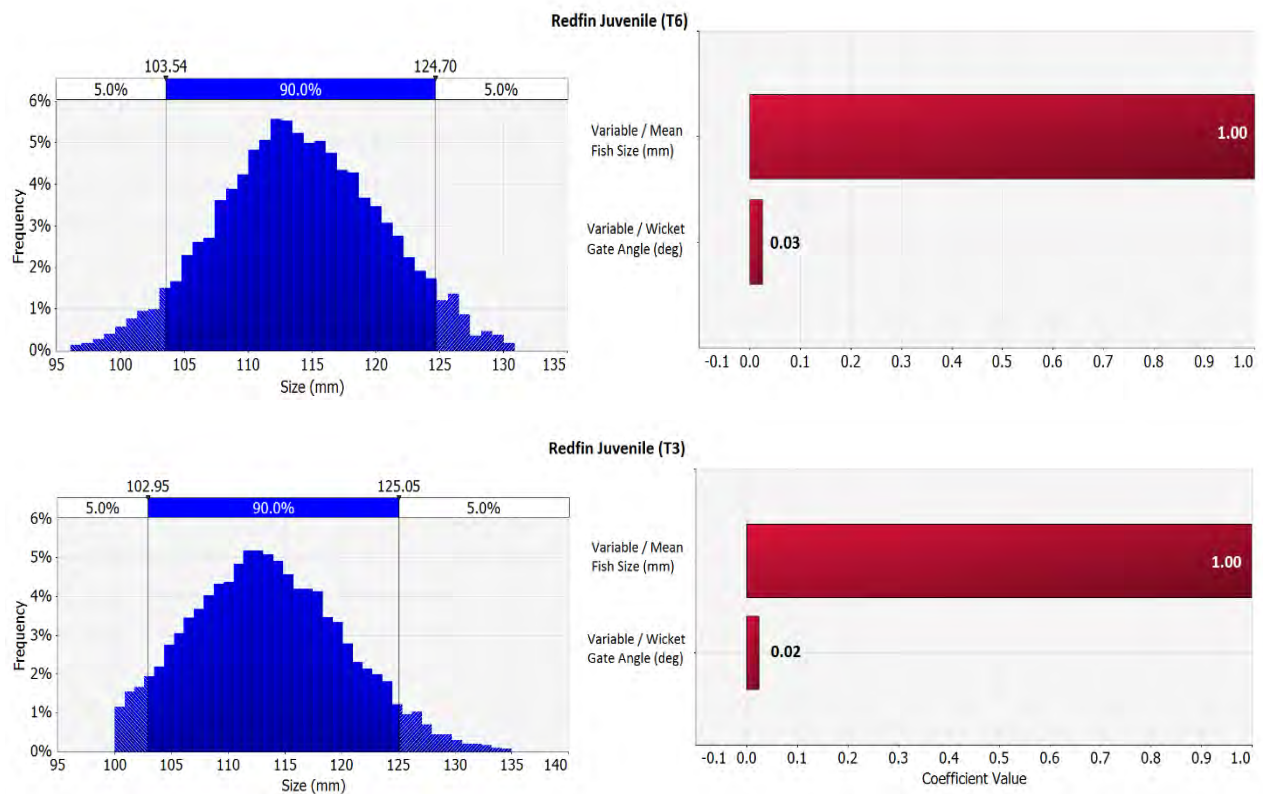


Figure 34. Input Distributions for Redfin Juveniles (T_6 and T_3) at two treatments (Left) and Standardised Regression Coefficients (Right) indicating the Sensitivity of Blade strike Predictions to Size and Wicket Gate Opening Angle.

Redfin adults

Redfin adults subjected to the T6 experiments ranged in FL size from 164–221 mm (mean \pm SD 189.5 ± 19.1 mm), while redfin adults subjected to the T3 experiments ranged in FL size from 164–209 mm (mean \pm SD 189.5 ± 13.1 mm) (Figure 35). The probability of blade strike for redfin adults ranged from 20.0% to 28.8%, with the mean value at about 25% for both distributions (Table 12). The standardised regression coefficients for fish size was 1.00 (Figure 35), indicating that fish size contributed more significantly to the probability of blade strike.

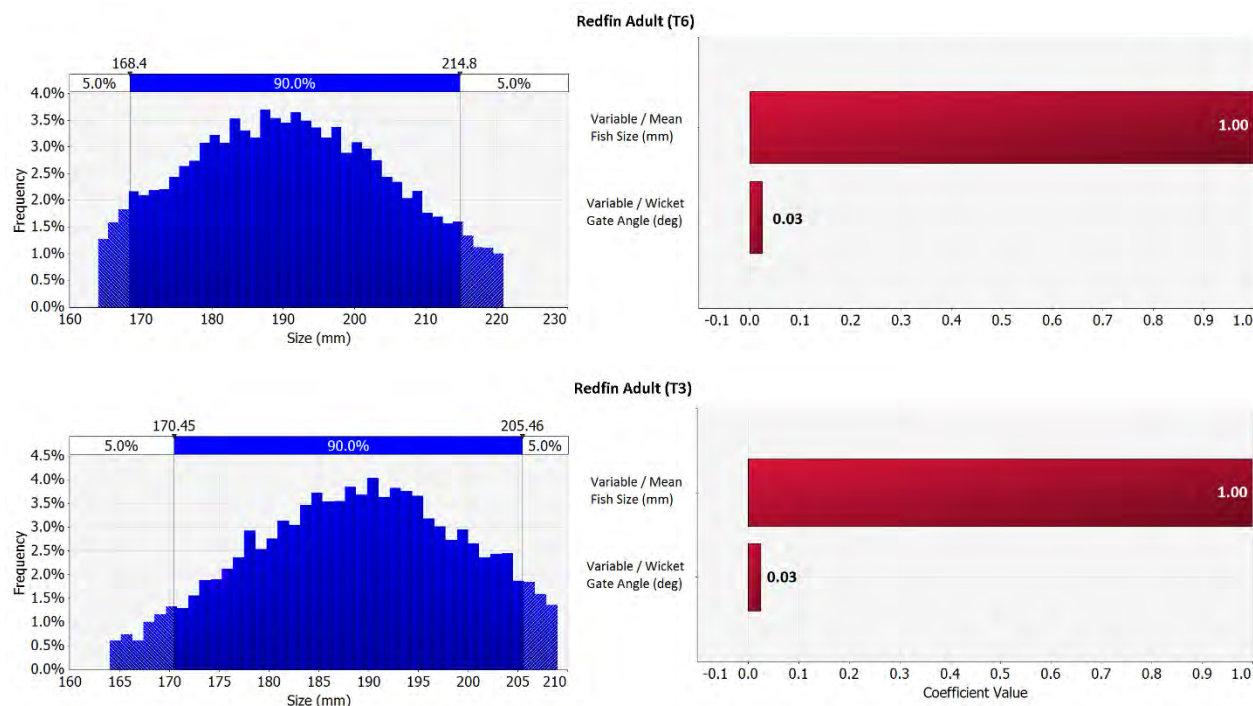


Figure 35. Input Distributions for Redfin Adults (T_6 and T_3) at two treatments (Left) and Standardised Regression Coefficients (Right) indicating the Sensitivity of Blade strike Predictions to Size and Wicket Gate Opening Angle.

Gambusia adults

Gambusia adults subjected to the T6 experiments ranged in TL size from 20–47 mm (mean \pm SD 30.5 ± 6.0 mm) (Figure 36). The probability of blade strike for gambusia adults ranged from 2.4% to 6.1%, with the mean value at 4.0% (Table 12). The standardised regression coefficient for fish size was 1.00 (Figure 36), indicating that fish size contributed more significantly to the probability of blade strike.

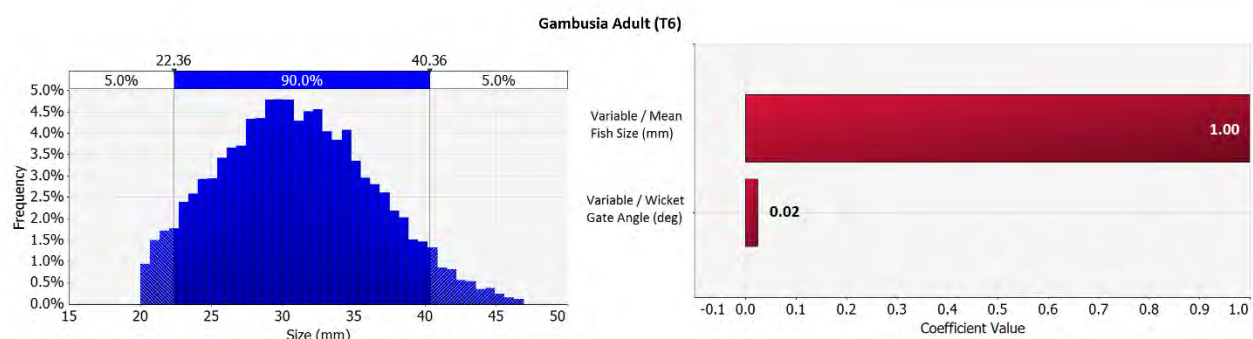


Figure 36. Input Distributions for gambusia Adults (Left) and Standardised Regression Coefficients (Right) indicating the Sensitivity of Blade strike Predictions to Size and Wicket Gate Opening Angle.

Table 13. Summary table of stochastic model results for the probability of blade strike (%) and the associated probability of not being struck (%) for T₆ and T₃ scenarios for redfin and gambusia.

Life stage / species	Pressure treatment	Probability of blade strike (%)	Probability of not being struck by a blade (%)
Adult	T6	29.00	71.00
	T3	27.00	73.00
Juvenile	T6	17.00	83.00
	T3	18.00	82.00
Larvae 28 – 30 DPH	T6	1.70	98.30
Larvae 12 – 18 DPH	T6	1.00	99.00
	T3	1.20	98.80
Egg	T6	0.25	99.75
Gambusia	T6	6.10	93.90

Pressure results for T₆ pressure profile (survivorship comparisons between experimental groups)

Redfin eggs

Instantaneous and 24 hour survival rates for eggs were both 55% (Figure 37). Paired comparisons showed no significant difference (t test, $T = 1.01$, $n = 5$, $P > 0.05$) in the survival of eggs after pressurisation, when compared to the control (Table 14, Figure 37).

Redfin larvae

Results for redfin larvae 12 - 18 DPH exposed to the T₆ pressure profile showed high mean survival rates of 96% and 95% for immediate and 24 hours after exposure, respectively (Figure 37). Generally, larval survival was lower after 5 days when compared to instant and 24 hours, reflecting daily larval die-off in both control and pressurised treatment groups (Figure 37).

For larvae 28-30 DPH mean survival rate was 76% for both instant and 24 hours after exposure (Figure 37). Survivorship decreased slightly after 5 days of exposure with both control and pressure treatments with mean survival of about 64% (Figure 37). There was no significant difference (two-tailed t-test, see Table 14 for P values) in the survival of larvae when pressurised for any of the ages when compared to the controls.

Redfin juveniles

All juvenile redfin (100%) survived pressure up to 5 days after exposure within the T₆ scenario (Table 14; Figure 37).

Redfin adults

All adult redfin (100%) exposed to the T₆ pressure profile survived exposure (Figure 37) exhibiting similar results to juveniles (Table 14).

Gambusia adults

Results for gambusia exposed to the T₆ pressure scenario also showed 100% survival rate up to 5 days after exposure in both the control and pressure treatment (Table 14; Figure 38).

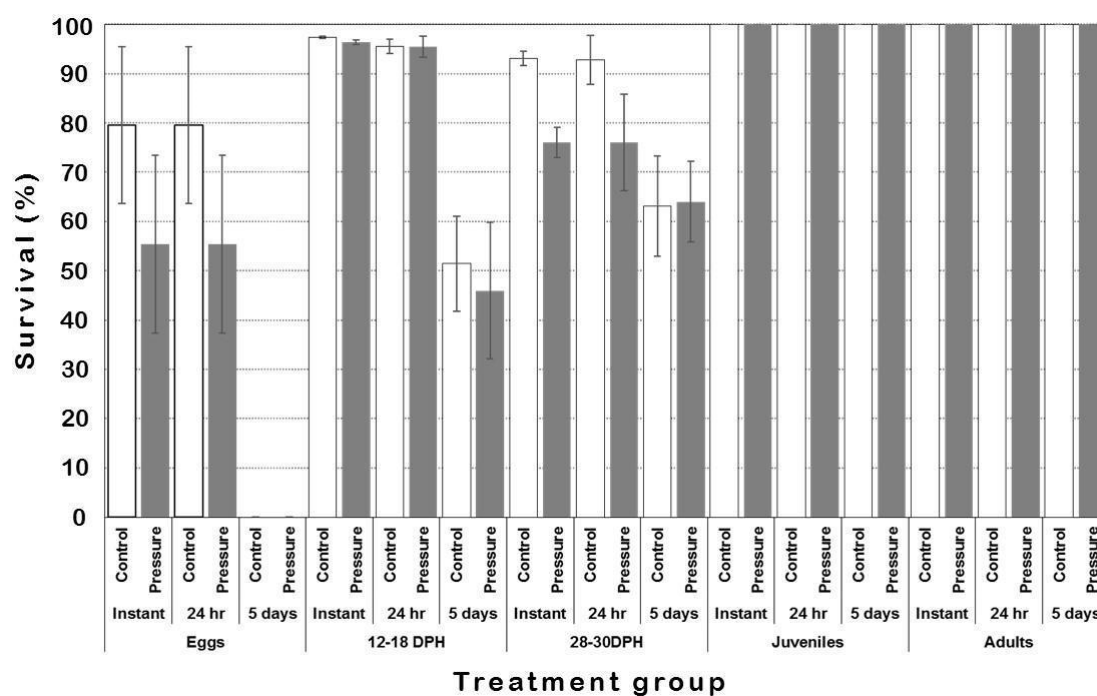


Figure 37. Cumulative mean \pm SE survival of various life stages of redfin instantaneously, 24 hours and 5 days after exposure to the T_6 pressure profile (all turbines operating at full capacity).

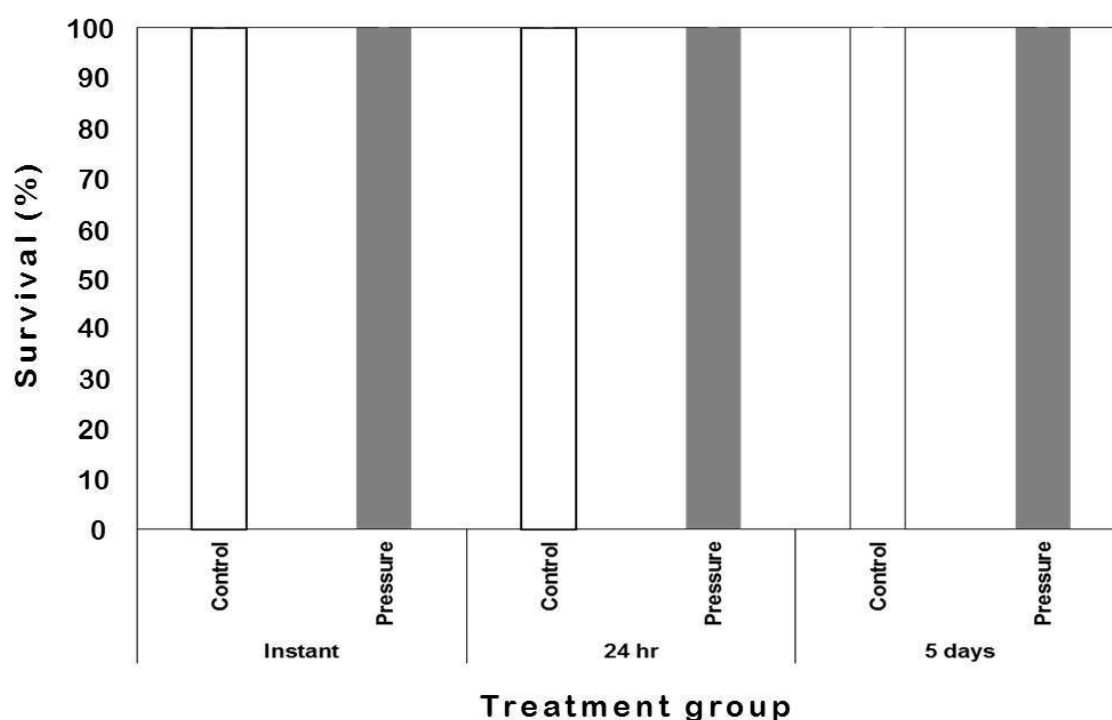


Figure 38. Cumulative mean \pm SE survival of adult gambusia instantaneously, 24 hours and 5 days after exposure to the T_6 pressure profile (all turbines operating at full capacity).

*Pressure results for T₃ pressure profile (survivorship comparisons between experimental groups)*Redfin larvae 12-18 DPH

Only larvae 12-18 DPH were exposed to the T₃ pressure profile. Mean survival was higher for larvae immediately after exposure (85%) and decreased to 70% within 24 hours and 45% within 5 days post exposure (Figure 39).

There was no significant difference in the survival of larvae when pressurised for any of the time after exposure when compared to the controls (two-tailed t-test; see Table 14 for *P* values).

Redfin juveniles

Immediately after exposure to the T₃ pressure profile, all juvenile redfin (100%) survived. Mean survival of juvenile redfin decreased after 24 hours following exposure (61%) and no further juveniles died in the days following (up to 5 days) (Figure 39). There was no significant difference between survival rates for fish exposed to pressure compared to the control (two-tailed t test; *T* = 1.61, *n* = 3, *P* > 0.05 for both 24 h and 5 day, Table 14).

Redfin adults

For adults exposed to the T₃ pressure profile mean survival rate was less (50%) than its paired control (83%) immediately after exposure (Figure 39). These survival rates remained the same for the first 5 days post exposure (Table 14; Figure 39). There were no significant pressure effects immediately after exposure, 24 h after exposure, or 5 days after exposure (for each two-tailed t test; *T* = 1.00, *n* = 3, *P* > 0.05).

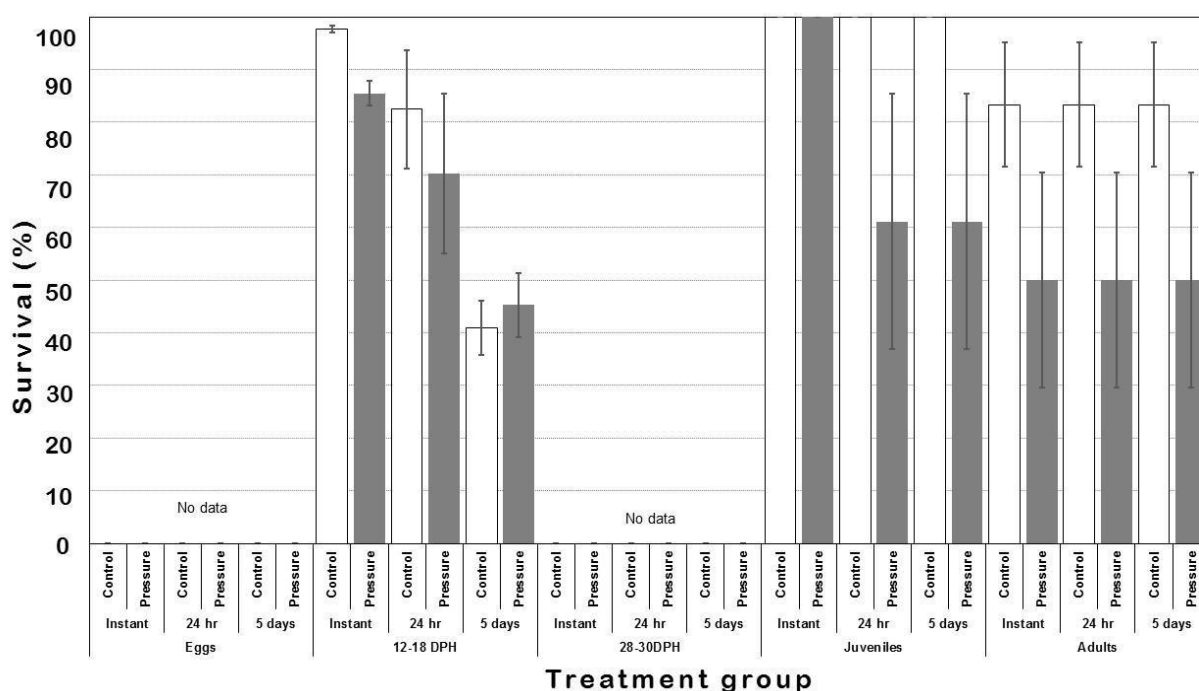


Figure 39. Cumulative mean \pm SE survival of various life stages of redfin instantaneously, 24 hours and 5 days after exposure to the T₃ pressure profile (half turbines operating at full capacity).

Table 14. T-test statistics and P values for redfin and gambusia survival analyses after exposure to each pressure profile scenario.

Species and life stage	Survival estimate	T ₆ T-stat	T ₆ P value	T ₃ T-stat	T ₃ P value
Redfin eggs	0 h	*Combined with the 24 h survival estimate		Fish not available for testing	
	24 h	1.005	0.344		
	5 d	Not assessed			
Redfin 12-18 DPH	0 h	0.146	0.889	1.000	0.423
	24 h	0.035	0.973	0.642	0.556
	5 d	0.324	0.754	0.494	0.655
Redfin 28-30 DPH	0 h	1.414	0.207	Fish not available for testing	
	24 h	1.529	0.165		
	5 d	0.064	0.951		
Redfin juveniles	0 h	All fish survived		All fish survived	
	24 h			1.606	0.184
	5 d			1.606	0.184
Redfin adults	0 h	All fish survived		1.000	0.374
	24 h			1.000	0.374
	5 d			1.000	0.374
Gambusia adults	0 h	All fish survived		Fish not available for testing	
	24 h				
	5 d				

Discussion

Shear stress impacts

A large proportion of all redfin life stages (eggs, larvae, and juveniles) and adult gambusia survived exposure to shear levels equivalent to the provided modelled data for the Snowy 2.0 facility. However, there was considerable variability in survival rates among redfin life stages. Egg survival was higher as shear levels decreased. None of the shear levels tested in this study resulted in no survival for juvenile redfin or adult gambusia. The impacts of shear could not be assessed for adult redfin, because the shear chamber was not designed to handle fish of that size. Nevertheless, shear-related mortality generally scales with fish size (Neitzel et al. 2000), so it would only be worthwhile investigating adult mortality if there was no survival among juvenile life stages. Given that we observed high survival in redfin juveniles in our laboratory experiments, then it could be expected that adults would experience low shear-related mortality through Snowy 2.0.

Neitzel et al. (2004) investigated the injury–mortality thresholds of juvenile rainbow trout (*Oncorhynchus mykiss*) and steelhead (anadromous rainbow trout), autumn (age-0 and age-1) and spring Chinook salmon (*O. tshawytscha*), and American shad (*Alosa sapidissima*) to shear strain rates in the laboratory. They reported an increase in injuries and mortalities for all species of fish at strain rates in excess of 495 1/s. However, they did not observe any apparent size-related pattern in vulnerability to high shear, apart from age-1 fall Chinook salmon being more vulnerable to shear environments than age-0 fall Chinook salmon (Neitzel et al. 2004).

The results for the survival rates observed after five days for both redfin and gambusia should be interpreted with caution since decreased values were observed also for the control group. Infection risks increase in fish kept in captivity due to various factors, including stress, and may affect survival (Portz et al. 2006). Therefore, the decreased survival rates observed in this study cannot be assumed to be likely to occur in the wild.

Blade strike impacts

When the blade strike effects were assessed using stochastic modelling, it was found that the modelled survival rate of fish was high. Similarly, the deterministic model predictions also suggested high survival. These values are likely to have underestimated survival rates in response to blade strike, since the model predictions were based on the assumption that all blade strikes would lead to mortality. But in reality not all blade strikes lead to mortality, and an empirical factor ('mutilation ratio') is often used to correct for this effect (Von Raben 1957). Blade strike is generally related to overall fish size. The bigger the fish, the more likely it will be struck by a rotating blade. For fish eggs and small fish, which have relative large surface area to mass ratio, their probabilities could be even lower due to the fact that they could be pulled around the blade rather than colliding with the blade edge. Considering the operating scenarios tested, overall survival rate from blade strike is also expected to be high.

Pressure impacts

Based on pressure testing using the modelled profiles provided by Snowy Hydro Ltd, (and similarly to that for the independent pressure and blade strike results), it seems that a large proportion of redfin eggs, larvae, juveniles or adults, if entrained at the intake, would survive the extreme pressurisation expected during passage through the Snowy 2.0 pumped hydropower facility. Under some operating conditions, survival rates of juveniles and adults were as high as 100%. Lampert (1976) subjected a redfin and a range of other species (Whitefish (*Coregoma* sp.), Rainbow trout (*Salmo gairdneri* Richardson), Grayling (*Thymallus* L.), Carp (*Cyprinus carpio* L.), Roach (*Leuciscus rutilus* L.) to a rapid increase in pressure 50 atm, and similarly reported high survival rates in all species. Indeed, the species exhibited differing pressure shock symptoms, but all fully recovered within 2 hours after the experiment. Also, Rowley (1955) simulated the pressure impacts of passage through a hydropower turbine by exposing Rainbow trout (*Oncorhynchus mykiss*) to rapid pressure rises of 244-1,277 kPa (i.e. in less than 1 minute), and then a sudden release of pressure again. The fish were immobilized while under pressure, but resumed normal activity immediately following the release of pressure (Rowley Jr 1955).

The lower survival rates observed for redfin juveniles and adults during the testing for the T₃ scenario than that for the T₆ scenario was probably largely due to temperature stress during the former scenario. The T₃ experiments for these life stages were run later in the spring (i.e. late September/early October) than those for the T₆ experiments, and water temperatures in the pressure chamber during the former experiments reached 28 °C (K. Doyle pers. obs). The chronic lethal temperature for Percid species has been reported to range from 29-35 °C (Hokanson 1977), so the water temperature reached within the pressure chamber would likely have placed substantial stress upon the animals when combined with the stress of being held in an experimental capsule for the 3.8 hour duration of the simulation. By contrast, the lower survival for the T₃ scenario than for the T₆ scenario was less likely to be due to a difference in pressure-related effects, because the lower survival rates for the T₃ scenario were evident in both control and treatment fish.

Potential effects of differing pressure profiles associated with differing turbine operating scenarios

The T₆ and T₃ scenarios had similar maxima and minima pressure values, but differed with regards to fish travel time and thus their overall rates of change between maximum and minimum pressure values. The high survival in redfin and gambusia for both the T₆ and T₃ scenarios, suggests that both species are capable of enduring the rapid compression component associated with each operating scenario. However, the response patterns of each species to the different fish travel times and rates of pressure change for each scenario remain unknown. Nevertheless, fish travel times and overall rates of pressure change are likely to vary greatly among Snowy 2.0 operating scenarios, and may have a strong influence on fish survival (see papers investigating the effects of the ratio of pressure change on fish survival during turbine passage (e.g. Brown et al. (2014) and Boys et al. (2016)). For example, the T₃ scenario had a longer fish travel time (approximately 3.8 hours) and (by association) a slower rate of change between maximum and minimum pressure values than the T₆ scenario (which had a fish

travel time of approximately 2 hours). A single turbine operating scenario would have an even longer fish travel time (in excess of 18 hours) than the T₃ scenario, and an even slower overall rate of change between maximum and minimum pressure values.

Despite the potential for Snowy 2.0 operating scenarios to have varying barotrauma impacts on redfin and gambusia, our study was not designed to allow for direct comparisons between the effects of the T₃ and T₆ scenarios, because the effects of each scenario were tested independently under slightly different experimental conditions. Further to the water temperatures in the pressure chamber being much higher during the T₃ experiments than during the T₆ experiments, the T₃ fish had been held in the pre-experiment enclosures for a much longer period of time than the T₆ fish. Consequently, the T₃ fish were potentially in a slightly poorer pre-experimental condition than the T₆ fish (Portz et al. 2006).

Indirect mortality effects on fish

Although the shear, blade strike and/or pressure stressors related to turbine passage may not necessarily be immediately lethal, there is a chance that fish may die from injuries or other impacts at a later stage (i.e. experience indirect mortality) (Čada 2001). For example, fish that become disorientated by shear, blade strike and/or pressure effects whilst passing through the Snowy 2.0 facility may be subsequently more vulnerable to predation once they reach Tantangara Reservoir (Čada 2001). Alternatively, passage through the Snowy 2.0 facility may lead to fish being physically injured (e.g. with an abrasion or haemorrhage), and consequently they may later succumb to the effects of the injury or disease (Čada 2001). Additionally, fish may also be injured during the entrainment phase. We observed that fish quickly became negatively buoyant (i.e. sank) once compressed. So these fish would generally be unable to maintain position in the water column whilst being transported to the turbine. They would likely impact continuously on the tunnel wall but the stress and impacts of such passage could not be tested within the current study.

The inclusion of a five day mortality assessment in the current study was designed to test for any potential delayed effects from injuries. The five day assessments revealed lower survival rates for several treatment-fish combinations, although the lower survival rates appeared to have been biased by enclosure- and/or handling-related factors in most instances because there were corresponding declines in the survival rates for the control fish (e.g. larval redfin for both the T₆ and T₃ pressure scenarios). Fish often experience confounding effects related to water quality issues and other synthetic factors when they are held in enclosures for an extended period of time (Portz et al. 2006).

Assumptions and caveats

There were a number of assumptions and caveats underpinning the results in this chapter for each of the stressors. The first is that we only tested one orientation for shear (head first). The impact of shear stress on fish is likely to be especially influenced by the orientation of the fish at the time of exposure (Neitzel et al. 2004). Given that we did not observe 100% mortality for that body orientation at all experimental shear levels, it was unnecessary to test the influence of other body orientations to demonstrate the potential for redfin and gambusia to survive the shear stress levels expected within the Snowy 2.0 facility. But for completeness, testing multiple orientations would provide a more comprehensive understanding of overall impacts. For blade strike trials, it was assumed that the likelihood of blade strike was directly reflective of the likelihood of mortality from blade strike because there was no 'mutilation ratio' literature to use for the target species. So our results likely underestimated survival rates.

Furthermore, there was difficulty in obtaining detailed expected turbine geometry and flow rates for the various operating scenarios. Blade strike is a function of fish size, rotation rate and flow, so the key to accurate blade strike modelling is accurate geometric data. Re-analysis would be possible once final operating parameters are known, but based on the high survival rates observed, vastly conflicting results would not be expected.

Given the aim of this project was to determine the likelihood of redfin surviving transport through the Snowy 2.0 facility, a conservative approach was taken by testing the pressure scenario with

Tantangara at MOL and Talbingo at FSL. This provided survival data under what could be considered the least lethal conditions. Survival rates for redfin and gambusia were high for this operational condition, even though fish were exposed to extreme high pressures. It was not possible to estimate the likelihood of redfin and/or gambusia survival for the most severe pressure scenario (i.e. Tantangara at FSL and Talbingo at MOL) using the results from the current study, but the hypothesis remains that lower survival rates would be expected.

The reason the most severe profile was not tested is because it was not a practical situation. When Tantangara Reservoir is at full supply level it cannot take any more water. When Talbingo is at minimum operating level, then it is unable to supply any more water for pumping. Even though this scenario provides the most extreme pressure differential, in reality it is not a scenario that will be regularly operationalised, and therefore was not tested. In any case, our results indicate that fish are likely to survive transport through the Snowy 2.0 facility under the least lethal pressure scenario.

Chapter 4: Survival estimation for the combined effects of shear, blade strike and pressure

Introduction

The independent assessments of hydraulic factors (shear stress, blade strike and pressure) on redfin and gambusia provide a useful indicator of proposed survival through Snowy 2.0 (Chapter 3). But it is important that the survival estimates be combined to provide an indication of likely survival for all three factors tested in this study. That most hydraulic factors were estimated in isolation introduces the problem of 'Multiplication of Probability' to calculate an overall figure. Within pumped hydropower facilities, the way in which fish experience different hydrological conditions can be considered sequential. For instance fish first become entrained into the system. They then pass through the draft tube and are exposed to increasing shear. Fish then pass through the turbine and become susceptible to blade strike. If both shear exposure and blade strike are survived, fish then experience a rapid compression (see Chapter 1). Individually, each event may not lead to mortality. So it could be expected that a percentage of fish survive shear stress exposure, then are exposed to blade strike, then compression in order to pass through the system.

It is important that the three mortality sources be combined in order to ascertain overall likelihood of survival. This chapter assesses the product of these three survival events to calculate an overall survival estimate.

Methods

General approach

Working within the bounds of multiplicative probability, the combined stressor analysis was based on the sequential survival rates for each stressor and multiplied to account for the proportion of fish surviving through each stage. All combined survival estimates were for immediate survival rates only, as it was not possible to model 24 hour or 5 day survival estimates for blade strike.

Two scenarios were tested; one based on the experimental survival rates for the shear ranges tested in the experiments, and another based on the experimental shear survival rates after they had been adjusted for the actual frequency distribution of shear ranges expected to occur within the Snowy 2.0 facility (i.e. after the experimental shear survival rates had been weighted with respect to the likelihood of occurrence of each shear range within the Snowy 2.0 facility).

This latter (i.e. adjusted) scenario was based on modelled shear data for the Snowy 2.0 spiral casing provided by SHL. Simulated values of the shear strain rates within the runner were modelled to reach a maximum of 60694 1/s, and the average shear strain in the runner was expected to be 6015 1/s (Figure 40). The vast majority of the expected shear strain rates (V95% of modelled numbers) were within the range of 25 to 1036 1/s (Figure 40). The ranges of the shear strain rates that fish were experimentally exposed to in the current study were compared to the computer simulated values (Figure 40). The majority of experimental data indicated a high likelihood of fish survival for shear stress values lower than 1036 1/s; which encompasses most of the shear values expected for the runner within the Snowy 2.0 facility. It is important that experimental data be combined with expected shear data to provide a realistic estimate.

Shear and pressure survival adjustment for fish handling mortalities (i.e. control adjustment)

Prior to undertaking the combined stressor analysis, we adjusted the shear and pressure survival rates to remove the influence of fish handling-related mortalities (hereafter referred to as the 'control-adjusted' survival rates) (see Appendix 1 for a full breakdown of these control-adjusted results). Control-adjusted (CA) shear and pressure survival rates were calculated by dividing the number of survivors in a treatment (T) by the number of survivors in the control associated with the treatment (C), for each time of survival assessment (i.e. immediately, 24 hours and 5 days after the experiment). The resulting value was then converted to a percentage by multiplying it by 100.

$$CA = 100 \times T/C$$

Control-adjusted survival rates more accurately reflect survival in response to shear strain and pressure impacts. Such an approach is commonly applied in laboratory studies where controls are used to remove any extraneous effects from the experimental variable under investigation (e.g. Schweizer et al. (2012)).

It should be acknowledged that this particular control adjustment approach can potentially result in survival estimates of more than 100% in situations where a higher number of individuals die in controls than in treatments. In these situations, the survival values are capped at 100%.

To accommodate the limitations of CFD data, two combined survival 'scenarios' were tested and calculated.

Scenario 1: Experimental shear data

For each experimental value of shear tested in this scenario, we were able to calculate an overall survival rate based on a certain level of shear. This involved, for each life history stage, using the survival value obtained from the experimental data. For instance, if our experiments suggested that 40% of larvae (across all treatments) would survive passage through the turbine; this was used in the overall calculation. The reason this calculation was performed is that not all CFD modelled data were available for all potential operating scenarios. Considering there are numerous operating permutations, such a simple mortality calculation will provide a "conservative survival estimate" averaged across all scenarios determined from laboratory trials.

Scenario 2: Experimental shear data adjusted for expected Snowy 2.0 CFD data

This scenario 'adjusted' the experimental shear rate by correcting for modelled CFD data (Figure 40; Figure 41). Data modelled for Kaplan turbines suggested that shear is not uniform within a turbine (Čada et al. 2006). There are areas of elevated shear, and similarly, areas of low shear. As such, whether or not critical levels will be exceeded will be completely dependent on the target fish's size and its flow path through the transfer system. CFD data was subsequently used to approximate expected survival estimates under expected operating conditions. International work, using time-averaged models, suggests that the areas of highest shear will be experienced within the turbine itself and immediately downstream (in the draft tube). But to understand the changes in shear through the turbine, it is useful to investigate different sections for analysis: inlet/outlet region (including spiral case, stay vanes, and wicket gates); runner (hub and blades); and draft tube region. Ideally shear should be simulated for three flow scenarios (1. Maximum operating flow, 2. Minimum operating flow, 3. A midpoint between the two). But for the purposes of this analysis, CFD data was only provided by SHL for the spiral casing and limited to the T6 operating scenario.

For both blade strike and pressure, we applied survival estimates calculated as part of laboratory trials.

Data analysis

The combined stressor analysis was based on the assumption that — during the pumping phase within the Snowy 2.0 facility — a fish will be:

- (1) exposed to high levels of fluid shear stress impacts in the draft tube as it approaches the turbine;
- (2) then be susceptible to blade strike impacts as it passes the turbine; and then
- (3) experience rapid compression impacts as it moves from the lower to the upper side of the turbine blades (Chapter 1).

Within Snowy 2.0, fish will be exposed to these events in a sequential order. Thus, the overall mortality probability can be expressed as:

$$SP_T = P_S \times P_{BS} \times P_P$$

Where SP_T is the overall survival probability, P_S is the proportional survival after experiencing shear events, P_{BS} is the proportional survival after experiencing blade strike and P_P is the expected proportional survival associated with rapid compression.

During actual turbine passage, fish will be exposed to these stressors instantaneously and in very quick succession (i.e. there will be no 24 hour or 5 day delay). Therefore all combined stressor survival estimates were modelled using instantaneous survival rates only.

Results

Simulated shear

Simulated values of the shear strain rates within the spiral casing were modelled to reach a maximum of 60694 1/s, and the average shear strain in the runner was expected to be 6015 1/s (Figure 41). The vast majority of the expected shear strain rates (V95% of modelled numbers) were within the range of 25 to 1036 1/s (Figure 41). These were below the lowest shear that was able to be generated by our experimental facility (Chapter 3). Thus, we combined our experimental mortality data with the expected shear ranges in the turbine and adjusted the mortality rate to Snowy 2.0 conditions. We made the assumption that mortality estimates between two tested shear ranges were the same. But in reality, survival would increase as shear decreases, as indicated by the results from the laboratory trials. The ranges of the shear strain rates that fish were experimentally exposed to in the current study were compared to the computer simulated values (blue box, Figure 41).

Scenario 1 survival

When the shear survival rates were adjusted for the frequency of shear ranges expected to occur within Snowy 2.0 (using the modelled shear data for Snowy 2.0), it was found that they would be between 15% and 94% for all life stages of redfin and adult gambusia (Table 16). Adult gambusia and juvenile redfin had the highest survival rates (94% and 83%, respectively), and survival rates generally declined in earlier life stages. Adults were not assessed as shear experiments were not done on this life stage (see Chapter 2).

Scenario 2 survival

The combined stressor analysis for scenarios 1 and 2 indicated that all life stages of redfin and adult gambusia would have survival rates of 36% or higher (Table 15 and Table 16). Adult gambusia were expected to have the highest combined stressor survival rate (86%), closely followed by juvenile redfin (75%). In comparison, redfin eggs were expected to have the lowest combined stressor survival rate (36%) (Table 16).

Survival range estimates based on both scenarios

When both scenarios were considered simultaneously, the results indicated that all life stages of redfin and adult gambusia would have survival rates ranging between 15% and 94% (Table 16). The survival ranges for the younger life stages (redfin eggs: 15-36%; 12-18 DPH redfin larvae: 44-66%; 28-30 DPH redfin larvae: 34-58%) were much broader than those for the older life stages (redfin juveniles: 83-75%; redfin adults: 71-63%).

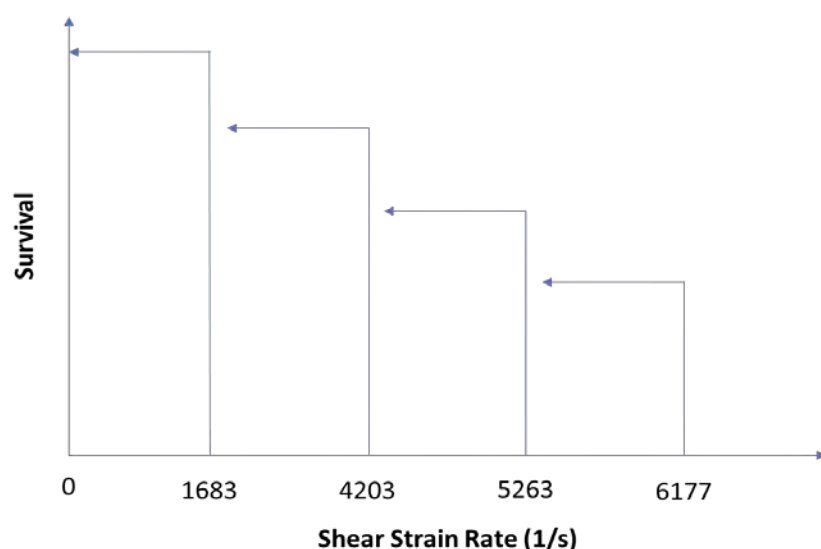


Figure 40. An example of how shear survival rates were interpolated for all potential shear scenarios. Here, the shear rates represent those tested in the egg trials. It was impossible to test all possible shear scenarios in laboratory trials. Thus, survival was estimated to be unchanged between the different values of shear tested.

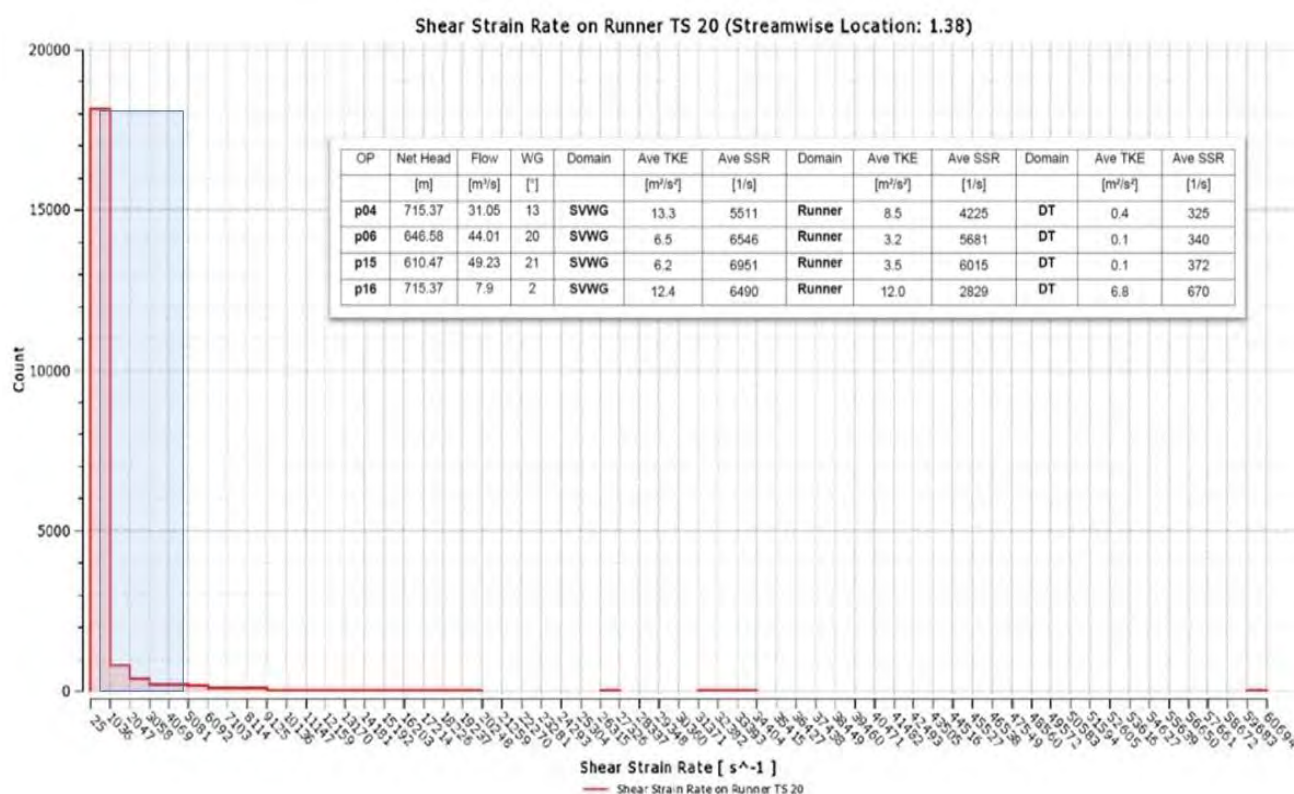








Figure 41. Expected shear strain rates on the spiral casing within the proposed Snowy 2.0 facility (based on modelled data from SHL). Shaded region depicts the range of shear stress experimentally tested during this study. Red bars show the frequency of shear strain rates expected to occur based on computer modelling. Although the average is high, it is skewed by some extreme values. The majority of modelled outputs suggest shear strain rates less than 1036 1/s.

Table 15. Fish survivorship immediately following exposure to experimental shear stress ranges after they had been adjusted for the frequency of shear ranges expected to occur within the Snowy 2.0 facility. All survival percentages have also been adjusted to account for control group mortalities.

Life stage / species	Survival (%) (shear stress and likelihood of exposure)
Redfin juveniles	90
Redfin larvae 28 - 30	71
Redfin larvae 12 - 18	69
Redfin eggs	51
Adult gambusia	91

Table 16. Combined stressor survival estimations with shear, blade strike and pressure effects for all life stages of redfin and adult gambusia. Scenario 1 relates to survival based on the experimental survival rates for the shear ranges tested in the experiments; and Scenario 2 relates to survival based on the experimental shear survival rates after they had been adjusted for the actual frequency distribution of shear ranges expected to occur within the Snowy 2.0 facility. All estimates have been adjusted for shear and pressure control group mortalities.

Combined stressor survival estimation			
Test group	Stressor	Scenario 1: Survival based on experimental data (%)	Scenario 2: Survival based on simulated probability of exposure (%)
Adult redfin 	Shear	100*	90*
	Blade strike	71	71
	Pressure	100	100
Combined survival range		71	63
Juvenile redfin 	Shear	100	90
	Blade strike	83	83
	Pressure	100	100
Combined survival range		83	75
Redfin larvae (28 – 30 DPH) 	Shear	42	71
	Blade strike	98	98
	Pressure	82	82
Combined survival range		44	66
Redfin larvae (12-18 DPH) 	Shear	46	69
	Blade strike	99	99
	Pressure	99	99
Combined survival range		34	58
Redfin eggs (with eyes formed) 	Shear	22	51
	Blade strike	100	100
	Pressure	70	70
Combined survival range		15	36
Adult gambusia 	Shear	100	91
	Blade strike	94	94
	Pressure	100	100
Combined survival range		94	86

*Likelihood of survival of adult Redfin to shear strain has been based on juvenile results since we did not expose adults to shear strain because they did not fit into the delivery tube.

Discussion

Irrespective of whether shear-adjusted or experimental data were applied, survival through conditions designed to replicate Snowy 2.0 was predicted for all species and life classes tested. These results correspond with laboratory studies (Chapter 3) and suggest that, if entrained, it is likely that at least some redfin and gambusia will reach Tantangara Dam alive if the system is operated under (a) a T₆ scenario; (b) with shear stresses that do not exceed those modelled for the spiral casing; and (c) if the geometry for blade strike calculations matches those provided.

The team modelled two scenarios, one using the actual laboratory data, and a second applying that data to CFD models. Considering accurate CFD data was not available, the two scenario model aimed to provide a suitable survival 'range'. The scenario using actual survival data applied from our laboratory study would have underestimated survival. This is largely because it averaged shear results across all treatments tested for each life history stage. This would lead to higher calculated shear values than might be expected within the turbine; because we specifically focused our experiments on previously-known lethal levels of shear. It provided an indicative estimate of what the maximum survival may have been if (a) shear, pressure and blade strike was constant within the turbine, and (b) the T₆ operating scenario was the most frequently used. These may not be the case, as it is likely that operation of Snowy 2.0 will be far more variable and utilize a range of operating modes.

Scenario 2 sought to apply the laboratory-calculated mortality through a realistic Snowy 2.0 operating scenario. The main difference between the two scenarios was that it sought to 'correct' the laboratory calculated shear data for expected flows within the pumped hydro scheme. It recognised that there will likely be areas of high and low shear under a normal operating scenario (Turnpenny et al. 2000, Čada et al. 2006). A fish may pass through the turbine along one of many possible flow paths. For instance, one that exposes it to lethal levels of fluid shear, or areas with significantly lower values. However, the CFD model was only based on modelled shear in the spiral casing which would be expected to be lower than the draft tube and within the runner itself; because this is all that was made available to the team. Consequently, this calculation is likely to result in an overestimate of survival; largely because there were significant areas of low shear in the modelled data that were provided. It could be expected that actual shear strain rates would be higher in the turbine and draft tube, but these data were not available. So rather than provide a definitive overall calculation; we could only present a conservative survival range. The precision of this range could be improved if a more complete set of CFD data became available.

It should be acknowledged that the combined survival estimation used in this study was limited by the assumption that a fish exposed to shear does not become more susceptible to blade strike. Similarly, the combined survival estimation assumed that a fish exposed to extreme compression had not been influenced by exposure to both shear and blade strike. It may well be that a fish exposed to shear or blade strike could be injured or stressed (Čada 2001). The added exposure to pressure could result in elevated mortality probability. But in order to assess the effects of such scenarios to multiplicative probability, it would be necessary to add experimental complexity to laboratory experiments. For instance, a fish could be exposed to shear then exposed to rapid compression and its survival compared against fish exposed to a single exposure to either shear or pressure. However, there are presently no examples from the literature where such an experiment aiming to expose fish simultaneously to shear, blade strike and pressure changes has been attempted. On the other hand, similar approaches exposing fish and other aquatic biota sequentially to different stressors have been undertaken (Čada et al. 1980; Bamber et al., 1994; Bamber et al. 1995; Bamber et al. 2004).

Transferring our findings to other life stages/species

The impacts of shear, blade strike and pressure stressors on fish associated with passage through pumped hydropower facilities will likely vary greatly among life stages. Only adult gambusia were assessed in the current study, but the expected impacts of the proposed Snowy 2.0 facility on juveniles may vary, particularly due to their smaller size. *Gambusia holbrooki*

reproduce through internal fertilisation, and their young develop inside the mother until they are born as small free-swimming fish (Wourms 1981). The smaller size of juveniles compared to that of adults could potentially make the former more vulnerable to shear stress (Neitzel et al. 2000, Silva et al. 2018), similar to the results observed in this study for redfin, since impacts would be concentrated over a smaller area, leading to a greater chance of injuries (Pyke 2005). Nevertheless, the relatively small size of juveniles could, instead, reduce their likelihood of being struck (Silva et al. 2018). Pressure effects, on the other hand, may not differ from those on adults since pressure changes affect fish from larvae through to adults, irrespective of size (Silva et al. 2018). These predictions are all speculative, and the only way of increasing confidence regarding the likely effects of the Snowy 2.0 facility on juvenile gambusia would be to undertake similar empirical experiments to those done for adult gambusia in the current study.

Evidence from conventional hydropower facilities suggests that the impacts of related stressors on fish will also vary greatly among species depending on a multitude of fish characteristics (Silva et al. 2018). Silva et al. (2018) argued that species-related differences in the response of fish to hydropower passage are particularly influenced by differences in body shape and size, swim bladder morphology, and spatial distribution within the water column. Consequently, results obtained for redfin and gambusia in the current experiments, in combination with results from previous studies (Boys et al. 2016, Pracheil et al. 2016, Silva et al. 2018) cannot be used to predict the impact of the proposed Snowy 2.0 facility on the passage other species, such as Climbing galaxias. Climbing galaxias, in particular, has a unique combination of characteristics (Table 17), and thus the impact of hydropower passage cannot be predicted based only on previous experiments or literature. Targeted experiments on Climbing galaxias, among different life history stages, would be necessary if an accurate estimate of the species' survival through the Snowy 2.0 facility was required; especially given the stark differences we observed among different sizes of fish and life stages for just two species tested in this study.

Overall conclusion

In the absence of control measures, the results of this study indicate that there is a risk that redfin and/or gambusia will survive a transfer from Talbingo Reservoir to Tantangara Reservoir through the proposed Snowy 2.0 facility. Survival through the Snowy 2.0 facility is likely because:

- there are actively recruiting populations of redfin and gambusia already present in Talbingo Reservoir
- entrainment of redfin into the intake is generally considered to be 'Likely', and entrainment of gambusia is generally considered to be 'Possible'.
- the results of this study indicate that it is likely that a large proportion of any redfin or gambusia entrained at the intake in Talbingo Reservoir would survive the shear, blade strike and pressure impacts expected to occur within the Snowy 2.0 facility.

Table 17. Morphological and physiological characteristics of redbfin, gambusia and climbing galaxiids and the consequence for fish passage through hydro structure related with each one of these characteristics.

Morphology and physiology	Redfin	Gambusia	Climbing galaxias	Consequence for fish passage through hydro structure
<i>Body size and shape</i>	Commonly <40cm Deep bodied	Females 6cm, males 3.5cm Flattened head	Max. size 28 cm, commonly 15 cm. Body tubular, scaleless.	<i>Blade strike</i> : Larger fish are more susceptible to this type of mechanical injury (Silva et al. 2018). <i>Pressure</i> : Pressure variation affects fish from larvae to adults, regardless of size (Silva et al. 2018). <i>Shear</i> : Shear stress affects smaller fish (based on body length) and early life stages more severely (Silva et al. 2018). However, shear stress impacts related to body shape (e.g. flat vs. tubular shape where flatter fish will be theoretically exposed to more shear stress compared to a tubular fish) are poorly understood.
<i>Swim bladder morphology</i>	Physoclistous	Physoclistous	Physostomous	<i>Pressure</i> : The capacity to inflate / deflate the swim bladder is influenced by swim bladder morphology. Physostomes and physoclists manage gas volume in the swim bladder differently: Physoclists: closed swim bladders, lack pneumatic duct. <ul style="list-style-type: none"> Inflation and deflation managed by a physiological process that uses circulatory system to input / output gas to swim bladder (Fänge 1983). Physostomes: opened swim bladder - connects to oesophagus via pneumatic duct. <ul style="list-style-type: none"> Expected that physostomes can quickly inflate or deflate the swim bladder by gulping or expelling air through pneumatic duct (Silva et al. 2018). Physoclists are more likely to be injured than physostomes as they cannot quickly release gas as the swim bladder expands during rapid decompression (Brown et al. 2012a). This remains to be fully investigated across the diversity of fishes, given the high diversity of swim bladder morphologies and physiological adaptations (Birindelli et al. 2009) with variations related to the number and size of the chambers (Brown et al. 2014), shape and presence of diverticular noted within the same family (Birindelli et al. 2009, Birindelli and Shibatta 2011) and location of pneumatic duct (Silva et al. 2018).
<i>Location in water column</i>	Shallow and Mid water column – pelagic schooling fish	Surface, shallow water specialist	Swims near bottom (benthopelagic), usually around cover of rocks and logs	<i>Pressure</i> : The depth, and thus pressure at which fish are acclimated to prior to infrastructure passage likely dictates the amount of gas a fish must have in its swim bladder to maintain neutral buoyancy. The ability of a fish to acclimate to deeper depths is dependent on functionality (morphology and physiology, see Fänge 1983) and the volume of gas that can be transferred to the swim bladder (Brown et al. 2012a), as well as the behaviour of the fish (nektonic v. benthic species; (Brown et al. 2014)). For example, a neutrally buoyant fish at deeper depths (higher pressures) will need to have a higher volume of gas in the swim bladder than its conspecific in shallower water (lower pressure; (Pflugrath et al. 2012). Fish at shallower depths require less gas to achieve same swim bladder volume for neutral buoyancy and may be less susceptible to barotrauma. For benthic fish, the initial acclimation pressure may be high and the lowest pressure experienced during hydroturbine passage is likely have a greater impact on swim bladder expansion (Brown et al. 2014). However, there are considerable knowledge gaps related to understanding the maximum depths occupied by benthic-oriented species in freshwater ecosystems, the determination of buoyancy achieved at different depths and consequently barotrauma effects (Silva et al. 2018).

Options for further work

Knowledge gaps

There are a number of possible studies that would provide a greater level of confidence of the likelihood of fish being transferred through the proposed Snowy 2.0 facility. These relate to both the risk of entrainment and the likelihood of post-entrainment survival during passage through the facility.

Entrainment

- Entrainment risk could be quantified by obtaining empirical data on:
 - the spatio-temporal distribution of redfin, gambusia and other target species within Talbingo Reservoir (e.g. depth preferences at different times of the year), using acoustic tracking, underwater sonar and/or baited remote underwater video (BRUV) stations.
 - the hydraulic conditions around the intake using CFD modelling
 - the swimming ability/rheotactic response of target species

Post entrainment

- Blade strike effects
 - Turbine blade strike impacts could be empirically assessed to validate the modelling predictions presented in this study. This would require a functioning pumped hydropower simulator, at a scale close to Snowy 2.0 with access to the target species.
 - This work could be extended to assess the (1) influence of blade thickness and (2) percentage of struck fish that actually end up dying (i.e. the mutilation rate) for the target species.
- Interactive effects of shear, blade strike and pressure
 - The modelling done for the present study was limited to treating shear, blade strike and pressure stressors independently to determine an overall survival probability.
 - A more realistic simulation would be to experimentally expose fish to the interactive effects of shear, blade strike and pressure. For instance, a controlled laboratory trial which exposes fish to shear, then strike, then pressure.
 - Fish subjected to one of these stressors (e.g. shear) might potentially be more susceptible to being negatively impacted by other hydropower-related stressors (e.g. blade strike and/or pressure changes).
 - For example, a fish that has become disorientated following exposure to shear stress could become more vulnerable to blade strike (similarly to the way in which it could become more vulnerable to natural ecological processes like predation).
- The likelihood of other life stages or species being unintentionally transferred through the proposed Snowy 2.0 facility
 - The shear and pressure tolerance experiments could be repeated for juvenile gambusia and all life stages of Climbing galaxias.

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Appendix

Shear and pressure experiment results after adjusting for control mortalities

All shear and pressure survival results were initially adjusted to take fish handling-related mortalities into consideration (i.e. were control-adjusted to account for non-experimental effects) prior to undertaking the combined stressor analysis in Chapter 4 for shear, blade strike and pressure impacts (see that chapter for more details about the justification and approach for applying the control-adjustment to these data). The complete breakdown of the control-adjusted shear and pressure survival results has been presented below.

Control-adjusted survivorship estimation for shear strain

There was a higher likelihood of survival for all life stages and species when they were exposed to lower shear strain rates, although the control-adjusted survival rates generally increased with the size of the fish being tested. The survival rate for redfin eggs decreased from 48% to 0% from the lowest (1683 1/s) to the highest (6177 1/s) shear strain rates (Table 18). Similarly, both redfin larvae (12-18 DPH and 28-30 DPH) survival rates measured instantly after exposure decreased from the lowest (1010 1/s) to the highest (3706 1/s) shear strain (Table 18). For juvenile redfin and gambusia, all fish initially survived exposure to shear strain (i.e. were alive immediately after being exposed to each shear strain treatment) (Table 18).

Survival rates also varied with regard to the time after exposure, and typically declined over time. The only exception to this pattern was for 28-30 DPH redfin larvae. The control-adjusted survival estimates for this life stage 24 hours and 5 days after exposure were actually higher than those immediately after exposure due to an anomalous die-off within the controls (Table 18).

Control-adjusted survivorship estimation for pressure exposure

Redfin eggs

After correction for control mortalities, it was estimated that survival of redfin eggs 24 hours post pressurisation was 70% for the T₆ pressurisation profile (Table 19).

Redfin larvae

Survival rates of 12 - 18 DPH larvae at 5 days post pressurisation were 89% and 100% for the T₆ and T₃ pressure profiles, respectively (Table 19).

In comparison, the survival rate of 28 - 30 DPH larvae at 5 days post pressurisation was 100% for the T₆ pressure profile (Table 19).

Redfin juveniles

Control-adjusted survival rates of juveniles after 24 hours were 100% and 61% for the T₆ and T₃ pressure profiles, respectively (Table 19). These adjusted survival rates remained the same even after 5 days.

Redfin adults






All adult redfin survived the first 5 days following exposure to the T₆ pressure profile (Table 19).

In comparison, the control-adjusted survival rate for adult redfin was 60% immediately following exposure to the T₃ pressure profile, and remained at this level for the first 5 days (Table 19).

Gambusia adults

One hundred percent of adults survived the first 5 days in both the control and pressure treatment (Table 19).







Table 18. Control-adjusted fish survivorship following exposure to experimental shear stress ranges. 'Control-adjusted' refers to the fact that survival percentages have been adjusted to account for control group mortalities.

Test group	Shear survival (%)			
	Treatment	Immediate	24 hr	5 day
<i>Juvenile redfin</i> 	337	100	100	100
	841	100	100	100
	1687	100	100	100
	1853	100	70	70
<i>Redfin larvae (Pre flexion; approx. 12-18 days post hatch)</i> 	1010	78	68	63
	2522	53	28	21
	3374	29	13	No survival
	3706	24	1	No survival
<i>Redfin larvae (approx. 28-30 days post hatch)</i> 	1010	82	100*	100*
	2522	80	100*	100*
	3374	6	No survival	
	3706	No survival		
<i>Redfin eggs (with eyes formed to confirm eggs are viable)</i> 	1683	48^	^	Not assessed
	4203	41^	^	
	5623	No survival		
	6177	No survival		
<i>Adult gambusia</i> 	505	100	98	98
	1261	100	74	55
	1687	100	86	No survival
	1853	100	76	53

100* = control-adjusted estimates of more than 100% due to their being a higher number of individuals die in controls than in the treatments. In such situations, the survival values were capped at 100%, because obviously it is impossible to achieve survival rates greater than that.

^ = egg survival was assessed during the 24 hour period after the experiment (i.e. immediate and 24 h mortalities were combined).

Table 19. Control-adjusted fish survivorship following exposure to the T_6 and T_3 experimental pressure profiles. 'Control-adjusted' refers to the fact that survival percentages have been adjusted to account for control group mortalities.

Test group	Pressure survival (%)			
	Operating scenario	Immediate	24 hr	5 day
Adult redfin 	T_6	100	100	100
	T_3	60	60	60
Juvenile redfin 	T_6	100	100	100
	T_3	100	61	61
Redfin larvae (Pre flexion; approx. 12-18 days post hatch) 	T_6	99	100	89
	T_3	88	85	100*
Redfin larvae (approx. 28-30 days post hatch) 	T_6	82	82	100*
Redfin eggs (with eyes formed to confirm eggs are viable) 	T_6	70	^	Not assessed
Adult gambusia 	T_6	100	100	100

100* = control-adjusted estimates of more than 100% due to their being a higher number of individuals die in controls than in the treatments. In such situations, the survival values were capped at 100%, because obviously it is impossible to achieve survival rates greater than that.

^ = egg survival was assessed during the 24 hour period after the experiment (i.e. immediate and 24 h mortalities were combined).

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Identification of galaxiid species (Teleostei, Galaxiidae) from the area of the proposed Snowy 2.0 Project

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Front cover photo: Individuals of *Galaxias* sp. collected from the Murrumbidgee River, upstream (top & middle images) and downstream (bottom image) of Tantangara Dam, May 2018. (Tarmo A. Raadik).

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Identification of galaxiid species (Teleostei, Galaxiidae) from the proposed alignment of the Snowy 2.0 Project.

Tarmo A. Raadik

Arthur Rylah Institute for Environmental Research
**Unpublished Client Report for EMM Consulting,
Department of Environment, Land, Water and Planning**

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Summary

As part of the planning and environmental documentation required for the proposed Snowy 2.0 Project, the potential presence of a threatened species of native freshwater fish, Stocky Galaxias (*Galaxias tantangara*), within the proposed alignment of the project is required to be determined. Sampling in early 2018 collected species of *Galaxias*, but the species could not be identified visually as they were part of the *Galaxias olidus* cryptic species complex.

To determine if these galaxiids were the threatened *Galaxias tantangara*, an additional narrow range species found nearby (Kosciuszko Galaxias *Galaxias supremus*), or the Mountain Galaxias (*Galaxias olidus*), collection of additional specimens, followed by a detailed morphological study, was initiated.

Galaxiid specimens were re-collected by backpack electrofishing from two of the three original survey sites: Murrumbidgee River just downstream of Tantangara Reservoir, and upstream of the reservoir near the junction with Tantangara Creek. Galaxiids, similar to those originally collected in early 2018, were not located from the Yarrangobilly River just upstream of Talbingo Reservoir (Tumut River system), despite sampling at four sites.

Study material was fixed in formalin for morphological examination and live images were taken of individuals from each study population for visual comparison. Fixed individuals were scored for 26 measured morphometric characters (e.g. body dimensions) and nine serially repeated meristic characters (e.g. fin ray counts), as well as a subjective assessment of some morphological characters found to be of secondary taxonomic importance. Morphometric values were converted into % ratios following previous taxonomic studies on galaxiids, and summary statistics of these, and of meristic values were compared against similar data, and the published descriptions for the described species.

The two study populations from the Murrumbidgee River were found to differ in 24 or more morphological characters to *Galaxias tantangara* and *Galaxias supremus*, similarly in meristic, and they differed considerably to the published descriptions. There was also substantial morphological differences in individuals between these two sites, and both sites were meristically different to other, nearby *Galaxias olidus* populations.

This study confirmed the two study populations of galaxiids in the area of the Snowy 2.0 project are not *Galaxias tantangara* or *Galaxias supremus*. They are confirmed as members of the *Galaxias olidus* complex, but tentatively as *Galaxias olidus*: this cannot be resolved on morphology alone.

Due to morphological variation between these two populations, between both and nearby *Galaxias olidus* populations, and some differences to the published description of *Galaxias olidus*, it is likely that previously unrecognised levels of within-species (*G. olidus*) variation, or additional, undiscovered species, may be present. This can only be resolved by undertaking genetic, and more detailed morphological, study.

1 Introduction

Snowy Hydro Limited (Snowy Hydro) proposes to develop the Snowy 2.0 project (Snowy 2.0), a pumped hydroelectric storage and generation project with the potential to provide storage for large scale, reliable and secure renewable energy to Australia. EMM Consulting Pty Limited (EMM) has been engaged by Snowy Hydro to prepare the planning and environmental documentation required for Snowy 2.0.

As part of this process, freshwater fish surveys were undertaken by Cardno along the proposed project alignment, from the upper Murrumbidgee River to the Yarrangobilly River, from the 29 January to 2 February 2018. Specimens of galaxiid fishes, tentatively identified in the field as belonging to the Mountain Galaxias cryptic species complex (Adams et al. 2014, Raadik 2014), were collected from three sites (Figure 1):

- Murrumbidgee River, downstream of Tantangara Dam (site code: Murrumbidgee DS);
- Murrumbidgee River, upstream of Tantangara Dam, at the junction with Tantangara Creek (site code: Murrumbidgee US); and,
- Yarrangobilly River, downstream of Wallace's Creek (site code: Yarrangobilly).

Whilst images of these fish were taken in the field (Figs 2–4), specimens were not retained and, therefore, accurate species identification is problematic as species in the Mountain Galaxias (*Galaxias olidus*) complex are very similar in external morphology (Raadik 2014). To identify this complex to species level requires a detailed examination of fresh voucher material. The failure to accurately identify the captured individuals to species is of concern, as at least one threatened species in the *Galaxias olidus* complex is potentially present within the proposed alignment: Stocky Galaxias (*Galaxias tantangara*). This species is listed as critically endangered in New South Wales (DPI 2017), and is found in a short reach of headwater tributary in Tantangara Creek (Figure 1). Further, taxonomy of this species complex has been only partly resolved in the streams in the Snowy, Murrumbidgee and upper Murray river catchments in the Snowy Mountains Region/Kosciuszko National Park, and this area potentially harbours additional, undescribed and narrow range endemic species (Raadik and Kuitert 2002, Raadik 2014).

Consequently, accurate identification of the galaxiid species present in the area of the Snowy 2.0 project is required to inform the environmental values, as part of the environmental impact assessment for the project.

EMM Consulting, on behalf of Snowy Hydro, contracted the Arthur Rylah Institute for Environmental Research to:

1. Collect fresh galaxiid material from the three sampling sites; and,
2. Undertake detailed morphological examination to confirm species identification.

Based on the published literature on the distribution of species in the *Galaxias olidus* complex (Raadik 2014), the following species may be present in these streams:

- *Galaxias olidus* (Mountain Galaxias) – high probability;
- *Galaxias tantangara* (Stocky Galaxias) – high probability;
- *Galaxias* sp., unidentified, possibly new, species in the complex – medium probability; and,
- *Galaxias supremus* (Kosciuszko Galaxias) – low probability (found in the headwaters of the Snowy River system about 65 km south-west, in high elevation alpine streams, and distribution may be more widespread).

The only other species of *Galaxias* recorded from higher elevation in streams draining the study area is the Climbing Galaxias (*Galaxias brevipinnis*). This native species is found in coastal catchments but appears to have recently invaded the inland upper Murray system (Morison and Anderson 1991) and was also found established in the Yarrangobilly River system (Murrumbidgee River drainage) in 2002 (Raadik 2003).

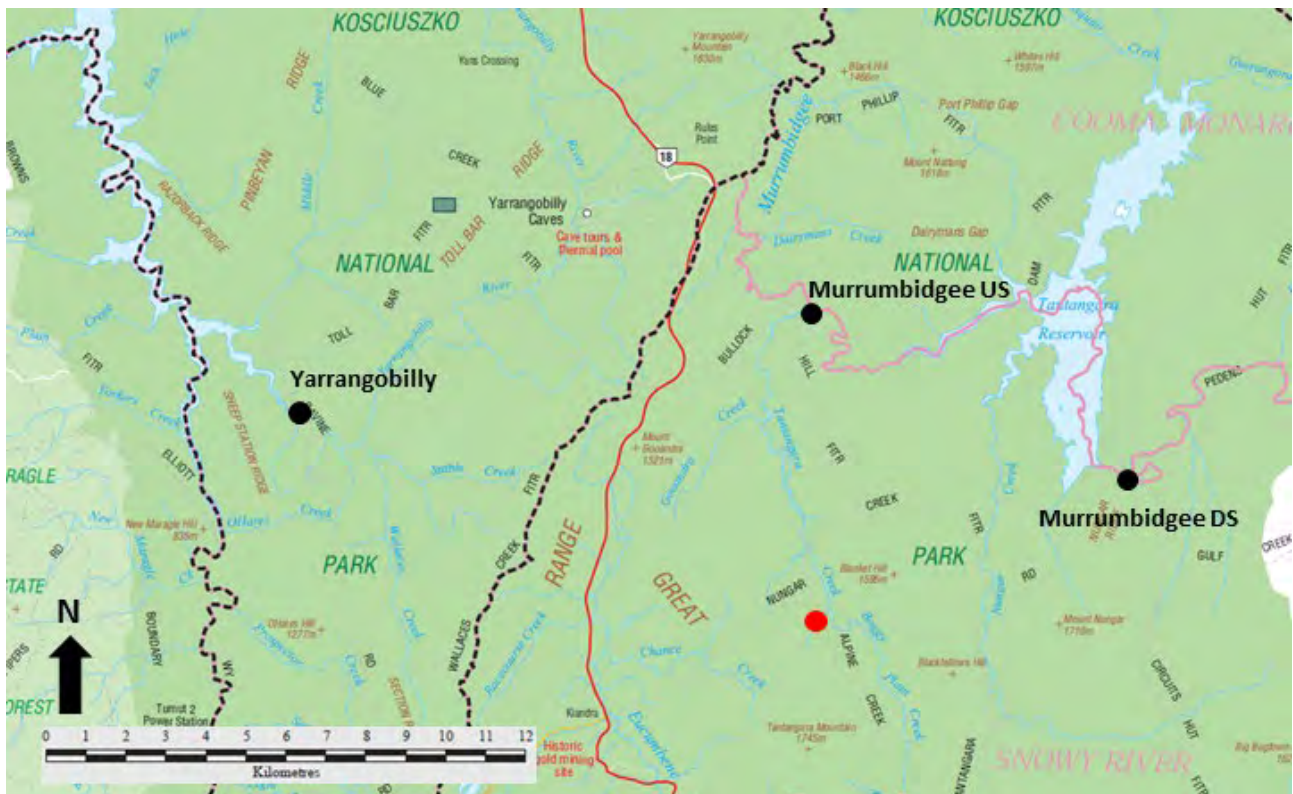


Figure 1. Location of sampling sites in the upper Murrumbidgee River catchment sampled by Cardno in early 2018 (black circles), in relation to the location of the only known population of *Galaxias tantangara* (red circle – not re-sampled).

Base map – modified from a NSW Department of Lands map of the proposed Tumut Local Government Area, 2003.



Figure 2. *Galaxias* sp. collected from the Murrumbidgee River, below Tantangara Dam, 30 January 2018.

Image: Daniel Pygas.



Figure 3. Two *Galaxias* sp. individuals collected from the Murrumbidgee River, upstream of Tantangara Dam, at the junction with Tantangara Creek. 30 January 2018.

Image: Daniel Pygas.



Figure 4. Two juvenile *Galaxias* sp. individuals collected from the Yarrangobilly River, 1 February 2018.

Image: Daniel Pygas.

2 Methods

A total of 15 individuals, preferably greater than 55 mm in length (length to caudal fork, LCF) of each potential species was set as a target for collection from each sampling site, as this number is considered adequate for detailed morphological examination (Raadik 2011, 2014). To allow for future genetic analysis to confirm species boundaries, if needed, a tissue clip (lower caudal fin lobe) from up to 10 individuals of each species at each site was also required.

2.1 Field Sampling

2.1.1 Location of Sites

The three sites at which galaxiids had been detected in early 2018 (above) were considered primary sampling sites (Table 1, Figure 5). We also surveyed secondary sites to check for the presence of additional taxa, and if not enough galaxiids were collected from primary sites.

Table 1. Details of the primary (bold) and secondary (asterisk) sampling sites, all within the Murrumbidgee River Basin, Kosciuszko National Park.

Primary sampling sites in bold; Elev – elevation.

Site	Waterbody	Location	Date	Latitude	Longitude	Elev (m)
1	Murrumbidgee R	upstream of bridge on Tantangara Road, 1.2 km downstream of Tantangara Dam	2/05/2018	-35.79976	148.6743	1200
2	Murrumbidgee R	Downstream to upstream of mouth of Tantangara Creek, upstream of Tantangara Dam	2/05/2018	-35.75417	148.56448	1255
3	Tantangara Creek	from junction with Murrumbidgee River to 50 m upstream	2/05/2018	-35.75259	148.56371	1260
4	Yarrangobilly River	downstream of ford on Lobs Hole Powerline Road	3/05/2018	-35.78541	148.39028	570
5	Yarrangobilly River	Downstream of stream gauge, off Lobs Hole Powerline Road	3/05/2018	-35.78959	148.40147	575
6	Yarrangobilly River	Off Mine Track, at old mine site	3/05/2018	-35.7930	148.40315	580
7	Wallace's Creek	off Mine Trail, just upstream of junction with Yarrangobilly River	3/05/2018	-35.79281	148.41288	600

2.1.2 Sampling methodology

Galaxiids were collected at each site during daylight hours using a portable, battery powered Smith-Root® Model 20B backpack electrofishing unit, operating at an output of 990 Volts, frequency of 90 Hertz and a duty cycle of 35 %. These settings, along with a triangle-shaped anode head, which condenses the electric field at its corners, is effective in stunning small galaxiids, particularly in very fresh water with low electrical conductivity (e.g. <100 EC) (Raadik, personal observation).

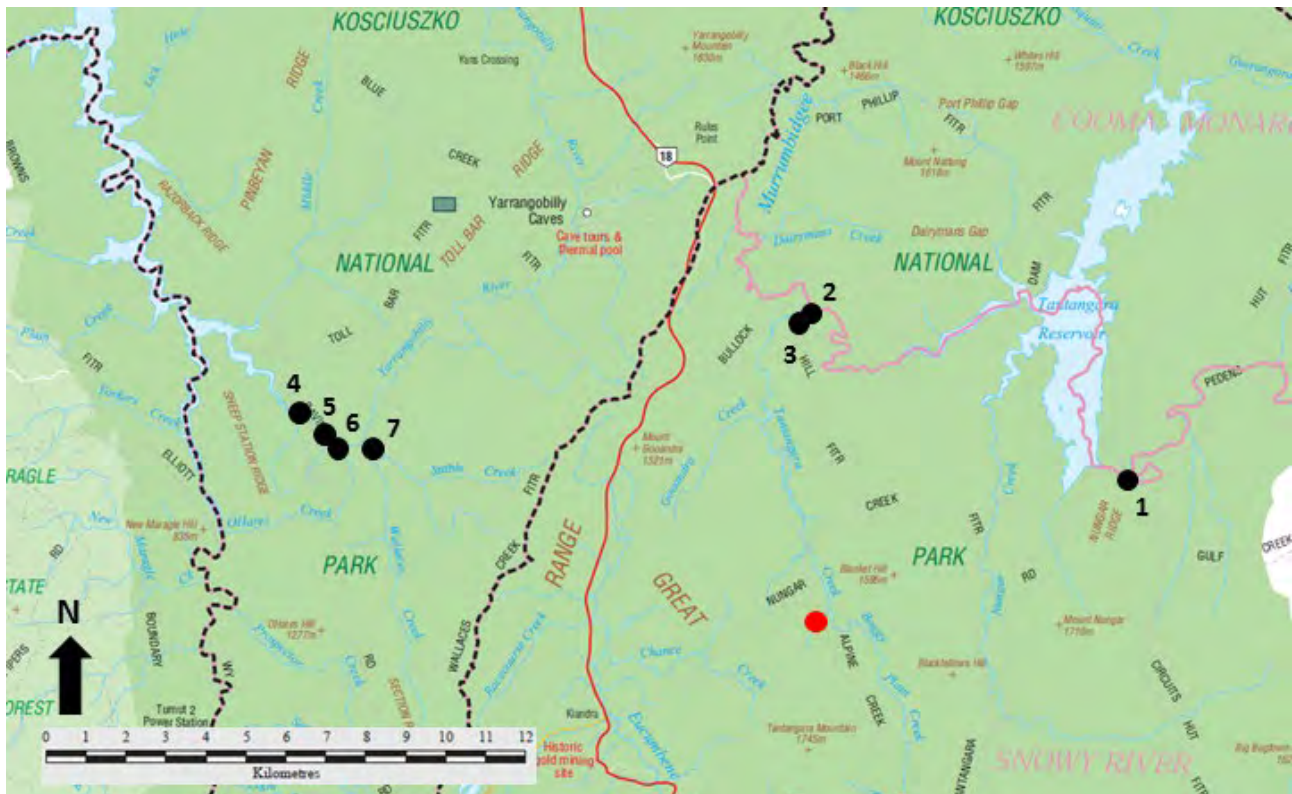


Figure 5. Location of sampling sites in the upper Murrumbidgee River catchment (black circles), and location of Stocky Galaxias (red circle – not sampled during this study).

Base map – modified from a NSW Department of Lands map of the proposed Tumut Local Government Area, 2003.

The operator of the electrofishing unit, wearing polarising glasses to reduce glare, slowly moved in an upstream direction targeting all instream habitat. Stunned fish were retrieved and placed into a bucket containing stream water, carried by an assistant, who also collected any missed fish using a large, fin-mesh dipnet. Electrofishing was undertaken during daylight hours. Each site was sampled until either the desired number of galaxiid specimens were obtained, or if no galaxiids were evident, until a minimum of 180 m of stream length had been sampled to maximise the probability of detecting and collecting all species of galaxiids present at a site.

For each sampling event/site, the following standard parameters were also recorded: sampling elapsed time (minutes), electrofishing power on time (seconds), sample reach length, stream width and average depth (in metres). Water temperature (°C) and electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$ @ 25 °C) were also recorded from a single location within the reach at 0.1 m below the water surface using a calibrated waterproof meter (Oakton® ECTestr 11).

2.1.3 Specimen processing

The processing of specimens followed Raadik (2011, 2014). In brief, a tissue clip (approximately 3–5 x 3–5 mm, depending on fish size) was taken from the lower lobe of the caudal fin for later genetic analysis from live individuals before they were vouchered for later morphological analysis. Tissue clips were then placed into separate 2 ml plastic vials containing 100% ethanol, stored in a zip lock bag and placed into a car fridge. Tissue vouchering was undertaken first to avoid the risk of DNA damage from formalin used to fix specimens. In the laboratory, the ethanol in the tissue voucher vials was replaced and vials were placed into longer term storage in a freezer at -10 °C.

Selected galaxiids were then euthanased in a bath of anaesthetic (Cove oil, 40 mg/L for 10 minutes) and species/morphotype identifications were double-checked before fixation. Whole fish were fixed in a 10 % solution of neutral buffered formalin, in a sufficiently large plastic container to avoid bending fish or fins which could alter specimen morphology and affect later analysis. After five days samples were washed in

freshwater to remove excess formalin and were transferred into 70 % ethanol for long term storage and to prevent decalcification of small bones.

Two to three, representative fish of each probable species from each site were also kept alive for later photography, to capture their natural live colouration. Fish from each site were placed into separate 20 L plastic buckets filled with approximately 4 L of river water which had been treated with ~0.7 g/L of salt (to calm the fish and reduce fungal infection), and aerated using an aquarium air pump. Water was changed each day, and on return to the laboratory, the fish were removed and photographed in glass aquaria.

2.2 Morphological examination

Morphological examination consisted of measuring (= morphometrics) and counting (= meristics) a conventional suite of body proportions or structures important in galaxiid taxonomy. This detailed process is a standard method in fish taxonomy, and has been used successfully to define and then resolve the *Galaxias olidus* species complex Raadik 2011, 2014). Raadik (2014) modified previous procedures used for galaxiid taxonomy (e.g. Frankenberg 1969, McDowall & Frankenberg 1981, McDowall 1997, 2001, 2003a), and developed new characters important for discrimination in the *Galaxias olidus* complex. Consequently, this method, described in detail in Raadik (2014), is followed here.

Measurements and counts were conducted on fish 55 mm or greater in length (LCF), however, meristic counts were also taken from smaller individuals when needed. Morphometric character measurements were taken to the nearest 0.1 mm with needlepoint digital vernier calipers, meristic characters were counted, and both were undertaken with the aid of a 3 x illuminated magnifier or under a dissecting stereomicroscope.

2.2.1 Morphometric characters

The morphometric character suite comprised the following 26 measured characters (Figures 6–7), including one calculated character:

AL (anal fin length) – distance from anterior end of anal fin base to posteriormost extremity of longest fin ray; **BDPec** (body depth at pectoral fin base) – vertical depth of body through pectoral fin base; **BDV** (body depth at vent) – vertical depth of body above vent; **CFFL** (caudal fin fork length) – calculated measurement (LCF – SL); **DCP** (depth of caudal peduncle) – vertical depth of caudal peduncle at posterior end of anal fin base; **DF–AF** – direct measure of the horizontal distance between two vertical lines, drawn through the origin of the dorsal fin and the anal fin; **DL** (dorsal fin length) – distance from dorsal fin origin to posteriormost extremity of longest fin ray; **ED** (eye diameter) – greatest horizontal distance between anterior and posterior margins of orbit; **GW** (gape width) – direct distance between junction of lower and upper jaws on each side of head, measured from ventral surface; **HD** (head depth) – maximum vertical depth measured from above middle of eye; **HL** (head length) – direct distance from tip of snout to most posterior extent of fleshy portion of gill cover above pectoral fin base; **HW** (head width) – maximum horizontal width measured at preopercular margin; **IOW** (interorbital width) – distance between mid-dorsal margins of eyes; **LAB** (length of anal fin base) – length of the fin base; **LCF** (length to caudal fork) – horizontal distance from tip of snout to caudal fin fork; **LCP** (length of caudal peduncle) – oblique distance measured from posterior end of base of last anal fin ray to midposterior edge of hypural joint; **LDB** (length of dorsal fin base – length of the fin base; **LJL** (lower jaw length) – distance from tip of lower jaw to posterior margin; **PecL** (pectoral fin length – distance from middle of fin base to posterior end of longest fin ray; **PecPel** (pectoral fin to pelvic fin length – direct distance from middle of pectoral fin base to origin of pelvic fin; **PelAn** (pelvic fin to anal fin length – direct distance from origin of pelvic fin base to origin of anal fin; **PelL** (pelvic fin length) – distance from dorsal origin of fin to proximal tip of longest fin ray; **PoHL** (post-orbital head length) – distance from posterior end of eye (orbit) to posteriormost extent of fleshy portion of gill cover above pectoral fin base; **PreA** (pre-anal fin length) – direct distance from tip of snout to anal-fin origin; **PreD** (pre-dorsal fin length – direct distance from tip of snout to dorsal-fin origin; **PrePel** (pre-pelvic fin length) – direct distance from tip of snout to origin of pelvic fin; **SL** (standard length) – horizontal distance from tip of snout to midposterior edge of hypural joint; **SnL** (snout length) – direct distance from tip of snout to anterior margin of eye; **UJL** (upper jaw length) – distance from tip of snout to posterior extent of upper jaw.

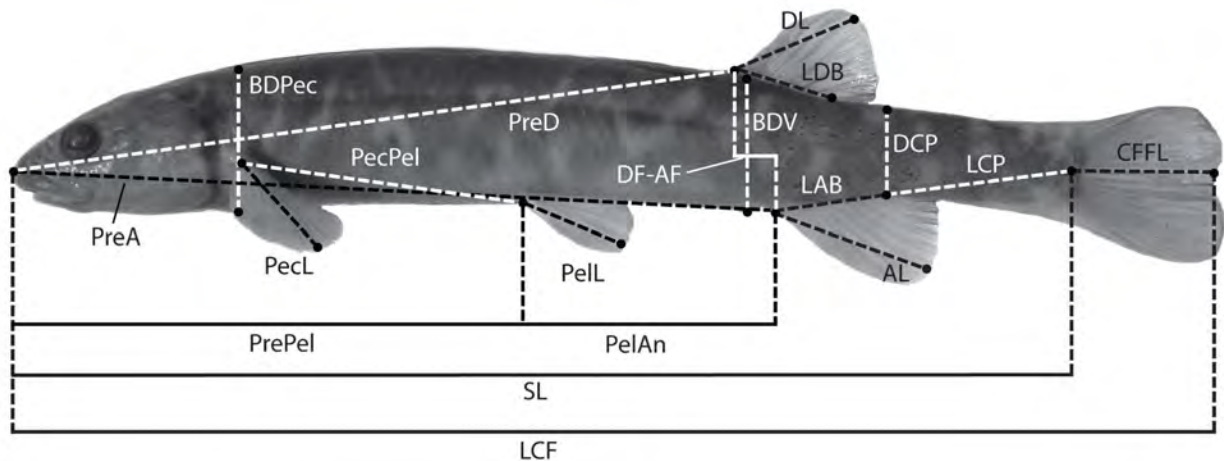


Figure 6. *Galaxias olidus* complex body and fin morphometric measurements.

See above for codes. From Raadik, 2014.

2.2.2 Meristic characters.

The following nine serially-repeated meristic characters were enumerated: **Dorsal Rays** – total number of segmented dorsal fin rays; last ray, if bifurcated at base, counted as a single element; **Anal Rays** – total number of segmented anal fin rays; last ray, if bifurcated at base, counted as a single element; **Caudal Rays** – principal caudal fin ray count (branched segmented rays plus one unbranched segmented ray above and below branched rays); **Pectoral Rays** – total number of segmented pectoral fin rays; **Pelvic Rays** – total number of segmented pelvic fin rays; **Gill Rakers (T)** – total number of gill raker elements on lower and upper limb of first gill arch, including any vestigial anterior rakers. Raker in bend of arch included in lower limb count. Counts made separately for each limb and total count derived by addition; **Gill Rakers (L)** – number of gill raker elements on lower limb of first gill arch, including any vestigial anterior rakers; **Gill Rakers (U)** – number of gill raker elements on upper limb of first gill arch, including any vestigial anterior rakers; **Vertebrae** – number of vertebral centra, excluding the hypural centrum (Figure 8).

Fin ray counts were made on the left hand side (LHS) of the specimen, under a dissecting stereomicroscope. The rays were viewed with reflected or backlight only (shining up through the fin) to more clearly define the ray structure. Procurrent rays, present in the dorsal, anal and caudal fins, anterior to segmented rays, were not enumerated (a modification to McDowall & Frankenberg 1981), nor was the small, unsegmented procurrent ray very occasionally present at the medial border of the pectoral and pelvic fins. Gill rakers, including small rudimentary or vestigial rakers present at the ends of each limb, were counted in situ on the first branchial arch on the LHS of the specimen and included all elements on the lower (L) limb or ceratobranchial, including the raker straddling the angle of the arch, and elements on the upper (U) limb, or epibranchial, to provide three counts (L, U, and Total (= L+U)).

Vertebral counts of preserved specimens were enumerated from x-rays. Wet specimens were laid on their sides on a water proof cardboard cartridge holding a sheet of industrial X-ray film (AGFA® Structurix D4 FW), and exposed for 30 seconds at 25 to 28 kV of power in a Faxitron® X-ray machine. The X-ray film was then developed, dried, and scanned at 600 dpi. Image files were enlarged to between 200–300X magnification on a computer screen and vertebrae counted.

2.2.3 Additional characters

Additional morphological characters important for taxon discrimination, not captured by the morphometric measurements, were objectively recorded (Raadik 2014). All varied in their occurrence between, but also to a degree within, taxa, and therefore are not considered robust, stable primary taxonomic characters. Some characters also varied in their degree of development. The characters included maximum size, shape of the dorsal and ventral trunk profiles, cross-section trunk shape, presence of a fleshy snout, shape of the lateral profile of the head and snout, and aspects of colour pattern morphology. Details of the four most prominent characters are given below.

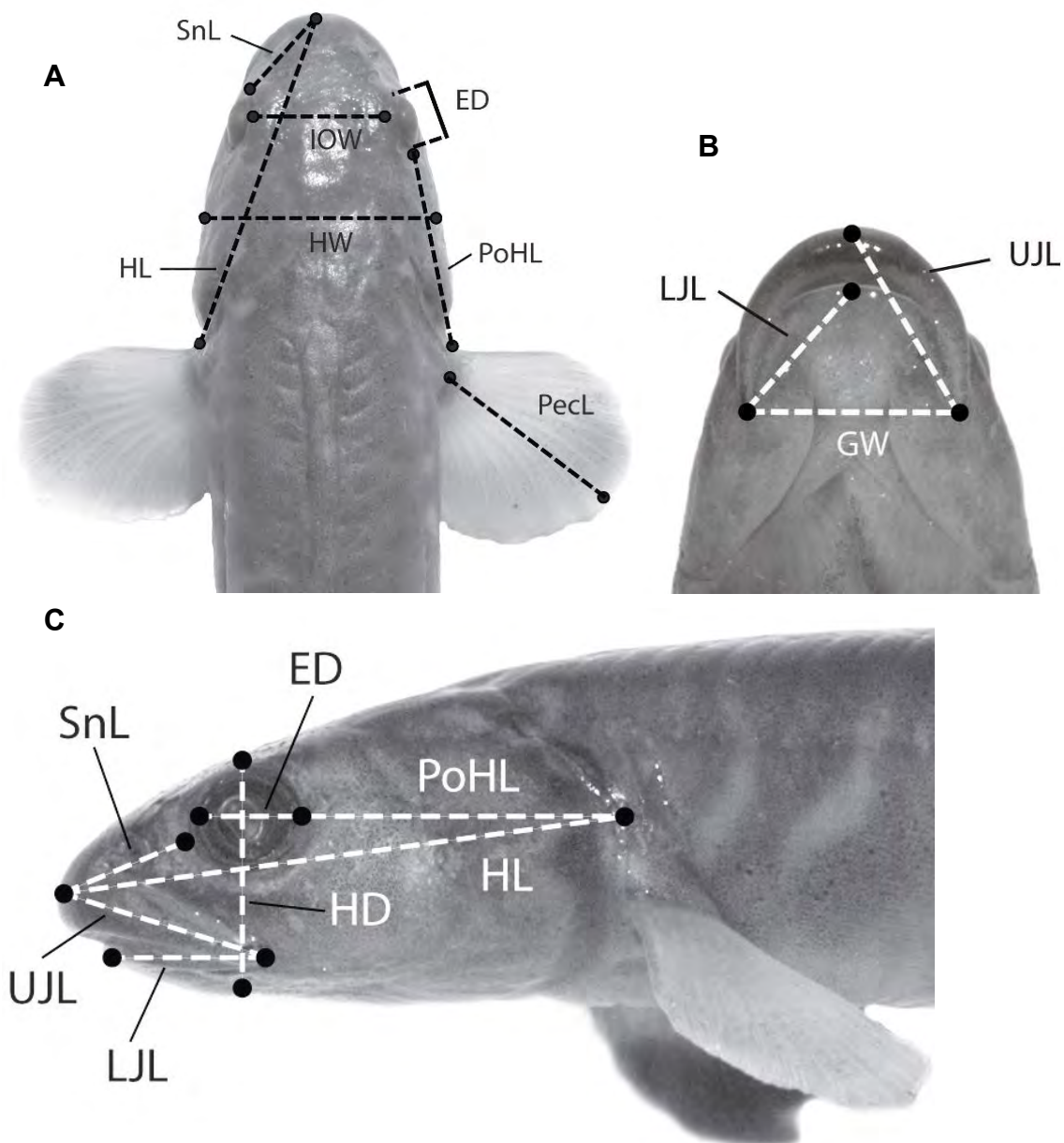


Figure 7. *Galaxias olidus* head morphometric measurements: A) dorsal; B) ventral; C) lateral.
See above for codes. From Raadik, 2014.

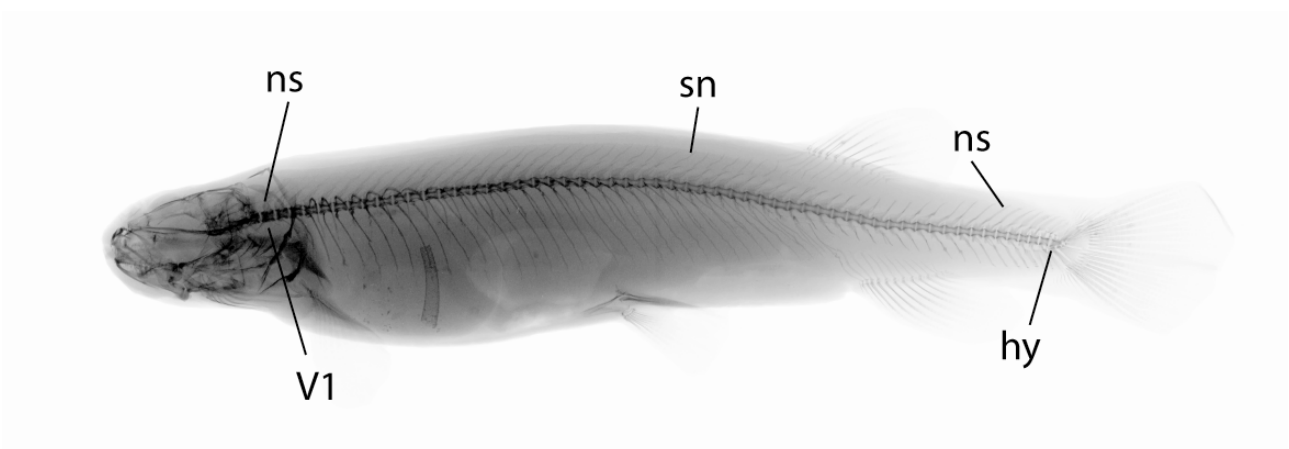


Figure 8. Example x-ray of a *Galaxias olidus* showing the vertebral column.
Hy – hypural; ns – neural spine; sn – supra neural; v1 – first vertebra. Adapted from Raadik 2014.

Pyloric caecae. Pyloric caecae, blind sacs located at the posterior of the stomach, are known to vary in presence, number and length between galaxiid genera and species. All caecae present were counted and measured for length. Where present, caecal length was expressed as a percentage of the standard length (SL) of the individual specimen. Where two caecae were present, they were always of unequal length and only the length of the longest was recorded.

Accessory lateral line. The presence or absence of accessory lateral line papillae, dorso-lateral to the primary trunk lateral line papillae, was also noted.

Paired fin ray lamellae. The presence of fin ray lamellae, a raised thin layer of skin forming a strong, backward facing longitudinal ridge of tissue along the ventral surface of a fin ray, was recorded when present. In the *Galaxias olidus* complex these are usually located on the ventral surface of anterior rays of the pectoral and pelvic fins, and they assist the fish when out of water in 'climbing' up the surface of wet rocks using water surface tension.

Mid-lateral bars. Colour and pattern (e.g. blotches, speckles and bars) can vary greatly in species of *Galaxias*. A distinctive character in the *Galaxias olidus* species complex, although not universally present, is a series of distinct, short, vertical, usually black, bars, often relatively uniform in shape and located over or very close to the mid-lateral region of the trunk. These contrast with blotches, dark grey to brownish markings, irregular in shape and with a more diffuse border, which are commonly also found on the dorsal surface or widespread over the lateral surface of the trunk and tend to be paler in colour and often coalesce with other blotches. When present, bars vary in distinctiveness, colour, shape, size, number, disposition (spacing between bars) and frequency of occurrence between species. Within species, these bars are relatively stable, although visual inspection of individuals from several populations is often necessary before a bar-pattern trend could be determined.

2.3 Comparative material

The morphological data collected above was compared against the species description for *Galaxias tantangara*, which is found nearby in a tributary of Tantangara Creek (Figure 5), and the species description for *Galaxias supremus* (Raadik 2014), another narrow range species also known from alpine areas in the Kosciuszko National Park, though currently only known from a location 65 km to the south-west of the area of the proposed Snowy 2.0 project in the upper Snowy River system. A diagnosis and line drawing of each species, taken from the original description, are provided in Appendix 1, and summarised morphometric data in Appendices 2 and 3, for reference. Specific data is also included in relevant tables in section 3.2, below.

The data was also compared to morphological data from six nearby (regional) populations of *Galaxias olidus* (three each from the Murrumbidgee River catchment (IV,10) and the upper Murray catchment (IV,01)) found in Raadik (2011, 2014) (Table 2), and more recent data from an additional population (Larrys Creek) in the Murrumbidgee River catchment.

Table 2. *Galaxias olidus* comparative material used in this study.

Museum registration numbers are all Museum Victoria (NMV).

Species	DD & RB	Waterbody	Latitude	Longitude	Elev (m)	Museum No., or Site Code
<i>G. olidus</i>	IV,10	Burns Crk	-36.01370	148.38070	1530	A30120-001
<i>G. olidus</i>	IV,10	Flea Crk	-35.28740	148.79080	820	A30040-001
<i>G. olidus</i>	IV,10	Happy Jacks Crk	-36.05775	148.49713	1420	A10385
<i>G. olidus</i>	IV,10	Larrys Crk	-35.89096	148.41943	1315	TR-18-222
<i>G. olidus</i>	IV,01	Three Rocks Crk	-36.33820	148.31810	1080	A29960-001
<i>G. olidus</i>	IV,01	Bogong Crk	-36.25310	148.25900	1120	TR-02-078
<i>G. olidus</i>	IV,01	L. Cootaputamba	-36.46650	148.26401	20320	A30010-001

2.4 Analysis

A detailed statistical analysis of the morphometric data (i.e. following Raadik 2011, Adams et al. 2014), including standardising data to remove the influence of size on shape, Principal Components Analysis, Discriminate Functions Analysis, etc. was not considered necessary for this study, as species diagnosis of study populations was based on comparison with described taxa, and not testing for, or defining, new species boundaries, nor identifying which morphological characters were important in species diagnoses.

The initial assumption, until otherwise disproved via analysis, was that the two study populations were assumed to be *Galaxias olidus*, the only other described species known from the catchment. Validation of this was achieved by coarse comparison of summary statistics of morphological character ratios or counts against those of *Galaxias tantangara* and *Galaxias supremus*, with multiple differences in character states between the study populations and the described species taken to be a lack of morphological similarity with those species.

3 Results

3.1 Specimen collecting

Fish sampling was undertaken on 2–3 May 2018. Details of the sampling effort (time elapsed, electrofishing power on time), area sampled (reach length, stream width and depth), and water temperature and electrical conductivity for each sampling site are provided in Table 3. Water temperature was cool, and electrical conductivity (a measure of salinity) was low <100 EC, typical of alpine streams.

Detectability of galaxiids was high due to the stream conditions typical of late autumn: low stream discharge, relatively shallow depth and clear water (no turbidity). Sampling results are provided in Table 4. Unidentified galaxiids, preliminarily conforming to the *Galaxias olidus* complex, were collected from site 1 and 2 on the Murrumbidgee River (Table 4). Additional sampling was undertaken at site 3 on the lower Tantangara Creek, though galaxiid individuals captured were of the same morphotype as those captured at site 2 in the Murrumbidgee River.

No galaxiids in the *Galaxias olidus* complex were found at the site 4, Yarrangobilly River, despite the species having been previously captured here, nor were they captured from three additional nearby sites (sites 5–7). The lack of galaxiids in the Yarrangobilly River, despite 3 hours electrofishing over 5,600 m² of stream at four sites indicates that they are very low in abundance in the reach upstream from Talbingo Reservoir (near Lobs Hole).

Two individuals, initially identified as the *Galaxias brevipinnis*, were collected from site 6 on the Yarrangobilly River (Table 4).

3.2 Identification of galaxiid species

The specimens of unidentified galaxiids collected from the Murrumbidgee River, below and above Tantangara Dam (sites 1 and 2 respectively; Table 3), were:

1. Confirmed to be of the genus *Galaxias* when compared to the key to Australian genera of Galaxiidae in Raadik (2014, p. 22).
2. Confirmed to be members of the *Galaxias olidus* complex, the *Galaxias olidus* cryptic species grouping, when compared to the key to species of *Galaxias* in south-eastern, mainland Australia (Raadik 2014, p. 23).

The identification of the two putative *Galaxias brevipinnis* collected from the Yarrangobilly River were also confirmed when compared to the key to species of *Galaxias* in south-eastern, mainland Australia (Raadik 2014).

3.2.1 Galaxiid images

Representative images of galaxiids collected from sites 1 and 2 in the Murrumbidgee River are provided in Figures 9 and 10, respectively, and images of three individuals of *Galaxias tantangara*, collected previously, are provided for comparison in Figure 11.

Table 3. Physical characteristics and survey effort expended at sampling sites.

Primary sampling sites in bold; m – metre; secs – seconds; Temp – water temperature °C; EC – water electrical conductivity, as $\mu\text{s.cm}^{-1}$ @ 25 °C.

Site	Waterbody	Gear Type	Reach Length (m)	Average Width (m)	Average Depth (m)	Maximum Depth (m)	Power On Time (secs)	Duration (min)	Temp	EC
1	Murrumbidgee R	EF/BP	220	9.0	0.35	0.9	3086	70	12.7	23
2	Murrumbidgee R	EF/BP	270	13.0	0.50	1.5	3288	115	9.1	30
3	Tantangara Creek	EF/BP	50	4.0	0.25	0.4	280	10	-	-
4	Yarrangobilly River	EF/BP	180	8.0	0.30	1.2	1873	50	9.5	172
5	Yarrangobilly River	EF/BP	250	9.0	0.30	1.2	1825	65	-	-
6	Yarrangobilly River	EF/BP	180	8.0	0.40	1.5	1880	65	-	-
7	Wallace's Creek	EF/BP	200	2.5	0.15	0.3	1201	30	9.8	182

Table 4. Number of fish and decapod crustacean species collected at each sampling site.

Primary sampling sites in bold; # - alien species. Numbers in square brackets refer to the number of galaxiid samples kept for morphology and genetics (in order); + - present but not counted as non-target fauna.

Site	Waterbody	Unidentified Galaxiid <i>Galaxias</i> sp.	Climbing Galaxias <i>Galaxias brevipinnis</i>	Spiny Crayfish <i>Euastacus</i> sp.	Yabby <i>Cherax destructor</i>	Brown Trout <i>Salmo trutta</i> [#]	Rainbow Trout <i>Oncorhynchus mykiss</i> [#]	Redfin Perch <i>Perca fluviatilis</i> [#]
1	Murrumbidgee R	48 [15, 10]			4	+		
2	Murrumbidgee R	22 [15, 7]		4		+	+	
3	Tantangara Creek	12				+	8	
4	Yarrangobilly River					+	+	+
5	Yarrangobilly River					+	+	
6	Yarrangobilly River		2 [2, 2]			+	+	
7	Wallace's Creek			1		+	+	



Figure 9. Two individuals of *Galaxias* sp. collected at Murrumbidgee DS (site 1), collected on 2/05/2018.

Image: Tarmo A. Raadik.



Figure 10. Two individuals of *Galaxias* sp. collected at Murrumbidgee US (site 2), collected on 2/05/2018.

Image: Tarmo A. Raadik.

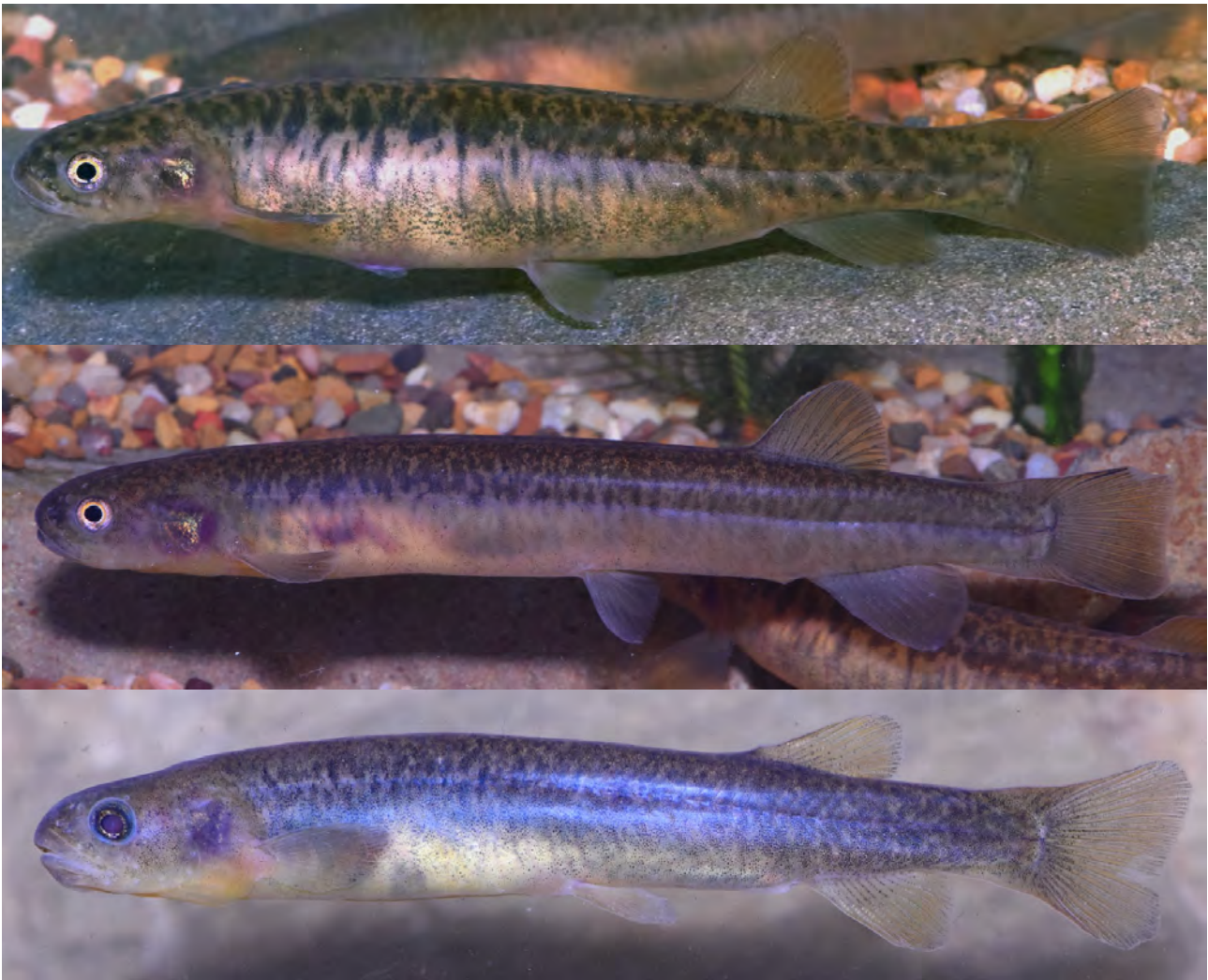


Figure 11. Three individuals of *Galaxias tantangara*, previously collected from Tintangara Creek (not collected during the current study, see red circle in Figure 1).

Image: Tarmo A. Raadik.

3.2.2 Morphological comparison

Detailed morphometric measurements were conducted on 12 individuals, and meristic counts on 15 individuals, from the two galaxiid populations in the Murrumbidgee River (site 1 and site; Figure 5). From here on these two sites will be referred to as Murrumbidgee DS (for site 1) and Murrumbidgee US (for site 2). Morphometric and meristic data for *Galaxias tantangara*, *Galaxias supremus*, and meristic data only for *Galaxias olidus* from seven nearby populations (see Table 2), were taken from Raadik (2014).

3.2.2.1 Morphometrics

Detailed morphometric data, expressed as % ratios, are provided for *Galaxias tantangara*, *Galaxias supremus*, and Murrumbidgee DS and US in Appendices 2–5. The morphometric characters which differed between the two Murrumbidgee populations (DS & US) and *Galaxias tantangara* and/or *G. supremus* are included in Table 5 and highlighted in yellow, and those which differed between Murrumbidgee DS and Murrumbidgee US are highlighted in orange.

Table 5. Morphometric character ratios differing between *Galaxias* sp. from two sites on the Murrumbidgee River (DS and US) and *Galaxias tantangara* and *G. supremus*.

Values are percentages of denominators in ratios. Differences in character ratios between one or both Murrumbidgee populations and either described species highlighted in yellow; differences in character ratios between the Murrumbidgee populations highlighted in orange. All morphometric data is presented for each group in Appendices 3–6.

Character	<i>G. tantangara</i>			Murrumbidgee DS			Murrumbidgee US			<i>G. supremus</i>		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
BDV / SL	14.1	12.6	15.6	10.9	9.8	12.1	12.2	10.6	13.1	10.1	8.4	12.4
BDPec / SL	16.6	14.9	17.9	13.2	11.8	15.1	14.5	13.9	15.5	13.6	11.8	16.4
BDPec / BDV	117.8	110.8	126.4	121.6	108.9	136.7	119.8	109.0	144.2	134.3	120.2	157.5
DCP / LCP	71.0	61.1	83.0	56.6	38.2	64.2	61.2	52.4	75.4	65.0	52.5	78.4
LCP/CFFL	95.8	81.0	107.6	100.6	84.2	120.5	96.6	78.3	128.0	85.1	71.6	101.0
DF–AF / LDB	45.0	25.9	84.2	68.6	54.7	90.6	70.3	54.4	93.5	85.0	66.0	121.3
LDB / LAB	91.2	79.9	100.0	90.3	76.7	111.8	96.0	83.1	112.8	95.4	79.8	108.7
DL / LDB	156.5	140.7	166.9	173.7	157.0	200.6	167.4	145.4	179.0	149.7	123.8	181.8
AL / LAB	149.7	136.0	171.7	170.0	151.0	208.9	168.4	155.4	202.9	144.1	128.1	169.4
PelL / PecL	84.2	75.8	91.7	92.0	83.0	100.3	84.5	71.6	90.4	87.5	79.7	96.6
PrePel / SL	51.7	49.5	54.2	20.7	19.5	21.8	21.6	20.6	22.7	53.2	49.7	55.6
PecL / PecPel	40.0	36.4	45.6	35.4	29.5	39.9	44.9	35.6	51.5	41.0	35.7	48.4
PelL / PelAn	45.7	41.1	52.4	45.1	39.3	50.7	50.9	41.2	62.8	47.0	39.7	56.5
HL / PelAn	92.9	79.3	103.1	89.9	79.2	103.3	95.3	81.4	114.8	91.5	78.5	109.9
HW / HL	68.3	63.4	72.8	58.8	54.6	61.7	61.6	56.9	66.5	63.0	59.4	67.5
HD / HL	44.7	41.4	48.2	37.9	35.4	42.4	38.7	34.9	41.8	40.4	36.5	49.1
HW / HD	152.9	142.8	165.3	155.3	140.9	167.5	159.5	150.0	174.0	156.6	136.9	174.4
SnL / ED	153.1	129.1	174.8	136.7	109.2	166.5	140.0	119.3	158.5	186.0	138.0	251.1
ED / HL	18.7	17.5	20.5	19.5	16.1	21.8	21.3	20.1	22.6	16.6	13.1	20.7
ED / HD	41.8	37.0	49.0	51.6	44.5	57.8	55.3	48.3	64.9	41.0	33.2	49.0
PoHL / HL	55.9	51.3	58.0	54.1	47.8	57.2	51.6	48.4	54.6	55.6	52.4	58.5
IOW / HL	37.5	34.6	40.9	35.6	32.4	38.9	38.3	35.6	41.6	38.0	34.1	40.2
ED / IOW	49.9	43.5	55.7	54.9	48.5	60.8	55.7	51.5	63.5	43.6	35.9	56.5
UJL / HL	38.1	35.4	42.7	34.6	32.4	36.6	37.4	34.8	39.8	37.0	31.6	42.1
LJL / HL	33.8	31.5	38.3	31.8	24.3	34.9	31.9	29.5	33.5	32.3	28.3	35.3
GW /HL	44.7	40.2	51.0	35.8	31.8	41.0	39.4	36.3	43.3	38.4	33.0	42.9
LJL / UJL	88.8	83.5	94.4	91.9	74.9	101.1	85.3	80.6	88.5	87.4	81.2	93.0
LJL / GW	75.8	67.8	82.4	89.4	69.7	109.7	81.1	74.7	87.0	84.5	76.6	93.3
GW / HW	65.4	59.6	72.2	60.8	52.9	67.1	64.1	59.4	71.8	61.0	50.7	67.0
SnL / UJL	74.9	71.1	86.1	76.8	63.3	85.4	79.5	71.3	87.8	81.5	72.1	89.8

The galaxiids from the Murrumbidgee River differed between site 1 (DS) and site 2 (US) by 3% or more in 26 and 24 mean character ratios respectively, based on the 26 characters measured, to *Galaxias tantangara* and *G. supremus*, respectively (Table 5), indicating that their external structural morphology differed to that described in these two species. Some of these differences are relatively small, and probably reflect the influence of local environmental factors on growth, whilst others are more pronounced and therefore most likely have taxonomic relevance.

Individuals from Murrumbidgee DS compared to US were also found to differ to each other in 17 mean character ratios, indicating external morphological differences between the fish at these two sites. This was unexpected given that these two populations of individuals are in the same river systems and are located only 20 km stream distance apart, though connectivity between them is now presumably blocked in an upstream direction by the wall of Tantangara Dam.

Differences in external morphology are also evident between the live images of individuals of Murrumbidgee DS & US and *Galaxias tantangara* (Figures 9–11), and between the two Murrumbidgee populations (Figures 9–10).

3.2.2.2 Meristics

Summarised meristic counts for seven characters are provided for the two Murrumbidgee populations (DS & US), and for *Galaxias tantangara* and *G. supremus* in Table 6. The frequency data for each character for these species, including for *Galaxias olidus* from local populations, is provided in Tables 7–11. The meristic characters which differed between the two Murrumbidgee populations (DS & US) and *Galaxias tantangara* and/or *G. supremus* are highlighted in yellow in Table 6, and those which differed between Murrumbidgee DS and Murrumbidgee US are highlighted in orange. The number of principal caudal fin rays in south-east Australian galaxiids, particularly in the *Galaxias olidus* complex, is relatively invariant, with all species possessing a mean of 16 rays. Therefore, this character is taxonomically unimportant.

Murrumbidgee DS differed to *Galaxias tantangara* based on four of the six variable meristic characters (dorsal fin rays, pectoral fin rays, gill rakers and number of vertebrae; Table 6). Murrumbidgee US also differed to *G. tantangara* in the number of dorsal fin rays, pectoral fin rays and gill rakers, the mean number of vertebrae was not different, except the modal count (Table 6). Murrumbidgee DS and US both differed to *Galaxias supremus* based on four of the six variable meristic characters (dorsal fin rays, pelvic fin rays, gill rakers and number of vertebrae) (Table 6). These are substantial differences between the two described species and the Murrumbidgee galaxiid populations.

Murrumbidgee DS was meristically similar to Murrumbidgee US, except one more vertebra was noted in some individuals from Murrumbidgee US. Vertebral number is a relatively stable character in between species in the *Galaxias olidus* complex (Raadik 2014).

Table 6. Summary of counts for the seven primary meristic characters assessed.

Character counts differing between the two Murrumbidgee galaxiid populations and the two described galaxiid species are highlighted in yellow. Gill raker values are total count only. Data for *Galaxias tantangara* and *G. supremus* are from Raadik (2011, 2014).

Species	Character	Dorsal Rays	Anal Rays	Caudal Rays	Pectoral Rays	Pelvic Rays	Gill Rakers	Vertebrae
<i>Galaxias tantangara</i>	Median	10	11	16	14	7	10	54
	Mode	10	11	16	14	7	10	54
	Mean	9.7	10.9	15.9	13.9	7.0	10.3	54.1
	Range	8–11	10–12	15–16	13–15	7	9–12	53–56
Murrumbidgee DS	Median	9	11	16	15	7	12	53
	Mode	9	11	16	15	7	11	53
	Mean	9.1	10.5	15.7	15.1	6.9	11.9	52.9
	Range	8–10	9–11	14–17	14–16	6–7	10–14	50–55
Murrumbidgee US	Median	9	11	16	15	7	12	54
	Mode	9	11	16	15	7	11	55
	Mean	9.2	10.6	16.0	15.1	7.0	12	54.3
	Range	8–10	10–12	16	14–16	7	11–14	53–56
<i>Galaxias supremus</i>	Median	10	11	16	15	8	13	56
	Mode	10	11	16	15	8	13	57
	Mean	9.9	10.8	15.8	15.4	8.0	12.2	56.0
	Range	9–11	10–12	15–16	14–17	7–9	10–14	52–59

The differences between meristic characters in Murrumbidgee DS & US and *Galaxias tantangara* and *G. supremus* are also evident in the following character frequency Tables 7–11. These tables also include frequency counts for characters in *Galaxias olidus* populations from nearby in the upper Murrumbidgee catchment (IV,10) and the adjacent upper Murray catchment (IV,01).

Table 7. Frequency distribution of total segmented Dorsal Fin and Anal Fin rays (branched and unbranched).

Modal frequency highlighted in bold.

Species	Dorsal Fin Rays				Anal Fin Rays						
	8	9	10	11	6	7	8	9	10	11	12
<i>Galaxias supremus</i>	-	9	16	7	1	-	10	14	7	-	
<i>Galaxias tantangara</i>	1	4	8	2	-	-	4	7	3	-	
Murrumbidgee DS	5	6	4	-	-	-	-	1	6	8	-
Murrumbidgee US	1	9	5	-	-	-	-	-	7	7	1
<i>Galaxias olidus</i> (IV,10)	-	6	32	20	-	-	-	1	8	33	15
Burns Crk	-	-	9	10	-	-	-	-	3	11	4
Flea Crk	-	5	8	4	-	-	-	-	2	12	3
Happy Jacks Crk	-	1	8	3	-	-	-	1	3	4	4
Larrys Crk	-	-	7	1	-	-	-	-	-	6	4
<i>Galaxias olidus</i> (IV,01)	2	16	14	2	-	-	-	-	14	16	4
Bogong Crk	1	5	7	2	-	-	-	-	3	9	3
L. Cootaputamba	-	1	1	-	-	-	-	-	1	1	-
Three Rocks Crk	1	10	6	-	-	-	-	-	10	6	1

The modal frequency of the dorsal fin ray counts in Murrumbidgee DS & US are like those in *G. olidus* from the upper Murray (IV,01) but one lower than for *G. olidus* in the upper Murrumbidgee (IV,10) (Table 7). Similarly, the modal frequency of the pelvic fin ray counts in Murrumbidgee DS & US are like those in *G. olidus* from the upper Murray (IV,01) but one lower than for *G. olidus* in the upper Murrumbidgee (IV,10) (Table 8).

Table 8. Frequency distribution of total segmented Pectoral Fin and Pelvic Fin rays (branched and unbranched).

Median frequency highlighted in bold.

Species	Pectoral Fin Rays						Pelvic Fin Rays			
	12	13	14	15	16	17	6	7	8	9
<i>Galaxias supremus</i>	-	-	3	17	11	2	-	1	31	1
<i>Galaxias tantangara</i>	-	4	9	2	-	-	-	15	-	-
Murrumbidgee DS	-	-	3	8	4	-	1	14	-	-
Murrumbidgee US	-	-	3	7	5	-	-	15	-	-
<i>Galaxias olidus</i> (IV,10)	1	5	6	24	19	5	2	23	32	-
Burns Crk	-	-	1	7	8	3	-	2	16	1
Flea Crk	-	-	-	13	4	-	-	14	3	-
Happy Jacks Crk	1	5	5	1	-	-	2	9	1	-
Larrys Crk	-	-	-	3	5	2	-	-	12	-
<i>Galaxias olidus</i> (IV,01)	-	-	2	14	14	4	-	23	11	-
Bogong Crk	-	-	1	7	6	1	-	15	-	-
L. Cootaputamba	-	-	1	1	-	-	-	2	-	-
Three Rocks Crk	-	-	-	6	8	3	-	6	11	-

The length of pyloric caecae are shorter in Murrumbidgee DS & US than in *Galaxias tantangara* and *G. supremus*, and the number of caecae in Murrumbidgee DS & US is also lower (Table 9). Neither Murrumbidgee population matches the frequency distribution of caecae in *Galaxias olidus* from the upper Murray (IV,01) or upper Murrumbidgee (IV,10) (Table 9).

The modal frequency of total gill rakers in Murrumbidgee DS & US is similar (11) but different to that (12) in *Galaxias olidus* from the upper Murray (IV,01) and upper Murrumbidgee (IV,10) (Table 10).

Table 9. Frequency distribution of Principal Caudal Fin Rays and Pyloric Caecae, including mean and range of caecal length (as % standard length).

Median frequency highlighted in bold; *N* – number.

Species	Caudal Rays							Pyloric C.		<i>N</i>
	14	15	16	17	0	1	2	Mean	Range	
<i>Galaxias supremus</i>	1	3	29	-	-	4	18	4.6	2.7–7.9	22
<i>Galaxias tantangara</i>	-	1	14	-	-	1	14	4.7	3.6–6.1	15
Murrumbidgee DS	2	1	11	1	1	8	3	0.9	0.1–1.6	13
Murrumbidgee US	-	-	15	-	8	6	0	0.7	0.3–1.3	6
<i>Galaxias olidus</i> (IV,10)	1	1	55	1	-	5	19			
Burns Crk	-	1	18	-	-	-	3			
Flea Crk	-	-	16	1	-	1	2			
Happy Jacks Crk	1	-	11	-	-	1	7			
Larrys Crk	-	-	10	-	-	3	7			
<i>Galaxias olidus</i> (IV,01)	-	3	29	2		5	2			
Bogong Crk	-	1	14	-	-	2	1			
L. Cootaputamba	-	-	2	-	-	-	1			
Three Rocks Crk	-	2	13	2	-	3	-			

Table 10. Frequency distribution of total number of Gill Rakers on the first gill arch, and lower and upper raker counts.

Modal frequency highlighted in bold.

Species	Total							Lower				Upper			
	9	10	11	12	13	14	7	8	9	10	11	1	2	3	4
<i>Galaxias supremus</i>	-	2	5	11	14	1	-	2	18	3	-	-	8	21	4
<i>Galaxias tantangara</i>	2	8	4	1	-	-	1	10	4	-	-	1	12	2	-
Murrumbidgee DS	-	1	6	2	5	1	1	6	6	1	1	-	-	11	4
Murrumbidgee US	-	-	6	4	4	1	-	6	8	1	-	-	1	8	6
<i>Galaxias olidus</i> (IV,10)	-	5	9	27	15	2	-	9	39	10		-	12	37	10
Burns Crk	-	3	6	8	2	-	-	5	12	2	-	-	8	12	-
Flea Crk	-	-	1	8	7	1	-	1	14	2	-	-	-	10	7
Happy Jacks Crk	-	2	2	7	1	-	-	3	7	2	-	-	4	8	-
Larrys Crk	-	-	-	4	5	1	-	-	6	4	-	-	-	7	3
<i>Galaxias olidus</i> (IV,01)	-	1	12	14	7	-	1	7	24	3	-	1	7	22	5
Bogong Crk	-	1	8	4	2	-	1	6	8	1	-	1	4	10	1
L. Cootaputamba	-	-	2	-	-	-	-	-	2	-	-	-	2	-	-
Three Rocks Crk	-	-	2	10	5	-	-	1	14	2	-	-	1	12	4

Finally, the modal count for vertebral number differs between Murrumbidgee DS & US (53 and 55 respectively), with that for Murrumbidgee US matching *Galaxias olidus* in the upper Murray (IV,01) (Table 11). The modal count for *G. olidus* in the upper Murrumbidgee (IV,10) is bimodal, with peaks at 53 and 57 vertebrae, corresponding to two populations each (Flea Crk and Happy Jacks Crk, and Burns Crk and Larrys Crk, respectively).

Table 11. Frequency distribution of Vertebrae (total).

Modal frequency highlighted in bold.

Species	Vertebrae										
	50	51	52	53	54	55	56	57	58	59	
<i>Galaxias supremus</i>	-	-	1	3	7	5	7	13	7	1	
<i>Galaxias tantangara</i>	-	-	-	3	8	3	1	-	-	-	
Murrumbidgee DS	1	1	3	6	2	2	-	-	-	-	
Murrumbidgee US	-	-	-	3	5	6	1	-	-	-	
<i>Galaxias olidus</i> (IV,10)	2	8	9	11	4	2	1	11	7	4	
Burns Crk	-	-	-	-	-	-	1	4	6	2	
Flea Crk	-	4	5	4	3	-	-	-	-	-	
Happy Jacks Crk	2	4	4	7	1	2	-	-	-	-	
Larrys Crk	-	-	-	-	-	-	-	7	1	2	
<i>Galaxias olidus</i> (IV,01)	-	-	-	5	9	10	8	3	1	-	
Bogong Crk	-	-	-	5	5	4	1	-	-	-	
L. Cootaputamba	-	-	-	-	2	-	-	-	-	-	
Three Rocks Crk	-	-	-	-	2	6	7	3	1	-	

3.2.2.3 Additional characters

A characteristic of *Galaxias tantangara* is the presence of a dark trunk pattern which extends over the dorsal and lateral surfaces of the head, extending down the cheeks, gill covers, snout and upper jaw, and sometimes onto the ventral surface (Figure 12). This was absent from all individuals examined from Murrumbidgee DS & US.



Figure 12. Detail of distinctive head pattern in *Galaxias tantangara*.

From Raadik (2014).

Midlateral bars were present on Murrumbidgee US (Figure 10), but absent from Murrumbidgee DS (Figure 9). Midlateral bars are absent from *Galaxias tantangara* (Figure 10, Appendix 1), and from *Galaxias supremus* (Appendix 1), however, *Galaxias olidus* can have either state.

The number of pyloric caecae can be a valuable, though secondary, taxonomic character in the *Galaxias olidus* complex, with five groups of species defined on the number of caecae present (Raadik 2014). Murrumbidgee DS conforms to Group 2, normally lacking caecae or possessing one, and Murrumbidgee US conforms to Group 4, along with *Galaxias supremus*, and *Galaxias olidus*, in possessing 1–2 caecae (Table 12). *Galaxias tantangara* is the only species from this group always with 2 pyloric caecae.

Table 12. Group membership based on the number of pyloric caecae.

Species	Caecae	Group 1 (0)	Group 2 (0 or 1)	Group 3 (1)	Group 4 (1 or 2)	Group 5 (2)
Murrumbidgee US			+			
Murrumbidgee DS					+	
<i>Galaxias supremus</i>					+	
<i>Galaxias olidus</i>					+	
<i>Galaxias tantangara</i>						+

Galaxiids in the *Galaxias olidus* complex can also be placed into five groups based on general body shape (Raadik 2011). Murrumbidgee DS & US conform to group 3, being moderately stout, as does *Galaxias olidus*. In comparison, *Galaxias supremus* is slender and elongate (group 2), and *G. tantangara* is stout (Group 5) (Table 13).

Table 13. Group membership based on general body shape.

Species	Caecae	Group 1 Diminutive and tubular	Group 2 Slender and elongate	Group 3 Moderately stout	Group 4 Moderately stout and elongate	Group 5 stout
<i>Galaxias supremus</i>			+			
Murrumbidgee US				+		
Murrumbidgee DS				+		
<i>Galaxias olidus</i>				+		
<i>Galaxias tantangara</i>						+

An accessory lateral line, and paired fin ray lamellae on pelvic and pectoral fins was present on individuals from Murrumbidgee DS & US. These characters are present in *Galaxias olidus* and *G. supremus*, and the accessory lateral line is present in *G. tantangara*, and lamellae may or may not be present (Raadik 2014). Therefore, these characters have no value in discriminating between these species.

3.2.3 Species identification

Murrumbidgee DS and Murrumbidgee US individuals were compared to the published key for species in the *Galaxias olidus* complex in Raadik (2014) (See Appendix 6). Neither keyed out as *Galaxias tantangara* or *Galaxias supremus* (Appendix 6). Both keyed out to *Galaxias olidus*, though did not match all characters in the couplet, differing in the number and length of pyloric caeca (Table 14). This, however, may be the result of the key not being as accurate when used at a more local (i.e. within the Murrumbidgee catchment) level,

as the couplet for *Galaxias olidus* was developed representing the species across its extensive range in south-eastern Australia from South Australia north-east to southern Queensland (Raadik 2014).

Table 14. Group membership based on general body shape.

Species	Progression steps through the key	Comment
Murrumbidgee DS	1), 2), 3).. <i>Galaxias olidus</i>	Does not fully match all characters in the couplet: specimens usually have 1 short pyloric caecum, <i>G. olidus</i> usually has 1–2 of medium length.
Murrumbidgee US	1), 4), 7), 8), 11), 12), 14), 15)... <i>Galaxias olidus</i>	Does not fully match all characters in the couplet: specimens usually have 0–1 short pyloric caecae, <i>G. olidus</i> usually has 1–2 of medium length.

The Murrumbidgee DS & US individuals were also visually compared against the detailed description for *Galaxias tantangara* and *Galaxias supremus* in Raadik (2014) (reproduced in Appendix 1) and were found to match each species poorly.

Therefore, the large difference between Murrumbidgee DS & US individuals and the diagnosis and detailed taxonomic description of *Galaxias tantangara* and *Galaxias supremus*, supported by the numerous differences between them in morphological (morphometric measurements and meristic counts) characters, confirms that they are not either described species, and that they conform, most closely to *Galaxias olidus*.

4 Discussion

The collection and detailed morphological examination of specimens of galaxiids in the *Galaxias olidus* species complex from two sites in the upper Murrumbidgee River, upstream and downstream of Tantangara Dam, has confirmed that neither population is *Galaxias tantangara* or the nearby, narrow-range endemic, *Galaxias supremus*. This is based on detailed examination of external body characteristics and counts of serially repeating external and internal body structures, such as fin rays, gill rakers and vertebrae. These were then compared against the taxonomic key and descriptions for *G. tantangara* and *G. supremus*, including summarised data on the morphological variables. Many inconsistencies with morphological characters between the Murrumbidgee galaxiid populations and the described species, including with the detailed taxonomic descriptions, were found, which supports this conclusion.

An additional biological feature which supports these populations as not *G. tantangara* and *G. supremus* is that they were found occurring with the non-native aquatic predators Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*). Neither galaxiid species is able to coexist with trout (Raadik 2014).

The identity of the galaxiid specimens collected previously by Cardno in early 2018 from the Yarrangobilly River just upstream of Talbingo Reservoir (Figure 4), could not be confirmed during this study as additional specimens were not re-collected. Specimens from the previous collection were not retained, however, based on the relative position of the origin of the dorsal and anal fins, and the length/depth of the body (stocky), of the individuals in the digital image (Figure 4), the species conforms to the *Galaxias olidus* complex, and is not the Climbing Galaxias (*Galaxias brevipinnis*).

The galaxiids at the two Murrumbidgee River sites were also found to differ morphologically to each other in many morphometric characters, and in one meristic character (number of vertebrae). They were also found in different habitats, with fish from the upstream population in shallower water (<0.2 m depth), with individuals amongst cobbles in faster flowing areas, compared to amongst structural habitat (timber debris and rock) and vegetation along margins of pools in the downstream population. This amount of variation was unexpected, given that captured individuals at the two sites are geographically very close, with only 20 km of stream distance (~ 11 km in a straight line) between them. Nevertheless, Tantangara Dam is located between these sites, and therefore connectivity has been severely reduced, or stopped, in the last 60 years since the dam was built. This, however, is too short a timeframe to expect genetic divergence to affect fish behaviour or diverge internal and external morphologies to such a degree.

The fish from these two sites conform more closely to the published re-description of *Galaxias olidus* (Raadik 2014), though they also vary in many morphological characteristics. This may be the result of the taxonomic key and description of *Galaxias olidus* being developed for the species across its large range in south-eastern Australia, and hence being less useful when substantial variation is present at a local scale. This is supported by *Galaxias olidus* being the most widely morphologically-variable taxon in the *Galaxias olidus* complex following the revision of its taxonomy from one species into 15 (Raadik 2014).

The galaxiids from the two Murrumbidgee River sites were also compared meristically to *Galaxias olidus* populations in alpine areas of the upper Murrumbidgee River system, and upper Murray River system further to the south-west. Overall, the fish from the two Murrumbidgee sites in this study varied in some meristic characters to fish from these other populations. This is not unexpected, at least for those comparison populations from the upper Murray catchment, as these are geographically distant, and in a different river basin to the Murrumbidgee fish. However, the comparison Murrumbidgee populations (Happy Jacks, Burns and Larrys creeks) are also in the Murrumbidgee River system, and ~13–25 km distant (in a straight line), from the two Murrumbidgee galaxiid sites in this study, and should therefore be morphologically more similar. These comparison populations, however, are all in the Tumut River catchment, and over 600+ km stream distance from the upper Murrumbidgee River galaxiid sites. This distance, suggestive of a high degree of genetic isolation in fish with poor dispersal ability (e.g. non-migratory), may explain the amount of morphological difference between these two groups of galaxiid populations, if the only mode of gene exchange between these two geographically close catchments which are widely separate by stream distance is along the stream system.

This seems logical for fish which are restricted to aquatic environments, though many species in the *Galaxias olidus* complex, including *Galaxias olidus*, possess fin ray lamellae on their pectoral and pelvic fins which assist them in climbing over and up wet rocks, out of the water (McDowall 2003b, Green 2008, Raadik 2014). This implies the potential ability to cross catchment boundaries during wet periods. Fin ray lamellae were present in the two Murrumbidgee galaxiid populations, and in the comparative populations in the Tumut catchment. Therefore, cross-catchment movement of individuals is a limited possibility which may not result in much morphological divergence between the two groups of populations.

A further explanation of the degree of divergence found between the two groups of galaxiid populations in the upper Murrumbidgee catchment may be the presence of additional, but unidentified species in the *Galaxias olidus* complex, and possible the presence of hybrid individuals between them and *Galaxias olidus*. As highlighted by Raadik (2014), the *Galaxias olidus* species complex has been taxonomically only partly resolved in the streams in the Snowy, Murrumbidgee and upper Murray river catchments in the Snowy Mountains Region/Kosciuszko National Park, and this area therefore potentially harbours additional, undescribed and narrow range endemic species. Lastly, this area of south-eastern Australia may contain additional, previously unrecorded levels of morphological diversity within *Galaxias olidus*.

Therefore, whilst the galaxiids from the two sites in the Murrumbidgee River are confirmed as part of the *Galaxias olidus* complex, they are tentatively considered *Galaxias olidus*. More definitive identification cannot be provided based on morphology alone of the currently available specimens. To resolve this issue, a more thorough collection of galaxiid material from across this specific sub-alpine to alpine area would be required, followed by detailed morphological study in combination with appropriate species-level genetic analysis (e.g. following Raadik 2011, Adams et al. 2014, Raadik 2014).

4.1.1 Climbing Galaxias

Two individuals of *Galaxias brevipinnis* were recorded for the first time from the Yarrangobilly River system. Whilst a native species, it is not indigenous to the catchments of the Murray-Darling River system, except in the lower Murray near Adelaide, and appear to have been introduced into the upper Murray catchment via the Snowy Hydro scheme (Morison and Anderson 1991, Waters et al. 2002).

Similarly, this species may have also be introduced into the Murrumbidgee River system via the Snowy Scheme, as it was first recorded from the Murrumbidgee River catchment in March 2002 (Raadik 2003). This site, in a tributary of Blowering Reservoir, is in the Tumut River catchment, as is the Yarrangobilly River site, but much further downstream than the Yarrangobilly River.

5 Conclusion

Based on the capture, and morphological examination, of specimens, this project was able to confirm that the threatened native fish *Galaxias tantangara*, and the native narrow-range species *Galaxias supremus*, were not identified at the two sites sampled in the upper Murrumbidgee River, in the area of the proposed Snowy 2.0 project. The galaxiids collected from the two sites are part of the *Galaxias olidus* cryptic species complex and are tentatively identified as *Galaxias olidus*. However, due to morphological variation between both sites, the nearby *Galaxias olidus* populations, and some differences to the published description of *Galaxias olidus*, it is likely that previously unrecognised levels of within species (*G. olidus*) variation, or additional, undiscovered species, may be present. This can only be resolved by undertaking genetic, and more detailed morphological, study.

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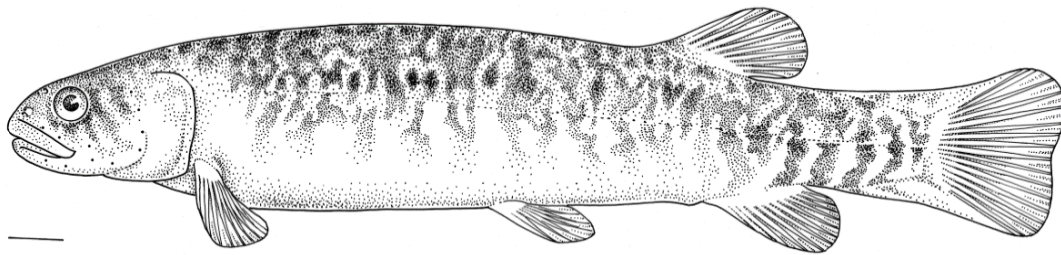
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Appendices

Appendix 1. Diagnosis and line drawing of A) Stocky Galaxias and B) Kosciuszko Galaxias from the original description (Raadik 2014).

Stocky Galaxias *Galaxias tantangara* Raadik, 2014

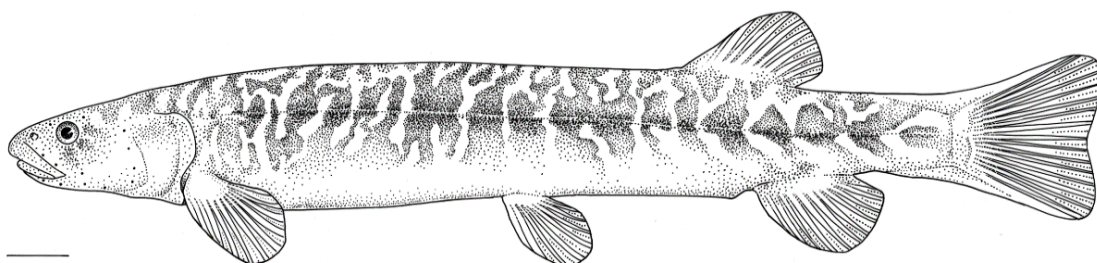
A)



Diagnosis: differs from all other species within the *Galaxias olidus* complex by a combination of the following characters: low mean total gill raker count of 10; body distinctly stocky and deep through vent and pectoral fin base (12.6–15.6 and 14.9–17.9 % SL); caudal peduncle deep (8.5–9.7 % SL); head obtuse to slightly bulbous in lateral profile, moderately deep (41.4–48.2 % HL) but wide (63.4–72.8 % HL); gape wide (40.2–51.0 % HL and 59.6–72.2 % HW); eye profiles usually not visible laterally from ventral view; nostrils short, not visible from ventral view; caudal fin weakly emarginate to truncate, about as long or slightly longer than caudal peduncle, vertical width of expanded rays usually equal to body depth through pectoral fin base; caudal peduncle flanges long, reaching more than half distance to anal fin base; anal fin long (16.3 % SL); most posterior extent of mouth 0.8 ED below ventral margin of eye; dorsal midline usually flattened anteriorly from above or slightly posterior to pectoral fin bases; raised lamellae absent from ventral surface of rays of paired fins; anal fin origin usually under 0.73 distance posteriorly along dorsal fin base; usually 2, occasionally 1, relatively thin and long (4.7 % SL) pyloric caecae; gill rakers short to very short; and, lack of distinct black bars along lateral line.

Kosciuszko Galaxias *Galaxias supremus* Raadik, 2014

B)



Diagnosis:

.... differs from all other species within the *Galaxias olidus* complex by a combination of the following characters: 8 segmented pelvic fin rays; very high mean vertebral count of 57, though range broad (52–59); mouth distinctly subterminal; dorsal, anal and pelvic fin origins set far back along trunk (70.4–75.1, 74.8–80.2 and 49.7–55.6 % SL respectively); distance between pectoral and pelvic fin bases long (27.8–35.7 % SL); body depth shallow (8.4–12.4 % SL) and much greater through pectoral fin base (11.8–16.4 % SL) than that through vent; caudal peduncle very short and shallow (10.0–12.7 and 6.3–8.9 SL respectively) and caudal fin much longer (120 %) than caudal peduncle; snout long (26.7–34.9 % HL) and eye small (13.1–20.7 % HL); nostrils short, not visible from ventral view; anal fin base short (8.7–10.9 % SL); dorsal and anal fins short (12.6–15.9 and 13.1–15.9 % SL respectively); dorsal midline usually flattened anteriorly from above or slightly posterior to pectoral fin bases; posterior extent of mouth about 0.8 ED below ventral margin of eye; usually 2, occasionally 1, pyloric caecae of moderate length (4.6 % SL); gill rakers short to moderately long; anal fin origin usually under 0.85 distance posteriorly along dorsal fin base, the greatest setback in all members of the species complex; lack of distinct black bars along lateral line though, very occasionally, dark patches on dorsal midline may have a very small, black bar in the centre; and, distinctive, mottled colour pattern.

Appendix 2. Summarised morphometric variation in specimens of *Galaxias tantangara*, from Raadik (2014).

Values are percentages of denominators in ratios, except for LCF and SL; N = 14.

Character	Mean	Min.	Max.	S.D.
LCF (mm)	86.6	74.6	102.7	
SL (mm)	76.5	65.5	91.5	
SL / LCF	88.3	87.7	89.1	0.51
BDV / SL	14.1	12.6	15.6	0.85
BDPec / SL	16.6	14.9	17.9	0.73
BDPec / BDV	117.8	110.8	126.4	4.50
LCP / SL	12.7	11.3	14.2	0.79
DCP / SL	9.0	8.5	9.7	0.41
DCP / LCP	71.0	61.1	83.0	5.87
CFFL / SL	13.3	12.2	14.1	0.65
LCP/CFFL	95.8	81.0	107.6	6.91
PreD / SL	71.4	69.8	73.2	0.78
PreA / SL	75.7	74.4	76.9	0.68
PreD / PreA	94.3	92.3	96.5	1.28
DF-AF / LDB	45.0	25.9	84.2	14.29
LDB / SL	9.9	9.0	10.9	0.57
LAB / SL	10.9	9.8	12.1	0.65
LDB / LAB	91.2	79.9	100.0	6.55
DL / LDB	156.5	140.7	166.9	7.70
AL / LAB	149.7	136.0	171.7	10.25
DL / SL	15.5	14.1	16.1	0.64
AL / SL	16.3	14.5	17.3	0.75
DL / AL	95.3	89.5	98.0	2.69
PecL / SL	12.8	11.2	13.8	0.88
PelL / SL	10.7	10.0	11.4	0.40
PelL / PecL	84.2	75.8	91.7	4.86
PrePel / SL	51.7	49.5	54.2	1.52
PecPel / SL	31.9	30.4	35.2	1.46
PelAn / SL	23.5	21.7	26.3	1.17
PecL / PecPel	40.0	36.4	45.6	2.60
PelL / PelAn	45.7	41.1	52.4	3.19
HL / SL	21.7	20.6	22.7	0.69
HL / PelAn	92.9	79.3	103.1	6.05
HW / HL	68.3	63.4	72.8	2.70
HD / HL	44.7	41.4	48.2	2.11
HW / HD	152.9	142.8	165.3	6.00
SnL / HL	28.5	26.4	31.5	1.52
SnL / ED	153.1	129.1	174.8	11.42
ED / HL	18.7	17.5	20.5	0.95
ED / HD	41.8	37.0	49.0	2.86
PoHL / HL	55.9	51.3	58.0	1.86
IOW / HL	37.5	34.6	40.9	1.62
ED / IOW	49.9	43.5	55.7	3.23
UJL / HL	38.1	35.4	42.7	1.96
LJL / HL	33.8	31.5	38.3	1.74
GW /HL	44.7	40.2	51.0	3.01
LJL / UJL	88.8	83.5	94.4	2.71
LJL / GW	75.8	67.8	82.4	3.72
GW / HW	65.4	59.6	72.2	3.20
SnL/UJL	74.9	71.1	86.1	4.66

Appendix 3. Summarised morphometric variation in specimens of *Galaxias supremus*, from Raadik (2014).

Values are percentages of denominators in ratios, except for LCF and SL; N = 29.

Character	Mean	Min.	Max.	S.D.
LCF (mm)	81.1	60.9	96.1	
SL (mm)	71.5	53.8	85.4	
SL / LCF	88.2	86.5	89.2	0.58
BDV / SL	10.1	8.4	12.4	0.86
BDPec / SL	13.6	11.8	16.4	1.28
BDPec / BDV	134.3	120.2	157.5	9.48
LCP / SL	11.4	10.0	12.7	0.69
DCP / SL	7.4	6.3	8.9	0.61
DCP / LCP	65.0	52.5	78.4	5.61
CFFL / SL	13.4	12.1	15.7	0.74
LCP/CFFL	85.1	71.6	101.0	7.63
PreD / SL	72.5	70.4	75.1	1.10
PreA / SL	77.5	74.8	80.2	1.41
PreD / PreA	93.7	90.1	96.5	1.71
DF-AF / LDB	85.0	66.0	121.3	9.93
LDB / SL	9.4	7.6	11.4	0.86
LAB / SL	9.9	8.7	10.9	0.58
LDB / LAB	95.4	79.8	108.7	7.58
DL / LDB	149.7	123.8	181.8	13.12
AL / LAB	144.1	128.1	169.4	8.39
DL / SL	14.0	12.6	15.9	0.79
AL / SL	14.2	13.1	15.9	0.69
DL / AL	98.7	87.4	110.9	4.91
PecL / SL	13.1	11.5	14.6	0.68
PelL / SL	11.5	10.2	13.2	0.63
PelL / PecL	87.5	79.7	96.6	4.69
PrePel / SL	53.2	49.7	55.6	1.45
PecPel / SL	32.1	27.8	35.7	2.03
PelAn / SL	24.4	21.4	26.7	1.39
PecL / PecPel	41.0	35.7	48.4	3.13
PelL / PelAn	47.0	39.7	56.5	3.61
HL / SL	22.3	21.0	24.3	0.80
HL / PelAn	91.5	78.5	109.9	7.24
HW / HL	63.0	59.4	67.5	1.94
HD / HL	40.4	36.5	49.1	2.89
HW / HD	156.6	136.9	174.4	8.98
SnL / HL	30.2	26.7	34.9	1.72
SnL / ED	186.0	138.0	251.1	31.11
ED / HL	16.6	13.1	20.7	2.28
ED / HD	41.0	33.2	49.0	4.19
PoHL / HL	55.6	52.4	58.5	1.75
IOW / HL	38.0	34.1	40.2	1.39
ED / IOW	43.6	35.9	56.5	5.98
UJL / HL	37.0	31.6	42.1	1.99
LJL / HL	32.3	28.3	35.3	1.67
GW /HL	38.4	33.0	42.9	2.54
LJL / UJL	87.4	81.2	93.0	3.02
LJL / GW	84.5	76.6	93.3	4.65
GW / HW	61.0	50.7	67.0	3.99
SnL/UJL	81.5	72.1	89.8	3.80

Appendix 4. Summarised morphometric variation in specimens of *Galaxias* sp. collected from the Murrumbidgee River, downstream of Tantangara Dam (site 1 or Murrumbidgee DS).

Values are percentages of denominators in ratios, except for LCF and SL; N = 15.

Character	Mean	Min.	Max.	S.D.
LCF (mm)	65.4	49.0	82.9	
SL (mm)	58.2	43.0	72.4	
SL / LCF	89.0	87.32	90.26	0.01
BDV / SL	10.9	9.8	12.1	0.01
BDPec / SL	13.2	11.8	15.1	0.01
BDPec / BDV	121.6	108.9	136.7	0.09
LCP / SL	12.3	10.7	13.9	0.01
DCP / SL	6.9	5.1	7.8	0.01
DCP / LCP	56.6	38.2	64.2	0.08
CFFL / SL	12.3	10.8	14.5	0.01
LCP/CFFL	100.6	84.2	120.5	0.12
PreD / SL	70.2	68.4	71.8	0.01
PreA / SL	75.2	73.2	77.3	0.01
PreD / PreA	93.3	89.5	95.3	0.02
DF-AF / LDB	68.6	54.7	90.6	0.10
LDB / SL	8.4	6.5	9.6	0.01
LAB / SL	9.3	7.7	11.1	0.01
LDB / LAB	90.3	76.7	111.8	0.11
DL / LDB	173.7	157.0	200.6	0.13
AL / LAB	170.0	151.0	208.9	0.18
DL / SL	14.5	12.8	15.6	0.01
AL / SL	15.7	14.2	17.6	0.01
DL / AL	92.0	85.8	105.7	0.05
PecL / SL	11.6	10.4	13.3	0.01
PelL / SL	10.6	9.6	11.3	0.01
PelL / PecL	92.0	83.0	100.3	0.06
PrePel / SL	20.7	19.5	21.8	0.01
PecPel / SL	30.2	28.4	34.2	0.02
PelAn / SL	23.6	21.3	25.4	0.01
PecL / PecPel	35.4	29.5	39.9	0.03
PelL / PelAn	45.1	39.3	50.7	0.04
HL / SL	21.2	19.8	22.6	0.01
HL / PelAn	89.9	79.2	103.3	0.07
HW / HL	58.8	54.6	61.7	0.02
HD / HL	37.9	35.4	42.4	0.02
HW / HD	155.3	140.9	167.5	0.08
SnL / HL	26.5	22.8	28.8	0.02
SnL / ED	136.7	109.2	166.5	0.16
ED / HL	19.5	16.1	21.8	0.02
ED / HD	51.6	44.5	57.8	0.04
PoHL / HL	54.1	47.8	57.2	0.03
IOW / HL	35.6	32.4	38.9	0.02
ED / IOW	54.9	48.5	60.8	0.04
UJL / HL	34.6	32.4	36.6	0.02
LJL / HL	31.8	24.3	34.9	0.03
GW /HL	35.8	31.8	41.0	0.03
LJL / UJL	91.9	74.9	101.1	0.08
LJL / GW	89.4	69.7	109.7	0.11
GW / HW	60.8	52.9	67.1	0.04
SnL / UJL	76.8	63.3	85.4	0.06
PectB Gap/GW	171.4	145.9	218.8	0.18

Appendix 5. Summarised morphometric variation in specimens of *Galaxias* sp. collected from the Murrumbidgee River, upstream of Tantangara Dam (site 2 or Murrumbidgee US).

Values are percentages of denominators in ratios, except for LCF and SL; N = 12.

Character	Mean	Min.	Max.	S.D.
LCF (mm)	64.92	44.13	82.28	
SL (mm)	57.2	39.2	73.0	
SL / LCF	88.1	86.8	89.9	
BDV / SL	12.2	10.6	13.1	0.01
BDPec / SL	14.5	13.9	15.5	0.01
BDPec / BDV	119.8	109.0	144.2	0.00
LCP / SL	13.0	11.2	14.3	0.10
DCP / SL	7.9	7.1	8.4	0.01
DCP / LCP	61.2	52.4	75.4	0.01
CFFL / SL	13.5	11.2	15.2	0.06
LCP/CFFL	96.6	78.3	128.0	0.01
PreD / SL	70.2	68.6	72.7	0.13
PreA / SL	75.3	72.3	76.9	0.01
PreD / PreA	93.2	91.5	96.1	0.01
DF-AF / LDB	70.3	54.4	93.5	0.02
LDB / SL	8.8	8.1	10.0	0.12
LAB / SL	9.3	7.8	11.2	0.01
LDB / LAB	96.0	83.1	112.8	0.01
DL / LDB	167.4	145.4	179.0	0.10
AL / LAB	168.4	155.4	202.9	0.12
DL / SL	14.7	13.9	15.6	0.13
AL / SL	15.5	14.3	17.4	0.01
DL / AL	95.1	85.9	100.0	0.01
PecL / SL	13.9	12.3	15.4	0.04
PelL / SL	11.7	10.6	12.8	0.01
PelL / PecL	84.5	71.6	90.4	0.01
PrePel / SL	21.6	20.6	22.7	0.05
PecPel / SL	31.1	28.0	34.4	0.01
PelAn / SL	23.2	19.2	25.6	0.02
PecL / PecPel	44.9	35.6	51.5	0.02
PelL / PelAn	50.9	41.2	62.8	0.04
HL / SL	22.0	20.8	22.9	0.06
HL / PelAn	95.3	81.4	114.8	0.01
HW / HL	61.6	56.9	66.5	0.09
HD / HL	38.7	34.9	41.8	0.03
HW / HD	159.5	150.0	174.0	0.02
SnL / HL	29.7	27.0	32.8	0.07
SnL / ED	140.0	119.3	158.5	0.02
ED / HL	21.3	20.1	22.6	0.12
ED / HD	55.3	48.3	64.9	0.01
PoHL / HL	51.6	48.4	54.6	0.05
IOW / HL	38.3	35.6	41.6	0.02
ED / IOW	55.7	51.5	63.5	0.02
UJL / HL	37.4	34.8	39.8	0.04
LJL / HL	31.9	29.5	33.5	0.01
GW /HL	39.4	36.3	43.3	0.01
LJL / UJL	85.3	80.6	88.5	0.02
LJL / GW	81.1	74.7	87.0	0.02
GW / HW	64.1	59.4	71.8	0.04
SnL / UJL	79.5	71.3	87.8	0.03
PectB Gap/GW	106.8	99.5	116.9	0.05

Appendix 6. Key to species of *Galaxias* in the *Galaxias olidus* complex. Adapted from Raadik (2014). Species potentially found in the study area highlighted in yellow.

- 1 One or more distinct, black, very dark grey or dark brown, elongate vertical bars (as opposed to diffuse and irregular shaped brown, brownish grey to dark brown blotches), usually much longer than wide and not centred inside a mid-lateral blotch, present along the lateral midline.....2
- Mid-lateral bars not as above, or if present, usually small and black, circular or slightly ovoid (as opposed to medium or large, distinct and elongate).....4
- 2(1) Mid-lateral bars large, black, generally long and wide, ovoid to inverted tear-drop shape, often surrounded by a lighter halo, relatively widely spaced and located between the pectoral and pelvic fin bases; pattern usually lacking from dorsal surface of trunk; gape wide ($> 41\%$ HL); head length typically greater than PelAn distance (HL/PelAn ratio usually $> 106\%$); body often orange, orange-red or orange-brown... ***Galaxias fuscus* Mack, 1936**
- Mid-lateral bars not as above; pattern usually often present on dorsal surface of trunk; gape width $< 41\%$ HL; head length typically shorter than PelAn distance (HL/PelAn ratio usually $< 106\%$); body usually brown to yellow..3
- 3(2) Mid-lateral bar shape variable, from elongate and very narrow, ovoid to almost round, small to moderate sized, becoming paler (and grey or brown) posteriorly, bars often closely spaced and extending posteriorly well past pelvic fin base; LDB/LAB ratio $> 85\%$; DL/AL ratio $> 90\%$; anal fin origin typically under $> 55\%$ distance posteriorly along dorsal fin base; 1, more often 2, pyloric caeca of moderate length.....
- Bars very short and narrow, usually restricted to anterior portion of lateral midline between the pectoral and pelvic fin bases, typically on or within lighter bars; LDB/LAB ratio $< 80\%$; DL/AL ratio $< 90\%$; anal fin origin typically under $< 55\%$ distance posteriorly along dorsal fin base; usually a single, short pyloric caecum, often absent..... ***Galaxias olidus* Günther, 1866 (in part)**
- Bars very short and narrow, usually restricted to anterior portion of lateral midline between the pectoral and pelvic fin bases, typically on or within lighter bars; LDB/LAB ratio $< 80\%$; DL/AL ratio $< 90\%$; anal fin origin typically under $< 55\%$ distance posteriorly along dorsal fin base; usually a single, short pyloric caecum, often absent..... ***Galaxias oliros* (in part)**
- 4(1) Mouth distinctly subterminal (lower jaw shorter than upper).....5
- Mouth usually terminal or nearly so (jaws about equal or lower slightly shorter than upper).....7
- 5(4) Snout and upper lip extended anteriorly as thick and fleshy protrubence; lower jaw distinctly shorter than upper; ventral profile of body straight; usually 7 segmented pelvic fin rays; caudal peduncle long (mean of 16.4% SL); anal fin origin usually under 0.4 distance posteriorly along dorsal fin base (known from the upper Murray River and tributaries in inland VIC and southern NSW)..... ***Galaxias arcanus***
- Snout and upper lip not as above; lower jaw not as above but shorter than upper; ventral profile of body arched; usually 8 segmented pelvic fin rays; caudal peduncle of moderate length (mean $< 13.5\%$ SL); anal fin origin under 0.5 or greater distance posteriorly along dorsal fin base.....6
- 6(5) Pyloric caecae absent; body depth at vent moderate (12.7 (11.2 – 14.3) $\%$ SL); anal fin long based (11.7 (11.0 – 12.5) $\%$ SL); segmented pectoral fin rays usually 14, sometimes 15; anal fin origin usually under 0.5 distance posteriorly along dorsal fin base; usually 55 or fewer vertebrae (known from the upper Roger River, lower Snowy River system East Gippsland, coastal VIC)..... ***Galaxias mcdowalli***
- Usually 2 pyloric caecae, occasionally 1; body depth at vent shallow (10.1 (8.4 – 12.4) $\%$ SL); anal fin based of moderate length (9.9 (8.7 – 10.9) $\%$ SL); segmented pectoral fin rays usually 15 or more; anal fin origin usually under 0.8 distance posteriorly along dorsal fin base; usually 57 vertebrae (known from upper tributaries of the Snowy River on Mount Kosciuszko, Snowy River system, NSW)..... ***Galaxias supremus***
- 7(4) Caudal peduncle, caudal fin and pectoral fins short, usually $< 12\%$ SL (known from the upper Tuross River system in coastal SE NSW)..... ***Galaxias brevissimus***
- Caudal peduncle, caudal fin and pectoral fins usually $> 12\%$ SL (found outside of the Tuross River system).....8
- 8(7) Length of dorsal fin base usually equal or greater than length of anal fin base.....9
- Length of dorsal fin base usually less than ($< 97\%$) length of anal fin base.....11
- 9(8) PelAn distance $> 24.5\%$ SL; anal fin base usually $< 10\%$ SL; dorsal fin length usually $< 14.5\%$ SL; dorsal and anal fins set back along body usually at > 71 and $> 76\%$ SL respectively; head depth $< 38.5\%$ HL; postorbital head length $> 58\%$ HL; usually 53 (52–53) vertebrae (known from the upper Dargo River system in the coastal Gippsland region of VIC)..... ***Galaxias mungadhan***
- PelAn distance $< 24.5\%$ SL; anal fin base usually $> 10\%$ SL; dorsal fin length usually $> 14.5\%$ SL; dorsal and anal fins set back along body usually at < 71 and $< 76\%$ SL respectively; head depth $> 38.5\%$ HL; postorbital head length $< 58\%$ HL; usually 54 (54–55) vertebrae.....10
- 10(9) Usually 1, less often 2, pyloric caecae; dorsal and anal fin bases long, $> 11.0\%$ SL; eye diameter relatively small ($< 17.5\%$ HL) and $< 48\%$ IOW; gape relatively narrow ($< 35.5\%$ HL); gill rakers moderately long; usually 9–10 segmented dorsal fin rays and 14 segmented pectoral fin rays; (known from a tributary of the lower La Trobe River system in the coastal Gippsland region of VIC)..... ***Galaxias longifundus***
- Pyloric caecae usually absent; dorsal and anal fin bases short, $< 11.0\%$ SL; eye diameter relatively large ($< 17.5\%$ HL) and $> 48\%$ IOW; gape relatively broad ($> 35.5\%$ HL); gill rakers short; usually 10–11 segmented dorsal fin rays and 15 segmented pectoral fin rays; (known from a tributary of the lower Thomson River system in the coastal Gippsland region of VIC)..... ***Galaxias lanceolatus***
- 11(8) Body stocky and deep, depth at vent $> 13.5\%$ SL, depth through pectoral fin base $> 15.5\%$ SL; typically 10 gill rakers on first gill arch; caudal peduncle depth usually $> 8.8\%$ SL; gape width $> 43\%$ HL and $> 64\%$ HW (restricted to the very upper Murrumbidgee River system in inland NSW)..... ***Galaxias tantangara***

- Body elongate and shallow to moderately deep, depth at vent < 13.5 % SL, depth through pectoral fin base < 15.5 % SL; usually 12 or more gill rakers on first gill arch; caudal peduncle depth usually < 8.8 % SL; gape width < 43 % HL and < 64 % HW.....12
- 12(11) Snout relatively long, > 29.5 % HL and > 157 % ED.....13
- Snout short or of moderate length, < 29.5 % HL and < 157 % ED.....14
- 13(12) Segmented pelvic fin rays 8; typically 11 segmented anal fin rays; gill rakers on first arch 14 (13–15); pyloric caecae absent; nostrils normally just visible from ventral view (known from the Arte River, Bemm River system, East Gippsland, in coastal VIC).....***Galaxias aequipinnis***
- Segmented pelvic fin rays 7; typically 10 segmented anal fin rays; gill rakers on first arch 12 (11–12); usually 1, sometimes 2, pyloric caecae; nostrils not visible from ventral view (known from an upper tributary in the Macalister River system, Gippsland, in coastal VIC).....***Galaxias gunaikurnai***
- 14(12) Accessory lateral line absent; dorsal and ventral trunk profiles straight or nearly so; anterior nostrils long and visible from ventral view; segmented pectoral fin rays 13; eye large, > 20 % HL and ED/HD and ED/IOW ratios > 50 and > 55 % respectively; pelvic fins small (< 9.8 % SL) and PelL/PecL ratio < 80 %; mean vertebral count 51 (diminutive species, average size 45–55 mm LCF; known from the Genoa/Wallagaraugh, Cann, and mid-Snowy River systems East Gippsland, coastal VIC/NSW).....***Galaxias terenusus***
- Accessory lateral line present; dorsal and ventral trunk profiles relatively evenly arched; anterior nostrils short to moderately long and not visible from ventral view; segmented pectoral fin rays 14 or more; eye moderate, < 20 % HL and ED/HD and ED/IOW ratios < 50 and < 55 % respectively; pelvic fins moderate (> 9.8 % SL) and PelL/PecL ratio > 80 %; mean vertebral count usually > 52; (average size 60–90 mm LCF; distribution not as above).....15
- 15(14) Interorbital width < 40.5 % HL; DCP/LCP ratio > 61 %; caudal peduncle length < 13 % SL; rakers on first gill arch usually 12 and typically 15 segmented pectoral fin rays; 1–2 pyloric caecae of moderate length.....***Galaxias olidus* Günther, 1866 (in part)**
- Interorbital width > 40.5 % HL; DCP/LCP ratio < 61 %; caudal peduncle length > 13 % SL; rakers on first gill arch usually 13 or more and typically 14 segmented pectoral fin rays; usually 1 pyloric caecum or caecae absent, if present, short to moderate length.....16
- 16(15) Anal fin base long (> 11.0 % SL), distinctly longer than dorsal fin base (LDB/LAB ratio < 83 %); BDPeC/BDV ratio < 114 %; anal fin origin typically under < 55 % distance posteriorly along dorsal fin base; AL/LAB ratio < 147 %; Pel/PelAn ratio > 48 %; LJJL/GW ratio > 90 %; usually 1 very short pyloric caecum, less often absent; typically 14 gill rakers on first arch with 9 segmented dorsal fin, and 12 segmented anal fin rays; caudal fin emarginate to weakly forked (found north of the Great Dividing Range, and west of the Otway Coast basin in coastal VIC, extending into SA).....***Galaxias oliros* (in part)**
- Anal fin base of moderate length (< 11.0 % SL), usually slightly longer than dorsal fin base (LDB/LAB ratio > 83 %); BDPeC/BDV ratio > 114 %; anal fin origin typically under > 55 % distance posteriorly along dorsal fin base; AL/LAB ratio > 147 %; Pel/PelAn ratio < 48 %; LJJL/GW ratio < 90 %; pyloric caecae usually absent, less often 1 present, moderately long; typically 13 gill rakers on first arch with 10 segmented dorsal fin, and 11 segmented anal fin rays; caudal fin emarginate (known from catchments draining to the middle of Bass Strait in central, coastal VIC).....***Galaxias ornatus* Castelnau, 1873**

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Tantangara Creek fish barrier design criteria – Snowy 2.0 Project

T.A. Raadik

May 2019



Arthur Rylah Institute for Environmental Research
Unpublished Client Report for EMM Consulting

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Front cover photo: Climbing Galaxias (*Galaxias brevipinnis*), adult. (Tarmo A. Raadik).

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Summary

As part of the planning and environmental documentation required for the Snowy 2.0 Project, a consideration is protection of the threatened Stocky Galaxias (*Galaxias tantangara*) from the invasive native freshwater fish Climbing Galaxias (*Galaxia brevipinnis*). Climbing Galaxias are native to coast river systems and have previously invaded across the Great Dividing Range, establishing and expanding their range into the upper Murray River system, and the Tumut River, a tributary of the mid Murrumbidgee River. Climbing Galaxias are currently not present within the habitat of Stocky Galaxias in the upper Murrumbidgee River/Tantangara Creek catchment but, given that the upper Tumut River and upper Murrumbidgee River systems are proposed to be connected via a water diversion tunnel as part of Snowy 2.0, a possibility exists for Climbing Galaxias to colonise upstream.

Climbing Galaxias are a highly vagile species which is adept at colonising upstream by swimming, as well as moving over or around instream barriers via climbing and thrashing out of the water column along moist connected pathways (e.g. seepage, very thin films of water). Consequently, construction of a fish barrier to protect Stocky Galaxias by preventing the upstream colonisation of Climbing Galaxias is seen as a potential pre-emptive mitigating option. However, designing a barrier effective in stopping the movement of a specialised coloniser first requires careful consideration of many factors additional to barrier height, in order to scope key preliminary criteria to guide barrier design/construction.

A crucial and primary consideration is barrier location. Based on three optimal characteristics (a natural constriction in the valley minimising or eliminating floodplain habitat, steepness of catchment slope, and steep stream gradient), a detailed contour analysis of landform along Tantangara Creek was undertaken to identify locations possibly suitable as barrier sites. A field inspection of each location identified the area immediately upstream of the waterfall on Tantangara Creek as the only suitable location in the Tantangara Creek system.

The overall objective of the fish barrier was defined as:

To prevent the upstream movement past the structure of all life stages of target fish species at the widest range of stream flows.

Sub-objectives were:

- Prevent target fish from climbing the barrier via the downstream face by a connected wetted pathway (slow-fast flowing water, seeping water, wet splash zone, or moist areas).
- Prevent target fish from jumping over the structure.
- Prevent target fish from swimming moving around structure outside of stream channel and during overbank flows.
- Reduce or eliminate splash and moist zones on downstream face of barrier.
- Eliminate low velocity or pool areas on the downstream face of the structure where target fish can rest.

Based on characteristics of the selected location preliminary design criteria and considerations were prepared, from a biological perspective, for use in the future engineering design and potential construction of an effective invasive fish barrier. These related to the following eight design features:

- Barrier strength and design life;
- Design flow;
- Barrier height, width, profile;
- Upstream and downstream zones; and,
- Wetted pathways for movement.

1 Introduction

Snowy Hydro Limited (Snowy Hydro) proposes to develop the Snowy 2.0 project (Snowy 2.0), a pumped hydroelectric storage and generation project with the potential to provide storage for large scale, reliable and secure renewable energy to Australia. EMM Consulting Pty Limited (EMM) has been engaged by Snowy Hydro to prepare the planning and environmental documentation required for Snowy 2.0.

A consideration is the protection of the native fish Stocky Galaxias (*Galaxias tantangara*) (Raadik 2014, DPI 2017), a threatened species with a small global population in the headwaters of Tantangara Creek, from impacts from the highly vagile native species Climbing Galaxias (*Galaxias brevipinnis*). Climbing Galaxias have been able to colonise inland across the Great Dividing Range from the coastal Snowy River catchment into the Murray-Darling Basin (Morison and Anderson 1991, Waters et al. 2002) and is now present in the Murray River catchment and the Tumut River system in the Murrumbidgee River catchment (Raadik 2003). This species grows to a large size (McDowall and Frankenberg 1981), can be aggressive, has been known to impact populations of smaller galaxiid species (e.g. McDowall and Allibone 1994, Chilcott et al. 2013, Raadik, unpublished data), and is adept at upstream movement, within the water column (swimming) and out of the water by climbing and jumping (Raadik 2019).

EMM Consulting, on behalf of Snowy Hydro, contracted the Arthur Rylah Institute for Environmental Research to:

1. Provide preliminary design criteria for a proposed instream barrier to be built on Tantangara Creek to prevent upstream movement of Climbing Galaxias into the population of the threatened Stocky Galaxias.

2 Preliminary design criteria for a proposed instream barrier on Tantangara Creek to prevent upstream movement of Climbing Galaxias

An option to increase protection for the threatened Stocky Galaxias if Climbing Galaxias were to colonise the upper Murrumbidgee River/Tantangara Creek system is to prevent Climbing Galaxias access upstream into Stocky Galaxias habitat. This can be achieved by an instream barrier.

A natural waterfall already exists at the downstream distributional limit of Stocky Galaxias in Tantangara Creek (Figure 1), which is effective at preventing the upstream migration of predatory trout (Raadik 2014). Trout can usually move upstream past barriers (weirs and waterfalls) by jumping, usually from a pool into the upstream channel. They can also jump from a pool into temporary side pools or low velocity flow areas created by overbank flows during higher discharge events, and by a combination of flipping and additional jumping, gain access to upstream habitat. These pathways are blocked by characteristics of the structure of the waterfall, i.e. its height and near vertical waterfall face, shallowness of the pool below, and the lack of a low-gradient floodplain on the edges of the stream at the falls.



Figure 1. Waterfall on Tantangara Creek, upstream of Alpine Creek Firetrail, 4 April 2019 (T.A. Raadik).

However, this waterfall is not considered an effective barrier to the upstream movement of juvenile or adult Climbing Galaxias, as there are many wetted pathways up and over the barrier which could be used by this climbing species. Consequently, one or more barriers, specifically designed to prevent the upstream movement of Climbing Galaxias are required.

Consideration of the number of barriers is outside the scope of this report, though an objective of previous projects to provide barriers to prevent target fish species reaching populations of threatened species (Raadik unpublished data) has been two barriers where possible, to:

- a) Increase the probability of success as more than one barrier needs to be breached by the invading species;
- b) Provide a monitoring area between the barriers for early detection of incursions (detection of invaders is improved as invading fish are constrained in the area between the barriers for a while); and,

- c) Allow for removal of invading fish from a constrained area before they spread widely up the system (improved efficiency in removal).

2.1 Potential instream barrier location

Irrespective of the type of an instream barrier for species which can flip, jump, thrash and move outside of the water, a fundamental consideration is the location. Carefully chosen, this can in many cases reduce the overall size and cost of the construction without affecting barrier efficiency in preventing upstream movement of fish. Optimal characteristics are:

- A natural constriction in the landscape which minimises or eliminates floodplain habitat on both sides of the stream channel (minimises area needed to be addressed to eliminate overbank flow movement pathways);
- Steep catchment slopes (minimises the overall height of the barrier required), and,
- A steep stream gradient – this increases water drainage below the structure to minimise pool depth and reduces the pool area on the upstream side (this needs to be infilled during construction to increase flow velocity over the barrier).

Consequently, EMM Consulting undertook a detailed contour analysis of the landform along Tantangara Creek and extending to about 10 km downstream of Gooandra Firetrail. This dataset was viewed to identify locations possibly suitable as barrier sites (i.e. matching the three characteristics above), which were then ground-truthed during a field inspection on 4 April 2019.

Whilst some areas of catchment constriction were identified, the narrowest were approximately 20 m in width, usually with a 10 m floodplain on each stream bank, relatively gently catchment slopes, and the stream had a very low gradient (e.g. Figure 2). These sites were considered unsuitable for a fish barrier due to the width and height required for an effective barrier (high construction cost), presence of additional flood channels on the floodplain, and other factors related to potential high water volumes and velocities which would further increase construction costs and design complexity.

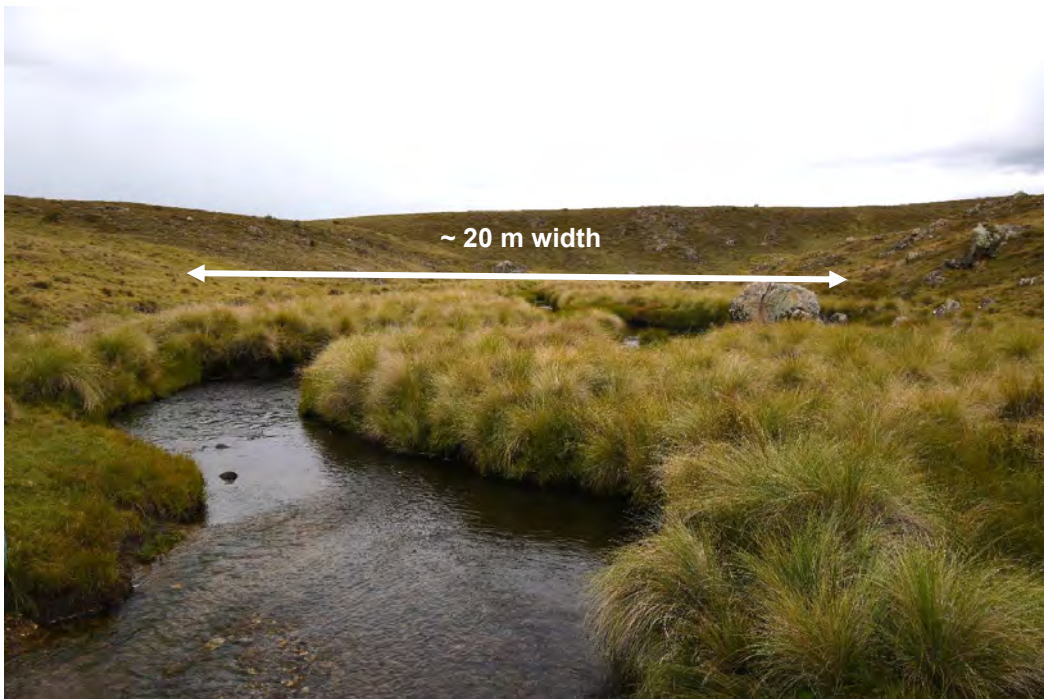


Figure 2. Example of an unsuitable potential fish barrier site at a 20 m wide valley constriction on Tantangara Creek, just downstream of Gooandra Firetrail, 4 April 2019 (T.A. Raadik).

The only suitable area, matching the above three characteristics, was at the location of the existing waterfall on Tantangara Creek, with a potential zone for selection of a suitable construction site extending approximately 70 m upstream (Figure 3). This zone had a narrow valley constriction with steeply sloping

valley walls, a relatively steep gradient, and a bedrock base and sides. Other benefits of this location are that it is upstream of the existing waterfall, and a new barrier would benefit by the extra protection this offers from trout invasion. The location is also around the bend from the existing waterfall and therefore out of sight from the Alpine Creek Firetrail, reducing aesthetic concerns with a built structure in a natural landscape and visitation by the general public. Further, it is located in a zone within the small distribution of Stocky Galaxias in which very few individuals are usually present due to the extensive areas of bedrock and fast flows, thereby minimising construction impacts on the population.



Figure 3. Potentially suitable area for a fish barrier (yellow rectangle), just upstream of the waterfall on Tintangara Creek (stream flowing to top right). (Google Earth).

2.2 Design criteria for a fish barrier

The following criteria are adapted from other projects related to installing barriers for the protection of threatened fish summarised in Franklin et al. (2018), and from learnings from work in Victoria since 1994 on constructing barriers to protect various threatened galaxiid species from predators (Raadik, unpublished data).

The **Objective** of the fish barriers is:

To prevent the upstream movement past the structure of all life stages of target fish species at the widest range of stream flows.

Sub-objectives are:

- Prevent primary target fish from climbing the barrier via the downstream face by a connected wetted pathway (slow-fast flowing water, seeping water, wet splash zone, or moist areas).
- Prevent primary and secondary target fish from jumping over the structure.

- Prevent primary and secondary target fish from swimming around structure outside of stream channel and during overbank flows.
- Reduce or eliminate splash and moist zones on downstream face of barrier.
- Eliminate low velocity or pool areas on the downstream face of the structure where target fish can rest.

The **primary target species** is Climbing Galaxias (*Galaxias brevipinnis*)

- Climbing ability: excellent, climbs out of water up moist to wet vertical to near vertical surfaces using a combination of water surface tension and body thrashing; also able to undertake small jumps.
- Size range for consideration: juveniles 40–75 mm long, adults 75-220 mm long.

The **secondary target species** are Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*)

- Climbing ability: none, but are good jumpers, and also jumpers and flippers out of water.
- Size range for consideration: 80–400 mm long.

Many intentional and unintentional common fish passage barrier features (those which prevent fish passage) can be used to aid design for successful built barriers to prevent the movement of specific target fish. Design criteria and considerations important to maximise the effectiveness of a barrier in preventing the upstream movement of Climbing Galaxias are presented in Table 1.

In general, overhang structures attached to barriers, such as grates, bars or pipes (Figure 4), do not limit climbing in smaller species such as Climbing Galaxias, however a solid plate can be successful in preventing climbing, as well as jumping (Figure 5).



Figure 4. An example of a built barrier in NZ with pipe extensions to remove wetted climbing pathways during low flow.

From Franklin et al. (2018)

Table 1. Design criteria and considerations

Caveat: The following design criteria and considerations are preliminary only and have been compiled from an aquatic biological perspective to aid further engineering interpretation and design.

Design Feature	Design Criteria	Design Considerations
Barrier strength and design life	<ul style="list-style-type: none"> - 1:100-year flood flows - 100 years longevity 	<ul style="list-style-type: none"> - Construct barrier on bedrock. - Anchoring barrier to bedrock (base, sides of wall) to prevent overturning, sliding, scour or undercutting. - Embed barrier walls into bedrock outcrops on each bank. - Grout below barrier to prevent seepage. - Backfill upstream pool to reduce risk of trout being translocated above the wall. - Minimise need for maintenance to barrier (mainly front face).
Design flow	<ul style="list-style-type: none"> - Minimum to maximum design flows from cease to flow up to 1:100-year flood flows for full exclusion. 	<ul style="list-style-type: none"> - Hydraulic profile over weir crest under varying flows. - Minimise opportunity for structure drown out.
Barrier height	<ul style="list-style-type: none"> - Minimum height of 1.8 m above low water level and bank/slope to be maintained along entire barrier width to prevent fish jumping. 	<ul style="list-style-type: none"> - Minimise rise in tailwater level in higher flows to maintain effective minimum barrier height (may need to remove rocks from the downstream side of the barrier in the slope, floodplain and stream channel area to reduce areas of slow water and ponding, increasing water drainage). - Barrier height at valley walls should be higher than 1: 100-year flow level (if possible).
Barrier width	<ul style="list-style-type: none"> - Barrier to extend from one valley slope to the other, above bank height. 	<ul style="list-style-type: none"> - Select narrowest location with bedrock on bed, banks and valley walls for efficient anchoring and to eliminate pathways around structure.
Barrier profile	<ul style="list-style-type: none"> - Top of barrier to maintain a concentrated, high velocity (target >1.7 m/s) body of flow towards the centre of the channel under low to high flow conditions. 	<ul style="list-style-type: none"> - V-shaped barrier crest to concentrate flow to mid channel to reduce wetted areas along the face of structure. - Top edge of barrier to act as a hydraulic barrier and maximise turbulence along its width.
Downstream zone	<ul style="list-style-type: none"> - Eliminate pooling and create a high velocity (target >1.7 m/s) and shallow water zone that inhibits jumping and swimming. 	<ul style="list-style-type: none"> - Scour protection on sides and downstream of barrier wall to ensure integrity of structure maintained long term and eliminate any opportunity for bypass around structure and undercutting. - Remove rock if required to eliminate pool formation and increase water flow downstream, also eliminating or reducing areas of slow water and back eddies.
Upstream zone	<ul style="list-style-type: none"> - Eliminate upstream habitat for invasive fish and create shallow, high velocity flow. 	<ul style="list-style-type: none"> - Backfill upstream pool to the lowest point of the wall of the barrier, increase stability of the structure and creating a shallow stream bed to remove habitat suitable for invasive fish.
Wetted pathways for movement	<ul style="list-style-type: none"> - Disrupt, reduce or eliminate wetted connected pathways along entire width of barrier wall, front apron area and valley sides to prevent opportunities for climbing. 	<ul style="list-style-type: none"> - Angle of downstream face of structure by 15° from horizontal, to increase effect of gravity on climbing fish, and potentially direct water away from the wall. - Smooth downstream face of structure along its height and length (no exposed aggregate, no indented or extruding seams, no spalling, pockets, etc.). - Treat downstream face of structure to prevent growth of algae, lichens or moss. - Treat downstream face of structure to reduce/disrupt water surface tension (may include attachment of hydrophobic substance). - Downstream face of structure to have two or more rows of metal angle (e.g. stainless steel, ~ 4 mm, 100 x 100 mm) running horizontally along its width with a minimum spacing of 250 mm, commencing 400 mm above the base of the structure, with at least two rows remaining 400 mm above highest tailwater level. - Prevent seepage from the structure in channel and overbank areas



Figure 5. Example of a v-notch built barrier from New Zealand, with a horizontal metal angle ‘lip barrier’ to prevent fish climbing up the wall.

From: Franklin et al. (2018), p. 106.

In addition, modifications to reduce or remove wetted surfaces from the downstream face of the barrier are crucial to disrupting connected wetted pathways which may be used by Climbing Galaxias. Further, increasing structural or flow characteristics on a barrier, which are usually reduced to increase fish passage for smaller native fish species (e.g. Baker and Boubée 2006, Doebling et al. 2012), can further reduce or eliminate climbing ability on a barrier.

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Review of Options to Prevent the Entrainment of Redfin (*Perca fluviatilis*) at the Proposed Snowy 2.0 Pumped Hydro-electric Generation Plant

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Executive Summary

Snowy 2.0 is a proposed pumped hydroelectric station that, if constructed, will generate up to 2,000 MW from connecting two existing reservoirs in New South Wales, Tantangara and Talbingo, via a 27 km tunnel and powerhouse. Water will be pumped to the upper Tantangara storage reservoir from an intake in the lower Talbingo reservoir.

Tantangara and Talbingo reservoirs are popular recreational fishing locations for non-native brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*), however Talbingo Reservoir is also inhabited by non-native European (redfin) perch (*Perca fluviatilis*), a declared notifiable species in NSW, under Schedule 1 of the Biosecurity Regulation 2017 that is considered to be absent in the upper Tantangara Reservoir.

Potential physical methods for preventing the entrainment of redfin at Talbingo intake include static screens, travelling screens and barrier nets. Where screens are fine enough, passage of small fish, including larvae and eggs, can be blocked. However, costs associated with blinding from debris and biofouling, hydraulic head loss and ongoing maintenance requirements may make these options impractical. Behavioural deterrents use stimuli such as light and sound to attract or repel fish from undesirable routes such as intakes. Although 100% exclusion using behavioural deterrents in isolation cannot be guaranteed, non-physical barriers can have relatively low capital and running costs and are not prone to debris fouling, flow constraint, increasing head loss or affecting the operation of the facility. Other potential options for preventing transfer of redfin from Talbingo Reservoir to Tantangara include stock management chemical control, and electricity to induce electronarcosis or cause electrocution.

The present study aimed to review the potential efficacy of options for the prevention of redfin entrainment at the proposed Talbingo intake as part of the environmental assessment for the Snowy 2.0 project. A systematic literature review and liaison with manufacturers identified that few studies have evaluated physical screens at high volume and bidirectional flow intakes, or the effectiveness of behavioural deterrents specifically for redfin. However, a number of potential entrainment prevention options were considered, including:

- Flat panel screens
- Through-flow and dual-flow travelling band screens
- MultiDisc screens
- Drum screens
- Water Intake Protection (WIP) screens
- Submerged Water Intake Fish-Friendly (SWIFF) screens
- Synchronised Intense Light and Sound (SILAS) – Bio-acoustic Fish Fence (BAFF) behavioural deterrent and physical barrier to eggs and larvae
- Stock management
- Electric euthanasia
- Electric deterrence combined with Acoustic Fish Deterrents (AFDs)

Given the design constraints and based on the findings of the current review flat panel wedge wire screens, drum screen and SWIFF screens were considered the most likely screening options to minimise the risk of redfin entrainment and warrant further investigation with manufacturers.

Flat panel wedge wire screens, drum screen and SWIFF screen would all require significant civil works that in the case of drum and SWIFF screens would include the construction of forebays and screen chambers. These civil works are likely to extend the footprint of the current scheme beyond the existing redline and require considerable volumes of additional excavation at each of the intakes with associated high construction costs. The construction and operational stages of intake screening may in themselves have adverse environmental impacts that would need to be assessed as part of the environmental impact assessment (EIA) process.

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1. Introduction

As an expansion of the Snowy Mountains Scheme, a hydroelectricity and irrigation complex in south-east Australia, Snowy 2.0 is a proposed pumped hydro station that aims to generate 2,000 MW from connecting two existing reservoirs in New South Wales, Tantangara and Talbingo, via a 27 km tunnel and powerhouse. At times of low electrical demand water will be pumped from an intake structure in the lower Talbingo Reservoir to the upper Tantangara storage reservoir. During periods of required energy generation, water will be released from Tantangara Reservoir back to Talbingo through turbines located in the powerhouse.

The turbines will be reversible (i.e. designed to act as both pumps and generators) and the intake heads and power waterways (tunnels that convey water between the two reservoirs), will be subject to bi-directional water movement of up to $410 \text{ m}^3 \text{ s}^{-1}$ generating flow and up to $300 \text{ m}^3 \text{ s}^{-1}$ pumping flow at design capacity (depending on operating head). The effect of abstraction and discharge will result in a fluctuation of surface water level within Talbingo Reservoir of up to 9 m.

The current design of the Talbingo intake structure incorporates a 10 m high trash screen at the head of the transition structure with the top of the racks 9 m below minimum operating level (MOL). The trash screen would comprise bar spacing not exceeding 120 mm with a gross area of 190 m^2 .

Nominal escape velocities (measured a short distance (c. 100mm) in front of the screen perpendicular to the screen face is defined as the velocity which a fish must exceed to avoid being entrained; also referred to by some authors as an approach velocity (Environment Agency 2005)). During periods of pumping from Talbingo Reservoir the approach velocity would not exceed 2.0 m s^{-1} .

An approach channel leading to the intake structure will convey water to and from the main body of the reservoir. The width of the approach channel will vary however at the entrance to the channel the wetted cross-sectional area at MOL will be around $1,000 \text{ m}^2$ providing for a much lower channel velocity.

Tantangara and Talbingo reservoirs are popular recreational fishing locations for *inter alia* non-native brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*). Talbingo Reservoir is also inhabited by non-native European perch (redfin) (*Perca fluviatilis*), a species presently considered absent in the upper Tantangara Reservoir. Redfin is listed as a notifiable species in NSW, under Schedule 1 of the Biosecurity Regulation 2017 (NSW DPI 2018). Redfin are regarded as rapacious piscivores that can carry the Epizootic Haematopoietic Necrosis virus (EHN), a virus that other recreational species and some native species have been shown to be susceptible to (Crane & Hyatt, 2011).

Water drawn into the intakes during the pumping phase risks the incidental capture of all life stages of fish and aquatic biota contained within the source water which can enhance the spread of non-desirable species, to the detriment of the more desirable species.

Methods for preventing the entrainment of fish into hydroelectric and power station cooling water intake structures include physical (positive) barrier screens, behavioural deterrents (that rely on the fish's behavioural response to hydraulic or other conditions produced by the guidance system), euthanasia and stock management and these methods have been described in numerous Good Practice guidance documents (DFO 1995; Environment Agency 2005, 2010; EPRI 1999, 2007; NIWA 2007; USBR 2006). However, whilst the proposed Snowy 2.0 abstraction shares some characteristics with conventional water intake facilities, pumped storage intake structures present some unique aspects with respect to flow and velocity characteristics, as well as operational regime (EPRI 2013).

Where physical screens are fine enough, the passage of small fish, including larvae and eggs, can be blocked. Whilst such methods may provide high levels of redfin exclusion, capital expenditures (CAPEX) and operating expenses (OPEX) can be high. Operating expenditure can include removal and disposal of impinged debris (and/or fish) which may result in hydraulic head loss if not addressed and maintenance/repair requirements, both of which may affect station efficiency and as a worse case require station outage.

Behavioural deterrents use stimuli such as light, sound and electricity to attract or repel fish from undesirable routes such as intakes (Pavlov 1989). Although 100% exclusion using behavioural deterrents in isolation cannot be guaranteed, non-physical barriers can have relatively lower operational costs as they are not as prone to debris fouling or as liable to induce flow constraint. Other potential options for preventing the transfer of undesirable species from Talbingo Reservoir to Tantangara include chemicals, electricity and ultraviolet light all of which can result in euthanasia.

The regulation (control) of redfin populations by stock or habitat management has been employed elsewhere (Ingram, 2016) and whilst typically employed on only small lentic habitats has the potential to reduce the risk of entrainment by decreasing the abundance of at risk life stages around the intake and has been included here for completeness.

THA Aquatic Ltd (THA) are independent consultants experienced in fish screening and deterrence solutions at power generation intakes in the UK and internationally and are the lead authors of a number of regulator (UK Environment Agency) and industry best practice guidance documents including:

- Environment Agency (2018) Protection of Biota from Cooling Water Intakes at Nuclear Power Stations: Scoping Study, RSR3 Phase 1, Document Reference: SC160009.
- Environment Agency (2010) 'Cooling Water Options for the New Generation of Nuclear Power Stations in the UK', Document Reference: SC070015/SR3.
- Environment Agency (2011) 'The Eel Manual: Screening at intakes and outfalls: measures to protect eel'. Document Reference: GEHO0411BTQD-E-E.
- Turnpenny and Coughlan (2003) Using Water Well? Studies of Power Stations and the Aquatic Environment. A report to the Joint Environmental Programme. Innogy Publication, Swindon.
- Environment Agency (2005) Screening for Intakes and Outfalls: Best Practice Guide. Document Reference: SC030231.

THA have drawn on this experience as well as other information derived from both peer-reviewed and grey literature to review the potential screening options for the exclusion of redfin from the intakes of the proposed Snowy 2.0 scheme.

1.1. Aims and objectives

The present study aims to review the potential efficacy of options to prevent the entrainment of redfin at the proposed Snowy 2.0 Talbingo intake as part of the environmental assessment for the project. Specific objectives were to:

- Produce a summary of the known behaviour of redfin, with particular reference to swimming ability, habitat and depth preferences in lakes and reservoirs;
- Conduct a structured literature review of fish intake entrainment prevention options with commentary on their applicability to pumped hydro of Snowy 2.0 magnitude;
- Attribute specific consideration to the effectiveness, maintenance requirements, and proposed intake design for any potentially feasible options.

1.2. Study sites

The Talbingo Reservoir is approximately 15 km long and 1 - 2 km wide with an area of approximately 1935 hectares. The reservoir is fed by the Tumut 2 Power Station and the Tumut and Yarrangobilly rivers. Talbingo is a popular location for leisure activities including boating and fishing with the reservoir known to contain a number of non-native fish species including rainbow trout, brown trout and redfin. The reservoir is stocked with brown trout, rainbow trout and the native trout cod (*Maccullochella macquariensis*) from hatchery programmes run by the Department of Primary Industries (DPI) (NSW DPI, n.d.).

Tantangara Reservoir is located 31 km north north-east of Talbingo Reservoir. Created by the construction of the Tantangara dam as part of the Snowy Hydroelectric scheme, Tantangara Reservoir collects flow from the Murrumbidgee and Goodradigbee Rivers and covers an area of approximately 2178 hectares. The reservoir is used for a number of leisure activities including a recreational fishery that targets non-native rainbow and brown trout (Snowy Mountains Fishing Information, c. 2018). The reservoir is not currently stocked, with stocks reliant on natural recruitment from streams that discharge into the reservoir (NSW DPI, 2016).

2. Redfin perch (*Perca fluviatilis*)

Redfin are a member of the Percidae family endemic to Europe and parts of Asia (Ceccuzzi *et al.* 2011). Individuals are reported to grow up to 60 cm in length and 4.8 kg in weight, however the species is most commonly observed to grow to around 25 cm (Fish Base, 2018). During the 19th century, redfin were deliberately introduced to other parts of the world outside its native range including Australia and New Zealand as a sport fish (Griffiths 1976; Jellyman 1980; Knight 2010; NSW DPI, 2017). Redfin have since become established in both Australia and New Zealand and although still a popular target for recreational fishing, they are regarded to have become a pest species, with the potential to impact upon the native ecosystems (Griffiths 1976; Jellyman 1980; Knight 2010; NSW DPI, 2017). In Australia, redfin spawn in late winter and early spring, laying ribbons of gelatinous egg masses with ribbons of between 0.1 m and 1.5 m in length (depending on female size) in aquatic vegetation (BIM 2008). Predation of egg masses is minimal owing to their unpalatable nature (NSW DPI 2017). Although redfin eggs are laid on substrate, events such as storms could cause pelagic dispersal making intake entrainment a risk. Reports of redfin egg sizes vary considerably, ranging from a minimum of 0.86 mm (Jellyman 1980) and 0.94 mm (Treasure 1981) to a maximum of 2 mm (Lintermans 2000; BIM 2008; Fish Base 2018) and 3 mm (Lintermans 2000). BIM (2008) recorded egg size at spawning ranges from 1.0 to 2.0 mm depending on the female size increasing after fertilisation and hydration to between 1.9 –2.8 mm. Egg development is temperature dependent, with redfin eggs hatching between 4 - 14 days after being spawned (BIM 2008). Recently hatched larvae are reported to be ~5 mm to 6.5 mm in length (Jellyman 1980; Mareš *et al.* 1996).

The diet of redfin shifts during the course of its lifespan, with juveniles (5 - 30 mm) feeding upon zooplankton in the pelagic zone, switching to benthic food sources at intermediate sizes (30 -80 mm), finally becoming piscivorous once maturity is reached (> 130 mm) (Hjelm *et al.* 2000; Closs *et al.* 2001; Ceccuzzi *et al.* 2011). Redfin are known to be opportunistic foragers which also exhibit cannibalistic behaviour (Closs *et al.* 2001).

Redfin shoaling behaviour is related to fish size, with smaller individuals (< 80 mm) more likely to form shoals of ten or more fish to assist with predator detection and evasion (Eklöv 1997). To increase feeding efficiency, larger individuals hunt in smaller groups or alone (Eklöv 1997).

Due to their voracious predatory nature and high fecundity they can significantly impact upon recreational fisheries and populations of native species and for this reason have been declared a notifiable species in NSW, under Schedule 1 of the Biosecurity Regulation 2017 (NSW DPI n.d.).

2.1. Swimming ability

Redfin swimming performance is related to water temperature, with higher swimming speeds reported at elevated temperatures (Clough *et al.*, 2004).

Swimming respirometer studies indicate that redfin are capable of sustaining critical swimming speeds of 6.3 (178 mm fish swimming at 1.13 m s⁻¹) and 7.9 (101 mm fish swimming at 0.80 m s⁻¹) body lengths per second (BL s⁻¹) (Tudorache *et al.*, 2008). Redfin maximum burst swimming speed is recorded at 12.6 BL s⁻¹ (115 mm fish swimming at 1.45 m s⁻¹) to 20 BL s⁻¹ (250 mm fish swimming at 0.5 m s⁻¹), however the duration over which this speed was maintained was not reported, although the standard convention assumes < 20 s (Clough *et al.*, 2004). Therefore, redfin swimming speed is not linear with regards to length, with smaller individuals capable of higher burst speeds in BL s⁻¹ than larger fish. Conversely, Davis (2000) regarded redfin to have a relatively weak swimming ability, with sustained swimming speeds of 0.15 m s⁻¹ and burst speeds of up to 0.32 m s⁻¹ (Knight, 2010). It is often the case that reports of substantially lower swimming speed result from poor experimental and fish handling techniques and the higher values reported are considered more reliable (Clough *et al.* 2004). Critical swimming speed of

coarse fish fry is reported as 6.5 BL s^{-1} (Thatcher, 1992) which equates to recommendations of water velocities of 0.15 m s^{-1} allowing escape (Solomon 1992). Therefore, the proposed nominal approach velocity of 2.0 m s^{-1} significantly exceeds the swimming abilities of all but the largest individuals.

2.2. Habitat and depth preference

Redfin habitat utilisation is dictated by fish size, with smaller individuals utilising more complex habitats compared to larger fish which inhabit more open environments, a result of smaller perch utilising highly vegetated areas to seek refuge from predation (Eklöv, 1997). Habitat complexity has also been shown to influence feeding strategy in larger individuals, with more complex habitats resulting in smaller group sizes and adoption of ambush tactics compared to larger group sizes which undertake active foraging in more open environments (*ibid.*). Depth and habitats occupied by redfin can also vary over a 24-hour period. Larger piscivorous redfin have been observed to forage in shallow areas (0.5 - 2 m) during daylight, moving to deeper water to feed at twilight (1.5 - 3.0 m) and resting on the bottom during darkness (Closs *et al.* 2001). Bathypelagic redfin fry also undertake diel vertical migrations with ranges of up to 11 m (Čech *et al.* 2005) and 13 m (Sajdlová *et al.* 2018), moving to the upper epilimnion layers at dusk (Čech *et al.* 2005; Probst *et al.* 2009).

Redfin are reported to predominately inhabit water depths of $\leq 10 \text{ m}$ (Thorpe 1977). Shoaling bathypelagic redfin fry in the Orlik Reservoir, Czech Republic, were recorded to inhabit depths of 6 - 10 m during the day however non-shoaling fry were observed to occupy depths of 12 - 17 m (Čech *et al.* 2007). Records from Lake Ontario, Canada, identify that perch (prob. yellow perch (*Perca flavescens*) a closely related species) inhabited depths to 56 m (Thorpe, 1977). Seasonal variations in redfin distribution have also been recorded, with perch inhabiting deep water in winter and moving to shallower areas in spring to spawn (Thorpe 1977). Redfin of all ages were recorded between depths of 35 - 70 m during winter in homothermal Lake Constance, central Europe (Imbrock *et al.* 1996). Vejřík *et al.* (2016) reported juvenile perch using deep hypoxic waters as a refuge from large predators with distribution better correlated with oxygen concentration than with depth.

The complexity of the observed distributional behaviour described above precludes making simple statements of entrainment exposure risk at the proposed Talbingo intake but it is clear that their various behaviours are likely to put them at risk of entrainment.

3. Options for preventing entrainment of redbfin perch at Talbingo intake

3.1. Methodology

To assess the extent of worldwide, peer-reviewed and governmental information on redbfin and physical barrier and behavioural deterrents, a structured literature review was conducted, outlined in Sections 3.2 and 3.3 below. For all searches, articles that were not readily available in the public domain or already held by THA, and not published in English, were not considered further.

A review was undertaken of documents in THA's research repository and those published by governmental research and regulatory bodies including Electric Power Research Institute (EPRI) and the Environment Agency, England.

The literature review considered research undertaken on *P. flavescens* as well as for *P. fluviatilis*. Yellow perch have similar morphology to European perch and an ability to crossbreed leading some to consider the two species as conspecific (Carney and Dick, 1999) and other to consider yellow perch as a "subspecies" (Brown *et al.*, 2009). Yellow perch are endemic to North America and the morphological and behavioural similarities of juvenile life stages (Post and McQueen, 1988) make them a suitable surrogate species for *P. fluviatilis*, increasing the body of work available to inform an assessment of intake screen efficacy.

3.2. Literature Review - Physical barriers

A Boolean search of article titles, abstracts and key words was performed in Web of Knowledge (ISI Inc.) between 12 and 15 November 2018 using the following search expression:

("mesh screen*" or "bar rack*" or "trash rack*" or "angle* screen*" or "v screen*" or "v-screen*" or "eicher screen*" or "modul* incline* screen*" or "incline* plane screen*" or "barrier net*" or "guide net*" or "aquatic filt* barrier*" or "wedge wire screen*" or "wedgewire screen*" or "wedge wire cylinder*" or "wedgewire cylinder*" or "wedge wire panel*" or "wedge wire panel*" or "travel* screen*" or "drum screen*" or "band screen*" or "rotary dis* screen*" or "Econoscreen*" or "Ristroph screen*" or "coanda screen*" or "spillway screen*" or "sub gravel intake*" or "subgravel intake*" or "porous dike*" or "marine life exclusion system*" or "labyrinth screen*" or "self-cleaning belt screen*" or "louver screen*" or "louvre screen*" or "velocity cap*" or "veneer intake*" or "fish pump*" or "filtrex filter system*" or "perforated pipe screen*" or "multi-disc screen*" or "Hydrolox screen*" or "water intake protection screen*" or "submerged water intake fish-friendly screen*" or "brushed cylinder screen*" or "brushed cone screen*" or "microfiltration barrier*")

Additional searches were performed in Google and Google Scholar. All records returned by Web of Knowledge were assessed. The first 50 results were reviewed for Google Scholar. Records with titles and abstracts referring to screening at intakes were retained for assessment of the full text.

3.3. Literature Review - Behavioural deterrents

A Boolean search of article titles, abstracts and key words was performed in Web of Knowledge (ISI Inc.) between 24 and 28 September 2018 using the following search expression:

'((perch* or redfin* or "red fin*" or "Perca fluviatilis" or "P. fluviatilis" or "P fluviatilis" or "P. flavescens" or "P flavescens" or percidae or perca) and ("non-physical barrier*" or "non physical barrier*" or "nonphysical barrier*" or "behaviour* barrier*" or "behavior* barrier*" or "guid* system*" or screen* or deterren* or bubble* or curtain* or "air diffuser*" or barrier* or *acoustic* or electric* or light* or strobe* or visual or "fish fence*" or BAFF)) not ("perchlor*" or "gutta-percha")'

Additional searches were performed in Google and Google Scholar coupling the search expression ('"perch" OR "perches" OR "redfin" OR "red fin" OR "redfins" OR "red fins" OR "perca" OR "Perca fluviatilis" OR "P fluviatilis" OR "P flavescens" OR "percidae"') with each of the following:

AND ("non physical barrier" OR "non physical barriers")
AND ("behavioural barrier" OR "behavioural barriers")
AND ("behavioral barrier" OR "behavioral barriers")
AND ("behaviour barrier" OR "behaviour barriers")
AND ("behavior barrier" OR "behavior barriers")
AND ("behavioural deterrent" OR "behavioural deterrents")
AND ("behaviour deterrent" OR "behaviour deterrents")
AND ("behavioral deterrent" OR "behavioral deterrents")
AND ("behavior deterrent" OR "behavior deterrents")
AND ("fish guidance system" OR "fish guidance systems")
AND ("bubble curtain" OR "bubble curtains" OR "bubble screen" OR "bubble screens")
AND ("bubble barrier" OR "bubble barriers")
AND ("acoustic barrier" OR "acoustic barriers")
AND ("acoustic fence" OR "acoustic fences" OR "fish fence" OR "fish fences")
AND ("acoustic deterrent" OR "acoustic deterrents")
AND ("bioacoustic fence" OR "bioacoustic fences")
AND ("electric barrier" OR "electric barriers") -bird
AND ("electric deterrent" OR "electric deterrents") -bird
AND ("strobe light")

3.4. All records returned by Web of Knowledge were assessed. The first 50 results were reviewed for each Google Scholar search string. Records with titles and abstracts referring to redfin (European or Eurasian) perch and behavioural deterrents or stimuli were retained for assessment of the full text. Searches in both Web of Knowledge and Google Scholar confirmed that relatively few studies had considered the effect of behavioural deterrents and associated stimuli specifically on redfin. Therefore, article selection criteria were broadened for both searches to include studies on similar species as appropriate e.g. closely related or anatomically similar species e.g. *P. flavescens*.

3.5. Physical barriers

Physical barriers predominately utilise mesh or bar screens to exclude fish from entrainment into intakes based on size. A number of screening options exist, including static screens which are fixed into position at an intake (Section 3.4.1), and modified static screens which are deployed on an incline and can pivot (Sections 3.4.2). Other screens are designed to rotate or travel (Section 3.4.3). Additional physical exclusion methods using nets and civil works design variations are also discussed (Section 3.4.4.).

3.5.1. Mesh size

Redfin larvae are pelagic making their entrainment a risk at the proposed Talbingo intake (Čech et al. 2005; Sajdlová et al. 2018). In order to ensure maximum protection from larval redfin entrainment, mesh size or slot-widths should be selected based on minimum anticipated larvae size. It is however the fish's cross-sectional dimension that will dictate larva exclusion from intake screens rather than total length and this varies with species (Schneeberger and Jude 1981). Body depth in particular is a crucial dimension when sizing mesh screens and the ratio between body length (standard length) and maximum body depth is termed the fineness ratio (Environment Agency 2005).

Turnpenny (1981) gave a formula for computing the rectangular mesh size needed to exclude fish of a given shape and size:

$$M = L / (0.0209L + 0.656 + 1.2F)$$

Where: M is the square mesh size in mm, L is the fish standard length in mm and F is the fineness ratio. The formula ensures that the calculated aperture size is small enough to exclude a fish by the bony part of the head, i.e. it is not the size at which a fish would just penetrate the mesh. Environment Agency (2005) provides fineness ratios for a number of marine and freshwater fish species however this excludes perch.

An assessment of yellow perch caught in Lake Michigan near the Campbell Power Plant, USA, estimated from body depth that a total body length of ≥ 4.7 mm would be excluded by a fine screen with a slot-width of 0.5 mm (Schneeberger and Jude 1981). Heuer and Tomljanovich (1978) considered a slot-width of 0.5 mm to be required for exclusion of larvae < 6.0 mm total length. As recently hatched redfin larvae have been reported to be as small as 5 mm in length (mean c. 6 mm) (Jellyman 1980; Mareš et al., 1996; Overton and Paulsen, 2005), a screen mesh or slot-width of 0.5 mm would be required to minimise the risk of perch larvae entrainment based on the literature. Increasing mesh size to 1 mm could facilitate passage of redfin larva of approximately 7.5 mm in length (Schneeberger and Jude 1981). A study by Bromley et al. (2011) estimated that redfin larvae of 29 and 67 days in age could physically pass through 1 mm and 2 mm screens, respectively. In a field study at Campbell Power Plant, Lake Michigan, USA, Patrick et al. (2018) recorded entrainment of yellow perch with 5.0 to 12.5 mm total length and head capsule sizes of 0.5 - 2.0 mm through mesh sizes down to 1.59 mm with a through-screen velocity of 0.15 m s^{-1} .

Redfin egg sizes are reported by some studies to be ≤ 1 mm in diameter (Jellyman 1980; Treasure 1981; BIM 2008) and although primarily laid on substrate (vegetation and branches etc) (BIM 2008), a screen mesh size or slot-width of 0.5 mm should prevent entrainment from water hardened eggs that have been inadvertently released into the water column.

At both the egg stage (before water hardening) and early stages of larval development most parts of both egg and larva are easily compressible and there is a risk, that these life stages could be extruded through mesh smaller than the cross sectional dimension of either egg or larva, where through screen velocities are sufficiently high.

An evaluation of wedge wire screens carried out by the Alden Research Laboratory at Chesapeake Bay (EPRI, 2006) determined that whilst the effect of slot velocity was variable among different species, a slot velocity of 0.15 m s^{-1} was generally more effective in reducing entrainment of eggs and larvae than a slot velocity of 0.30 m s^{-1} . These results supported the findings of earlier laboratory and field studies that entrainment increased with both slot size and slot velocity and decreased with channel velocity and larval length (EPRI, 2003b & 2005).

Based on available literature, the required mesh size (including all seals, abutments and fixings) for physical exclusions screens to minimise the risk of redbfin entrainment is considered to be 0.5mm. It should be noted that there is no known precedence for fine mesh screens of 0.5mm at the scale of the required Snowy 2.0 abstraction.

3.5.2. Bi-directional flow

The Snowy 2.0 pumped hydro-electric development will be designed so that the turbines will pump water through a series of power waterways from Talbingo to Tantangara during periods of low electrical demand, with water being conveyed back through the same tunnels, turbines, intake and outlet structures in the reverse direction during the generation phase. This risks the transfer of undesirable fauna and flora between the two water bodies that may impact upon the receiving water course. Consideration has been given to the use of separate intake and out fall structures however the introduction of separate structures within Talbingo introduces significant operational and safety risks. Operational risks include the excessive time it would take to transition between intake structure during the pumping and generation modes which would not align with performance requirements of the station which is required to account for the National Electricity Market (NEM) moving into a volatile decarbonised state. These operational risks could also affect SHL's flexibility to provide 'hydraulic contingency'. A safety risk is that in the event of a plant, systems or communications failure the plant may operate against a closed gate, which could result in dramatic consequences such as tunnel failures/ gate structure failures. The current design is therefore for a common intake and outfall structure.

There is however no known precedence for the use of fine mesh screens employed to filter bi-directional flow, such as is required in the proposed Snowy 2.0 pumped hydro-electric station (EPRI, 2013).

This means that even though the primary need identified is to prevent fish transfer from Talbingo to Tantangara during pumping, screens of a compatible mesh size would also be required at Tantangara to ensure that fish and debris were not caught on the inside of the mesh screens in Talbingo, blinding the screen and reducing its hydraulic efficiency.

3.5.3. Additional requirements

As well as being able to be constructed using 0.5mm mesh with seals, abutments and fixings also being able to meet this criterion, any technology considered suitable for installation at Snowy 2.0 must be capable of the following:

- installation and operation in a lentic (reservoir) environment as opposed to a flowing, lotic (river) environment;
- operating under a range of reservoir operating levels (ranging from 9m at Talbingo and 24m in Tantangara);
- accommodating the peak flow requirements of up to $410 \text{ m}^3 \text{ s}^{-1}$ generating flow and up to $300 \text{ m}^3 \text{ s}^{-1}$ pumping flow at design capacity;
- be able to be maintained in such a way as to ensure that eggs, larvae or fish are not able to move through the structure.

A consideration of how the available technologies could adequately meet these requirements is discussed in the sections below.

3.5.4. Static screens

3.5.4.1. Flat Panel Screens - Mesh, bar and passive wedge-wire screens

Static mesh, bar and passive wedge-wire screens are constructed of flat bar or mesh panels mounted on a stiffening frame which can be installed onto support frames in front of an intake, either perpendicular or angled to the direction of flow. Flat plate screens are typically installed at small low volume intakes (EPRI 2005) however a large flat plate wedge-wire screens (1.75 mm slot opening) was recently installed (2012) by the Bureau of Reclamation to protect fish within the Sacramento River from entrainment to the Red Bluff pumping plant (Figure 1). The screen consisted of a number of plate sections for a total screen length of 350 metres, with each screen 10.6m in height. The screen was designed to accommodate a flow of up to $c.71 \text{ m}^3\text{s}^{-1}$ with a through slot velocity of $c. 0.1 \text{ ms}^{-1}$.

Screens are typically manufactured from corrosion-resistant materials dependent on the environment in which it is to be deployed and copper-nickel units are available where biofouling may be an issue.

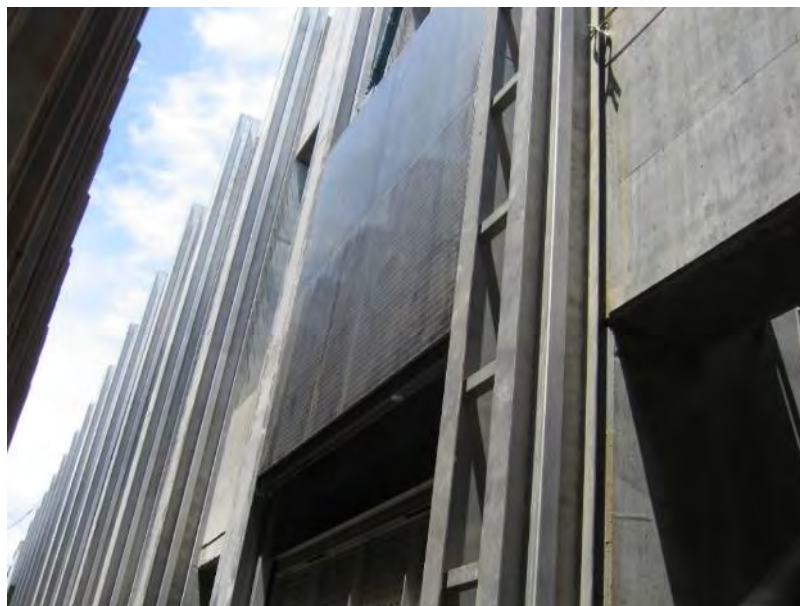


Figure 1 A flat panel fish screen installed at Red Bluff Pumping Plant (photograph: Hendrick Screen Company <https://www.hendrickcorp.com/screen/>)

The key points for consideration of this technology's applicability to Snowy 2.0 are as follows.

At a slot width of 0.5 mm, debris accumulation and hydraulic head loss will be a consideration for the high pumping and generating flows (Environment Agency 2010) proposed. It is estimated that just 10% blinding of a coarse trash rack could result in a 100 mm gradient loss, resulting in considerable energy waste (Walczak et al. 2006). The impact of fine screens on head loss can be minimised by effective screen cleaning processes (Tsikata et al. 2014; Walczak et al. 2016). For meshes, woven wire is more challenging to clean than welded wedge wire (Environment Agency 2005) and the properties of wedge wire bars mean that this is the preferred option for static screens. Wedge wire consists of wire triangular in cross section, TIG welded to support profiles. The wide edge of the wedge-wire faces outwards, into the oncoming flow and offers a flat surface that offers good filtering

performance (Figure 2). Stainless-steel wedge-wire is reported to be particularly effective for excluding both adults and juvenile fish from intakes as they are less prone to blinding than woven mesh panels with equivalent spacing (Environment Agency 2005).



Figure 2 Wedge-wire panel (Progress Industry Group 2018).

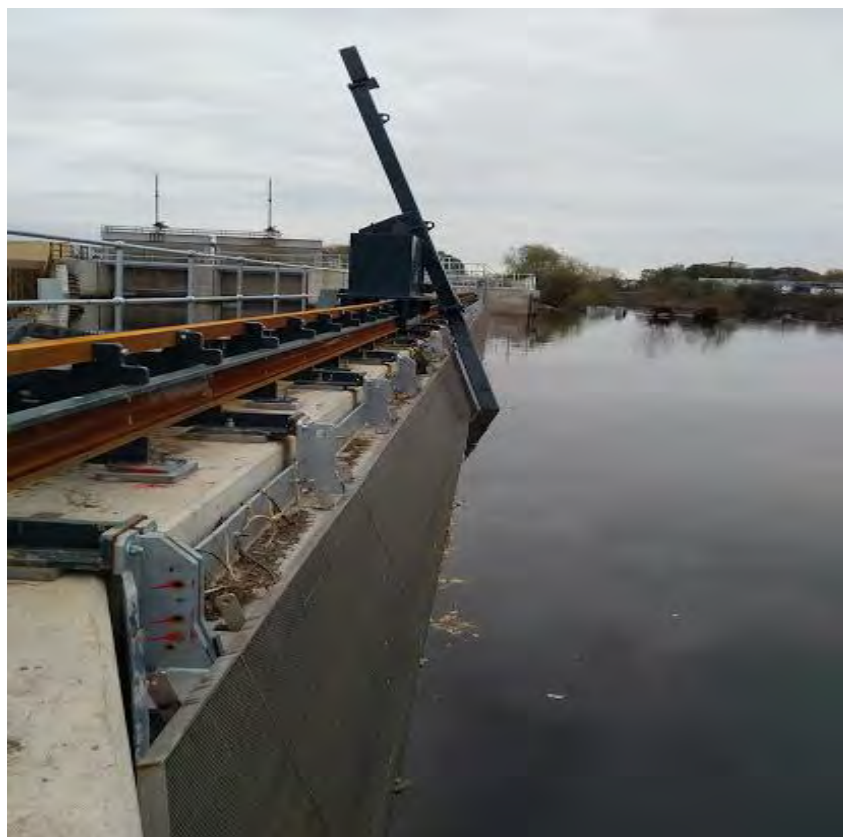


Figure 3 Horizontal wedge wire screen (6mm slot width) with a mechanical brush cleaner at Knottingley Hydropower Station, River Aire, UK - intake flow capacity of $34 \text{ m}^3 \text{ s}^{-1}$ (THA Aquatic).

Screen cleaning maintains hydraulic efficiency and assists with maintaining a consistent approach (escape) and through-screen velocity (Environment Agency, 2005). Screen approach velocities of approximately 0.3 m s^{-1} are recommended for adult fish and 0.15 m s^{-1} for fry to prevent impingement (and entrainment of any fish small enough to physically penetrate the screen) (Environment Agency 2005). Impingement can result in injury and scale

loss from abrasion, physical exhaustion and suffocation (Swanson *et al.* 1998, 2004; White *et al.* 2007) and should therefore be avoided to prevent loss of native or desirable species. Guidance advises that screens should be designed with at least an additional 20% surface area to allow for the effects of partial blinding on increased slot velocities as well as head loss (Environment Agency 2005). Siltation immediately in front of the screen can have similar effects to debris and de-silting procedures may also need to be implemented as part of a static screen maintenance schedule (Environment Agency 2005).

In lotic environments static screens can be deployed at an angle to the flow, rather than perpendicular, so that the sweeping flow (the component of flow parallel to the screen face) minimises debris load which also serves to guide fish towards any bypass facility (Ebel *et al.* 2015). This flow component is however absent in lentic environments and fish impingement is best reduced by locating intakes away from juvenile fish refugia and spawning habitat and maintaining escape velocities to below $0.15\text{m}^3\text{s}^{-1}$.

Flat panel mesh, bar and wedge-wire screens would need to be constructed with a fine slot width (0.5mm) to prevent entrainment of redfin larvae and eggs. Fine meshes and slot-widths however increase risk of blinding, resulting in head loss (Boettcher *et al.* 2016) and inconsistent flow velocities across the screen (velocity hotspots), which can cause individual fish and other aquatic life forms theoretically large enough to be physically excluded by the bar separation to be extruded through the screen (EPRI 2003b; Bromley *et al.* 2011), if not maintained.

The porosity of submerged screens can however be maintained by periodic lifting, for manual cleaning (requiring a secondary screen to be installed behind the primary screen to avoid station outage), back flushing of the screen face *in situ* or by mechanical sweeping or raking technologies; with bar screens and wedge-wire screens more effectively maintained by mechanical sweeping or raking technologies than woven wire mesh panels.

Hydraulically operated, articulated arm raking systems have been designed to clean screens to a depth of 30m (e.g. www.lakeside-equipment.com/hydro-power/). These raking systems are available as either stationary or traversing units with the latter travelling horizontally along a monorail system (Figure 3). Alternatively, impinged debris may be removed from the screen face by a hoist operated rake. The rake head typically comprises of a trough shaped bucket fitted with upwardly orientated tines or brushes on its leading edge to facilitate debris removal. The rake is drawn up the face of the screen, by means of a cable hoist system, collecting material impinged on the screen surface. When the rake reaches the top of the screen, it continues to rise through the upper water column guided by rails until it reaches deck level where hydraulic ramps tip the trough to empty into a launder which along with the debris may be flush to a perforated skip for disposal.

To accommodate a mechanical brush or rake, the screen must be designed to withstand the additional loading (EPRI 2005). The drivers that control the rake systems are located above deck level.

The frequency of cleaning would be dependent on debris loading and should be aimed at maintaining escape velocities across the full face of the screen at or below $0.15\text{m}^3\text{s}^{-1}$. A wetted screen area in the order of $2,400\text{m}^2$ (inclusive of c. 20% redundancy to accommodate debris blinding) would be required to ensure approach velocities during the pumping phase of approximately $0.15\text{m}^3\text{s}^{-1}$.

Horizontal alignment of wedge-wire bars is noted to pass a greater proportion of flow compared to vertical screens with equal bar spacing (de Bie *et al.* 2018) however may be impractical to maintain (clean) when fully submerged.

Flat panel screens are typically designed to receive uni-directional flow, with cleaning mechanisms (e.g. brushes) typically only servicing one screen face. There are currently no facilities that afford dual face washing facilities to accommodate flow reversal. In theory however cleaning of the reverse face would not be required should a comparable screening arrangement be installed at the Tintangara Reservoir intake.

Flat panel screens can provide the level of screening required for Snowy 2.0 and warrant further investigation with manufacturers however the large screen area and cleaning facility may both entail significant cost and the extensive civil structure would need to be assessed as part of the environmental impact assessment (EIA) process.

3.5.4.2. Passive Wedge-Wire Cylinder (PWWC) screens

Passive Wedge-Wire Cylinder (PWWC) screens comprise a cylinder of wedge-wire through which water is abstracted. Screen ends are blanked off with either a flat plate or conical end for streamlining when orientated into the flow (Environment Agency 2005) (Figure 4; Figure 5). The V-shaped wire is helically wound around a mandrel of longitudinal supporting bars to which the apex of the V is welded at each point of contact. The benefit of PWWCs screens is lower hydraulic resistance and risk of debris blocking (when compared with conventional screening materials) over the same amount of open screen area.

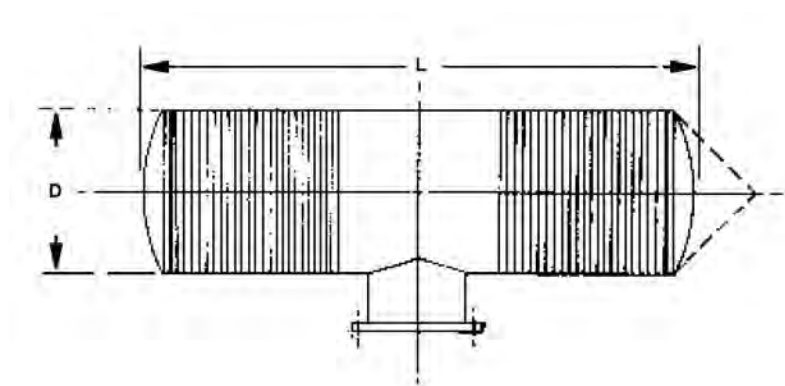


Figure 4 Passive Wedge-Wire Cylinder (PWWC) (Johnson Screens 2018).



Figure 5 Visualisation of a PWWC screened intake (Aqseptence Group 2018).

In the UK, PWWC screens are regarded as Best Available Technology (BAT) for preventing entrainment of juvenile and larval fish and considerable experience exists in their use at small power plants and at water utilities in both the UK and US (EPRI 1999; Environment Agency 2005). PWWC screens have not been designed for bi-directional flow applications lacking suitable washing capacity (debris removal facilities) during the reverse flow cycle. No examples of PWWC screens being operated with bidirectional flow have been identified.

As for flat panel screens (Section 3.4.1.1), a slot-width of 0.5 mm would be required to minimise risk of entrainment of percid larvae at Snowy 2.0. Similarly, it is recommended that escape velocities, 100 mm in front of the screen face, should not exceed 0.15 m s^{-1} however where larval/post larval life stages ('pinhead fry') are anticipated through-slot velocity should not exceed 0.075 m s^{-1} (Environment Agency 2005). To achieve these slot-velocities for the proposed Talbingo abstraction a significant array of screens (in excess of 90 units; Paolo Franchi, Head of Sales – Water Intake Systems, Aqseptence Group GmbH pers. comm.) would be required linked to a complicated manifold/piping system connecting screens to the main intake pipes. The area occupancy of the system would also be large, estimated at approximately c. 8,500 square metres. For this reason, PWWC screens are not typically used for large flow applications (Environment Agency 2005).

The submerged depth and fluctuating water level anticipated at Talbingo Reservoir would not affect the performance of PWWC screens. Maximum screen diameter is between one-third and half the water depth at MOL (this is not considered a constraint at Snowy 2.0) and minimum submergence depth is half the screen diameter, to minimise the risk of entraining surface debris (Environment Agency 2005). PWWC screens should be installed far from the forebay walls to avoid entrapment of large debris and maintain laminar flow around the screens (Environment Agency 2005).

Regular cleaning of the screen face to prevent blinding by debris would be required to minimise velocity hot-spots and minimise head loss. EPRI (2003b) found that slot velocity through a PWWC influenced the entrainment of yellow perch. In this laboratory study, entrainment was < 1% of yellow perch ranging from 4.9 to 7.8 mm with a body width of 0.4 to 1.1 mm at a 0.5 mm PWWC with a slot-velocity of 0.15 m s^{-1} , however this significantly increased at a slot-velocity of 0.30 m s^{-1} . Increases in slot velocity also risks extrusion of small fish and eggs considered too large to pass through the slots under the recommended slot velocity. For example, Bromley *et al.* (2011) found that a PWWC screen with a 1 mm slot-width excluded fish $\geq 10 \text{ mm}$ in length with a through-slot velocity of 0.15 m s^{-1} under laboratory conditions. However, during field assessments of a 1 mm PWWC screen, fry (including redfin) up to 14 mm in length were entrained, larger than the size that should be able to physically penetrate the screen. The authors considered partial screen blinding by debris increasing slot velocities to be the cause, distorting the shape of fry and subsequently forcing them through the screen. The 1 mm PWWC screen was observed to be covered in more debris than 2 mm and 3 mm PWWC screens located nearby, which did not entrain any fish above the size that should physically fit through the slots suggesting behavioural avoidance.

Impinged debris may be cleared from the screen face by an air-blast backwash system. The release of compressed air from a perforated pipe housed within the screen lifts debris that has become impinged on the outer surface of the screen back into the water column where it can be carried away by sweeping flows ($>0.3 \text{ m s}^{-1}$) (Environment Agency 2005). However, under normal operating conditions, sweeping flows will not be present at the Talbingo intake. Within lentic environments there is no means of removing the debris once displaced and debris would be rapidly impinged back on to the screen face following each air burst. PWWC screens recessed into the River Dee bank at the Deeside CCGT Station, UK, and out of the main channel flow experienced periodical blockages whereas consistently good operational performance was reported for screens in the main estuary channel at Connahs Quay CCGT also located on the river Dee, UK (Environment Agency 2010). Although biofouling and debris within oligotrophic (nutrient poor) waters is generally low, additional screen area of up to 25% (redundancy) is recommended within good practice to allow for anticipated blinding (Environment Agency 2005).

The high number of screen units required, the large occupancy area and the lack of a suitable cleaning mechanism in the reservoir environment, where suitable flow conditions are unlikely to prevail, means that PWWC screens are not considered a suitable option for screening the proposed Snowy 2.0 intakes.

3.5.5. Inclined, pivoting screens

3.5.5.1. Eicher screens

The Eicher screen consists of a flat, wedge-wire panel, elliptical in shape, placed within a pipe at an upwardly inclined shallow angle so that fish and debris passing through are swept up along the face of the screen toward a bypass route (Figure 6). The screen is supported in the midpoint by a shaft that runs perpendicular to the pipe around which the screen can pivot to allow back flushing (cleaning cycle). Fish and debris entering the bypass pipe may be recovered to either the source water or elsewhere. A key advantage to the Eicher screen design is the relatively high approach velocities at which it can operate, with a maximum mean flow velocity of $\sim 2.4 \text{ m s}^{-1}$ in the pipe (Larinier and Travade 2002).

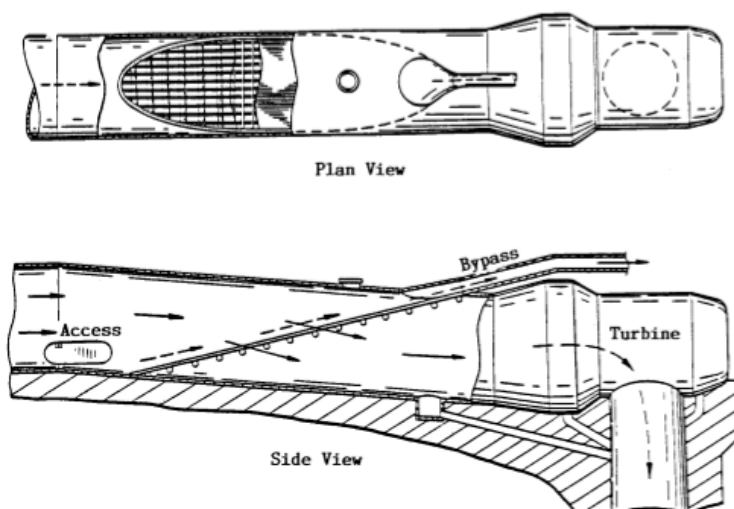


Figure 6 Eicher screen (EPRI 2002).

The Bureau of Reclamation (2006) identified three hydroelectric stations where Eicher screens have been installed (Puntledge, British Columbia; Elwha, Washington and T.W. Sullivan, Oregon) with the largest screen accommodating $15 \text{ m}^3 \text{ s}^{-1}$. Given the limited number of installations, the Bureau of Reclamation (2006) consider the design to be experimental although they cite low associated maintenance and running costs and consider the back-flush cleaning design to be effective and mechanically simple. Where Eicher screens have been installed, assessments of the effectiveness for screening salmonids under high velocity flows have been promising. High survival rates were observed for a number of Pacific salmonid smolt species at approach velocities up to 2 m s^{-1} pipe (Larinier and Travade 2002). A study by Cramer (1997) reported that 94.9 to 99.6% of spring and fall chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) smolts were effectively diverted by an Eicher screen at the T. W. Sullivan Plant, Willamette River, Oregon, USA, with insignificant injury or scale loss. The screen was constructed of 2 mm wedge-wire slot-widths. Mean penstock and screen face water velocities were 1.5 m s^{-1} and 0.45 m s^{-1} with the screen included at 19° to the direction of flow. In another assessment, conducted over two years at Puntledge Hydroelectric Project, Puntledge River, British Columbia, bypass efficiency

was $\geq 96\%$ for coho (*Oncorhynchus kisutch*) and chinook salmon smolts and steelhead, sockeye and chum salmon fry at a bar spacing of 2.5 mm and a design approach velocity of 1.83 m s^{-1} and a screen inclination of 16.5° (EPRI 2005).

A 1:4.5 hydraulic scale-model was constructed for an Eicher screen at the Elwha Hydroelectric Project, Elwha River, Washington, USA to improve flow distribution (EPRI 2005). The study identified that screens with a gradual transition in porosity towards the downstream end increased flow distribution uniformity and decreased the maximum velocity approaching to the screen by approximately 10% compared to an Eicher screen installation at the hydroelectric project. Modifications to the screen support structure were also recommended to decrease head loss. Following these alterations, more than 98% of steelhead, coho, and chinook smolts at the Elwha Hydroelectric Project survived following passage at an Eicher Screen installed in a 2.7 m diameter penstock (EPRI 1992b). Passage survivals exceeding 91.5% were also reported for fingerling pre-smolts of all species (average lengths of 44 to 73 mm). De-scaling increased with greater velocities but did not exceed 6.7%. Bar spacing ranged from 3.2 mm in the upstream section to 0.9 mm at the downstream end. A separate flume study indicated that channel velocities greater than 1.5 m s^{-1} were less likely to cause fish impingement on 2 mm slot-width wedge wire Eicher screens. Reducing the slot-width of the screen to 1 mm at the downstream end further decreased impingement.

The Puntledge and Elwha Hydroelectric Projects noted no routine maintenance tasks to the screens and recorded no operational problems (EPRI 2005). Whilst the Eicher Screen can accommodate reverse flow when rotated into a backwash position they have not been designed to facilitate the effective screening of bi-directional flow and there are no examples of screens operating in this configuration. In conclusion whilst this design has been observed to be effective for screening of salmonid smolts at relatively small installations (up to $15 \text{ m}^3 \text{ s}^{-1}$) the design, is still considered concept. Fine screening located within the confinement of the pipe may result in significant flow constraint and the velocities predicted within the pipes (of up to 6m/s) risk extrusion of small fish and eggs through the screen which may otherwise be considered too large to pass through the slots under lower slot velocity. Further given the screen being located in situ within the tunnel, maintenance or removal of any significant blockage may require station outages resulting in production loss. Eicher screens are not considered appropriate technology for the proposed Snowy 2.0 scheme.

3.5.5.2. Modular Inclined Screens (MIS)

Modular Inclined Screens (MIS) (Figure 7) follow a similar design concept to the Eicher screen. The screen is typically deployed at an angle of approximately 10° to 20° to the direction of flow (EPRI 1999). Fish and debris passing along the face of the screen are diverted to a transport pipe for return to source water or to waste. Screen face typically comprise wedge wire flat panels with bar spacings generally in the region of 1.9 mm to enable 50% porosity (Environment Agency 2016). MISs are designed to operate at water velocities ranging from 0.6 to 3.1 m s^{-1} (EPRI 2005). Early trials noted a relationship between diversion efficiency and survival and test velocity suggesting smaller life stages may be extruded through the screen at higher velocities.

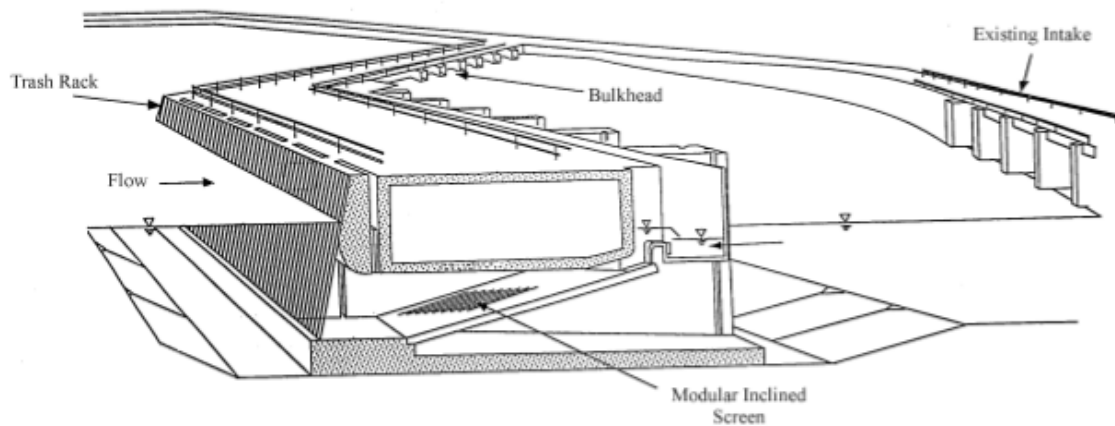


Figure 7 Schematic of a Modular Inclined Screen (EPRI, 2002).

Laboratory experiments have indicated > 99% survival for juvenile coho salmon, brown trout, and Atlantic salmon (*Salmo salar*) following MIS passage at velocities ranging from 0.6 to 3.1 m s⁻¹ (EPRI 2005). In general, the screen's effectiveness at passing fish was not compromised by accumulation of debris. Further experimental study, recorded diversion efficiencies of > 96% for a number of species, including coho salmon, rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon where approach velocities ranged from 0.6 to 3.0 m s⁻¹ (Amaral *et al.* 1999). Subsequently, a prototype MIS was constructed at the Green Island Hydroelectric Project, Hudson River, USA. Field tests were undertaken at the Project at velocities of 0.6 to 2.4 m s⁻¹. Passage survival and diversion was > 95% for a number of different riverine species assessed (Amaral *et al.*, 1999). No other assessments of MIS installations in the field were identified and the design is still considered concept.

The design is not considered to warrant further investigation due to deficiencies in proof of concept at the scale proposed at Snowy 2.0 and concerns over extrusion of eggs and fry through the screen resulting from high operating velocities.

3.5.6. Rotating and traveling screens

3.5.6.1. Brushed cylinders

Brushed cylinders are a variant of PWWC screens, whereby the screen cylinder continuously rotates enabling brushes located in fixed positions outside and inside to clean the screen (Figure 8). Brushing helps to minimise debris loading and head loss and maintain even flow across the screen. Wedge wire slots widths range from 0.5 mm to 9.0 mm. Cylinders are designed to be retrievable for maintenance via a vertical track system. Each unit has a maximum capacity of 3.42 m³ s⁻¹ and similar to PWWC screens, a large screening array would be required for the proposed Snowy 2.0 scheme. The largest known application is for the Lower Yellowstone Irrigation District, Yellowstone River, Glendive, MT where flows of 41.1 m³ s⁻¹ is drawn through 12 tee screens. Each tee screen is measures approximately 2m in diameter by 2.5 m in width (two screens per tee) and is formed of wedge-wire with a 1.75mm slot width.

As for PWWCs, sweeping flows are required to carry fish and debris away from the screen face once displaced by the brush system. This requirement restricts the suitability of screen deployment to a lotic environment and in combination with the high number of screen units required and the large occupancy area the design is not considered suitable for this application.

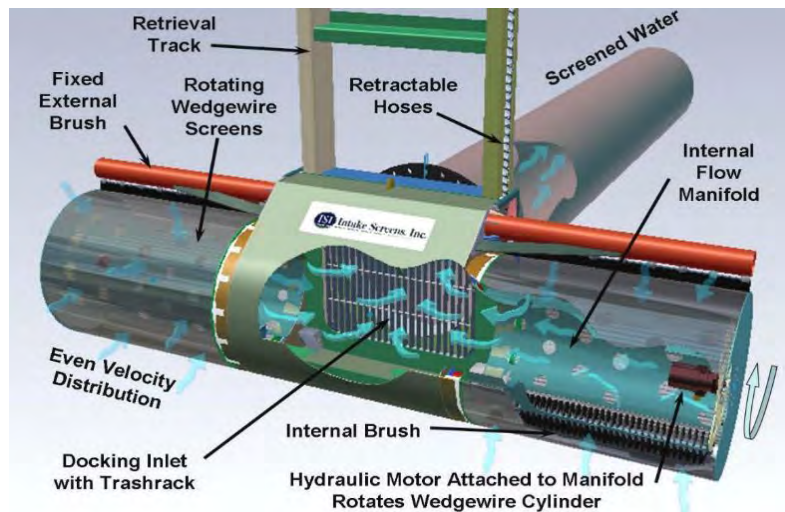


Figure 8 Brushed cylinder (ISI 2018).

3.5.6.2. Travelling band screens

Band screens comprises a continuous ‘band’ of fine filtration mesh which travels in a conveyor-like motion around a spindle at the top and bottom of the band. Vertical guides mounted in the screening chamber walls are fitted with contact seals which prevent debris and or biota larger than the filtration threshold from bypassing the band screen. The screen is rotated (typically) by a chain, driven by an electric motor situated within a head frame directly above the band screen and located at deck level (Figure 10). The moving mesh band lifts debris from the water to service deck level where it can be cleaned by water jets. There are three principal configurations of band screen design: through-flow, dual-flow and centre-flow. Through flow screens currently being provided to Hinkley New Nuclear Power station are approximately 25m high and 2.5m wide with mesh formed of stainless steel with a mesh aperture of 5 mm × 5 mm. Each screen filtering approximately $2.81 \text{ m}^3 \text{ s}^{-1}$ (EDF, 2016). Modern engineered polymer traveling screens can filter down to 1.75 mm and are compliant with 316(b) of the Clean Water Act (USA) and considered Best Available Technology (BAT) in the UK.

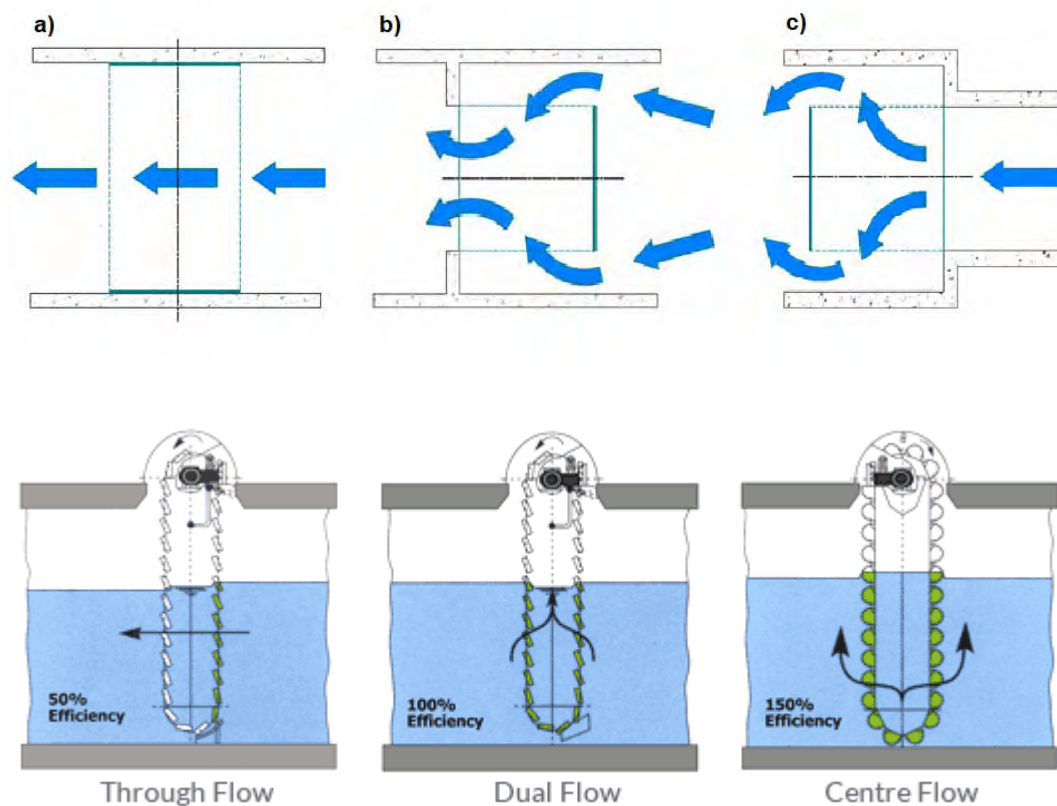


Figure 9 Plan and side views of a) through flow, b) dual flow and c) centre flow band screen designs (Environment Agency 2010; MACMET 2018).

Through-flow band screens

Through-flow band screens are deployed perpendicular to the direction of flow and water passes through both the ascending and descending sides of the band (Figure 9). Fish and debris are prevented from entering through the base of the screen by patented boot seals with a static shoe. The boot seals do not require regular maintenance and have a service life in excess of 15 to 30 years (K.Bousfield, Hydrolox, pers. com.).

The screens, which are designed to withstand debris impact, can be fitted with fish recovery and return facilities and/or debris handling flights.

Through-flow band screens are installed at a number of UK water utility intakes. Thames Water, the largest water and wastewater services provider in the United Kingdom installed 11 Hydrolox Series 1800 traveling water screens for their advanced water treatment works in Walton, London. The screens, with 1.75mm openings and accommodating a total flow of up to $10.5 \text{ m}^3 \text{ s}^{-1}$ were installed in order to comply with the U.K. Eels Regulations.

In the USA through flow screens are installed by a number of industries including thermal and hydro-electric power stations and irrigation and water abstraction plants. Flows up to $140 \text{ m}^3 \text{ s}^{-1}$ are typically screened (EPRI 2005) with the largest intake applications reported at Surry Station, James River (4.5 mm screen mesh, $111 \text{ m}^3 \text{ s}^{-1}$ intake flow), Oyster Creek, Barnegat Bay (9.5 mm mesh, $116 \text{ m}^3 \text{ s}^{-1}$), Indian Point Units 1 (2.5 mm mesh, $132 \text{ m}^3 \text{ s}^{-1}$) and 2 (9.5 mm mesh, $133 \text{ m}^3 \text{ s}^{-1}$), Hudson River (combined flow for all units $133 \text{ m}^3 \text{ s}^{-1}$) and Salem Station, Delaware River ($6.3 \text{ mm} \times 12.7 \text{ mm}$ rectangular mesh, $140 \text{ m}^3 \text{ s}^{-1}$) (EPRI 2005). Of these, fish entrainment has only been monitored at

the Unit 1 intake of the Indian Point Generating Station. Screening at Indian Point Unit 1 consists of a 9.5 mm fixed screen followed by a bar rack and a 2.5 mm nylon mesh band screen. Each individual screen accommodates flows of up to $17.6 \text{ m}^3 \text{ s}^{-1}$. Average approach velocity to the screens is 0.27 m s^{-1} . The screen, which has been fitted with a fish recovery and return facility is subject to heavy seasonal debris loading and the screen rotation speed varies between 0.8 to 6.1 m min^{-1} dependent on debris loading. The screen was not successful at preventing entrainment of 14-day-old post-yolk-sac striped bass (*Morone saxatilis*) larvae of 7 to 9 mm length. However, entrainment of late post-yolk-sac larvae (length = 10 to 18 mm) was prevented. Estimated survival of the late post-yolk-sac larvae impinged on the screen was 69%, falling to 47% after 96 h and survival of impinged juveniles was high. It was estimated that screen retention would near 100% as juveniles exceeded 19 mm in length for this species (EPRI 2005).

With the exception of the Indian Point Unit 1 intake, through flow band screen mesh sizes for the high capacity intake stations reported above are relatively coarse (4.5 and 9.5 mm) and would allow entrainment of redbfin eggs, larvae and some juveniles if used at the Talbingo intake. Smaller through flow mesh sizes (0.5 - 1.0 mm) have frequently been used at lower volume intakes ($12.3 \text{ m}^3 \text{ s}^{-1}$ to $39.7 \text{ m}^3 \text{ s}^{-1}$) in the USA (EPRI 2005) and UK. For example, at Brunswick Station, Carolina, (intake flow $17.1 \text{ m}^3 \text{ s}^{-1}$) entrainment at a through flow screen with 1.0 mm polyester mesh was compared to a 9.4 mm mesh. The fine mesh screen decreased the total number of fish entrained by 84% over the period of a three-year study. Species included gobies (*Gobiosoma spp*), croaker (*Scianidae*), spot (*Leiostomus xanthurus*) and anchovies (*Anchoa spp.*).



(photo Hydrolox™)

Figure 10 Series 6000 Hydrolox™ traveling water screen fitted with a fish recovery & return facility. The screen dimensions are 2.5m wide by 17.7m in height. The unit is designed to filter a flow of approximately $1 \text{ m}^3 \text{ s}^{-1}$.

Rather than considering the effectiveness of fine mesh through flow screens for preventing 100% exclusion, research has primarily assessed the impact of impingement on the condition and survival of fish. In a laboratory

study, yellow perch impingement mortality was assessed for a 0.5 mm synthetic through flow travelling band screen mesh at velocities ranging from 0.15 to 0.91 m s⁻¹ and for durations of 2 to 16 minutes. Yellow perch pro-larvae of 5.8 - 6.0 mm in length were impinged on the screen and showed increasing mortality with increasing velocity and impingement duration, with mean mortalities ranging from 6.8% at 0.15 m s⁻¹ to 31.5% at 0.61 m s⁻¹. Yellow perch post larvae ranging from 6.3 - 6.5 mm in length experienced high impingement mortalities (> 85%) under all test conditions including the control (EPRI 2005).

A comparative study of 3.2 mm mesh through flow band screens at the Hanford and 100-N Generating Plants, Columbia River, Washington, found that yellow perch fry and chinook salmon were the most commonly impinged species at both stations (EPRI 2005). The plants have an intake flow of 35.6 m³ s⁻¹ and 26.4 m³ s⁻¹, respectively. One-hundred percent mortality was observed for all impinged fish assessed at 100-N, whereas 97% and 92% of chinook salmon and yellow perch fry survived at Hanford Generating Plant. Differences in impingement survival are considered by the authors to have arisen from plant locations relative to one another (i.e. upstream or downstream), orientation of the forebay, intake configuration and variation in the trash rack and curtain wall design which altered the hydrodynamic conditions in front of the trash rack. Further trials were conducted that released live and dead fish in front of the trash racks and traveling screens. Cadavers were retained more often at the 100-N screens, and six times more live fish were recorded at Hanford, suggesting that hydrodynamic conditions in front of the trash rack were inducing avoidance reactions in live fish.

High survival (69% to 98%) was reported for impinged young-of-the-year white perch (*Morone americana*) during three 9.5 mm through-flow band screen operation modes (continuous screen rotation and washing, screen rotation and washing for 20 minutes once every 2 hours and screen rotation and washing for 20 minutes once every 4 hours) and two pressure wash modes (at Bowline Point Generating Station, Hudson River, New York, USA (EPRI 2005). However, continuous operation led to the highest survival rate.

Dual-flow band screens

Dual-flow band screens run perpendicular to the intake opening and parallel to the flow, with a blanking plate opposite the intake opening to divert the flow equally through the descending and ascending bands (Figure 9b). The filtered water exits the band screen well via a conduit in the rear wall called the suction eye. Such screens are used for filtering cooling water at thermal and nuclear power stations in the UK, France and USA, for example at the Calvert Cliffs Station, Chesapeake Bay (8 mm mesh, 30.5 m³ s⁻¹), Arthur Kill Generating Station, Arthur Kill Tidal Strait (6.4 mm x 13 mm and 32 mm mesh, 28.6 m³ s⁻¹), Roseton Generating Station, Hudson River (9.5 mm mesh, 40.4 m³ s⁻¹) and Big Bend Station, Tampa Bay, Florida (flow 45.6 m³ s⁻¹). At Big Bend Station, dual-flow screens had a 0.5 mm mesh and 100% and 97.9% immediate and 96h post-trial survival, respectively, was reported for silver perch (*Bairdiella chrysoura*) eggs but only 19.2% larvae survival (EPRI 2005). Spray wash pressures were 10 psi and approach velocities ranged from 0.15 to 0.31 m s⁻¹. As a result of the high survival observed for other species at Big Bend Station, six 0.5 mm screens were installed at Unit 4, Big Bend Station. In the UK, it is proposed that a dual-flow band screen will be installed at Hinkley Power Station to filter the safety water supply system. Proposed screen dimensions are 2.5 m wide and 25.0 m high, with each unit (eight in total) filtering 3.7 m³ s⁻¹. Both through flow and dual-flow screens are well suited to fish recovery and return facilities (FRR).

Centre-flow band screens

As for dual-flow screens, centre-flow screens are also positioned parallel to the flow; however, water passes in between the bands and is then diverted by a blanking plate through the ascending and descending sides of the band (Figure 9c). Centre-flow screens are regarded as BAT for German nuclear power stations (Environment Agency 2010).

Four Passavant, fine-mesh (0.5 mm) centre-flow screens were installed at the Barney M. Davis Power Station, upper Laguna Madre, Texas (total screened flow = $21.5 \text{ m}^3 \text{ s}^{-1}$). Initial survival for all individuals recovered from the screen (15 species of invertebrates and 37 species of vertebrates) was 86%. Algal biofouling had little impact on survival of impinged fish compared to filamentous debris such as marine grass which could cause entanglement (EPRI 2005).

Comparative study of travelling band screen design

A comparative study of entrainment at two types of band screen found that fewer fry (56 fry ML^{-1}) of a number of species (including roach) were entrained at through-flow screens compared to a centre-flow screen (362 fry ML^{-1}) at a test facility in the lower River Thames, UK (Bromley *et al.* 2011). The proportion of fry entrained by the centre-flow screen did not differ significantly from control assessments, thought to be a result of screen alignment perpendicular to the flow. Further, the central-flow pattern was considered to create a low velocity area inside the screen structure causing fry to congregate and increasing entrainment risk. The configuration of dual-flow screens means that they would be unlikely to create these disadvantageous hydraulic conditions. Compared to dual- and centre-flow band screens, through-flow bands have comparatively simple civil works requirements, however the fine mesh required for screening perch eggs and larvae would mean greater head loss as flow passes through ascending and descending screen face before entering the intake (Environment Agency 2010). Historic concerns of debris carry over into the intake with some through-flow screens has been addressed with the use of high-pressure spray bars coupled with cantilevered head section, although system performance guarantees would need to be sought from the supplier.

Preliminary discussions with suppliers concluded that further investigation of travelling band screens were not recommended due to the difficulties in designing and operating such large screening facilities down to the required mesh size (0.5 mm) and/or achieving compatible seal tolerance requirements to prevent exclusion of all life stages of roach.

3.5.6.3. Drum Screens

Drum screens comprise a large rotating cylindrical structure with woven wire filtration panels attached to the periphery and supported by a horizontal, revolving centre shaft (Figure 11). They are driven by a motor located on the service deck. The screens are typically situated behind a coarse primary screen that provides protection from damage or fouling by large debris items. Primary screening typically comprises of vertically inclined parallel bars or rods, often referred to as a rack (EPRI 2005). Drums can be designed with either in-to-out or out-to-in flow patterns. In-to-out screens draw water into the drum and then out through the screen, retaining debris on the inside (Figure 12a). The converging flow created by this configuration minimises turbulence entering the intake. Additional benefits include a hydraulically balanced screen where hydraulic loads push downwards onto the concrete foundation and preventing risk of the drum floating free from its foundation (Ovivo 2018). Out-to-in drums draw water through the screen into the drum, collecting debris on the outside (Figure 12b). Diverging flow results from water movement through out-to-in drums, meaning that the flow must converge again before entering the intake. For this reason, in-to-out drums are the favoured option for screening at many intakes,

including installations in the UK where drum screens are considered BAT for large water intakes (Environment Agency 2010).

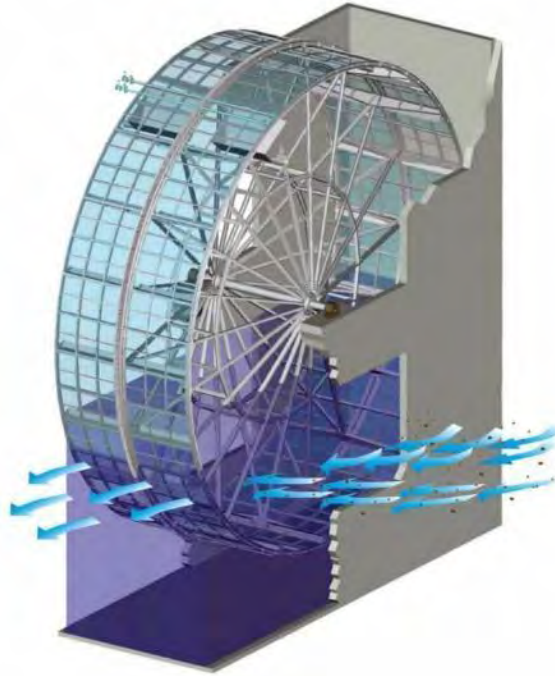


Figure 11 Brackett Green in-to-out drum screen (Ovivo 2018).

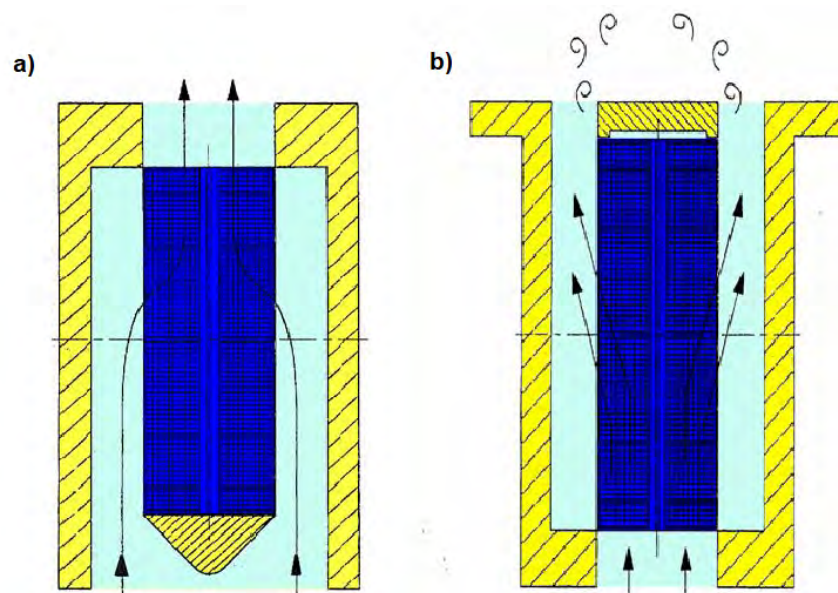


Figure 12 a) In-to-out and b) out-to-in flow patterns for drum screen designs (Environment Agency 2010).

For coastal power stations where debris loadings are high, drums are typically covered with woven wire at 6 mm spacings. Mesh spacing can be reduced to 0.5 mm, particularly in locations where debris load is lower, which would be effective at screening juvenile redfin and eggs, however such fine tolerance can be difficult to achieve (guarantee) between screen seals and the supporting frame. For in-to-out drums, debris is cleaned off the screen

face using high pressure spray jets located outside the screen with dislodged debris falling by gravity into a collection hopper and flushed along launders to a perforated skip. Out-to-in drums are washed from the inside that requires higher jet wash pressures (~ 3 bar) because debris removal from screens is not gravity assisted. This results in elevated running costs compared to in-to-out screens and means that screen downtime is required for maintenance which would rule the out-to-in configuration out for Snowy 2.0.

Flow into in-to-out drums can be either single- or double-entry, whereby flow either enters the drum from one or both sides, respectively. Double-entry flow drums are suitable for higher flow applications (up to $\sim 35 \text{ m}^3 \text{ s}^{-1}$) than single-entry drums ($\sim 10 \text{ m}^3 \text{ s}^{-1}$). Therefore, fine mesh double entry drum screens can filter considerably higher water volumes than band screens.

At least thirteen $24 \text{ m}^3 \text{ s}^{-1}$ drum screens (18 m drum diameter, with an inlet channel width of 3.5 m) would be required for screening the pumped flow at the Talbingo intake (OVIVO UK Limited, pers. comms.).

Drum screens have been widely installed throughout the UK, Europe, Middle East and North America. Hinkley 'C' new nuclear power station proposes to install four 27.0 m diameter, 6.5 m wide drum screens (OVIVO UK Limited) fitted with 5 mm mesh to filter up to $125 \text{ m}^3 \text{ s}^{-1}$. Once built, these will be the largest drum screens ever installed. At Ras Laffan Common Cooling Water Intake, Qatar, 14 drum screens are installed with a combined flow capacity of $333 \text{ m}^3 \text{ s}^{-1}$ (Ovivo, pers. comm.).

The smallest mesh routinely fitted is 3 mm, as used at French nuclear stations such as the Gravelines station which has a combined cooling flow of around $240 \text{ m}^3 \text{ s}^{-1}$ filtered through twelve, 15m diameter drums. Smaller scale drum screen applications are reported for the USA, the largest to our knowledge being Glenn-Colusa Irrigation District irrigation diversion, Sacramento River, California and White River Hydroelectric Plant (EPRI 2005). Forty drum screens measuring 5.2 m in diameter and 2.4 m wide were installed at Glenn-Colusa Irrigation District irrigation diversion. Screens consisted of 4.3 mm woven stainless-steel wire cloth. Screens were operated continuously from April to November each year with design approach velocities of 0.2 m s^{-1} . Mark-recapture studies indicated that fingerling chinook salmon were lost through the screens, potentially due to mesh size or leaking seals around the screens. At the White River Hydroelectric Plant, $57 \text{ m}^3 \text{ s}^{-1}$ was diverted through 6 mm mesh drum screen to prevent entrainment of chinook, coho, and steelhead smolts. Variable survival rates were observed, ranging from 10% - 90%. The screen operated continuously between March and November with approach velocities of approximately 0.5 m s^{-1} . Screens at both these two sites have however subsequently been replaced with flat panel static screen as a result of poor biological performance, considered to be either as a result of poor orientation and or lack of suitable fish recovery/escape facilities (EPRI 2005).



Figure 13 Drum screen screening installation at Ras Laffan Common Cooling Water Intake, Qatar. 14 drum screens are installed with a combined flow capacity of $333 \text{ m}^3 \text{ s}^{-1}$ (courtesy of Ovivo).

Although the requirement for civil works costs may be greater, drum screens have fewer moving parts than band screens, enhancing reliability and minimising maintenance requirements, particularly in high sediment load waters (Environment Agency 2010). Drum screens can also accommodate considerably higher flow capacity than band screens with wide panels and the circular frame design minimising head loss while drive assemblies decrease energy consumption (Ovivo 2018). However, FRR methods are currently less advanced for drum screens and require careful design. Other considerations, applicable to all travelling screens, include maintaining submergence and approach velocities throughout pumping flow periods at Snowy 2.0 as water depth will vary by 9 m at Talbingo and 23 m at Tantangara. As the Talbingo Reservoir water depth will decrease during pumping, screen and approach velocities will increase. Designing screens so that approach velocities are suitable for minimising fish and debris impingement at MOL risks excessively low velocities at high water levels that could cause adverse effects from siltation or fouling. A potential option to combat this is to have a redundant screen(s) that can be brought online as reservoir levels drop.

As a concept, this design could be considered feasible if tolerance of contact seals sufficient to prevent exclusion of all life stages could be guaranteed. Compatible screens would be required in both reservoirs and as with all types of travelling screens the associated civil structures e.g. forebays and screen wells, would be significant due to the variation in water levels that would need to be accommodated, particularly at Tantangara. There are no examples of drum screens having been installed to accommodate bi-directional flow.

3.5.6.4. MultiDisc Screen

The Geiger MultiDisc screen (**Figure 14**) is a relatively new travelling screen design that complies with US Environmental Protection Agency (USEPA) BTA (Best Technology Available) requirements. Sickie-shaped mesh panels, oriented perpendicular to the direction of flow, descend and ascend through the water along a revolving carrier chain (Aqseptence Group 2018). Screens are typically constructed of woven stainless-steel mesh or perforated plastic of sizes from 0.5 mm to 10 mm. Built-in debris exporters transport debris to the service deck where it is removed via backwash spray.



Figure 14 Geiger MultiDisc screen (Aqseptence Group 2018).

Worldwide, MultiDisc screens have been installed at > 200 intakes (Aqseptence Group 2018). The largest installation is located at the DC Cook Nuclear Plant, Lake Michigan, USA, where 15 9.5 mm mesh screens are installed with a total flow capacity of $15.3 \text{ m}^3 \text{ s}^{-1}$ (Peltier 2004). A 12-unit installation is currently in operation at Power Plant Moorburg, Germany (Aqseptence Group 2018).

Impingement of fish at MultiDisc screens has been evaluated at Potomac River Generating Station, Alexandria, Virginia, USA (EPRI 2007). Screens were deployed at a single screen bay with a flow of $19.2 \text{ m}^3 \text{ s}^{-1}$ and approach velocities of 0.18 m s^{-1} . Survival of white (*Morone americanus*) and yellow perch impinged on the screen was 30 - 56 % and 100%, respectively, however the authors note that the majority of white perch were impinged during storm events which may have independently influenced survival (EPRI 2007). Survival was considered comparable to results for other traveling screen assessments. In a laboratory study, impingement of six juvenile and adult freshwater fish species was assessed at a MultiDisc screen using 2.0 mm stainless-steel mesh and 9.5 mm perforated plastic (EPRI 2013). Combined species and treatment survival was 95.7%. For most species, injury and survival decreased as velocity increased, and larger fish generally suffered lower mortality than smaller fish. For some species, the 2.0 mm mesh resulted in lower mortality and less scales loss than the 9.5-mm perforated mesh. Higher velocities significantly increased the number of fish injured with individuals predominately displaying scale loss, bruising and fin damage. Conversely, a MultiDisc screen with 8.0 mm perforated plastic panels and a fish collection and return system has also been tested at Salem Generating Station (Strait 2018). Compared to existing Ristroph-modified travelling band screens installed at the station (6.35 mm x 12.7 mm woven wire mesh), latent impingement mortality was higher at the MultiDisc screen.

Large MultiDisc units (width c.3.5 m, depth c.25 m) with wide aperture mesh can filter flows of up to $13.89 \text{ m}^3 \text{ s}^{-1}$. Finer mesh or perforated screens would have lower flow capacities. A minimum of 22 units would be required at the Talbingo intake to filter pumped flow. MultiDisc screens are however not designed to filter bi-directional flow. To accommodate a bi-directional flow of up to $410 \text{ m}^3 \text{ s}^{-1}$ a minimum of 30 units would be required to screen the intakes in both Talbingo and Tantangara Reservoirs.

Compared to through-flow travelling band screens, MultiDisc screens have less impact on head loss as flow is only required to traverse one screen. MultiDisc screens also create less turbulence than dual and centre flow band screens. Operational costs of the MultiDisc are relatively low due to the only moving part being a single centrally guided chain, resulting in low maintenance costs (Aqseptence Group 2018).

Although screens can be provided with 0.5mm mesh and are fitted with contact seals, the Aqseptence Group felt they could not offer a guarantee for a screen c. 25m in height on seals conforming to a tolerance of 0.5mm. As a result, this technology is not considered suitable for application at Snowy 2.0.

3.5.6.5. Fish Recovery and Return (FRR)

Both band and drum screens can be fitted with a fish recovery and return (FRR) facility to reduce the risk of fish mortalities. Eggs, larvae and adults impinged on the screen are recovered to water-retaining fish collection buckets compliant with UK and US fish impingement and entrainment regulations (Figure 15) which are fitted to the screen face in place of trash elevators. Ristroph-style buckets fitted to some band screens have been hydraulically designed to retain fish in a water pocket with minimal turbulence. Fish and debris are flushed from the buckets into fish return gullies by low pressure spray bars designed to reduce damage to fish (Environment Agency 2010). The buckets are treated to prevent fish adhering to the steel alloy during the flushing process. Through-flow band screens may be fitted with cantilevered head section, designed to ensure accurate fish delivery into the return gullies. High pressure spray bars fitted post fish removal clear tougher debris from the screen face and are designed to eliminate debris carryover.

Buckets and recovery systems should be designed in compliance with criteria set out for FRR systems. It should be noted that FRR methods for drum screens are currently less advanced than for band screens, as the fish bucket tipping radius is much larger on drum screens risking fish falling from buckets at height back into the screenwell which, for species such as eel, can occur on multiple occasions (Environment Agency 2010).

Band and other travelling screens may be required to be deployed with approach velocities that exceed the sustainable swimming ability of fish larvae (0.15 m s^{-1} [Solomon 1992]) and some juveniles due to the additional cost that would otherwise be required to purchase, operate and maintain the number of screens required to screen the equivalent area. In such scenarios FRR facilities should be a requirement to reduce loss/impact of desirable species.



Figure 15 Fish buckets fitted to a dual-flow band screen (photo THA Aquatic Ltd).

Should FRR be required at the Snowy 2.0 scheme, it would be recommended that the return pipe outlet discharges to below the Talbingo Reservoir MOL. Research on North American freshwater fish species identified that survival rates were higher for individuals released from a fish return pipe at a submerged discharge when compared to heights ranging from 0.61m to 1.22m (EPRI 2013). The inside of the return pipe should be smooth with a minimum diameter of 0.3 m diameter with at least ≥ 0.5 m diameter where the main return channel exceeds 30 m in length (Environment Agency 2010). A radius of 3 m is suggested for swept bends and a continuous supply of wash water should always be maintained through the return pipe (Environment Agency 2010). Return pipes should also be covered to minimise predation risk and algal growth, although access is required for periodic cleaning (Environment Agency 2010). Fish-friendly pumps can be employed if FRR facilities cannot be designed to operate under gravity alone at the Snowy 2.0 scheme.

A review by EPRI (2003a) of 71 studies at 35 power plants with angled, dual-flow, and through flow traveling screens found fish impingement survival rates of 70–80% or higher where adequate screen design and operation was employed. Many of the studies included assessment of Ristroph-style modifications to band screens and modifying operational procedures such as screen wash periods. Overall, increased screen wash frequency, continuous movement, increased screen travel time, modifications for separating fish, and improved debris handling all influenced survival rates. Ristroph-style fish recovery adaptations have improved survival of impinged fish at large power generating stations with coarse mesh, such as Surry Station ($111 \text{ m}^3 \text{ s}^{-1}$ flow, 4.5 mm mesh), Salem Station ($140 \text{ m}^3 \text{ s}^{-1}$ flow, 6.3 mm x 12.7 mm rectangular mesh) and Indian Point Generating Station Unit 2 (12.7 mm mesh, combined unit flow $133 \text{ m}^3 \text{ s}^{-1}$) and for finer mesh band screens at lower intake flows, for example Somerset Station, Lake Ontario, New York (flow $12.3 \text{ m}^3 \text{ s}^{-1}$, 1 mm mesh) and Dunkirk Station, Lake Erie (flow unspecified, 3.2 mm mesh) (EPRI 2005). Fish survival can be optimised by operating band screens continuously and at a higher speed setting when increasing head loss occurs (>100 mm) across the screens (Environment Agency 2005). This will minimise damage to the fish through impingement by hydraulic shear, asphyxiation or exhaustion where screens are not operated continuously (Environment Agency 2005). Guidance recommends that screens are rotated at a constant speed of 1.5 m min^{-1} to minimise fish contact with the screen or buckets (Environment Agency 2010). Lower pressure spray washes can also decrease mortality resulting from impingement (EPRI 2005).

Further, forebays and screenwells should be designed to dissipate energy and turbulence to minimise stress to fish to enhance survival (Environment Agency 2018).

3.5.6.6. Water Intake Protection (WIP) Screens

The Water Intake Protection (WIP) system is formed of a circular screen with a fish-friendly NOCLING™ anti-fibre panel (**Figure 16**) (Beaudrey 2018). The screen is deployed perpendicular to the direction of flow. As the screen rotates, radial compartments retain fish and debris and divert them to a scoop where they are removed from the screen via a fish-friendly Hidrostal screw-centrifugal backwash pump. The NOCLING™ panel minimises skin damage and surface trauma to fish. Compared to traditional band screens, WIP fish recovery buckets are never exposed to air. The screen has few moving parts decreasing maintenance costs and removes the risk of debris carry over (Filon 2011). WIP screens can be supplied with mesh size from 0.5 mm however flow rate capacities are low; flow capacity of $0.7 \text{ m}^3 \text{ s}^{-1}$ through a 2800 mm diameter screen (Beaudrey 2018).

An example of a WIP installation is Omaha North Power Station, Missouri River, Nebraska, USA, (intake flow $32 \text{ m}^3 \text{ s}^{-1}$), which is serviced by one 6.1 mm^2 mesh WIP and five 9.5 mm mesh travelling screens (Bigbee *et al.* 2010). Survival rates (48 h) of a number of native species were close to 100% for the WIP screen (*ibid.*).

The low flow capacity of this technology means that this method is not considered suitable for installation at the Snowy 2.0 intakes.

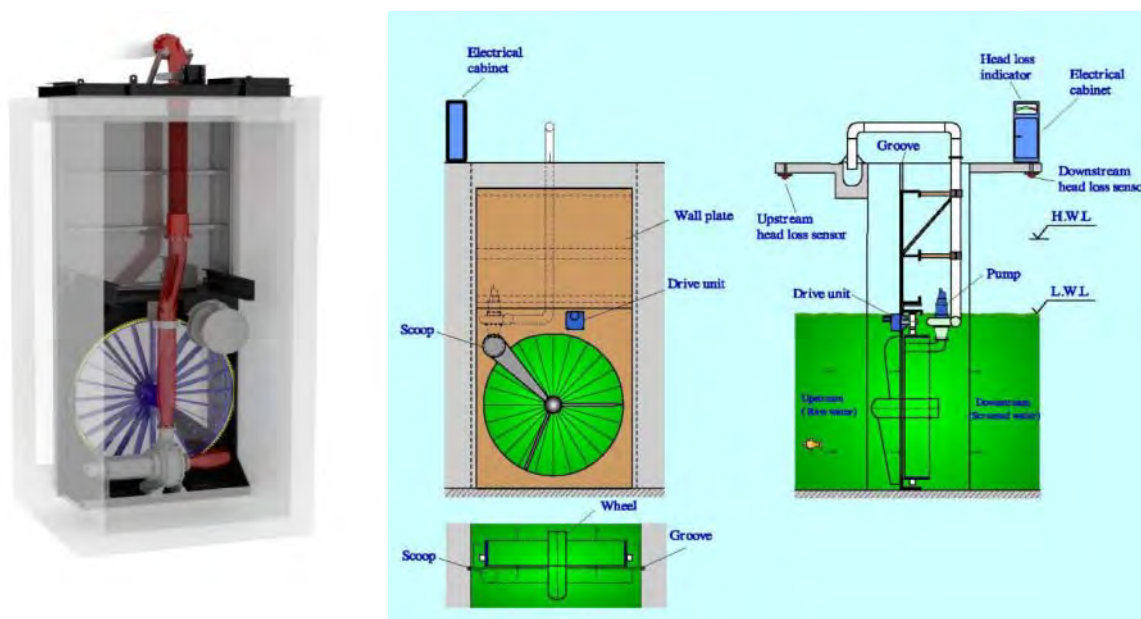


Figure 16 Water Intake Protection (WIP) screen system (Filon 2011).

3.5.6.7. Submerged Water Intake Fish-Friendly (SWIFF) Screens

Submerged Water Intake Fish-Friendly (SWIFF) screens are formed of a cylindrical drum covered in mesh around the curved outer surface (Beaudrey 2018) (Figure 17). The screen continuously rotates, similar to a drum screen however the fully submerged nature of the screen allows water to be drawn through 360° of the outer curved screen. Fish and debris impinged upon the submerged cylinder's external surface passes in front of a backwash suction scoop as the drum rotates which removes the debris by reversing the flow through the mesh. Suction is created by a special fish and eel-friendly pump that sends the fish and debris back to the source water (or to trash)

via a return pipeline. Additional suction scoops can be installed for fine mesh applications. The screen normally rotates at a low speed setting with the pump in service however if a head-loss is detected the drum speed can switch to a higher speed.

The SWIFF screen is mounted in a box structure and slid into position over the intake via wall guides. The entire assembly can be lifted out of the pit for major inspections, refits (every few years). The box slides are fitted with seals to prevent any by-pass through the guides. The sealing between the rotating drum and the box structure uses head-loss-applied total seals. SWIFF screens comprise fewer moving parts than traditional travelling band screens, allowing less frequent and lower-cost maintenance, (Beaudrey 2018).

As for all forms of physical exclusion screens SWIFF screens are not suited for reverse flow as there is no mesh backwashing which may result in the head-loss rising to dangerous levels (P. Jackson, Beaudrey, pers. com.). Options include installing similar screening facilities at the Tantangara Reservoir intake or design engineering solutions that would permit reverse flow to by-pass the mesh without significantly increasing the size of the structure (P. Jackson, Beaudrey, pers. com.).

It was considered that SWIFF screens warranted further consideration for the proposed Snowy 2.0 intake.

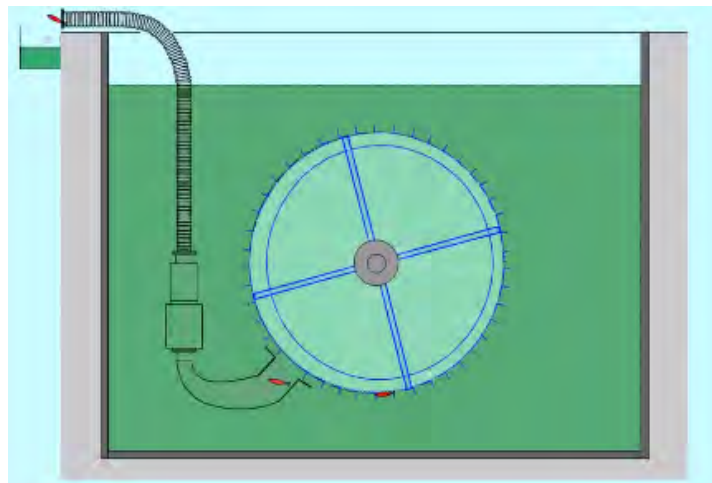


Figure 17 Submerged Water Intake Fish-Friendly (SWIFF) screen (Beaudrey 2018).

3.5.7. Other physical barriers

3.5.7.1. Barrier nets

Barrier nets are large nets rigged with a float line and a heavily weighted bottom to maintain a vertical profile throughout the entire water column. Nets can be anchored or deployed from piles approximately 3 - 12 m apart to assist with vertical positioning and prevent movement towards the intake, which has been observed in some lower abstraction applications (Edwards and Hutchison 1980). The net is deployed in an arc around an intake at a distance that ensures an approach velocity of $< 0.08 \text{ m s}^{-1}$ and can result in the net being many kilometres in length (Environment Agency 2005). Mesh size is dependent on the species and life stages requiring exclusion and should be small enough to avoid gilling fish (Environment Agency 2005). Figure 18 provides the fish-mesh size relationship (Environment Agency 2005) for exclusion. Diamond meshes are recommended over square meshes which deform more easily (*ibid.*).

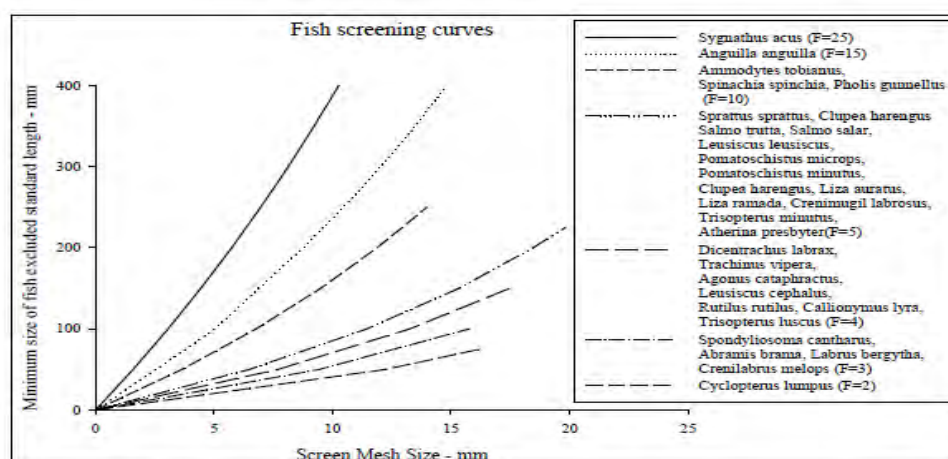


Figure 5.6 Mesh size curves for screening fish of different body shape, plotted from equation 1 (after Turnpenny, 1981, with additional coarse fish data from Table 5.1).

Figure 18 Mesh size curves for screening fish of different body shape (Environment Agency 2005).

Coarse mesh barrier nets have been successfully used at a pumped storage plant to reduce fish entrainment into the station. A 2.5-mile-long, ½-inch bar wing mesh and ¾-inch bar central mesh barrier net set in open water around intake jetties, has been used to reduce entrainment of fish species in the vicinity of the 1,872 MW Ludington Pumped Storage Plant intake, Lake Michigan (Guilfoos 1995). The net was first deployed in 1989 and modifications to the design in subsequent years led to a net effectiveness for target species retention of > 80% since 1991 and 96% in 1995 (Alden Research Laboratory 2015). The annual maintenance cost was > \$2.8M USD for 6 months of operation. The process to install or remove the net requires barges, cranes, SCUBA divers and several days of assembling and/or cleaning the various panels it is comprised of. While in operation, net cleaning is an ongoing process that also requires boats, divers and pressure washers. Permanent, bottom-anchor piles and anchor chains are used to keep the barrier net in place.

At Bowline Point Generating Station, Hudson River estuary, USA, 9.5 mm and 12.7 mm mesh barrier nets decreased impingement young-of-the-year and yearling (50 – 100 mm total length) white perch (*Morone americana*) and striped bass (*Morone saxatilis*) by up to 90%. Coarse mesh barrier nets could be deployed in the Talbingo Reservoir to minimise the effects of debris in conjunction with other fish transfer prevention technologies. Periods of excessive debris and clogging can however cause even coarse nets to be lifted from the riverbed while wave action can enable fish to pass over the top. These factors were identified by Patrick *et al.* (2014), who recorded a screen efficacy resulting from the use of barrier nets of between 75% and 100% at Pickering Nuclear Generating Station, noting significant reductions in net effectiveness with degradation from fouling. Such permeability is less of a concern in power plant applications, where an 80 - 90% reduction in fish entrainment is typically targeted, however the method would not be considered effective where complete exclusion is required, as is the case for the Snowy 2.0 project.

Nets typically require clearing and maintenance every few weeks, however local debris and biofouling levels will influence maintenance requirements (Environment Agency 2005). Although mesh sizes of 9.5 mm and 12.7 mm have been noted to show no difference in clogging or debris accumulation in a field application at Bowline Point Generating Station (Edwards and Hutchison 1980), much smaller mesh sizes will increase the likelihood of clogging and frequency of maintenance. Although shore mounted drum winches can be used to assist retrieval for maintenance and cleaning, maintenance of large barrier nets is intensive, as demonstrated at the Ludington

Pumped Storage Plant. THA (A.W.H. Turnpenney) have observed a 2,000 ft fine mesh barrier net operated at Pickering Nuclear Generating Station Fish Diversion System in Ontario, Canada, where at certain times of the year maintenance requirements were considerable, necessitating a large team of approximately 16 staff that operated from boats in a 3-shift pattern round the clock to maintain the anchorage and air back flushing systems.

Although problems from biofouling and blockage will be reduced in oligotrophic (nutrient-poor) waters, barrier nets are considered to be a labour and cost intensive method that would not guarantee a high level of inter-catchment transfer prevention, particularly where fish entrainment prevention is required year-round (Environment Agency 2005). Other examples of barrier net applications at larger pumped storage projects and power stations, such as Ludington, only operate the net seasonally (April 15 to October 15 annually) and prevention of the transfer of eggs and larvae is not required for the project.

Barrier nets are not considered to warrant further investigation for intake screening at Snowy 2.0.

3.5.7.2. Aquatic Filter Barrier (AFB) or Marine Life Exclusion System (MLES)

Aquatic Filter Barriers (AFBs) are perforated mats made of pressed polyester fibres. The Marine Life Exclusion System (MLES) is a variant of the AFB specifically designed for exclusion of aquatic organisms rather than pollutants. The MLES system is considered a method of Best Technology Available under the Clean Water Act, Section 316(b) (USA) and comprises two layers of treated polypropylene/polyester fabric which can be suspended from expanded polystyrene billets, anchored to the bed of the water course and sealed around intake structures (Gunderboom 2018) (**Figure 19**). The large surface areas of MLESs ensure that water velocity at the barriers can be low to minimise entrainment and impingement. MLESs can be cleaned by bursts of compressed air that shake debris from the fabric panels, controlled by sensors and a computer system (Gunderboom 2018).

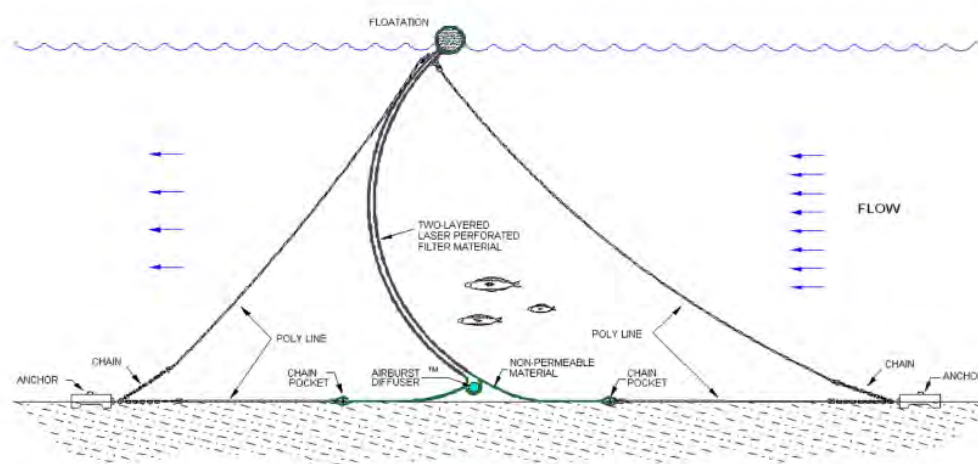


Figure 19 Marine Life Exclusion System (MLES) (Gunderboom 2018).

Studies have shown improvements in the technology's potential for minimising entrainment. Early laboratory studies found that AFBs with perforation sizes of 0.5, 1.0 and 1.5 mm were less successful at retaining early life stage rainbow smelt at the higher flow rate tested (20 gpm/ft²) compared to the lower rate (10 gpm/ft²). Larger perforation sizes also decreased the number of common carp, rainbow smelt and striped bass that were retained (EPRI 2005). In the field, a 500 ft AFB deployed at Lovett Generating Station, Hudson River, New York, USA (flow capacity 17 m³ s⁻¹) captured eggs, larvae and juveniles, lowering the daily mean densities of organisms entrained when compared to a control intake without an AFB deployed at the same station at the start of the study (EPRI 2005). However, assessments during the latter phase of the study found no difference in the ichthyoplankton

entrained at both intakes, considered to be the result of an unknown failure of the AFB. Further adaptations were made to the barrier and subsequent trials of MLES at the station with airburst cleaning showed an 80 - 95% decrease in fish entrainment and resulted in full deployment of a ~425 m long and ~18 m deep MLES at the station (Gunderboom 2018). A MLES has also been installed at Taunton River De-salination Plant, Taunton River, Massachusetts, USA ($1.13 \text{ m}^3 \text{ s}^{-1}$) (Gunderboom, 2018), however no other applications have been found.

Given the labour (and therefore cost) intensive requirements for maintenance, the risk of overtopping by wave action and the relative concept status of the technology it is considered that AFBs are unlikely to guarantee prevention of inter-catchment transfer for eggs and larval life stages and are therefore not considered suitable for application at the Snowy 2.0 scheme.

3.5.7.3. Porous Dikes

Porous dikes can be constructed in front of intakes to prevent passage of organisms. Limited research has been conducted on this method for preventing fish entrainment at intakes. Tests in the field have been conducted at Brayton Point Station, Narragansett Bay, Massachusetts, USA, for a 6.4 m wide, 18.3 m long, and 6.1 m deep dike. The study assessed two dike constructions: the first was constructed of two rows of gabions filled with 7.6 cm stones, the second was formed of three rows of gabions filled with 20.3 cm stones. Water was drawn through the dike at a rate of $2.9 \text{ m}^3 \text{ S}^{-1}$. Decreased dike pore size reduced filtration of larval bay anchovy from 94% for 20.3 cm stones to 99% for 7.6 cm stones and winter flounder by 23% from 100 to 87%. However, a number of fish larvae still penetrated the barrier. Entrainment avoidance was 100% for all juvenile and adult fish. There is a high risk of blockage and head loss at porous dikes (EPRI 2005), making the method unsuitable for Snowy 2.0.

3.5.7.4. Sub-Gravel Intakes and Wells

Sub-gravel intakes and wells abstract water through the riverbed or aquifer. For sub-gravel intakes, a screen can be laid over the horizontal intake opening and covered with layers of gravel separated by geomembrane sheets (Environment Agency 2005). Cleaning is achieved by backwashing. Collector wells extend into bedrock and may abstract water through lateral perforated intake pipes situated at depth (Environment Agency 2005). Both methods are only suitable for low flow capacity applications and would therefore not be applicable to Snowy 2.0.

3.5.8. Behavioural Deterrents

3.5.8.1. Introduction

A number of behavioural deterrents have been developed to prevent fish from becoming impinged and entrained at water intakes (Pavlov 1989). These non-physical barriers utilise the behavioural response of fish to a number of stimuli including light, sound and vibration, pressure, touch, electrical currents, hydraulic shear and acceleration, and olfaction (Pavlov 1989; Turnpenny and O’Keeffe 2005). Such stimuli may attract or repel fish depending on signal properties and strength, fish species, life stage and environmental conditions (Pavlov 1989). Sections 3.4.8.2 to 3.4.8.7. explore the application of light, acoustic, bubble, hybrid and electric deterrents for diverting fish away from water intakes. As deterrent effectiveness is often species-specific, particular consideration has been given to research into their effect on redfin or comparable species.

3.5.8.2. Light

Light transmits well in clear, calm waters (Popper and Carlson 1998) and can be used as either a fish deterrent or attractant, depending on fish species, light intensity and whether the source is continuous or discontinuous (Pavlov

1989). Artificial light sources used as behavioural modifiers can be split into two broad categories; continuous and strobe (flashing) lights.

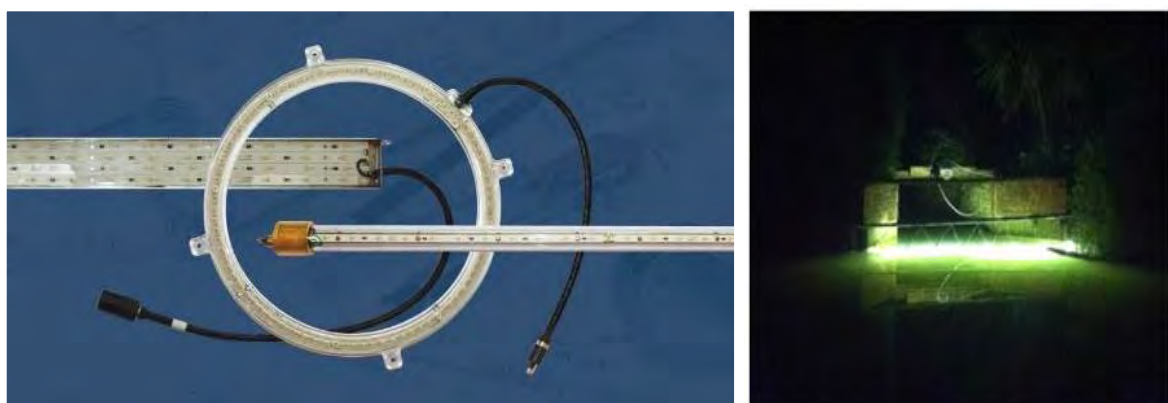
3.5.8.3. Continuous light

Research by Fore (1970) found that a number of freshwater fish species including clupeids, atherinids, serranids and cyprinids were attracted to a constant light source. For percids such as roach, avoidance has been observed to continuous illumination of structures from unsubmerged lamps. For example, continuous nocturnal illumination of the cooling water intake at Bergum Power Station, the Netherlands (flow range = 12.5 - 23.9 m³ s⁻¹), repelled 98% of 0+ and 1+ age class roach (Hadderingh 1982). Entrainment of other percids including Eurasian ruffe (*Gymnocephalus cernua*) and pike perch (*Sander lucioperca*) was reduced by 99% and 97%, respectively (Hadderingh 1982), while Pavlov (1989) was able to decrease entrainment of juvenile percids and cyprinids by 91% using continuous light. A postulated reason for entrainment reduction using continuous light was that fish were better able to navigate away from hazards, especially at night.

Despite some studies showing high efficacy, continuous light will not provide a total exclusion barrier for all life stages under all environmental conditions therefore do not warrant further consideration for Snowy 2.0.

3.5.8.4. Strobe lighting

Compared to continuous sources, strobe lighting has been reported to induce a greater avoidance response in a wider range of fish species (e.g. salmonids, Brett and MacKinnon 1953; Craddock 1956; Patrick *et al.* 1985; Puckett and Anderson 1987; EPRI 1990; Nemeth and Anderson 1992). Older strobe lights used high-voltage xenon discharge tubes with limited life expectancy and potential electrical safety issues. Modern strobe light systems consist of submersible low-voltage flash heads (e.g. sealed LED panels: Figure 20) wired to an external power supply and signal control unit to create abrupt changes in light levels that are unlike natural fluctuations e.g. from atmospheric and water-surface effects such as waves or cloud-cover (Dera and Gordon 1968; McFarland and Loew 1983). Fish can be slow to adapt to changing light levels (Li and Maaswinkel 2006), creating the potential of strobe



lights for behavioural deterrence.

Figure 20 Three types of FGS (Fish Guidance Systems Ltd) HIL strobe light units. Inset: intake illuminated by strobe lights (courtesy FGS Ltd).

Strobe lights have been demonstrated to be an effective deterrent for a wide range of non-percid fish species including: kokanee (*Oncorhynchus nerka*) (Maiolie *et al.* 2001), chinook salmon, yearling coho salmon (Ploskey and Johnson 2001; Amaral *et al.* 2018), alewife (*Alosa pseudoharengus*), rainbow smelt (*Osmerus mordax*), gizzard shad

(*Dorosoma cepedianum*), spot (*Leiostomus xanthurus*), Atlantic menhaden (*Brevoortia tyrannus*) (Patrick *et al.* 1985), American eel (*Anguilla rostrata*) (Patrick *et al.* 2001) and white fish (*Coregonus lavaretus*) (Königson *et al.* 2002). However, a number of studies have also reported ineffectiveness of strobe lights as deterrents (e.g. Ichthyological Associates 1994; Ichthyological Associates 1997; EPRI 1998a).

Adult redbfin are visual predators (Ali *et al.* 1977). No research specific to redbfin responses to strobe lights was identified and hence no firm conclusions can be drawn for this species, however avoidance responses have been observed at flash rates of 86 flashes per minute (FPM) in the closely related yellow perch when exposed to strobe lights (Richards *et al.* 2007) as well as other fish species (e.g. chinook salmon, white perch and spot) (Sager *et al.* 2000). Large-scale field application of the technology has also shown promise. During pump-back discharges of approximately $1,869 \text{ m}^3 \text{ s}^{-1}$ at the Ludington Pumped Storage Plant, Michigan, USA, strobe lights operated during the hours of darkness over a 4 month period significantly decreased total entrained fish abundance of yellow perch as well as rainbow trout (*Oncorhynchus mykiss*), brown trout, lake trout (*Salvelinus namaycush*), coho salmon, chinook salmon, alewife, rainbow smelt and various cyprinids compared to periods when the lights were off (Taft *et al.* 2001).

In a different application, yellow perch were significantly deflected by strobe lights operated at 300 FPM from December to mid-July at a cooling water intake at Milliken Steam Electric Station, New York, USA, which drew a flow of $10.7 \text{ m}^3 \text{ s}^{-1}$ at 12.2 m depth (Ichthyological Associates 1994; Ichthyological Associates 1997). Conversely, yellow perch were not significantly attracted to the lights during late summer and autumn at the same station, so the effectiveness may be seasonal (Ichthyological Associates 1994; Ichthyological Associates 1997). Weak reactions were also observed for yellow perch in strobe light (200 - 600 FPM) cage tests conducted at the Kingsford Hydroelectric Project on the Menominee River, Wisconsin, USA, although a stronger response was reported for the percid species walleye (*Sander vitreus*) (Winchell *et al.* 1997; EPRI 1998a,b; Michaud and Taft 2000). At the White Rapids Hydroelectric Project on the Menominee River, Wisconsin and Michigan, USA, no difference was observed between yellow perch entrainment during controls and periods of strobe light operation at 300 FPM (EPRI 1998a). Discharge rates for the three White Rapids Hydroelectric Project Francis turbines ranged from $25.8 \text{ m}^3 \text{ s}^{-1}$ to $43.7 \text{ m}^3 \text{ s}^{-1}$.

As a second-order effect, strobe lights have also been shown to significantly decrease the density of zooplankton, a food source of juvenile redbfin, throughout the water column (Richards *et al.* 2007). This effect may be worth exploring further, since if attraction to an entrainment-risk area is food related then it would be helpful to reduce the cause.

Variations in the deflection efficiency of strobe lights reported for percids in different cases could be a result of differences in strobe flash rate, duty cycle, wavelength or environmental parameters such as turbidity or flow. Intake velocity and the ability of fish to avoid entrainment is often a factor in the performance of any behavioural deterrent. For best performance, flash rates should be optimised for species-specific repulsion, although there is an upper limit: the rate must not exceed the critical flicker fusion frequency of the species, beyond which an organism is unable to distinguish individual flashes and instead perceives a constant light source. For example, flash rates of 300 FPM have previously been found to be most effective at repelling Atlantic salmon (Patrick *et al.* 1982) and rainbow trout (Johnson *et al.* 2001). Further investigation would be required to identify the most effective flash rates for redbfin and to identify the optimum settings for other flash characteristics such as pulse length and duty cycle.

Strobe light effectiveness can also be influenced by environmental factors such as daylight penetration (Nemeth and Anderson 1992; Johnson *et al.* 2005) and turbidity (McIninch and Hocutt 1987; Maiolie *et al.* 2001). For example, dark-acclimated white perch and menhaden often showed greater avoidance than light-acclimated individuals (Sager *et al.* 1987). Maiolie *et al.* (2001) observed that strobe light avoidance by kokanee occurred at a

greater distance when water clarity was higher. However, a little turbidity can be helpful in reflecting (backscatter) the light. The estuarine species Atlantic menhaden, spot and white perch displayed increased avoidance to a strobe light deterrent during elevated turbidity levels (102 - 138 NTU) (McIninch and Hocutt 1987). Alewife also displayed high avoidance (> 90%) to strobe lights in turbid waters, although avoidance did decrease at even higher turbidity as attenuation increased (Patrick *et al.* 1985). Therefore, information from pre-construction monitoring would benefit from turbidity data, and possibly on-site evaluation of strobe lights.

The majority of strobe light deterrent studies discussed have concentrated on white light. Wavelength of light that elicits the greatest avoidance response can be species specific depending on eye pigments (Sager *et al.* 1985). Loew and Wahl (1991) reported a visual pigment absorbance maximum in yellow perch the region of 400 nm. It is now technically feasible to select LED emission spectra to match visual pigments of the target species.

For non-migratory fish such as redbfin, habituation to behavioural deterrents must also be considered. Unlike for continuous light sources, acclimation to strobe lights has been reported less frequently for many fish species (e.g. salmonids (Puckett and Anderson 1987); rainbow smelt (Hamel *et al.* 2008)). For yellow perch, plasma cortisol levels in those exposed to strobe lights over a 7 hour period did not differ from controls (Richards *et al.* 2007), however the authors propose that confounding factors such as confinement stress may have prevented identification of differences after the initial few hours. Random flash patterns can be used to alleviate the possibility of habituation (Hamel *et al.* 2008).

Fish health may also affect behavioural response to an environmental stimulus. Ocular parasites may influence the effective light frequency and/or flash rate to which perch respond (Munoz, Jenny Carolina Vivas; Staaks, Georg; Knopf, Klaus 2017).

Finally, attraction of non-target species must also be considered in light deployment (Hadderingh 1982).

In conclusion efficacy of strobes for deflection of redbfin may be affected by individual health history (parasite load), water quality and ambient light conditions and will not provide a total exclusion barrier for all life stages under all environmental conditions therefore do not warrant further consideration for Snowy 2.0.

3.5.8.5. Acoustic fish deterrents

Unlike light, transmission of low frequency sound in water is not affected by turbidity, ambient light levels and time of day, and propagation over long distances can be achieved where required (Popper and Carlson 1998). In recent years, acoustic fish deterrents (AFDs) have been widely and effectively used for repelling fish from water intakes. AFDs typically comprise a signal generator feeding a bank of high-powered amplifiers and underwater sound projectors to create a fish-repellent sound field (Figure 21). Modern systems incorporate much of the electronics in the submerged sound projector units, minimising costly cabling requirements when used at remote intake structures (Lambert 2014). To effectively deflect fish away from an intake, the repellent sound field must extend far enough from the intake to ensure water velocity does not exceed fish swimming ability.



Figure 21 FGS Synchronised Intense Light and Sound (SILAS) sound projectors raised for inspection at Pembroke Power Station, UK. LED lights can be seen illuminated (courtesy FGS Ltd).

Frequencies emitted by AFDs must be within the audible detection range of the target species. In many fish species, the lateral line and inner ear are responsible for hearing (Bennett *et al.* 1994). Mechanosensory hair cells in the lateral line are sensitive to low-frequency (< 100 Hz) particle motion and can only detect signals originating within a number of body lengths distance from the fish (Popper *et al.* 2003). The inner ear is sensitive to vibration from further afield at frequencies of up to 1-3 kHz (Popper and Carlson 1998; Popper *et al.* 2003). Noise and vibration sensitivity is species-specific and largely related to the presence and anatomy of a swim bladder. The swim bladder operates as a transducer, converting pressure waves into vibrations. Species without (e.g. elasmobranchs) or with a reduced (e.g. flatfish) swim bladder typically possess low auditory sensitivity (Hawkins 1986).

Species with high sensitivity hearing, also known as hearing specialists, possess anatomical structures that couple the swim bladder with the inner ear. For example, a pneumatic duct and Weberian ossicles connect the swim bladder and inner ear in clupeid and cyprinid species, respectively (Hawkins 1986). Species that possess a fully functional swim bladder that is formed independently of the inner ear, such as percids and salmonids, are regarded as hearing generalists that are of medium sensitivity to sound (Hawkins 1986). Figure 22 shows how fish may be divided into 3 distinct classes according to hearing ability. Lower sensitivity does not mean that AFDs will not be applicable, but that they may require more audio power to achieve a similar degree of response.

Fish Sensitivity Classes

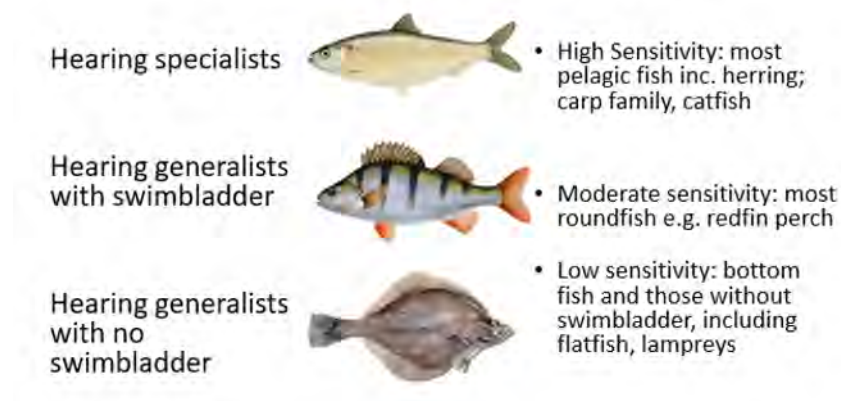


Figure 22 Fish hearing can be classified into 3 classes according to anatomical specialisations of different species (see text).

It has been demonstrated for roach that the inner ear and not the lateral line is responsible for detecting infrasound frequencies < 20 Hz (Karlsen 1992). Roach respond to infrasound vibrations from 0.3 Hz with threshold values of approximately $2 \times 10^{-4} \text{ m s}^{-2}$ particle acceleration (Karlsen 1992) and an upper frequency limit of approximately 300 Hz (Wolff 1967; Sand 1974). Amoser and Ladich (2005) recorded hearing thresholds of 80 dB re $1 \mu\text{Pa}$ for roach, with highest sensitivity in the frequency range of 0.1 - 0.3 kHz (Figure 23).

Although a considerable amount of research has shown promising results using AFDs to deflect hearing specialists (Ross *et al.* 1993, 1996; Schilt and Ploskey 1997), good results have also been observed for hearing generalists. For example, 83% of chinook salmon smolts were observed to be diverted from entering Georgiana Slough at its confluence with Sacramento River, California (Cramer *et al.* 1993) and 94% (maximum 100%) and 81% (maximum 83%) fewer steelhead and chinook salmon smolts were diverted at the Buchanan Hydroelectric Project on the St. Joseph River, Michigan, USA (Loeffelman and Tamms 1991; Loeffelman *et al.* 1991; Klinec *et al.* 1992).

For roach specifically, acoustic deterrent results are limited. In long-term field trials, 88% of 0-group roach were deflected by sound projectors emitting a 50 - 500 Hz chirp signal set at source levels of $8 \times 152 \text{ dB @ 1 m}$ to prevent entrainment into a raw water intake at Farmoor, River Thames, UK (Turnpenny *et al.* 1998). The intake had a maximum pumping capacity of $2.66 \text{ m}^3 \text{ s}^{-1}$ and the effective sound field range was designed to extend a maximum of 10 m. In other trials, at the River Foss Pumping Station, York, UK ($32 \text{ m}^3 \text{ s}^{-1}$ intake), an AFD system emitting a 100 - 500 Hz acoustic signal reduced the entrainment of roach by 56% (Wood *et al.* 1994). A field evaluation of an AFD system emitting sounds at a 0.2 s repetition rate at between 20 and 600 Hz at the Doel Nuclear Power Plant, Belgium reported a 51.2% decrease in entrainment of roach (Maes *et al.* 2004).

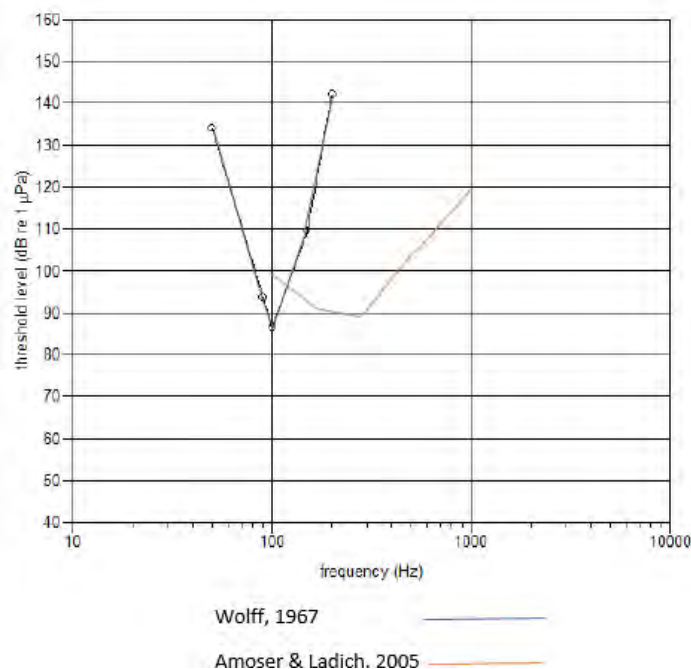


Figure 23 Audiograms of redfin perch from two studies. Both show similar sensitivity, with optimum hearing in the 100 - 300 Hz range.

For species closely related to redfin, the effect of acoustic deterrent studies to date also differs. Yellow perch and walleye displayed moderate avoidance to a sound system during cage trials at the Kingsford Hydroelectric Project on Menominee River, Wisconsin and Michigan (Winchell *et al.* 1997; EPRI 1998a,b; Michaud and Taft 2000). Other species tested, including the hearing generalist black crappie (*Pomoxis nigromaculatus*), did not display any avoidance to a number of acoustic signals. Yellow perch deterrence occurred at centre frequencies of 673, 953, 1,000, and 2,000 Hz. In the same study, effective signals for walleye centred at 566, 673, 1,350, and 2,990 Hz. A hydroacoustic survey of fish distribution in the forebay of Lennox Generating Station, Lake Ontario, Canada, assessed the dispersion of a number of species in the vicinity of an acoustic source ('fishdrone') emitting frequencies of 27, 64, 99, and 153 Hz at a pulsed signal of 3 s on and 1 s off, and an underwater hammer device operating at a frequency of 28 Hz at a rate of 15 - 20 sounds per minute (McKinley *et al.* 1988; Patrick *et al.* 1988). Species surveyed comprised hearing generalists yellow perch, pumpkinseed (*Lepomis gibbosus*), black crappie, rock bass (*Ambloplites rupestris*) and rainbow trout, and hearing specialists alewife and golden shiner (*Notemigonus crysoleucas*). Fish movement away from the forebay walls was recorded using the fishdrone at 153 Hz. A response was not observed for species other than alewife to the hammer. Similarly, acoustic transducers deployed on the trash racks at an intake of the White Rapids Hydroelectric Plant, Wisconsin and Michigan, USA, did not significantly decrease the number of yellow perch entrained (Winchell *et al.* 1997; EPRI 1998a,b; Michaud and Taft 2000). An infrasound device emitting 50 - 60 Hz tested at Kingsford Hydroelectric Project, Menominee River, Wisconsin and Michigan, USA, also revealed little or no response from yellow perch and walleye as well as largemouth (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*) (Winchell *et al.* 1997; EPRI 1998a, 1998b; Michaud and Taft 1999 from EPRI 2005).

Many of the studies discussed are salmo-centric or devised for multi-species deterrence. Whereas high levels of ambient noise, such as from pumped water intakes, may impair the hearing of specialists and mask the output of acoustic deterrents, the hearing of generalists such as redfin is only marginally or not at all affected in this way (Amoser and Ladich, 2005). To ensure the level of the sound is high enough to elicit a reaction in redfin at Talbingo intake, it may be necessary to model the underwater noise spectra under typical operating conditions (Lambert *et*

al., 1997). At the intended location of deflections, the sound level experienced by the fish should be maintained adequately above the ambient noise level, which is species-specific and dependent on the type of signal (Nedwell *et al.* 2004).

Dissimilar to some strobe light and bubble curtain (Section 4.5) research, habituation has rarely been observed using acoustic deterrents (e.g. Sonny 2007; Turnpenny *et al.* 1993). Acoustic signals are typically designed to minimise acclimation risk by using artificially generated waveforms that rapidly cycle in amplitude and frequency content (Turnpenny *et al.* 1993).

It may be concluded that responses of redfin and other hearing generalists to acoustic deterrents are variable, with promise shown by high percentages from some studies (Turnpenny *et al.* 1998). High variation in efficiencies between studies is likely a result of site-specific differences in bottom morphology, hydrology, angle of sound waves (Katopodis *et al.* 1994) and temperatures boundaries (Bergmann and Spitzer 1969). For example, low-frequency sound waves do not propagate well in shallow water and across hard substrates (Popper and Carlson 1998). Therefore, not all environments are effective at transmitting the same sound frequencies (Rogers and Cox 1988; Popper and Carlson 1998) and acoustic modelling, e.g. using the PrISM acoustic model (Fish Guidance Systems (FGS) Ltd), to take account of bathymetric properties of the intake and wider reservoir should be considered prior to installation of an acoustic deterrent.

Currently AFD's will not provide a total exclusion barrier for all life stages of redfin under all environmental conditions therefore AFD's, in isolation, do not warrant further consideration for Snowy 2.0. Should however AFD's be considered in combination with other deterrents to reduce the risk of fish entrainment, further research will be required to determine optimal frequency and pulse patterns for the principal species of concern.

3.5.8.6. Air-bubble screens

Bubble screens are formed from a curtain of air bubbles generated via compressed air pumped through a perforated tube or diffuser laid along the bed of the water body (**Figure 24**). In flowing water, the curtain is typically set at an angle to the flow (Turnpenny, 1998a) or in a loop around an intake entrance to deflect approaching fish and guide them away; air flow rates of 1 - 4 L s⁻¹ are recommended (Environment Agency 2005). Fish deterrence is believed to occur as a result of a combination of visual, auditory or shear-current stimulus caused by the rising bubbles Solomon 1992.



Figure 24 Surface appearance of a fish deflection bubble curtain, Head of Old River, California, USA (courtesy FGS Ltd).

Bubble curtain fish deflection efficiency results for hearing specialist species have shown relatively high values. Zielinski and Sorensen (2016) reported 73 - 80% deflection for Asian and common carp. Measurements of sound pressure and acoustic particle motion fields in the curtain indicated that the bubbles were producing sound at frequencies between 100 and 1000 Hz at 145 dB; this led the authors to conclude that sound was the primary stimulus to which fish were responding in this case.

For less sensitive fish, including percids and other hearing generalists, results with bubble curtains have been less encouraging. A bubble curtain installed at an intake canal at the White Rapids Hydroelectric Project, Menominee River, Michigan, USA, was not effective in reducing entrainment of yellow perch and the percid walleye (Detroit Edison 1975). In laboratory studies with the percid Eurasian ruffe (> 10 cm length), significant deflection by bubble curtains was reported, however each fish passed the screen multiple times and so the method was not consistently effective (Dawson *et al.*, 2006). The extent to which multiple passage events was a result of confinement or habituation is not understood, such factors always being difficult to interpret from laboratory

studies. In another study, less than 50% of white perch were observed to avoid an air-bubble barrier (McIninch and Hocutt 1987). No research was found on the effect of air-bubble curtains on juvenile or adult redfin.

To date, research on redfin has concentrated on the use of bubble curtains for exclusion of eggs and fry rather than adults. In this case, bubble curtains do not function as behavioural deterrents, as eggs and even larvae do not possess the ability to react. Instead, air bubbles rising through water lift discrete particles, including eggs and larvae, to the surface via floatation resulting from microbubble formation and localised vertical water movements (Pavlov 1989). Surface water currents generated by the bubble curtain then serve to repel the eggs and larvae. Low levels of protection (12 - 36%) have been recorded with the use of bubble curtains alone at facilities with high volume intakes such as the Kakhovskaya irrigation system in Russia which has a discharge of up to $530 \text{ m}^3 \text{ s}^{-1}$ (Pavlov 1989). Larger, weakly swimming organisms such as shrimps (*Crangon crangon*) have been excluded from UK thermal power stations using this method with an efficiency of around 60% (Turnpenny *et al.* 1993), indicating that bubble curtains have the potential to lift a range of juvenile fish sizes larger than eggs and small fry. Redirection of the rising bubble plume by the addition of special jet-guiding elements or baffles located in the upper water column may considerably increase effectiveness by directing the surface flow in a more appropriate direction away from the intake (Pavlov 1989; Turnpenny 1998b). In trials at the ZIL power plant (intake flow $10 \text{ m}^3 \text{ s}^{-1}$), Moskva River, USSR, $81.8\% \pm 3.6\%$ of redfin fry (14 - 32 mm length) and zander (*Stizostedion lucioperca*) fry (14 - 32 mm length) were diverted from an intake using a bubble screen (Pakhorukov 1984; Kolesnikova 1985).

There is evidence that bubble curtains can be injurious to some organisms. Small scale turbulence from bubble screens has been shown to increase the mortality of zebra mussel (*Dreissena polymorpha*) veligers (Rehmann *et al.* 2003), opossum shrimp (*Mysis relicta*) (Gregg and Bergersen 1980) and American paddlefish larvae (*Polyodon spathula*).

Variation in effectiveness for larger juveniles and adult fish are likely a result of species- and environmental-specific variations been studies. Visibility can make bubble curtains less effective, with darkness (Patrick *et al.* 1985) and turbidity (McIninch and Hocutt 1987) having been shown to limit their effectiveness.

A further limitation for bubble screen deployment in the Talbingo Reservoir with respect to older juvenile and adult life stages of redfin is the risk of habituation. In a UK trial, installations provided to exclude resident mixed species populations from a water intake were shown to decline in effectiveness within a few weeks of installation as a result of this effect (Turnpenny 1998a). Combinations with other stimuli such as light and sound do however present options for reducing habituation risk (Section 4.6). Bubble curtain performance can also decrease with increasing water depth, because the bubble plume becomes unstable as it rises further in the water column (Environment Agency 2005), although this will be less of an issue with a deeply submerged intake as proposed for Talbingo, provided a uniform curtain can be achieved in front of the intake openings. Consideration should also be given to understanding possible unwanted attraction of non-target species (Patrick *et al.* 1985).

Currently bubble curtains, in isolation, will not provide a total exclusion barrier for all life stages of redfin under all environmental conditions at Talbingo and are therefore not considered further.

3.5.8.7. Electrical barriers

Electrical barriers comprise submersed electrodes which pass an electrical current from anode to cathode to produce an electric field in the water. Electrodes can be mounted in concrete to reduce the potential for human contact and to prevent build-up from debris. Pulsed direct current electric fields are recommended for greatest behavioural effect and minimal damage to fish (Hocutt 1980; Reynolds 1996). Depending on the current and voltage of the barrier, the electric field can involuntarily attract fish towards the anode inducing electrotaxis (i.e. attraction to repulsion from an electrical current) (Hocutt 1980), however the field should be designed to startle

fish by stimulating the neuromuscular system but not inhibiting the ability to swim away (Parasiewicz *et al.* 2016). A graduated spatial electrical field, such as that produced by a graduated field fish barrier (GFFB), can be created to ensure the stimulus progressively increases towards the barrier and avoids electronarcosis, where the fish becomes stunned, immobilised and is subsequently carried by water flow through the barrier. Threshold voltage gradients that alter behaviour in fishes were assessed by Kolz and Reynolds (1989), who exposed common goldfish (*Carassius auratus*) to fields of constant DC, household AC and 50-Hz PDC. Table 1 provides a summary of the power transfer studies conducted by these authors on goldfish. The voltage gradients necessary to induce a behavioural change using a constant DC waveform ranged from 0.18 to 1.46 V cm⁻¹ and from 0.15 to 0.92 using a pulsed DC waveform. These kinds of data provide perspectives on the electric field intensities necessary to deter freshwater fish species from areas where their presence is not wanted. Moreover, the research by Kolz and Reynolds (1989) was conducted at water conductivity ranges not much greater than those found in Talbingo Reservoir.

Table 1 Power-density thresholds (μW/cm³) converted to equivalent voltage gradients (V cm⁻¹) for an evaluation of goldfish threshold responses to three electrical waveforms typically used by fisheries managers during electrofishing surveys (from Kolz and Reynolds 1989). Values for the PDC waveform used a pulse frequency of 50 Hz and were averaged for three electrical duty cycles in this research.

Gradient Response and Waveform	Peak Power Density (μW/cm ³)	Equivalent Voltage Gradient (V/cm)	Effective Conductivity (μS/cm)
Twitch			
DC	2.4	0.18	69
AC	2.1	0.13	119
PDC	2.3 – 2.7	0.15 – 0.17	93
Anodic Attraction			
DC	41	0.70	82
Narcosis (Stun)			
DC	179	1.46	83
AC	126	0.89	156
PDC	103 – 127	0.83 – 0.92	147

Species specific differences from electric pulse magnitude, rate, shape and duration, fish size and water conductivity will influence the extent of galvanotaxis (i.e. attraction to/ repulsion from an electrical current) (Hocutt 1980, Popper and Carlson 1998), leading to variable barrier effectiveness. Assessments of the effect of electrical barriers on percids have shown a variety of results. A study by Novotny and Priegl (1974) determined an optimal pulsed DC frequency of 15 - 40 Hz for yellow perch. However, the purpose of the study was to determine the most efficient electrical settings for catching fish by boat electrofishing. For the percid Eurasian ruffe (*Gymnocephalus cernual*) (≥ 10 cm length), an invasive species in the Great Lakes, USA, the most effective setting out of four tested used 5 ms pulses at 6 Hz, preventing only approximately half the number of passes through the barrier when compared to a control (Dawson *et al.*, 2006). Electrical barrier effectiveness has also been reported

for the percid walleye (Weber *et al.* 2016). Prior to construction, threshold values for electrical deterrence of redbfin perch would need to be tested and evaluated by experimental means (e.g. a flume study).

For non-percids such as bighead carp and silver carp, effectiveness of an experimental electric barrier deployed in the Chicago Sanitary and Ship Canal between the Great Lakes and Mississippi River drainage basins has been tested. Only one tagged carp out of 118 passed through the electrical barrier, which was thought to occur as a result of electrical shadowing from a passing barge (Stainbrook *et al.* 2005). Similar results have been found for other carp species (Verrill and Berry JR 1995; Bullen and Carlson 2003; Sparks *et al.* 2010; Parasiewicz *et al.* 2016). The Chicago barriers output 0.9 V cm^{-1} of PDC at a pulse frequency of 30 Hz (Culver and Chick 2015). Research into electric-gradient protocols for Chicago barrier operation provides additional information on fish taxis responses, from “first response” and “flight response” to immobilization thresholds (Holliman 2011) (Table 2). The results presented by Holliman (2011) are considered robust and offer unique information on behavioural thresholds of fish to electric gradients. Results are based on a range of fish barrier operating simulations (where pulse frequencies, pulse widths, and voltage gradients were all varied) on treatment groups comprising not less than 20 naïve individuals per test. Bighead carp juveniles averaged 5.6 cm in length whereas Silver carp were larger and averaged 19.5 cm in length. Holliman (2011) found that “righting” behaviour (i.e. recovery) for the bighead carp was 47 s in fish exposed to 1.02 V cm^{-1} but only required 7 s in fish exposed to 0.79 V cm^{-1} , although such recovery data was not reported for silver carp.

Table 2 Response of invasive carp species at electric deterrence gradients designed to determine “first response,” flight, and immobilization thresholds in support of research for the Chicago Sanitary and Ship Canal electric barriers (from Holliman 2011).

Mean Voltage Gradient (V/cm) for:					
Carp Species	Total Length (Mean cm)	First Response	Flight	Immobilization	Ambient Water Conductivity ($\mu\text{S/cm}$)
Silver	19.2	0.12	0.16	0.40 – 1.90	687 – 765
Bighead	5.6	0.10	0.38	0.81	980 – 1,050

Table 3 summarises peer-reviewed literature and technical reports concerning the efficiency of electric barriers to deter various fish species at hydropower-related projects and other deterrence applications, worldwide. Whilst the majority of barriers are configured to prevent upstream movement of fish, some studies have reported on the efficacy of electric streams to prevent downstream movements. An electric barrier in the Shiawassee River, Michigan, was reportedly successful at preventing invasive round gobies from moving downstream in this system (Savino *et al.*, 2001). Similar results were achieved with downstream electric barriers used by Maceina *et al.* (1999) at a slow-moving lake outlet in the State of Georgia and in the Sacramento River, California, where an electric barrier reduced juvenile salmon entrainment at a pump intake by 79% (Demko *et al.*, 1994).

The effectiveness of electrical barriers has also been found for some salmonids and clupeids (Barwick and Miller 1996). However, poor results were observed in one study for Atlantic salmon smolts (Larinier and Travade, 2002).

Table 3 A summary of some of the results of studies and evaluations conducted on Electric Fish Barriers from both peer-reviewed publications (the first six entries) and agency reports. Barriers designed to deter upstream-moving fish are designated (U) while (D) denotes barriers used to guide or control downstream-moving fish (list supplied by Smith-Root).

Barrier Location and Type	Citation	Author-Reported Conclusions on Electric Barrier Efficiencies
Heron Lake MN (U)	Verrill & Berry 1995	"None of 1,600 tagged fish were among the 3,367 examined above the barrier."
Shiawassee R. MI (D)	Savino et al. 2001	"The only marked round goby found below the barrier were dead."
Lake Seminole GA (D)	Maceina et al. 1999	"After (the) electric barrier was in place, no verified escapes occurred."
Jordan River MI (U)	Swink 1999	"No unmarked and none of the 1,194 tagged lamprey were found above the barrier."
Chicago Ship Canal IL (U and D)	Sparks et al. 2010	"Of 130 radio-tagged carp, one (a dead fish?) was found above the electric barrier."
San Joaquin R. CA (U)	CDFG 1993	Only 1% of fish were observed upstream of the barrier.
Wilkins Slough CA (D)	Demko et al. 1994	"Downstream juvenile Chinook entrainment (from Sacramento River) was reduced by 79%."
Vessy Switzerland (U)	GREN Biologie 2009	"None of 339 marked trout were found in the hydropower tailrace after GFFB installation."

Particular caution would need to be taken when applying electrical barrier technology to pumped intakes as any fish immobilised by the screen will neither be able to swim away from the intake nor will they be carried passively away from the intake by prevailing currents, instead being drawn into the intake. Specifically, electric fields strong enough to elicit avoidance in small fish are often high enough to stun large individuals (Turnpenny 1998a) which may result in entrainment into an intake. Graduated electric fields can decrease this effect providing there are suitably low velocities at the barrier (Pugh *et al.* 1970; Demko *et al.* 1994) however in lentic environments there is no flow cue to allow fish to move out of the electric field. The use of electrical currents in low flow environments, in which an incapacitated individual would not be passively carried out of the electrical field would preclude its use under 'best practice' in the UK (Environment Agency 2005). If the voltage of an electric barrier is set too high, fish death may occur and whilst this may be desirable for species such as redfin this may not be the case for indigenous, native species.

To construct an electric barrier, electrodes are placed across the channel so that the electric current is parallel to the prevailing streamlines. The electrodes can be mounted vertical, suspended from a cable or structure, attached to piling, or they can be laid flush to the bed or side wall. Electrodes that are mounted flush to the surface do not present an obstruction to flow and offer no impediment to the movement of debris through the electrical field and are therefore most commonly deployed. Configurations of this type are well-suited to culverts or tunnels. Examples of bottom-mounted open-channel electrical barriers and a culvert/tunnel barrier are shown in Figure 26 & Figure 27.

The shape of the electrical field is determined by the electrode configuration and the water depth. The field is not affected by water flow, turbidity, debris, or modest sediment (although thick layers of sediment that are less conductive than the water can reduce the field in the water above bottom mounted electrodes).

To produce the most efficient electric field pattern for blocking fish, it is desirable to produce a field with electric lines running head-to-tail through the fish (Figure 25). This orientation transfers the maximum power from water into the fish. In flowing water of 1.5 to 2 fish body lengths per second or greater, fish instinctively swim with their heads into the flow. Therefore, the most effective field pattern is one with the electric field lines running parallel to water flow.

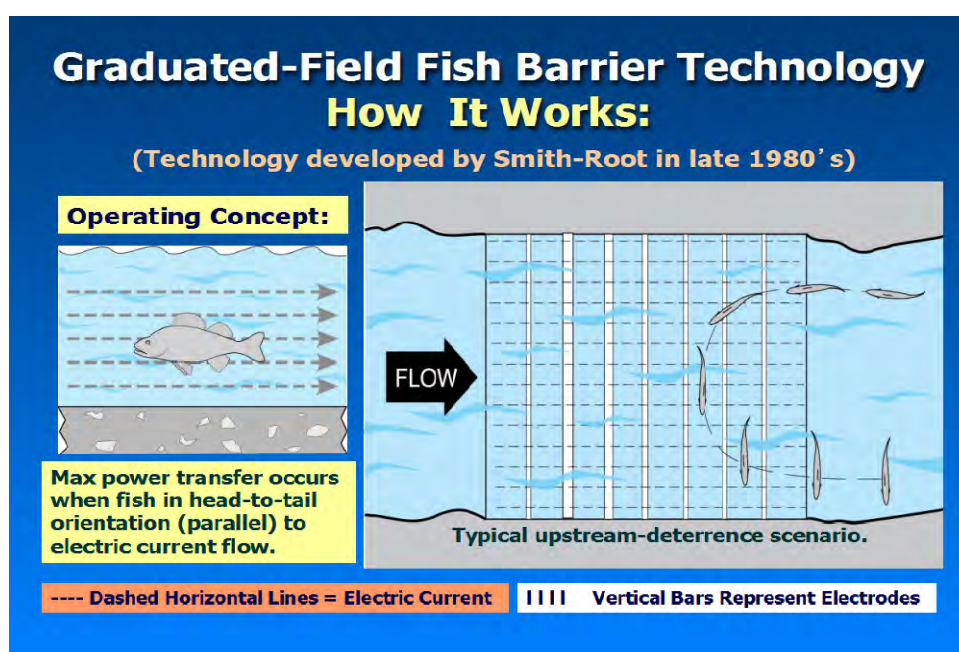


Figure 25 Conceptual views of how Graduated-Field Fish Barriers operate to deter fish attempting to move upstream. In these depictions, the horizontal dashed lines represent the flow of electric current; vertical bars represent electrodes. Fish experience maximum energy transfer from snout to tail when moving perpendicular to electrodes (parallel with electric field lines). The graduated field intensifies the further that fish move into it, causing sideways deflections and/or deterrence. (Smith-Root).

Fish learn very quickly that by turning sideways to the flow they can minimize the effects of the electric field (as the potential gradient across the fish is reduced). In the case of an electric barrier being used in a 'downstream' context such as pump mode within Talbingo where the flow of water is towards the intake, this risks fish being drawn through the electric current and being entrained without being significantly harmed. This may have significant implications when considering the feasibility and risk of fish transfer from Tantangara Reservoir when considering the deployment of an electric behavioural barrier at Snowy 2.0.



Figure 26 Example of bottom-mounted, open channel electrical barrier in channel with water drawn down. Okoboji Lake outlet in Iowa, USA (Smith-Root).



Figure 27 Example of culvert electrical barrier at lake outlet. White Lake, Minnesota, USA (Smith-Root).

Vertical electrodes: An electrical field can be applied by electrodes positioned vertically in the water column. The main advantage of vertical electrodes is that a uniform electrical field can be applied to any depth of water. For deeper waterbodies, the electrical field can be created with much higher efficiency and less input power than that used for bottom-mounted electrodes. Multiple rows of vertical electrodes can create graduated electrical fields as well as static electrical fields. In vertical configurations, electrodes can be either suspended from fixed lines at or near the surface or employed as “risers” (from fixed piles or weighted, bottom-mounted cables). An example of a vertical electrode barrier system with pile risers is presented in Figure 28.

Electrical deterrence has the potential to be highly effective at preventing upstream movement of fish, however it would be unable to prevent the movement of non-motile stages of fish during the ‘downstream’ pumping phase of operation at Talbingo. As such, it is not recommended for application at Snowy 2.0 in isolation

3.5.8.8. Electric screens for euthanasia

The use of electric gradients to euthanise fish has been deemed an acceptable, humane method by the Royal Society for the Prevention of Cruelty to Animals in the UK (RSPCA 2014) and is widely employed as a humane method at fish farms. Electric barrier technology can be used to create electric fields sufficiently strong enough to kill any unintended organisms either prior to entrainment or within the entrainment viaduct of Snowy 2.0 itself. Support for this premise comes from several published accounts in the fisheries literature. For example, Henry and Grizzle (2006) demonstrated that 60 Hz PDC electrical gradients of 2.5 to 8 V/cm for 20-second exposures were sufficient to induce very high mortality among newly transformed juveniles of three fish species. Their trials used water conductivities that varied from 10 to 1,020 $\mu\text{S}/\text{cm}$. Test fish and size ranges included 14 - 19 mm bluegill, 34 - 44 mm largemouth bass and 34 - 43 mm channel catfish. The newly transformed bluegill juveniles were just 15 - 20 days old (those of the other two species were 45 - 90 days old). In addition, the optimum conductivity for achieving mortality was found to range from 65 to 175 $\mu\text{S}/\text{cm}$ – values not greatly dissimilar to those of Talbingo and Tantangara reservoirs.

Newly transformed juvenile fishes are most susceptible to mortality induced by electricity (Henry *et al.* 2003). Larvae and juveniles of bluegill (19 - 21 mm), largemouth bass (29 - 32 mm), channel catfish (27 - 30 mm), and 21 mm Nile tilapia (*Oreochromis niloticus*) exposed to 60 Hz PDC fields having voltage gradients from 2 to 16 V/cm in 100 $\mu\text{S}/\text{cm}$ water. Immediate mortality was documented at the time of transformation from larvae to juveniles, confirming the susceptibility of larval fish life stages to electric gradients. As far back as the 1920's, research on salmonid fishes established that mortality can occur among juvenile, 8-cm chinook salmon in constant DC fields of 0.65 to 1 V/cm if exposure times exceed 1 minute (McMillan 1928). For these reasons and based on the Henry and Grizzle (2006) paper cited above, there is good reason to believe that a sufficiently strong electrical gradient could prevent the transport of live Redfin perch larvae to Tantangara Reservoir. No data is however available that elucidates susceptibility to mortality of redfin eggs exposed to an electrical current and further research would be required to confirm euthanasia outcomes for this life stage, (coupled with power transfer theory to compensate for different water conductivity).

As some juvenile fish are susceptible to mortality induced by electricity (60-Hz pulsed direct current) at voltage gradients from 2 to 16 V cm⁻¹ in 100 $\mu\text{S cm}^{-1}$ water, consideration needs to be given to health and safety however. This voltage gradients equates to 360 to 2,880 volts potential difference from head to toe for a 180 cm human entering this field. At these voltages, infrastructure may need to be within the confines of the tunnels to ensure safety. This option would not be practical however, as this would require the draining of the tunnel in order to undertake maintenance or repairs. A benefit of such a system however is its ability to accommodate bi-directional flow.

Whilst advice from Smith-Root in the USA has indicated that the technology is technically feasible, estimations of ongoing power requirements for a deterrence system backed up with a secondary higher voltage lethal system to euthanase eggs, larvae and any fish that are unable to escape the deterrence zone has been calculated to have an ongoing power requirement of 21 MW (Smith-Root). There are also no known applications of upstream electrical barriers targeting complete exclusion via the use of lethal voltages. As a result, this technology is not considered suitable for Snowy 2.0.



Figure 28 Example of vertical electrode barrier system at power plant tailrace to prevent the movement of fish upstream into the tailrace. Battle River Generating Station, Alberta, Canada (Smith-Root).

3.5.9. Hybrid fish deterrents

Hybrid deterrent systems combine two or more behavioural deterrent technologies to create a multi-stimulus behavioural barrier. Such methods can reinforce the biological response and may have specific advantages for multi-lifestage repulsion and creating equipment redundancy (Popper and Carlson 1998; Welton *et al.*, 2002).

3.5.9.1. Light and bubble barriers

Combined light and bubble systems have shown increased efficiency over either stimulus alone in a number of studies. The addition of light improves the effectiveness of bubbles as a visual barrier (Sager *et al.* 1987). For example, Sager *et al.* (1987) found that bubble barriers illuminated by strobe lights (300 FPM) were more effective at inducing avoidance behaviour in the fish species spot (up to 100%), menhaden (up to 68%) and white perch (up to 36%) in both clear and turbid conditions. Patrick *et al.* (1985) found that strobe lighting was more effective in deterring alewife, rainbow smelt, and gizzard shad when combined with a bubble barrier. In an experimental flume, strobe lights and bubble curtains elicited avoidance responses of 3 - 58% for white perch, 21 - 85% for spot and 9 - 81% for menhaden (Sager *et al.* 2000). Conversely, the addition of light to bubble curtains did not sufficiently decrease entrainment of sockeye salmon (*Oncorhynchus nerka*) in field studies at the Seton Hydroelectric Station, British Columbia, Canada (McKinley and Patrick 1988), and the White Rapids Hydroelectric Project (EPRI 1998b).

As this technology is considered unlikely to provide a total exclusion barrier for all life stages of redbfin under all environmental conditions, it is not considered further for application at Snowy 2.0.

3.5.9.2. Sound and bubble barriers

Bubble curtains with acoustic devices have shown high levels of deterrence compared to studies of bubble curtains alone (Atlantic salmon smolts (Welton *et al.* 2002); Asian carp species (Pegg and Chick 2004); common carp (*Cyprinus carpio*) (Zielinski *et al.* 2014)). A unique design known as a 'Bioacoustic Fish Fence' (BAFF™) can be used to produce an evanescent sound field using a dense curtain of bubbles to trap a pneumatically or electronically produced sound signal by refraction (Larinier and Travade 2002). Sound levels inside the bubble curtain can achieve 170 dB re 1mPa (around 20 times higher than levels reported above for bubble curtains alone), decaying to approximately 5% of this value within 0.5 - 1 m from the bubble curtain (Figure 29). The specific sound signal used can be manipulated to target the species of interest by a sound projector array. BAFF barriers have elicited high guidance efficiencies away from undesirable routes for both hearing specialists (e.g. bighead carp (*Hypophthalmichthys nobilis*), Taylor *et al.* 2005) and generalists (e.g. Atlantic salmon smolts, Nedwell and Turnpenny 1997; Welton *et al.* 2002; Spiby 2004).

As this technology is considered unlikely to provide a total exclusion barrier for all life stages of redfin under all environmental conditions, it is not considered further for application at Snowy 2.0.

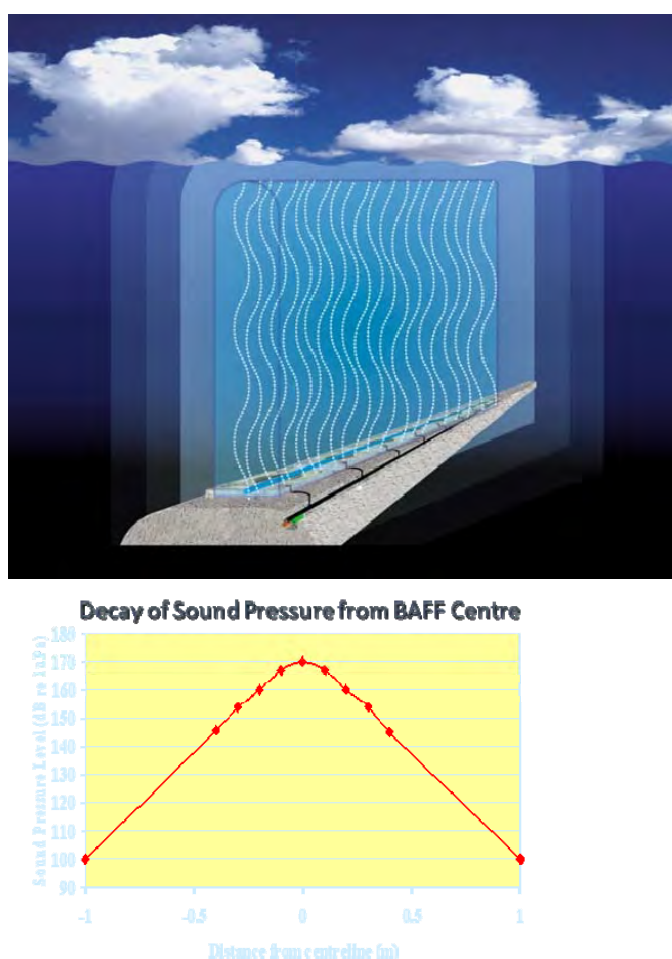


Figure 29 (Above) Schematic of a Bioacoustic Fish Fence (BAFF) showing a rising bubble plume into which sound is injected by sound projectors set into the base, becoming trapped by refraction within the bubble curtain to form a 'wall of sound'; (below) plot of upstream to downstream sound decay from the midline of the BAFF (courtesy FGS Ltd).

3.5.9.3. Sound and light

A hybrid strobe light/popper system was found to be marginally more repellent than strobe lights alone for sockeye salmon at the Seton Hydroelectric Station, British Columbia, Canada (McKinley and Patrick 1988). Sound and light hybrid technologies have received less attention than studies combining light with BAFF (Section 4.6.4) however as this technology is considered unlikely to provide a total exclusion barrier for all life stages of redbfin under all environmental conditions, it is not considered further for application at Snowy 2.0.

3.5.9.4. Sound and electric barrier

A number of fish species have demonstrated the ability to learn sound association. For example, grass carp have been trained to come to sounds in the range of 600 - 1000 Hz to feed (Willis *et al.* 2002). Behavioural studies with goldfish and tilapia found that fish responded to sound and light in conjunction with electric shock (Yliff *et al.* 2008). Conditioning with sound has also been found in non-hearing specialist salmonid species (Tlustý *et al.* 2008). There is potential therefore for graduated electric barriers to be combined with AFDs to prevent fish entrainment. Fish experiencing the electric field for the first time would also hear the AFD signal. On subsequent approaches to the barrier when hearing the AFD signal could exhibit a Pavlovian response and turn away before experiencing the electric field. In this way the effect of multiple challenges of the electric barrier is mitigated (Turnpenny 2011). Further research is required to optimise signals for target fish (sound frequencies, strobe rate, electric field strength) and understand the learning ability of redbfin and salmonids over a period long enough to assess learning or habituation effects.

As this technology is considered unlikely to provide a total exclusion barrier for all life stages of redbfin under all environmental conditions, it is not considered further for application at Snowy 2.0.

3.5.9.5. BAFF and light

For the percid walleye (*Sander vitreus*), a bio-acoustic bubble-strobe light barrier reduced escapement rates from 89.3% with the barrier off to 44.1% with low and medium sound, whereas up to 100% of the fish escaped with the addition of light (Flammang *et al.* 2014). A sound-bubble-strobe light barrier preventing upstream passage of a number of freshwater fish species in Quiver Creek, Illinois, USA, diverted the movement of 99% of marked silver carp (*Hypophthalmichthys molitrix*), and 97% of other marked species including bluegill (*Lepomis macrochirus*), common carp, grass carp (*Ctenopharyngodon idella*), green sunfish (*Lepomis cyanellus*), largemouth bass, sauger (*Sander canadensis*), white sucker (*Catostomus commersonii*) and yellow bullhead (*Ameiurus natalis*) (Ruebush *et al.* 2011). BAFF systems using Synchronised Intense Light and Sound (SILAS) technology have been successfully trialled at Head of Old River and Georgiana Slough in California, USA, to reduce the risk of acoustic tagged chinook salmon entering irrigation intakes (Bowen and Turnpenny 2009). SILAS-BAFF systems are currently being installed at a number of other sites in the USA to control the spread of invasive Asian carp species.

As this technology is considered unlikely to provide a total exclusion barrier for all life stages of redbfin under all environmental conditions, it is not recommended for application at Snowy 2.0.

3.5.10. Hydraulic deterrents

3.5.10.1. Veneer intake

A unique submerged intake known as the veneer intake can be found at Darlington Nuclear Power Station on Lake Ontario (Figure 30). The veneer intake has a central non-porous section located over a vertical riser pipe

surrounded by a porous section with 140 mm wide slot openings. Fish protection is provided by a combination of low and uniform velocities and unwillingness of fish to pass through them. Entrainment sampling conducted at Darlington indicated that the veneer intake resulted in an 80% reduction in juvenile and adult fish and a 60% reduction in eggs and larvae when compared to a shoreline surface intake with no fish protection (SENES 2011). A large orifice area would be required to achieve approach velocities of 0.15 m s^{-1} making the method unlikely to be feasible for Snowy 2.0. As this technology is considered unlikely to provide a total exclusion barrier for all life stages of redfin, however it is not recommended for application at Snowy 2.0.

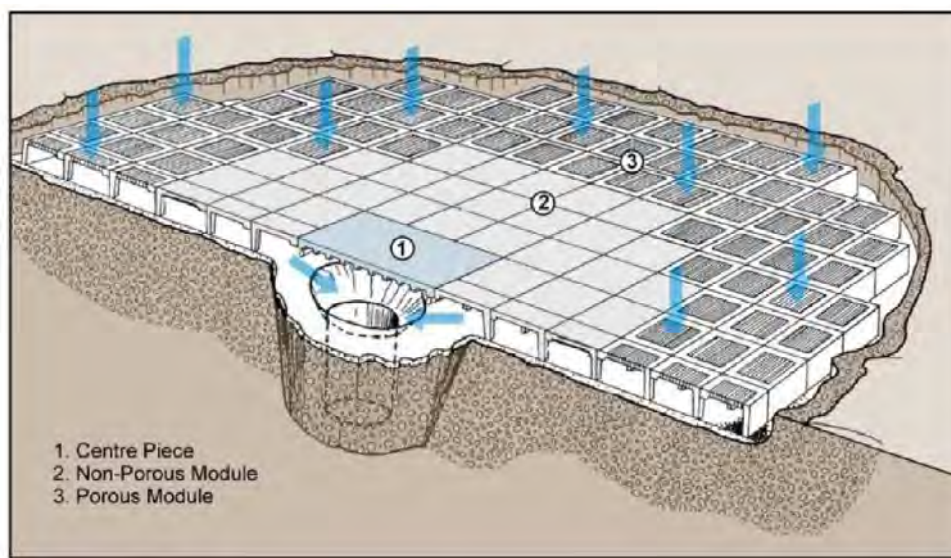


Figure 30 Design for Darlington Veneer Intake (Christie *et al.* 1984).

3.5.11. Chemicals

Chemicals can be added to or infused into the water to create behavioural or physiological barriers to fish passage. Chemicals applied as behavioural barriers cause avoidance (or attraction) responses in fish whereas physiological chemical barriers prevent passage by creating conditions that fish cannot physically tolerate, generally resulting in mortality if concentrations are high enough and persistence in the environment is long enough.

Chemical deterrents such as carbon dioxide and nitrogen bubbled into water through a diffuser to decrease oxygen levels (nitrogen sparging), can be used to create a behavioural avoidance response in fish (Noatch and Suski 2012). Fish can detect and avoid low oxygen concentrations (Miranda and Hodges 2000; Burleson *et al.* 2001), with hypoxic water known to preclude fish from entering an area (Maes *et al.* 1998; Hasler *et al.* 2009). Fish can also sense high carbon dioxide levels (Perry and Gilmour 2002). For example, elevated carbon dioxide has been shown to elicit avoidance responses in juvenile silver carp, bighead carp, bluegill and largemouth bass (Dennis *et al.* 2015) as well as yellow perch (Donaldson *et al.* 2016). Laboratory experiments also showed that fish avoided water enriched with carbon dioxide (Kates *et al.* 2012; Cupp *et al.* 2017). However, impacts from such chemical deterrents are complex. Potential impacts at the population, community and ecosystem level are poorly understood when compared to the physical properties of other behavioural stimuli. For example, weak acidification can occur in freshwater under increased free carbon dioxide levels (Hasler *et al.* 2018).

Chlorine is commonly used as a biocide to prevent fouling in power station cooling water discharges. The chemical is toxic to fish because it damages gill tissue (Brungs 1973). A number of fish species have been found to be capable of detecting and avoiding sublethal chlorine gradients (Brungs 1973; Giattina *et al.* 1981; Wilde *et al.*

1983). The chemical can also be lethal in high enough doses. Chlorine degrades naturally over time; however, persistence is dependent on sunlight, aeration and availability of nitrogenous compounds with which it combines to form stable residuals (Katz *et al.* 1977).

Pheromones can also be used to modify fish behaviour and prevent passage through undesirable routes. Pheromones are chemical odours secreted by organisms, for example to attract mates or for predator avoidance (Sorensen and Stacey 2004). Research has found that approximately 50% of marked female sea lamprey (*Petromyzon marinus*) released downstream of a trap containing a synthesized pheromone were successfully attracted (Johnson *et al.* 2009). Conversely, evasive responses can be induced in fish by releasing pheromone-like 'alarm substances' that simulate those naturally produced when fish are injured (Brown *et al.* 2000). This literature review did not identify pheromones specifically designed to target redfin.

Chemical deterrents such as ozone can also act as a physiological, rather than a behavioural, barrier to invasive fish entrainment, with lethal doses requiring only short exposure times. Ozone also has a short half-life in freshwater and is considered to have minimal environmental impacts when compared to other chemicals (Buley *et al.* 2017).

Piscicides such as antimycin A, rotenone and salicylanilide I, are regarded in some countries as reliable and cost-effective methods for removing invasive fish species from the environment (Marking 1992; Donnelly 2018). An example of rotenone treatment is a temporary application in the Chicago Sanitary and Ship Canal, Great Lakes, USA, to prevent the transfer of invasive carp species while the electric barrier was undergoing maintenance (Buck *et al.* 2010). However, the toxicity of piscicides can also impact non-target species abundance both directly via toxically induced mortality or indirectly by decreasing numbers of susceptible prey species (Donnelly 2018). A study on the songbird American Dipper (*Cinclus mexicanus*) found that antimycin A and rotenone decreased the availability of benthic invertebrate prey, leading to significantly reduced body condition lasting up to 9 months post application (Donnelly 2018).

For both physiological and behavioural chemical deterrents and barriers, chemicals are difficult to confine to a selected area and to maintain at the required concentration over time and under all conditions as effectiveness is often site, season, weather and water quality specific (Donaldson *et al.* 2016; Cupp *et al.* 2017). Therefore, chemicals are best applied in confined environments and not large rivers and reservoirs such as the Talbingo system. In addition, the application of biocides such as sodium hypochlorite may result in the production of more persistent by-products and dosed chemical concentrations would be required to decay below a Predicted No-Effect Concentration prior to discharge into the upper Tantangara Reservoir, or pumping discharge would need to be treated adding additional cost. Therefore, chemical barriers and deterrents are not a recommended method for preventing the entrainment of redfin at the Talbingo intake.

3.5.12. Stock Management

Redfin are naturalised throughout the Tumut River system and Talbingo reservoir and whilst options exist for managing populations adjacent to the water intake, eradication of the species from the catchment would be a significant and costly undertaking with no guarantee of success or of future reintroductions.

Whilst eradication is a feasible consideration for small enclosed water bodies removal from larger, online water bodies present significant challenges and best endeavours may only result in reducing local populations over relatively short temporal periods. Stock control however may have benefits in that the risk of entrainment of fish from relatively depauperate populations may be significantly reduced.

Options for the management of redfin populations include:

- Physical removal
- Chemical control

- Biological control

Physical removal

Physical removal may include electric fishing, netting, trapping and the use of artificial spawning substrate. Electric fishing involves placing electrodes in the water and generating an electric field to stun fish within range. Target species can be removed to bins using hand nets whilst non-target species can remain within the water course to recover. Fishing should be undertaken by trained personnel by either wading using back-pack equipment or 'wander' leads or from a boat. Fishing is most effective in shallow water (<3m) in complex habitats (lake margins) and larger fish, >100 mm, are more susceptible to capture. Efficiency of electric fishing can vary according to environmental conditions e.g. water conductivity, temperature, turbidity, etc.

Seine netting is most effective when deployed from gently sloping beaches with smooth substrate with no bed obstructions to hinder the net. Suitable netting stations within Talbingo are likely to be limited due to the large volumes of standing timber and allochthonous material within the margins. Fish are encircled by a wall of net deployed from a boat or by wading and the net closed and drawn to the shore where fish are removed. Efficiency is however low in large bodies of water. Freshwater trawls can be deployed behind boats to intercept shoals identified by echo sounders (fish finders). Mesh size will depend on target size however the smaller the size of mesh employed the slower the tow speed. Thus, fine mesh may allow larger specimens to evade capture whilst larger mesh will not intercept some juveniles.

Other forms of net include fyke netting which can be effective for smaller perch (<150 mm in length) and gill netting which can be effective for larger specimens.

Generic net traps such as portable pound nets or more targeted 'Windermere perch' or minnow traps can be effective a certain times of the year e.g. during the spawning period. Traps are however highly selective for species and size of fish with the mesh size determining the lower length limit of fish trapped (Craig, 1980).

Perch typically spawn in the shallow margins on coarse medium and allochthonous material. Whilst spawning may be impaired by fluctuating water levels (eggs may be desiccated if exposed for sufficient periods) egg mass may remain viable if periods of inundation are relatively short. Artificial spawning substrates such as bundles of brushwood may be deployed along the lake perimeter at depths between 2 and 4 m throughout the spawning period and checked regularly for the presence of egg strings which can be removed when observed (Ingram, 2016).

Controlling redfin perch in Talbingo Reservoir would require the development of a long-term management strategy which may entail excessive costs for the benefits derived as population reduction may be short-lived if not maintained. The presence of just a small number of redfin perch in the lake and the risk of reintroduction from feeder streams, may lead to rapid recovery of the population when conditions are favourable (Ingram, 2016).

Chemical control

Rotenone is an inorganic pesticide that can be used, typically under licence, to remove undesirable or nuisance fish from water courses. Rotenone is sprayed in liquid form into the water course and is rapidly broken down in the environment with low persistence. The pesticide works by inhibiting cellular respiration leading to reduced cellular uptake of oxygen from the blood stream and effects most gill breathing organisms. Rotenone is however non-specific with regards to its target organism (fish) and invertebrates and other native fish species will be similarly affected. The presence of Murray Crayfish (*Euastacus armatus*) (listed as vulnerable under the NSW Fisheries Management Act, 1994) and other valuable recreational fish species precludes the use of rotenone in Talbingo even on a local scale.

Biological control

Large redbfin are cannibalistic on smaller redbfin and removal of larger specimens, which are easily screened from water intakes and returned to the source water, may regulate the number of smaller fish. Higher recruitment of small juvenile perch has been recorded where larger cannibalistic adult fish were absent (Closs *et al.*, 2001). Predation of the young-of-year redbfin by adults may also have a positive effective on the surviving fish, which may have increased growth rates due to reduced intraspecific competition. Increasing the number of large redbfin perch may be achieved by the introduction of a maximum size regulation (Ingram 2016) or returning larger specimen captured during physical removal exercises. Maintenance of the larger specimens may however increase the rate of reproduction and recruitment which may be counter productive.

3.5.13. Euthanasia and disinfection

3.5.13.1. Ultraviolet light

Ultraviolet (UV) light can be used to remove bacteria and other microorganisms from water. UV disinfection is typically used on relatively low water volumes, for example at fish hatcheries and for domestic and industrial water supplies, removing > 99.9% of bacteria and preventing the transmission of diseases between fish. UV light is also used in water pipes to prevent invasive and nuisance bivalve larvae settlement. UV light penetrating the upper depths of water bodies can also have long term effects on the survival of fish larvae and juveniles during early life stages.

Yellow perch egg survival in lake conditions with high, moderate, and low solar UV radiation found that all eggs exposed to high UV perished (Williamson *et al.* 1997). In the same study, a survey of yellow perch eggs in multiple lakes identified that eggs were deposited deeper in environments with higher solar UV radiation penetration (Williamson *et al.* 1997). In a more recent study, the effects of the UV-A/UV-B ratio and irradiance of natural UV radiation on the survival of yellow perch larvae was assessed (Boily *et al.* 2011). Results indicated that incident UV-A radiation could impact the survival of yellow perch larvae.

A UV system rated to provide a wall dose (UV intensity at the furthest location from the UV lamp) of approximately 40 mJ/cm² is recommended to achieve effective disinfection of water in domestic and industrial applications (Defra, u.d.; atg UV Technology, 2018). Lethal doses of UV required for redbfin egg and larvae euthanasia may therefore be expected to fall close to this value, however exact doses would require experimental assessment.

The actual UV dose required to be delivered to ensure euthanasia of redbfin eggs and larvae would be dependent on water quality and flow. Water flowing to UV disinfection systems should be clear and relatively free from dissolved substances (Defra, u.d.). Low levels of both turbidity and total suspended solids within both Talbingo and Tantangara reservoirs may increase the feasibility of successful treatment however water quality may vary seasonally and only a slight drop in ultraviolet transmittance (UVT) may double the amount of UV intensity required to provide disinfection.

The unprecedented capacity of the UV system required and the need to guarantee total exclusion of all life stages of fish from the intake means that ultraviolet light disinfection is not recommended for application at Snowy 2.0.; this recommendation is informed in part by the unknown efficacy of UV light as a method of euthanasia for egg and larval stages of fish that would require extensive dosage and exposure trials, as well as high estimated capital, operation and maintenance costs.

4. Discussion and conclusions

Snowy 2.0 is a unique scheme, proposing the development of a pumped hydro station that aims to generate 2,000 MW from connecting two existing reservoirs through a 27 km bi-directional flow tunnel. Where other schemes approach the intake flows proposed for Snowy 2.0, such as Ludington Pumped Storage Plant, Lake Michigan, complete prevention of inter-catchment transfer of fish species is not required, and the methods employed for minimising entrainment reflect this. For example, barrier nets are used seasonally at Ludington to reduce, but not eliminate entrainment of fish. The present report has drawn on industry experience, peer-reviewed and grey literature to advise on potential redbfin intake entrainment prevention options for the Snowy 2.0 scheme.

Physical screening options to prevent the entrainment of redbfin into the intakes located within Talbingo Reservoir include flat panel screens, travelling screens, drum screens and barrier nets/filters.

Physical exclusion screening options would require a mesh size or bar separation of $\leq 0.5\text{mm}$ and a low approach velocity to provide confidence around complete exclusion of all life stages of fish. In order to accommodate bi-directional flow, any screens installed at Talbingo would need to be replicated at the Tantangara intake.

Flat panel screens can be manufactured in wedge-wire or profile bar construction with slot widths down to $<1\text{mm}$. Such fine screens however are at risk from blinding from debris and biofouling which can result in hydraulic head loss and increase through-slot velocities (velocity hot-spots) (Environment Agency, 2005). Screens would require to be fitted with automated trash rakes or brushes with multi-speed operation linked to a head-loss monitoring system. Additional ancillary equipment would be required that includes debris collection and disposal systems (baskets, etc). The wetted screen surface area required to ensure escape velocities of 0.15 m s^{-1} c. 100mm in front of the screen face and the wetted surface area would be large, in excess of 2400 m^2 (including allowance of c. 20% redundancy to accommodate debris blinding). The screens would be supported by concrete civils with a service deck set at an elevation above reservoir full supply level.

Flat panel screens could accommodate the level variation anticipated at Snowy 2.0. however due to the screens not being able to accommodate bi-directional flow, similar screening would be required to be installed at Tantangara Reservoir intake as well. The array and associated civil structure required to achieve the screening criteria would be immense and larger than any of the other alternatives investigated.

Alternatives to vertical panel screening includes the Eicher screen, an inclined wedge-wire panel screens located within the tunnel system. The screen can pivot on a central axle that allows it to be cleaned by back flushing. Despite studies having shown good fish and debris handling credentials, the design is unproven for such fine bar separation and the large volume abstractions as is required for the Snowy 2.0 scheme. It is unlikely that the wetted screen area required for the Snowy 2.0 scheme, with the narrow slot widths stipulated could be accommodated. Further, given the installation of the screening facility within the intake tunnel itself access for repairs and maintenance would require station down time. It is considered that neither Eicher screens nor modular inclined screens warrant further investigation.

Both PWWC screens and brushed cylinder screens can be manufactured to provide an appropriate level of screening to exclude all life stages of non-native species (0.5mm bar separation) and sized to ensure low approach and slot velocities ($< 0.15\text{ m s}^{-1}$) in line with best available technology requirements.

Debris impinged onto submerged PWWC screens may be removed by fitting an air backwash cleaning system. A short blast of compressed air from a compressor located shoreside, delivered through a pipe fitted within each cylinder head, lifts impinged debris off the screen surface displacing it back into the water column where it can be swept away from the screen face by the prevailing currents. The cleaning cycle can be operated by a manual valve, a programmable timer or a programmable logic controller (PLC) system which enables cleaning from a remote,

central control system. Brush cylinder screens (a variant of PWWC) continuously rotate enabling fixed brushes located outside and inside the screen cylinder to clean the outer face. Both variants require debris dislodged from the screen face to be removed by the prevailing current before it can become re-impinged. Such sweeping flows however are not present within Talbingo Reservoir and displaced debris may be rapidly drawn back on to the face of the screen. The potential therefore for screen fouling along with the large area occupancy (scheme footprint) required by the screen array (which would be considerable, with the need to accommodate 90+ screens) and the complicated and extensive manifold/piping system required to connect the screens to the main intake pipe mean that neither screen option warrants further investigation.

Barrier nets are coarser in mesh than AFBs and are not designed to exclude larvae or egg life stages. They are potentially labour and cost intensive and if not maintained may start to sink or become over topped by wave action reducing their effectiveness. They are not considered suitable technology to guarantee exclusion of the early life stages of redfin from the proposed Talbingo intake.

AFBs are manufactured *inter alia* from polypropylene/polyester fabric and have the potential to screen eggs, larvae and juveniles. However, like barrier nets they require constant manual maintenance, risk overtopping by wave action and the lack of peer reviewed studies suggests they are as yet unproven in the 'field' for such an application as Snowy 2.0. The design is not considered to warrant further investigation at this time. Other aquatic filter designs such as the Filtrex filter system are also considered concept and unproven for large abstractions.

The three principal design configurations of travelling band screens: through-flow, dual-flow and centre-flow, have all been fitted and operated with fine mesh screening down to 0.5 mm mesh (Barney M. Davis P.S. Texas, USA; Big Bend P.S., Florida USA; Celestin 1 and Marcoule 2), however are more typically fitted with coarser mesh down to 1.75 mm. Whilst the mesh panels may conform to the 0.5mm mesh specification suppliers contacted could not provide guarantee that seals and fixing points, could meet the 0.5mm mesh specification for screens with such a large overall length as required at Snowy 2.0.

Travelling screens are typically located behind coarse trash racks (with bar spacing of between 50 – 120 mm) to exclude large debris from fouling the screens and operating mechanisms. These coarse screens would require to be fitted with automated raking mechanisms and debris collection and disposal facilities. All three travelling screen configurations are a proven design at large volume water intakes and considered best practice compliant by both the US and UK regulators. Through-flow and dual-flow screens have however been shown to perform more effectively than centre flow screens if fish recovery and return facilities are required to be fitted.

The screens can be manufactured to cope with significant reservoir surface level variations however with a service deck set at an elevation above reservoir full supply level the facility would require extensive concrete civil works.

In conclusion the manufacturers of the three band screen designs were unable to provide the level of contact seal tolerance required to achieve complete exclusion of all life stages from the intakes at Talbingo and as such these options do not warrant further investigation.

In common with band screens MultiDisc screens are also proven technology at power stations throughout the US and their design eliminates any concern regards carry over whilst also reducing hydraulic head loss. The sickle-shaped panels can be manufactured with a mesh size between 0.5mm and 10mm however as with band screens the manufacturers are unable to warrant contact seal tolerances of 0.5 mm for the chain driven Multidisc that would provide complete exclusion, so this option does not warrant further investigation.

Drum screens have a high load capacity meaning they are better able to accommodate additional loads and with fewer moving parts, drum screens may require less maintenance than band screens. Their existing deployment for large scale water management applications such as Ras Laffan Common Cooling Water Intake, Qatar, which has 14

drum screens installed with a combined flow capacity of $333 \text{ m}^3 \text{ s}^{-1}$ (Ovivo, pers. comm.), means there is experience to draw from, however forebay design, cost and space would require consideration. FRR can be incorporated into drum screen designs where required. As a concept, drum screens could be considered feasible, with at least one manufacturer considering the contact seal could be manufactured and guaranteed to 0.5 mm (Beaudrey pers. comm.), however immense civil structures would be required in both reservoirs to accommodate the variation in water levels, particularly within Tantangara.

The manufacturer of SWIFF screens has asserted that the screening technology can screen out biota to 0.5mm with all seals and attachment points meeting this criterion. The fully submerged screens can accommodate level variations of c. $\geq 10\text{m}$ and proposed intake flows could be accommodated. The screens are not designed to accommodate reverse flow. The lack of a cleaning mechanism for removing and conveying debris impinged on the inner screen face during reverse flow cycle means that the same screening installation would need to be replicated at both intakes. SWIFF fine mesh screens are considered to be the technology with the highest potential to successfully screen all life stages of fish through Snowy 2.0, although given the size of the array required, would still be expected to be technically challenging.

Non-physical barriers offer an alternative to physical screening. They are not as prone to fouling by allochthonous debris however may become fouled by algal growth. They have minimal effect on flow rates, with little to no hydraulic head loss and can accommodate reverse flows. Few studies have evaluated the effectiveness of behavioural barriers specifically for redfin. Where research exists on redfin and closely related or anatomically similar species, the levels of deterrence reported are mixed, possibly a result of species, site or even methodological variations. Nonetheless, good results have been reported in some studies for AFD methods (e.g. Turnpenny *et al.* 1998) while bubble curtains have been shown to work effectively as a non-behavioural method for entrainment reduction of eggs and fry (Pavlov 1989). Where strobe lights have been deployed as a single means of deterrence, they have been observed to effectively deflect some species and life stages of fish however they may be more effectively deployed in combination with other behavioural deterrents (bubbles, acoustic). A multi-method approach utilising AFD, strobes and bubble curtains is therefore considered to have the potential to minimise entrainment to Talbingo intake by non-physical means although it is unlikely that manufacturers would guarantee total (100%) exclusion of all life stages of redfin from the intakes and as such, this option is not recommended for application at Snowy 2.0.

Electric barriers offer a further non-physical option for preventing redfin entrainment as well as euthanasia of eggs and larvae. The effects of electric fields to prevent the upstream movement of fish is well documented in peer reviewed papers, however consideration needs to be given to risk of immobilisation of fish in the vicinity of a barrier at the Talbingo intake which would cause fish to be entrained. Euthanasia offers potential however, capital and power costs are high, and health and safety concerns may rule this option out. There are no known applications of electrical barriers targeting complete exclusion via the use of lethal voltages and no data on the susceptibility to mortality of redfin larvae or eggs exposed to an electrical current which would require further research; for these reasons electric barriers are not recommended for application at Snowy 2.0.

Other non-physical methods for minimising redfin entrainment were also considered. Redfin are recorded to inhabit a variety of depths in lakes and reservoirs, making variations to the Talbingo intake depth a limited option for redfin exclusion. Methods such as chemical barriers are not a recommended solution at the Talbingo intake because they are difficult to confine to a selected area and to maintain at the required concentration over time and under all conditions as effectiveness is often site, season, weather and water quality specific. Additional risks include the potential for persistent by-products and impacts to non-target species.

Other measures, such as provision of unfavourable habitat near, and favourable habitat away from, the Talbingo Reservoir intake could further supplement behavioural deterrence.

Stock management e.g. undertaking a concentrated program of netting, electric fishing or trapping program, may have an impact on numbers and has been seen to successfully reduce or eradicate redfin populations in small isolated still waters (Ingram 2016). However, in large waters such as Talbingo Reservoir and the wider Tumut catchment, stock management will require significant resources and finances with no guarantee of success and is unlikely to be effective in the long term if carried out in isolation.

UV treatment of water is not considered to be a viable option due to the volume of water to be screened and the high TOTEX (whole life cost of the asset).

A summary of the methods of redfin entrainment prevention discussed in the present review is presented in Table 4.

Table 4 Summary of potential options for preventing entrainment of redfin at Talbingo intake.

Key	Description	Recommendation
	Meets (manufactures claim) criterion for exclusion of all life stages of redfin from water intake.	Options warrants further investigation
	Option does not meet biological criterion for exclusion of redfin from water intake.	Option does not warrant further investigation

Technology	Report Section	Description	Minimum Mesh Size	Screen Seals ≤ Mesh Size (0.5mm)	Accommodate Lake Level Fluctuation >10m	Approximate Number of Units	Precedence	Bi-directional Flow	Maintenance	Comment
Flat panel screens.	3.4.4.1.	<p>Stainless-steel or Z-alloy, vertically orientated wedge-wire flat panels fitted to supporting frame (manufactured from steel and fitted with lifting lugs) with side and base contact seals. Horizontal tie bars maintain equal bar separation. The screen is lowered in front of the intake aperture via a vertical slot or slide rails. Wear strip may be attached to the frame to facilitate fitting and removal.</p> <p>Screen approach velocity should not exceed 0.15 m s^{-1}. Impinged debris and biota may be cleaned by an automated front-brush system although this would not facilitate fish recovery and return if required</p> <p>Where an automated screening mechanism is employed screen surface area should allow for up to 20% redundancy (dependent on-site debris loading) to ensure velocity hot spots do not form on screen face.</p> <p>Fine screen may require to be located behind a coarse, vertical-bar debris screen (c. 50mm bar separation). Woven mesh screens are difficult to clean that may necessitate frequent removal for cleaning and is therefore not recommended.</p>	≤0.5 mm	Yes	Yes	<p>Allowing for 10% redundancy, to achieve c. 0.15 m s^{-1} approach velocity would require a wetted screen area of approximately 2,200 sq. m at lowest reservoir operating levels</p> <p>Allowing for 20% redundancy, to achieve c. 0.15 m s^{-1} approach velocity would require a wetted screen area of approximately 2,400 sq. m at lowest reservoir operating level</p>	<p>Red Bluff pumping plant, Sacramento River, USA.</p> <p>Flat plate wedge-wire screens (1.75 mm slot opening) with a total screen length 350 m comprising a number of plate sections, with each screen 10.6 m in height.</p> <p>The screen was designed to accommodate a flow of up to $c.71 \text{ m}^3 \text{ s}^{-1}$ with an approach velocity of c. 0.1 m s^{-1}.</p> <p>No precedence for screening of bi-directional flow.</p>	<p>Horizontal tie bars are fitted to the back of the screen to ensure maintenance of fine bar separation. Screen frame and tie bars prevent automated brushing of reverse side that excludes bi-directional flow unless identical screening provided at other end of a 'closed pipe' system.</p> <p>No precedence for screening of bi-directional flow.</p>	<p>Periodic inspection.</p> <p>Other than variable speed, cable operated cleaning brush no moving parts.</p> <p>May require two sets of slots one behind the other to allow a second screen or blanking plate to be inserted prior to the primary screen being removed for maintenance.</p>	<p>Screen could provide level of protection required however screen array would be vast although cleaning facility proven technology, O&M costs could be high.</p>

Technology	Report Section	Description	Minimum Mesh Size	Screen Seals ≤ Mesh Size (0.5mm)	Accommodate Lake Level Fluctuation >10m	Approximate Number of Units	Precedence	Bi-directional Flow	Maintenance	Comment
Inclined Plane Screens (MIS and Eicher Screen)	3.4.5.1 3.4.5.2	Both Eicher screen and Modular Inclined Screen are considered high velocity (up to 3 m s^{-1}) closed conduit screens designed for hydroelectric schemes. Screens comprise an inclined, flat, wedge-wire panel set at a shallow angle ($10^\circ - 20^\circ$) within pipe work or conduit. Screens divert fish passing along the face to a bypass pipe. Eicher screens installed with different bar spacings in the upper and lower sections of the panel to create an even flow field across the screen face with bar spacings varying from 0.9mm to 3.6mm. Bar spacing in MISs typically remain uniform at around 2mm, although could be manufactured with a reduced porosity (necessitating a greater screen area). Eicher streams are located within an enclosed pipe. The screen is supported in the midpoint by a shaft that runs perpendicular to the pipe around which the screen can pivot to allow back flushing (cleaning cycle). Modular Inclined Screens are installed within a rectangular conduit and may be fitted with a mechanical cleaning mechanism such as an automated brush.	0.9mm	Information not available	Yes	Largest screens to date trialled at Puntledge Hydroelectric Project, BC with each screen accommodating a flow of $7.5 \text{ m}^3 \text{ s}^{-1}$. Scaling this up to the proposed Snowy 2.0 project would require 44 screens.	There are no full-scale applications of an MIS screen installed to date. Eicher screens have been installed at three relatively small ($<15 \text{ m}^3 \text{ s}^{-1}$) hydroelectric stations in BC, Washington and Oregon). Results for Eicher screens suggest fish deflection efficiencies of up to 95 - 99% although currently installed at only a limited number of small hydro stations. Both designs are still considered concept. Neither design has been evaluated for fish eggs of larvae and the high flows ($> 3 \text{ m s}^{-1}$) may extrude these life stages through the screen face. Expected velocities within the tunnel up to 6m/s make this option impractical.	Whilst closed conduit screens have been designed to accommodate back-flushing during the cleaning cycle they have not been designed to operate effectively for bi-directional flows. The Bureau of Reclamation (USA) note that the screens do not exclude fish during the back-flush cycle.	Access to screens for maintenance or inspection is limited and would require unit outage.	No
Passive Wedge-Wire Cylinder (PWWC) Screens	3.4.4.2	Passive Wedge-Wire Cylinder (PWWC) screens comprise a cylinder formed of vee-wire closely wound around the circumference with each end blanked off. Flow is drawn at uniformly low velocity ($<0.15 \text{ m s}^{-1}$) through the screen surface. The low hydraulic resistance results in reduced debris blockage and reduces the risk of biota entrainment. The passive intake screen has no moving parts reducing servicing requirements or the need for seals that could be subject to wear. The passive screens are cleaned using an air burst back wash system that flushes impinged material from the screen face back into the water column. This facility requires a compressor, high pressure storage tank, piping valves and	< 1 mm	Yes	Yes	90 units	No precedence for PWWCS installed at large water intakes $>30 \text{ m}^3 \text{ s}^{-1}$	No precedent of bi-directional flow through a PWWC screen.	Airburst cleaning is effective at moving debris off of screen face however requires sweeping flow to remove debris from draw zone to avoid re-impingement. Cu-Ni alloys can reduce biofouling. Screen surface area should allow for 25% redundancy to allow	No Whilst screens could provide level of filtration required the array would be both vast and complicated. Whilst air blast would remove debris from the screen face, material may be rapidly re-impinged due to lack of

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		control units. In lentic environments however, with an absence of a sweeping flow, this material could be readily and rapidly re-impinged. PWWC screens are not typically used for large scale applications due to the vast screening arrays required and associated manifold and piping system that would be required. Coarse screening measures i.e. piles may be required to protect the screen array from large debris items.							for the effects of partial blinding on increased slot velocities as well as head loss.	sweeping flows in the reservoir.
Brushed Cylinder or Cone Screens	3.4.6.1	Brushed cylinder or cone screens are a variant of PWWC screens, whereby the screen cylinder continuously rotates enabling brushes located in fixed positions outside (and inside) to clean the screen. Brushing helps to minimise debris loading and head loss and maintain even flow across the screen. Wedge-wire slot width ranges from 0.5 mm to 9.0 mm. Cylinder screens are designed to be retrievable for maintenance via a vertical track system. Cone screens are not retrievable for routine inspection and maintenance. Screens typically manufactured from wedge-wire with a slot width of 1.75mm although can be manufactured down to 0.5	0.5	Yes	Yes	The largest standard cylinder screens have an overall dimension of approximately 1.8 m diameter by 7 m in width. Screens manufactured from wedge wire with a slot width of 1.75mm with an approach velocity of $\leq 0.15 \text{ m s}^{-1}$ would have a flow of $3.4 \text{ m}^3 \text{ s}^{-1}$ requiring 98 units . The largest standard cone screens have an overall dimension of approximately 1.2 m in height by 4.25 m in diameter. Screens manufactured from wedge wire with a slot width of 1.75mm with an approach velocity of $\leq 0.15 \text{ m s}^{-1}$ would have a flow of $1.7 \text{ m}^3 \text{ s}^{-1}$ requiring 195 units . The number of units will increase for mesh widths less than 1.75 mm	Intake Screens Inc. Yellowstone River, Glendive, MT, USA. 12 x 2m diameter; 2.54 length, retrievable brush-cleaned cylindrical Tee-screens installed with a max flow of $41 \text{ m}^3 \text{ s}^{-1}$. See: http://intakescreensinc.com/projects/intake-dam/	No precedent of bi-directional flow through a brushed cylinder screen.	The cleaning cycle can be triggered by a differential water level across the screen face. The screen rotor requires a motor flexible hoses, hydraulic, pumps, tanks and controls. Additional warnings alarm may alert operators to when the screen requires to be recovered for additional cleaning. The retrievable brush-cleaned cylindrical screen is track mounted and is recoverable using a hoist.	No. Large and complex facility required at both reservoirs with no precedence for size. Lentic environment of the intake locations would create substantial maintenance demands due to ineffective automated cleaning.

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Travelling Screen	3.4.6.2	<p>Vertical travelling screens are formed from articulated mesh panels connected to form a continuous belt. The belt may rotate either continuously or intermittently, lifting impinged debris and biota from the water to the service deck where it is removed by spray bars. The drive system comprises the motor, chain, sprocket and tensioning mechanism. The drive mechanism is located above deck level.</p> <p>The screens may be fitted with fish buckets to ensure recovery of impinged biota back to the source water via a Fish Recovery and Return facility. Screens modified to improve survival of impinged fish will require to operate continuously otherwise screens rotate on a timer or in response to a level switch.</p> <p>Travelling screens are compliant with 316(b) of the Clean Water Act (USA) and are considered Best Available Technology (BAT) in the UK.</p> <p>Travelling screens may be configured as either through-flow, dual-flow or centre-flow screens.</p> <p>Head-loss associated with through-flow screens as water passes through screen face twice and there is a risk (although small) of carryover.</p> <p>Dual-flow are preferred configuration due to improved hydraulic conditions in front of the screens, less head-loss as flow only passes through one screen, no carry over of debris into the intake and production of converging flow into the intake.</p>	0.5mm	Through-flow screens - concern with carry over. Due to the height/size of the screen Aqseptence Group, Beaudrey & Ovivo felt they could not offer a guarantee on seals conforming to a tolerance of 0.5mm.	Yes	-	<p>Fine mesh screens (0.5 – 1mm) have been fitted at a number of stations in the US including.:</p> <p>Big Bend Stn, FL, Dual-Flow 0.5 mm $30.5 \text{ m}^3 \text{ s}^{-1}$.</p> <p>Prairie Island Station, MN Through-Flo, 0.5mm $39.7 \text{ m}^3 \text{ s}^{-1}$.</p> <p>Barney Davis, TX, Centre-flow, 0.5 mm, $21.5 \text{ m}^3 \text{ s}^{-1}$</p>	No precedent of bi-directional flow through a travelling screen.	Maintenance includes servicing the drive motor, bearings, chain, tensioning unit and spray bar and seals. Chain life site specific however c. 5 – 10yrs.	No
Modified Travelling Screen	3.4.6.2	<p>Hydrolox offer a modified travelling screen that is compliant with 316(b) of the Clean Water Act (USA) and considered Best Available Technology (BAT) in the UK.</p> <p>Hydrolox's engineered polymer screen is driven by a chainless positive drive system that distributes load across the entire screen width reducing uneven wear and increases considerably the recommended service intervals.</p> <p>Screens can be manufactured to any width or length. Current screens are manufactured with a minimum slot width of 1.75mm although the company is looking to reduce this down to 1mm for the US market.</p>	1.75 mm	No (only to 1.75 mm) Patented boot seal prevents aquatic life & debris from entering through the bottom of the screen; no opening in the boot	Yes	17 off S6000 through-flow screen 3.987m wide x 29.7 m OAL.	<p>Numerous installations at nuclear cooling water intakes throughout US with up to 18 units installed single site.</p> <p>Alabama Power station: Energy Corp, Little Gypsy, Mountz, Louisiana: Agrilectric Power, Lake Charles, Louisiana.</p>	No precedent of bi-directional flow through a Hydrolox travelling screen	No moving parts below water level reduces servicing requirements. All servicing carried out a deck level. Intralox offers a 4yr warranty on workmanship and components.	No Min. mesh size currently >1mm (1.75 mm) Through-flow screen small risk of carry-over (Hydrolox claim this to have been eliminated). Potential headloss through through-flow screen

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				seal is larger than the mesh size.						
MultiDisc Screen	3.4.6.4	<p>The Geiger MultiDisc is a travelling screen formed of sickle-shaped mesh panels, oriented perpendicular to the direction of flow, descend and ascend through the water along a revolving carrier chain. Screens are typically constructed of woven stainless-steel mesh or perforated plastic of sizes from 0.2 mm to 10 mm. Built-in debris exporters transport debris to the service deck where it is removed via backwash spray. Max flow capacity per unit is $13.89 \text{ m}^3 \text{ s}^{-1}$ (1mm mesh).</p> <p>Unlike standard through-flow screens the single flow through multi-disc screen reduces head loss with no risk of carry over.</p> <p>Two chain (rotation) speeds c. 0.12 and 0.24 m s^{-1}</p>	0.5 mm (can go to 0.2mm)	Although screen fitted with contact seals the Aqseptence Group felt they could not offer a guarantee for a screen c. 25m in height on seals conforming to a tolerance of 0.5mm.	Yes	<p>24 units</p> <p>Screen width up to 3.5m</p> <p>Screen height up to 15m</p>	<p>KPONE Independent Power Plant, Ghana 4 x Geiger® Cable-Operated Bar Screen with Grab Cleaner 4 x Geiger MultiDisc® 650 Technical Data: Flow rate per channel: $15,000 \text{ m}^3/\text{h}$ Bar screen spacing: 60 mm MultiDisc® mesh spacing: 9.5 mm Channel Depth: 19.70m Nuclear Power Plant D.C. Cook - USA 15 MultiDisc® Screens – MDS 700 Channel Width 3.40 m Installation Depth 13.71 m Design Flow Rate $55,100 \text{ m}^3/\text{h}$ Mesh size 9.5 mm</p>	No precedent of bi-directional flow through a Multidisc screen.	Service interval dependent onsite specifics and whether fish recovery required. FRR requires continuous operation, normal operations based on a timer and or head loss alarm. Chain c. 10 yr life, can be replaced from deck level. Operational lifetime c. 35 yrs.	No Manufacturer concerned at issuing guarantee for seals to conform to a tolerance of 0.5mm for such a large, chain driven screen

Technology	Report Section	Description	Minimum Mesh Size	Screen Seals ≤ Mesh Size (0.5mm)	Accommodate Lake Level Fluctuation >10m	Approximate Number of Units	Precedence	Bi-directional Flow	Maintenance	Comment
Drum Screen	3.4.6.3	Drum screens comprise a large rotating cylindrical structure with woven wire filtration panels attached to the periphery and supported by a horizontal, revolving centre shaft. The screen is driven by a motor on the service deck and operated most efficiently at ~70% to 80% submergence. In-to-out screens draw water into the drum and then out through the screen, retaining debris on the inside. The converging flow created by this configuration minimises turbulence entering the intake. Additional benefits include a hydraulically balanced screen where hydraulic loads push downwards onto the concrete foundation and preventing risk of the drum floating free from its foundation. Flow capacity per drum is up to $24 \text{ m}^3 \text{ s}^{-1}$. 12 would be required to screen Talbingo intake (Ovivo, pers. comms.). In UK, 5-6 mm square mesh is currently favoured for drum screens, while some French stations use ~2 mm plastic mesh (Turnpenny et al., 2010). Both drum and band screens use a water backwash system and, generally, fine-mesh screen systems have proven to be reliable in operation and have not experienced unusual clogging or cleaning problems as a result of the small mesh size. However, seals may not filter down to < 1 mm.	0.5 mm	Ovivo could not guarantee seals to tolerance of <2mm. Aqseptence Group could not guarantee seals to a tolerance of 0.5 mm Beaudrey 0.5 mm contact seals compatible with mesh.	Yes	14 units - 18m diameter, 5.6 m width drums with each screen passing a flow of $24 \text{ m}^3 \text{ s}^{-1}$	Ovivo Drum screens are currently in use at large intakes, such as Ras Laffan, Qatar ($333 \text{ m}^3 \text{ s}^{-1}$) (5mm mesh). Beaudrey French nuclear power plants use drum screens fitted with either 3x3 mm aperture mesh or 1x1 mm. The twelve drum screens in GRAVELINES are 15 m in diameter and use 3x3 mm mesh. BUGEY power plant, located on the Rhone river, 15 m drum screens use 1x1 mm mesh. 1.5 x 1.5 mm mesh apertures are often used for irrigation purposes and LNG regasification plants.	No precedent of bi-directional flow	Low maintenance when main shaft (axel) fitted with roller bearings.	Yes Considered technically feasible but the size of the required array at each reservoir would be considerable.
Submerged Water Intake Fish-Friendly (SWIFF) Screen	3.4.6.7	Submerged Water Intake Fish-Friendly (SWIFF) screens are formed of a cylindrical drum with a mesh (NOCLING™ anti-fibre panel) circumference. Water is drawn through 360° of the outer curved screen. Deep compartments on the screen collect fish and debris as the drum continuously rotates. As each compartment passes in front of the backwash suction scoop, the contents are diverted to a return pipe by reversing the flow through the mesh (fish-friendly Hidrostral screw-centrifugal backwash pump). SWIFF screens are designed to be retrievable for maintenance via a vertical track system.	0.75mm (possible to go to 0.5mm)	Beaudrey Contact seals compatible with mesh openings	Yes	c. 3 units Drum screen diameter c. 15.3 m Overall drum screen width 11.0 m	BLAYAIS nuclear power plant – 15 m diameter drum – 3 fish pumps $24 \text{ m}^3 \text{ s}^{-1}$ at LLW CORDEMAIS 600 MW coal-fired power plant – 15 diameter drum – 3 pumps $22 \text{ m}^3 \text{ s}^{-1}$ at LLW SERAING power plant (Belgium) – one 10 m diameter drum with one fish pump – $12 \text{ m}^3 \text{ s}^{-1}$ SINES power plant (Portugal) – one 12 m diameter drum with two pumps – $12 \text{ m}^3 \text{ s}^{-1}$	No precedent of bi-directional flow	5 yrs between maintenance cycles. 10 yr haul out inspection if required (site specific)	Yes

Technology	Report Section	Description	Minimum Mesh Size	Screen Seals < Mesh Size (0.5mm)	Accommodate Lake Level Fluctuation >10m	Approximate Number of Units	Precedence	Bi-directional Flow	Maintenance	Comment
Water Intake Protection (WIP) Screen	3.4.6.6	The Water Intake Protection (WIP) system is formed of a circular screen with a fish-friendly NOCLING™ anti-fibre panel on the downstream side. As the screen rotates, radial compartments retain fish and debris and divert them to a scoop where they are removed from the screen via a fish-friendly Hidrosta screw-centrifugal backwash pump. The NOCLING™ panel minimises skin damage and surface trauma to fish. Compared to traditional band screens, WIP fish recovery buckets are never exposed to air.	0.5mm	Contact seals compatible with mesh openings	Yes	2800 mm diameter screen has a flow capacity of $0.7 \text{ m}^3 \text{ s}^{-1}$.		No precedent of bi-directional flow	5 yrs between maintenance cycles. 10 yr haul out inspection if required (site specific)	No No precedent for screens of required dimensions. Manufacturer considers this option inappropriate for Snowy 2.0 scheme.
Barrier Nets	3.4.7.1	Barrier nets are large nets rigged with a float line and a heavily weighted bottom to maintain a vertical position across the entire water column. Nets can be deployed from piles approximately 3 - 12 m apart to assist with vertical positioning and prevent movement towards the intake. The net is deployed in an arc around an intake at a design approach velocity of $< 0.08 \text{ m s}^{-1}$ and can be many kilometres in length (Environment Agency 2005). Mesh size is dependent on the species and life stages requiring exclusion. Considerably finer mesh nets generally require more maintenance. The use of barrier nets requires suitable hydraulic conditions and light debris loading that requires site specific evaluation. Barrier efficiency can be compromised in conditions of high wind and waves or if net becomes fouled. Barrier nets are composed of Dyneema or knotless nylon in sizes down to 3mm stretch mesh.	3mm Minimum mesh size would not impede passage of larvae or egg drift.	Both flotation, and bottom skirts need to be sized sufficiently to ensure adequate seal in inclement weather / flow conditions.	Lower Baker Dam lake level fluctuates by 15 – 20 m.	Net should be set where flow $< 0.15 \text{ m s}^{-1}$ requiring modelling of draw zone to determine velocity profile.	Lower Baker Dam, Washington -Full Exclusion Nets Upper Baker Lake: 600 m long X 90 m deep, constructed from 3 mm x 3 mm and 6.5 mm x 6.5 mm Dyneema netting. Lower Baker Lake: 730 m. long X 90 m deep, constructed from 3 mm x 3 mm and 6.5 mm x 6.5 mm nylon netting Designed for lake level fluctuations of 15 to 20m. Designed for single direction water flow velocities $0.03\text{--}0.15 \text{ m s}^{-1}$ Deployed year round.	Barrier nets could be designed and installed to accommodate bi-directional flow	Debris cleaning and biofouling control can be labour-intensive and entail significant OPEX. Net, inspection, repair and maintenance is undertaken by dive teams or net can be recovered using electric winches, cables, and pulleys to raise haul and subsequently setting and lowering each net. This latter method requires two nets, one laid inshore of the other allowing one net to be removed for cleaning whilst maintaining an effective barrier The anticipated lifespan of the barrier nets are 3 to 10 years	No Unable to exclude all life stages of fish
Aquatic Filter Barrier (AFB) or Marine Life Exclusion System (MLES)	3.4.7.2	Aquatic Filter Barriers (AFBs) are perforated mats made of pressed polyester fibres. The Marine Life Exclusion System (MLES) is a variant of the AFB specifically designed for exclusion of aquatic organisms rather than pollutants. The MLES systems is considered a method of Best Technology Available under the Clean Water Act, Section 316(b) and	Mesh size n/a however reported to screen fish eggs	Seals with bed and shoreline compatible with filter	No data	Based on Lovett Gen. Stn. a barrier 8.25 km would be required to filter $330 \text{ m}^3 \text{ s}^{-1}$	Lovett Generating Station, Hudson River, NY - ~425 m long and ~18 m deep ($\sim 17 \text{ m}^3 \text{ s}^{-1}$) Taunton River Desalination Plant,	MLES could be designed and installed to accommodate bi-directional flow	The barrier is cleaned of impinging material and biofouling by an Automatic AirBurst™ system. The system routinely releases	No Unable to exclude all life stages of fish under all environmental conditions.

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		comprises two layers of treated polypropylene/polyester fabric which can be suspended from expanded polystyrene billets, anchored to the river bed and sealed around intake structures. Computer controlled airburst systems assist with cleaning. Few existing examples of deployment. The length of barrier required would result in a large volume of water inside of the net that would have to be 'sterilised' and maintained prior to and during operation.	down to 0.6 mm.	medium.			Taunton River, MA (~1.3 m ³ s ⁻¹).		compressed, high-pressure air at the boom's base to disturb impinged material. Routine inspection and maintenance of, screen integrity ground anchor and flotation chambers as well as Airburst system (compressor, high pressure storage tank, piping valves and control units).	
Porous Dike	3.4.7.3	Porous dikes (leaky dams) are designed to allow water to pass through the pores in the structure while preventing passage of juvenile and adult fish. Such structures have been shown to be effective at reducing entrainment of juvenile and adult fish but not smaller organisms that may move passively through the dam. Adversely the dike may provide spawning medium and or encourage colonisation by species that seek hard substrate and rocky habitats. Unsuitable for high capacity flows.	n/a	n/a	yes	n/a	Wisconsin Electric Power Plants, Lake Michigan, Wisconsin- study showed porous dike structure would lower but not eliminate entrainment of motile larvae.	-	-	No Unsuitable for high capacity flows.
Sub-Gravel Intakes and Wells	3.4.7.4	Sub-gravel intakes and wells abstract water through the riverbed or aquifer. For sub-gravel intakes, a screen can be laid over the horizontal intake opening and covered with layers of gravel separated by geomembrane sheets. Cleaning is achieved by backwashing. Collector wells extend into bedrock and may abstract water through lateral perforated intake pipes situated at depth.	n/a	n/a	yes	n/a	No precedence for sub-gravel intakes on medium or large intakes.	-		No Unsuitable for high capacity flows.
Light	3.4.8.2 3.4.8.3 3.4.8.4	Submerged strobe lights have been demonstrated to be an effective deterrent for a wide range of fish species, however results are highly variable, and further work is required to determine flash rates, duty cycles and wavelength to optimise signal for redbfin. As a second-order effect, strobe lights have been shown to significantly decrease the density of zooplankton, a food source of juvenile redbfin. Efficacy of strobes for deflection of redbfin may be affected by individual health history (parasite load), water quality and ambient light conditions and will not provided a total	n/a	n/a	yes	-	No research specific to redbfin responses to strobe lights was identified.	Yes	Lens requires to be kept free of microalgae. Lamps can be fitted with automatic brush mechanism or fitted with UV lamps to reduce fouling or removed periodically and wiped clean. Service intervals less in	No Unable to guarantee exclusion

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		exclusion barrier for all life stages under all environmental conditions therefore not considered further. Strobe lights can enhance the effectiveness of other technologies such as bubble curtains and BAFF.							oligotrophic waters (6 mths – 1 yr).	
Acoustic Fish Deterrents	3.4.8.5	Acoustic Fish Deterrents (AFDs) are formed from submerged sound projector arrays that transmit low frequency sound signals. Responses of redfin and other hearing generalists to acoustic deterrents are variable, however some studies have shown relatively high deflection efficiencies. High variation in efficiencies between studies is likely a result of site-specific differences in bottom morphology, hydrology, angle of sound waves, temperatures boundaries and signal not being optimised for repelling redfin. Further research would be required to ascertain optimal signal. Currently AFD's will not provide a total exclusion barrier for all life stages of redfin under all environmental conditions therefore AFD's not considered further in isolation.	n/a	n/a	yes	-	Farmoor Raw Water Intake, River Thames, UK, (2.66 m ³ s ⁻¹). Source levels of 8 x 152 dB @ 1 m extending out to 10 m (50 - 500 Hz). 88% of 0-group redfin deflected. River Foss Pumping Station, York, UK (32 m ³ s ⁻¹), redfin entrainment reduced by 56% (AFD emitting a 100 - 500 Hz). Doel Nuclear Power Plant, Belgium, AFD emitting a signal of between 20 and 600 Hz reported a 51.2% decrease in entrapment of redfin.	Yes	Internal monitoring systems provided continual remote feedback as to status of hydrophones. Service requiring hydrophone removal every 4 yrs. Hydrophones designed to be retrievable for maintenance via a vertical track system.	No Unable to guarantee exclusion
Air Bubble Screens	3.4.8.6	Bubble screens are formed from a curtain of air bubbles generated via compressed air pumped through a perforated tube or diffuser laid along the bed of the water body. Curtain is typically set in a loop around an intake entrance to deflect approaching fish and guide them away. Results for hearing generalists such as perch but have been less encouraging than for hearing specialists. Air bubbles can be used to lift discrete particles, including eggs and larvae, to the surface via floatation. Fouling of perforated tube may result in gaps in the curtain allowing penetration. Currently bubble curtains will not provide a total exclusion barrier for all life stages of redfin under all environmental conditions therefore not considered further in isolation.	n/a	n/a	yes	The leaky hose should be laid on the bed far enough from the intake to ensure water velocity does not exceed fish swimming ability (< 0.15 m s ⁻¹). This may be informed by hydraulic modelling.	White Rapids Hydroelectric Project, Menominee River, Michigan, USA, bubble curtain was not effective in reducing entrainment of yellow perch and the percid walleye. Laboratory studies showed no significant deflection of Eurasian ruffe (percid) (> 10 cm length), and < 50% of white perch were observed to avoid an air-bubble barrier. No research was found on the effect of air-	yes	Labour-intensive. Routine inspection and maintenance of, leaky hose as well as routine and annual maintenance of compressor, high pressure storage tank, piping valves and control units.	No Unable to guarantee exclusion

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							bubble curtains on juvenile or adult redbfin. Low levels of protection (12 - 36%) afforded by surface water currents generated by a bubble curtain in repelling the eggs and larvae.			
Electrical Barriers	3.4.8.7	<p>Electrical barriers comprise submersed electrodes which pass an electrical current from anode to cathode to produce an electric field in the water. Pulsed direct current electric fields are recommended for greatest behavioural effect and minimal damage to fish. A graduated spatial electrical field, such as that produced by Smith-Root's Graduated Field Fish Barrier (GFFB), should be created to ensure the stimulus progressively increases towards the barrier and avoids electrocution, where the fish becomes stunned, immobilised and is subsequently carried by water flow through the barrier.</p> <p>Susceptibility of individual to electrocution dependent on both size and orientation of fish in the electric field. Currently electric barriers will not provide a total exclusion barrier for all life stages of redbfin under all environmental conditions therefore not considered further in isolation.</p>	n/a	n/a	Yes – (when installed in confined tunnel)		No research specific to the response of redbfin to electrical barriers were identified.	Yes	Access to electrodes situated in the confines of the intake chamber for inspection and maintenance is limited and would require unit outage. High TOTEX.	No Unable to guarantee exclusion
Hybrid Deterrents: Synchronised Intense Light and Sound (SILAS) & Bio-Acoustic Fish Fence (BAFF)	3.4.9	<p>A Bio-acoustic Fish Fence (BAFF™) can be used to produce an evanescent sound field using a dense curtain of bubbles to trap a pneumatically or electronically produced sound signal by refraction. Sound levels inside the bubble curtain can achieve 170 dB re 1mPa (around 20 times higher than levels reported above for bubble curtains alone), decaying to approximately 5% of this value within 0.5 - 1 m from the bubble. The specific sound signal used can be manipulated to target the species of interest by a sound projector array. BAFF barriers have elicited high guidance efficiencies away from undesirable routes for both hearing specialists and generalists.</p> <p>Although 100% exclusion using behavioural deterrents in isolation cannot be guaranteed, non-physical barriers can have relatively low capital and running costs and are not prone to blocking with debris or biofouling, constraining</p>	n/a	n/a	SILAS – Yes	Both the SILAS and BAFF array should be laid on the bed far enough from the intake to ensure water velocity does not exceed fish swimming ability (< 0.15 m s ⁻¹). This may be informed by hydraulic modelling.	No research specific to the response of redbfin to SILAS or BAFF deterrents were identified.	Yes	As for strobe lights, AFD and bubble curtains.	No Unable to guarantee exclusion

Technology	Report Section	Description	Minimum Mesh Size	Screen Seals ≤ Mesh Size (0.5mm)	Accommodate Lake Level Fluctuation >10m	Approximate Number of Units	Precedence	Bi-directional Flow	Maintenance	Comment
		flow rates and causing head loss. Suitable designs, such as SILAS-BAFF (sound, light and bubbles) could potentially offer a greatly reduced risk of inter-catchment fish transfer. Air bubbles can be used to lift discrete particles, including eggs and larvae, to the surface via floatation.								
Hybrid Deterrents: Sound Projector Array (SPA) and Electric Deterrent	3.4.9	There is potential for graduated electric barriers to be combined with AFDs to prevent fish entrainment. Fish experiencing an electric field for the first time whilst receiving an AFD signal may subsequently exhibit a Pavlovian response and turn away on detecting an AFD signal before experiencing the electric field potentially mitigating the effect of multiple challenges to the electric barrier. Further research is required to optimise signals for target fish (sound frequencies, strobe rate, electric field strength) and understand the learning ability of redfin over a period long enough to assess learning or habituation effects. Hybrid AFD and electric barriers however will not provide a total exclusion barrier for all life stages of redfin (eggs and larvae) or other life stages under all environmental conditions therefore this system is not considered to meet the criteria set out by the Snow 2.0 project.	n/a	n/a	yes	n/a	No research specific to the response of redfin to hybrid AFD / electrical barriers were identified.		As for AFD and electrical screens.	No Unable to guarantee exclusion
Piscicides	3.4.11	Methods such as chemical barriers are not a recommended solution at the Talbingo intake because they are difficult to confine to a selected area and to maintain at the required concentration over time and under all conditions as effectiveness is often site, season, weather and water quality specific. Additional risks include producing more persistent by-products and impacting non-target species. Chemical barriers are not recommended for further consideration.	n/a	n/a	yes	n/a	n/a	yes	Labour-intensive	No Significant negative side effects. Difficult to maintain effective concentrations under all flow and environmental conditions

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Ecology, Monitoring, Conservation

The status of Murray Crayfish in Talbingo Reservoir, 2019

Sylvia Zukowski and Nick Whiterod



CONFIDENTIAL report to EMM Consulting



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Disclaimer

Although reasonable care has been taken in preparing the information contained in this publication, neither Aquasave–NGT, nor Snowy Hydro or EMM Consulting accept any responsibility or liability for any losses of whatever kind arising from the interpretation or use of the information set out in this publication.

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Background

Murray Crayfish *Euastacus armatus* (von Martens 1866) is an iconic freshwater crayfish endemic to the waterways of the southern Murray-Darling Basin (MDB). This long-lived and slow-growing species was once widespread, but has experienced substantial declines in range and abundance over the second half of the twentieth century (Furse and Coughran 2011; Gilligan *et al.* 2007). Recent population decline across sections of its range (McCarthy *et al.* 2014; Noble and Fulton 2017; Whiterod *et al.* 2018) indicate that the species is experiencing a declining trend in population status. Murray crayfish are thought to be locally extinct in South Australia and are protected under the Fisheries Act. The species is listed as vulnerable in the ACT and NSW, and threatened in Victoria, with concerns over its long-term sustainability.

In response, there has been dedicated research and monitoring focusing on the sustainability of recreational fishing (Zukowski *et al.* 2011; Zukowski *et al.* 2012; Zukowski *et al.* 2013), the impacts of extreme hypoxic blackwater (King *et al.* 2012; McCarthy *et al.* 2014; Whiterod *et al.* 2018) and habitat degradation (Noble and Fulton 2017) as well as comprehensive genetic assessment (Whiterod *et al.* 2017) and the development of a stochastic population model to provide a framework to address management and conservation scenarios (Todd *et al.* 2018). Recently, translocations have been instigated on the Murray River near Echuca, with a translocation method defined by Whiterod and Zukowski (2019). This collective work has improved understanding and facilitated effective management of the species. It also emphasised the requirement for research and monitoring to conserve known populations of this threatened species that are potentially impacted by disturbance.

The species was historically abundant in the region of the Tumut River impounded by Talbingo Reservoir (Gilligan *et al.* 2007). Whilst a flow specialist, Murray Crayfish has persisted following the construction of the reservoir. Importantly, the species is totally protected from recreational fishing in the reservoir. The population has also been shown to be genetically distinct from a broad interbreeding lowland population across the Murray-Murrumbidgee region (Whiterod *et al.* 2017). Targeted annual surveys within Talbingo and Blowering Reservoirs over 2008–2010 provided the first assessment of the status of the species in the reservoirs (Zukowski *et al.* 2013). In Talbingo Reservoir, Zukowski *et al.* (2013)

demonstrated a healthy population with a 1:1 sex ratio, and a good population structure (including regular recruitment and long-term survival). Whilst sampling of a single site in 2013 in Talbingo Reservoir (NSW DPI, unpublished data) demonstrated a similar abundance, a considered reduction in catch was observed in 2018 surveys (Cardno Consulting, unpublished data), raising concern about the status of the species in the reservoir.

This present project aims to resolve the present status of the Murray Crayfish populations in Talbingo Reservoir to inform considerations for the species in the Snowy 2.0 project.

Description of the species

Murray Crayfish is a member of the *Parastacidae* family, and recognised as the second largest freshwater crayfish in the world (Riek 1969). It is characterised by a hard carapace (exoskeleton) and robust sharp spines on its abdomen and chelae (claws) with smaller spines on the pereopods (walking legs) and the carapace



(Morgan 1986; Morgan 1997). It can be variable in colour; typically, the carapace and abdomen is brown-green to brown (and sometimes slightly tinged blue). Its spines and chelae are white or cream, except for juveniles which have mottled green and yellow chelae (Morgan 1986; Morgan 1997). Murray Crayfish are easily distinguished from other spiny crayfish by its large size, white claws and absence of male cuticle partition (Gilligan *et al.* 2007; Morgan 1986).

Life history

Murray Crayfish is a long-lived (potentially up to 28 years) and slow-growing species with a maximum length 174 mm occipital carapace length (OCL, in mm) observed (Gilligan *et al.* 2007; Morison 1988). The species is a late maturing (i.e. 8–9 years) annual spawner with a winter-spring brooder strategy (Gilligan *et al.* 2007). Typically, females are first observed with eggs as water temperatures decline in late autumn, and fertilised eggs and then larvae (through a succession of moults) remain attached to the pleopods under the abdomen until

independent juveniles are released during late spring to early summer (Geddes *et al.* 1993; Gilligan *et al.* 2007; O'Connor 1986; Zukowski *et al.* 2012).

Habitat requirements

The species occurs across a wide range of mesohabitats – cool, clear upland streams, impoundments, and turbid lowland river sections – with a strong preference for cool, oxygenated and flowing water (Gilligan *et al.* 2007; Riek 1969). Flow velocity (and its link to higher dissolved oxygen concentrations) is considered an important overriding habitat feature guiding both the species' mesohabitat (i.e. the species is largely absent from slow-flowing impoundments and weir pools with lower dissolved oxygen levels) and microhabitat preferences (i.e. where the species occurs it prefers medium to high flows) (Gilligan *et al.* 2007; McCarthy 2005; Noble and Fulton 2017; Zukowski 2012). Other microhabitat features include physical structure such as rocks and woody structure that provide shelter and refuge, as well as clay banks believed to be necessary for burrowing (Gilligan *et al.* 2007; Noble and Fulton 2017; Zukowski 2012). Overhanging riparian vegetation and deeper pools appear to be important microhabitat features for upland populations of the species (Noble and Fulton 2017).

Diet and feeding

Freshwater crayfish form important links in the transformation of energy through aquatic food webs (Reynolds and Souty-Grosset 2011). Murray Crayfish are opportunistic omnivores, consuming decomposing vegetation, algae, aquatic macrophytes, invertebrates and fish and other meat (Gilligan *et al.* 2007). Starrs *et al.* (2015) recently demonstrated Murray Crayfish feeding on terrestrial materials (i.e. fine woody debris and leaf litter), emphasising the importance of these food resources. More comprehensive dietary studies are necessary.

Movement and home ranges

Murray Crayfish is considered to have lower dispersal abilities and occupy small home ranges (O'Connor 1986). Consistently, a radio-telemetry study of upland populations did not detect large-scale movements, but rather activity was restricted to small home ranges (i.e. single pools: Ryan *et al.* 2008). For lowland populations, movement may occur across a broader home range as revealed through a mark-recapture study (O'Connor 1986) and

intimated by genetic analyses (Whiterod *et al.* 2017). Whilst the genetic analyses suggest home ranges may be up to 50 km, exploration of movement and home ranges across lowland populations, guided by recent advances in telemetry, is required. This work would be particularly useful to assess how the fragmented nature of existing populations and potential barriers to dispersal (e.g. weir pools) may influence the ability of the species to recolonise areas impacted by disturbance events.

Environmental tolerances

The species is broadly tolerant; however, mortality is experienced during adverse environmental conditions that occasionally prevail across its range. For water temperature, short-term exposure to 30°C resulted in 50% mortality of the test individuals (i.e. 12 hour LC₅₀) with peak movement performance occurring at 18°C (Geddes *et al.* 1993; Stoffels *et al.* 2016). The species can tolerate salinities greater than 16 mgL⁻¹, but not low dissolved oxygen (DO) concentrations (i.e. hypoxia) with the 12-hour LC₅₀ estimated at 2.2 mgL⁻¹ (Geddes *et al.* 1993). An understanding of the sensitivity of the species to agricultural chemicals and pesticides remains lacking (Gilligan *et al.* 2007).

Conservation status

Murray Crayfish is considered *threatened* in Victoria under the *Flora and Fauna Guarantee Act 1988* and *vulnerable* in NSW (*Fisheries Management Act 1994*) and the ACT (*Nature Conservation Act 1980*). In South Australia, it is classed as *protected* under the *Fisheries Management Act 2007*. The species is listed nationally as *indeterminate* (under the *Environment Protection and Biodiversity Conservation Act 1999*) and internationally as *data deficient* (under *International Union for Conservation of Nature Red List of Threatened Species 2010*) (Alves *et al.* 2010).

Project objectives

The specific objectives of this project were to:

- Undertake a comprehensive survey of sites that have been previously sampled as well as extra sites in the Southern end of the reservoir;
- Record habitat (physical structure, aquatic vegetation, water depth and location (GPS coordinates) for each net deployment and site-based water quality parameters);
- Collect data on population abundance and demographics (length, weight, sex ratio, reproductive stages of individuals); and

- Assess (a) the temporal trend in abundance and demographics, and (b) presence in Southern areas of the reservoir.

Field surveys

Study region

Talbingo Reservoir (35°37'59.95 S, 148°18'05.76 E) occurs within and adjacent to the Kosciuszko National Park just south of Tumut in the Murrumbidgee Valley on the Tumut River, south-eastern Australia. Construction of the reservoir was completed in 1970. The reservoir creates a head storage for the operation of the Snowy Hydro Tumut 3 hydroelectric pump-storage project. Water released from the reservoir is used to supply water for irrigation and industry, hydro-power and environmental flows. The reservoir is also used for recreational activities including waterskiing, sailing, boating, and fishing. Talbingo Reservoir has a gross capacity of 920,600 GL and surface area of 1936 hectares (www.ancold.org.au). It is primarily fed by releases from Tumut 2 power station tailrace at the southern end of the reservoir. Other waterways provide additional inflows, including Tumut and Yarrangobilly rivers, and Long, Honeysuckle, Plain and Middle creeks.

Survey methods

The targeted surveys were undertaken 3–10 June 2019. Surveyed sites included the seven existing sites of Zukowski *et al.* (2013) (sites 1–7) (this includes site 3 (Honeysuckle) also sampled by NSW DPI and Cardno Consulting) and seven additional sites located towards the Southern end of the reservoir, especially in the Yarrangobilly River and Middle Creek arms (Table 1, Figure 1. (sites 8–14) (total 14 sites). Sites 6, 8, 9 and 13 were also sampled by Cardno in 2018. Replicating some of the sites previously surveyed in 2008–2010, 2013 and 2018 was used to provide temporal comparison of previously surveyed sites.

The field survey employed two types of sampling gear (hoop and munyana nets) to maximise the probability of detection. Predominately, the standardised hoop net sampling protocol, was employed: at each site (sites 1–14), 20 replicate single hoop nets (700 mm diameter with a mesh size of 13 mm) baited with approximately 300 g of ox liver were set and checked hourly (and deployed at the same location) for a total of three hours (60 hoop

net hauls per site) during daytime hours (0800–1700) (McCarthy et al. 2014; Whiterod and Zukowski 2017; Whiterod et al. 2018; Zukowski et al. 2013).

Munyana nets were used in addition to hoop nets at sites 6 and 8–14 to increase the rate of capture (see McCarthy 2005). At each of these sites, commercially available crab nets (Munyana net, Wishart, Queensland: 60 mm mesh, two 0.76 m diameter steel hoops and two 0.18 m × 0.12 m openings) were baited with approximately 300g of ox liver and set overnight. This type of method is designed to have greater retention of crayfish unlike hoop nets from which crayfish can enter and exit.

For each net, the location (easting, northing), time set and retrieved, sampling level (in metres above sea level, mAHD, to account for variation in reservoir level) and water depth, distance from bank and habitat descriptors was recorded. Water quality parameters (water temperature, pH, dissolved oxygen concentration and electrical conductivity) (YSI 556 multi-probe) and percentage cover of submerged aquatic vegetation was recorded at each site.

Table 1. Summary of sites sampled across Talbingo Reservoir in 2019

Site no.	Site description	Date sampled	Sampling level (mAHD)	(Zone 55)	
				Easting	Northing
1	Adjacent to car park	4/06/2019	527.92–537.22	618230	6056485
2	Near swimming area	4/06/2019	519.72–537.12	617167	6056496
3	Honeysuckle Creek arm	3/06/2019	513.31–538.01	617645	6050419
4	Landers Creek arm	3/06/2019	519.61–538.71	620251	6053220
5	Lick Hole Creek arm	9/06/2019	526.08–535.98	620413	6048075
6	Long Creek arm/Cascade Bay	9/06/2019	515.48–537.98	620126	6044117
7	O'Hares Campground (Sue City)	8/06/2019	529.05–537.55	623186	6036163
8	Plain Creek arm	8/06/2019	513.55–534.95	621316	6041933
9	Open site at rivers junctions	5/06/2019	504.76–536.06	622401	6042059
10	Middle Creek arm	7/06/2019	522.30–535.80	623942	6041691
11	Yarrangobilly River upstream	6/06/2019	525.03–535.33	624124	6040423
12	Yarrangobilly River middle	6/06/2019	530.43–536.73	624445	6040388
13	Yarrangobilly River bottom	9/06/2019	516.10–538.40	624840	6039835
14	Open site at rivers junction	5/06/2019	519.96–537.46	623720	6041749

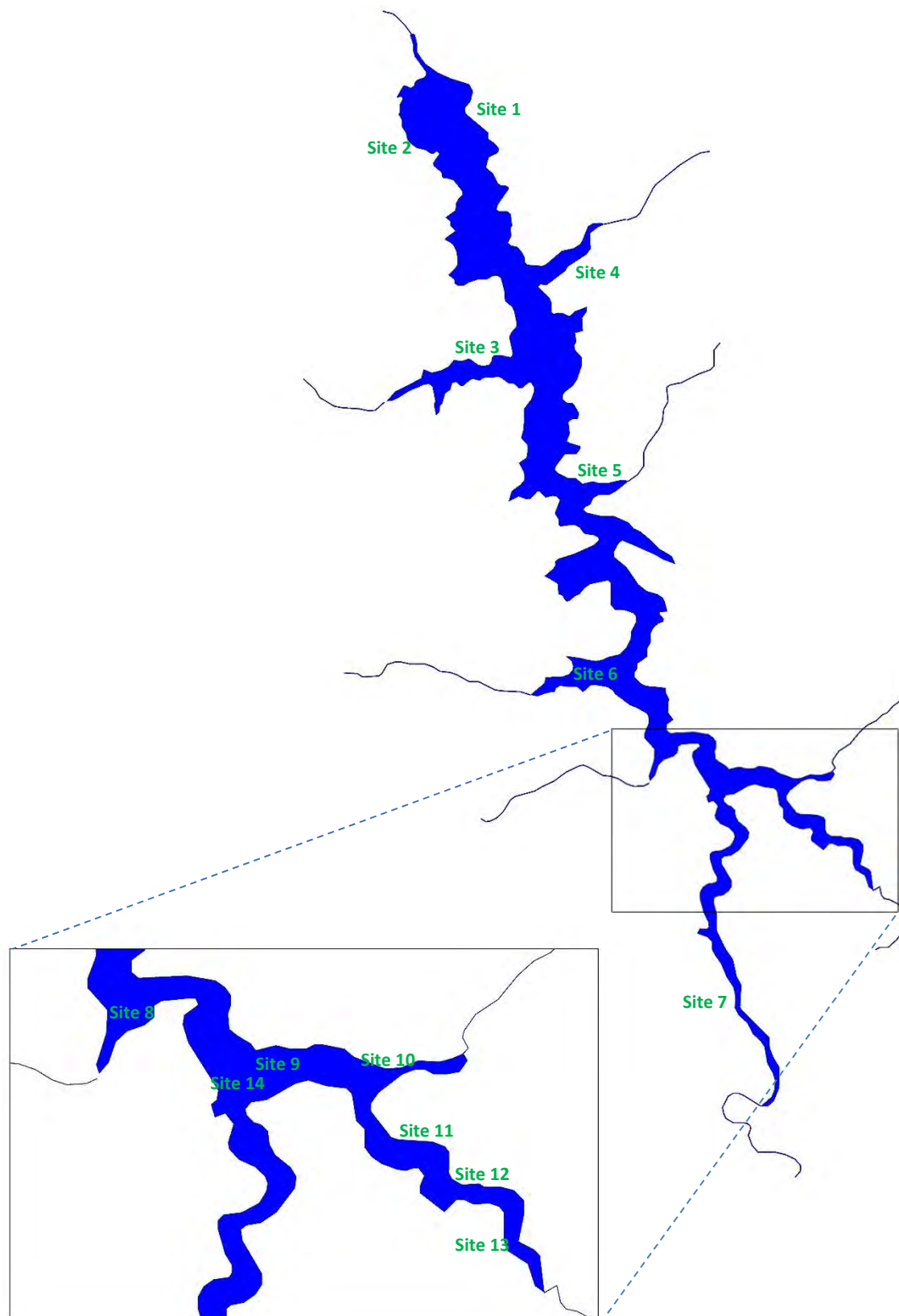


Figure 1. Survey sites included the seven existing sites of Zukowski et al. (2013) (sites 1–7) (this includes site 3 (Honeysuckle) also sampled by NSW DPI and Cardno Consulting) and seven new sites (sites 8–14) (total 14 sites).

Data collection and treatment

Sampled Murray Crayfish were sexed, weighed (W, in g) using waterproof scales (A&D weighting, Tokyo, Japan) and occipital carapace length (OCL, measured from the rear of the eye socket to the middle of the rear of the carapace, to the nearest 0.1 mm) was measured (using Vernier callipers Kinchrome, Scoresby, Victoria, Australia) (Figure 2). The stage of maturity (stages 1–3) was recorded for females as was the presence of eggs. Additionally, each crayfish was marked using a Uni PAINT PX-20 marker (Mitsubishi Pencil Co. Ltd, Milton Keynes, UK: see Ramalho et al. 2010) to identify potential recaptures (during sampling event) before being returned to the water at the point of capture. These marks persist for months (potentially until the next moult which occurs annually in adult crayfish) and have been employed successfully for a medium-term mark-recapture study on the species (Zukowski *et al.* 2018). At each site, the total number of Murray Crayfish caught and the sampling effort (number of net replicates) was used to compute the catch-per-unit-effort abundance (as crayfish net⁻¹ h⁻¹; hereby referred to as relative abundance).



Figure 2. Weighting (left) and measuring length (right) of Murray Crayfish sampled from Talbingo Reservoir in winter 2019 (note: temporary 'X' mark to help identify recaptures).

Data supplied in excel format

In addition to this current report, the following data is attached in an excel spreadsheet.

- Data on surveys completed (date, method);
- Descriptive information (GPS location, sampling level and water depth, bank habitat, distance from bank) for each hoop and Munyana net deployment;
- Number of individuals caught; and
- Demographic information (length, weight, sex and life stage) for all sampled crayfish.

Results

Catch summary

In total, 19 Murray Crayfish (8 females, 11 males) were sampled from 880 net retrievals during 1,528 fishing hours (hoop nets (all sites) 840 hours (9 crayfish), Munyana nets (sites 6 and 8–14) 688 hours (10 crayfish)) in the Talbingo Reservoir in June 2019. All Murray Crayfish sampled appeared in good health with no obvious deformities, disease or parasite infestations apparent (Figure 3). Individuals were recorded from 11 sites during the present survey (Tables 2 and 3, Figures 4 and 5). For comparison, a total of 188 individuals (95 females (13 berried), 93 males) were recorded over the three years of sampling between 2008 and 2010 (Table 2).

Table 2. Summary of Murray Crayfish numbers across the 14 sampled sites from combined 2008–2010 (Zukowski et al 2013) data and current survey data.

Site	2008–2010 Hoop Nets	Present study Hoop Nets	Present Study Munyana Nets	Present Study Total
1	19	3		3
2	3	1		1
3	13	1		1
4	73	-		-
5	47	-		-
6	12	-	1	1
7	21	-		-
8	-	-	3	3
9	-	2	3	5
10	-	1		1
11	-	1		1
12	-	-	1	1
13	-	-	1	1
14	-	-	1	1



Figure 3. Murray Crayfish captured in Talbingo Reservoir in June 2019.

Changes over time

Alarming, the overall relative abundance dropped considerably from 0.18 ± 0.01 crayfish $\text{net}^{-1} \text{h}^{-1}$ over 2008–2010 to only 0.01 ± 0.01 crayfish $\text{net}^{-1} \text{h}^{-1}$ during the present study, when comparing across the seven sites sampled with hoop nets over time. This reduction was experienced at all sites, except site 2, the relative abundance of sites dropping as high as 0.43 ± 0.04 crayfish $\text{net}^{-1} \text{h}^{-1}$ (site 4) to zero (sites 4–7) during the present study with the species not detected at sites 4–7 during the present study (Table 3).

Table 3. Comparison of Murray Crayfish relative abundance (mean \pm standard error) across the 14 sampling sites between 2008–2010 (Combined) and the present study. Sites not sampled over 2008–2010 indicated with -. Hoop net data only

Site	2008–2010	Present study
1	0.11 ± 0.02	0.05 ± 0.03
2	0.02 ± 0.01	0.02 ± 0.03
3	0.13 ± 0.03	0.02 ± 0.02
4	0.43 ± 0.04	0
5	0.28 ± 0.04	0
6	0.10 ± 0.03	0
7	0.17 ± 0.03	0
Sites 1–7	0.18 ± 0.01	0.01 ± 0.01
8	-	0
9	-	0.08 ± 0.04
10	-	0.02 ± 0.02
11	-	0.02 ± 0.02
12	-	0
13	-	0
14	-	0

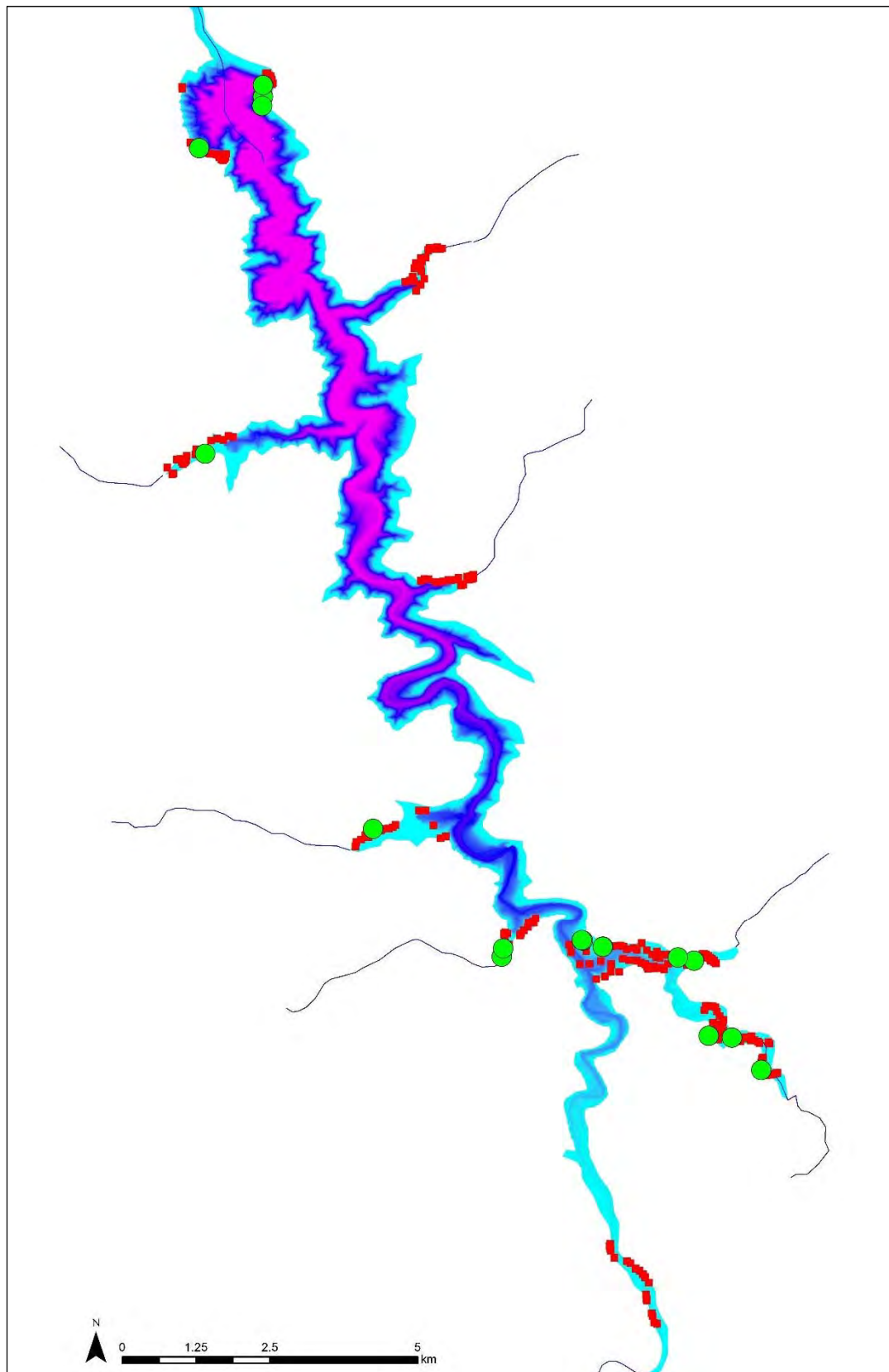


Figure 4. Individual net deployments (red circles) and captured crayfish locations (green circles) in Talbingo Reservoir June 2019. The bathymetry of the reservoir is also shown, with shallower (<20 m) areas indicated in light blue).

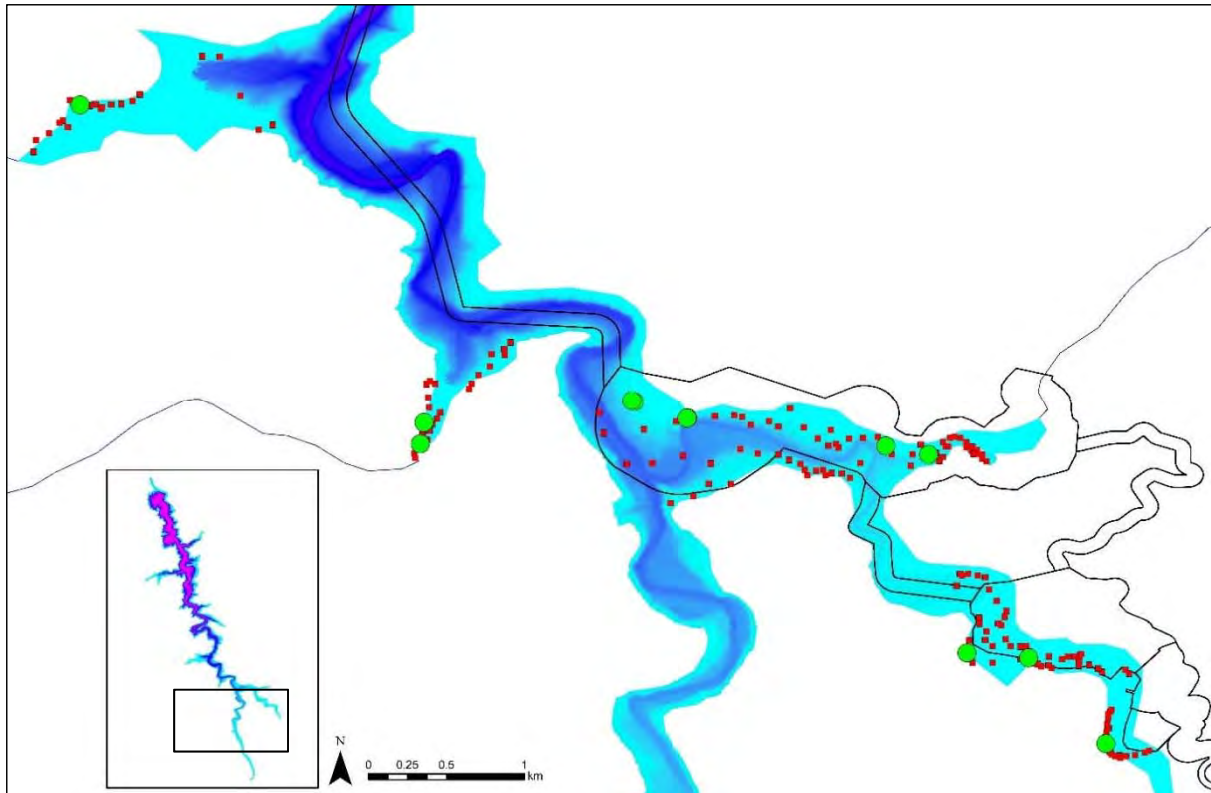


Figure 5. Close up map of the locations of net deployments and crayfish captures in the southern section Talbingo Reservoir. The current potential impact area (black outline) of activities associated with Snowy 2.0 and bathymetry of the reservoir is also shown, with shallower (<20 m) areas indicated in light blue.

Length structure

Sampled Murray Crayfish ranged from 53 to 127 mm, and 53 to 927 g, with the majority of individuals being adults ≥ 100 mm (5 females, 9 males) but there were five advanced juveniles also recorded (Figure 6). Eight females were recorded of which five were sexually mature (63%). Of the sexually mature females, only three were carrying eggs (i.e. in berry) at varying egg abundance (one was in full berry, one was $\frac{3}{4}$ full of eggs, one was $\frac{1}{4}$ full of eggs) with eggs absent from the remaining two crayfish.

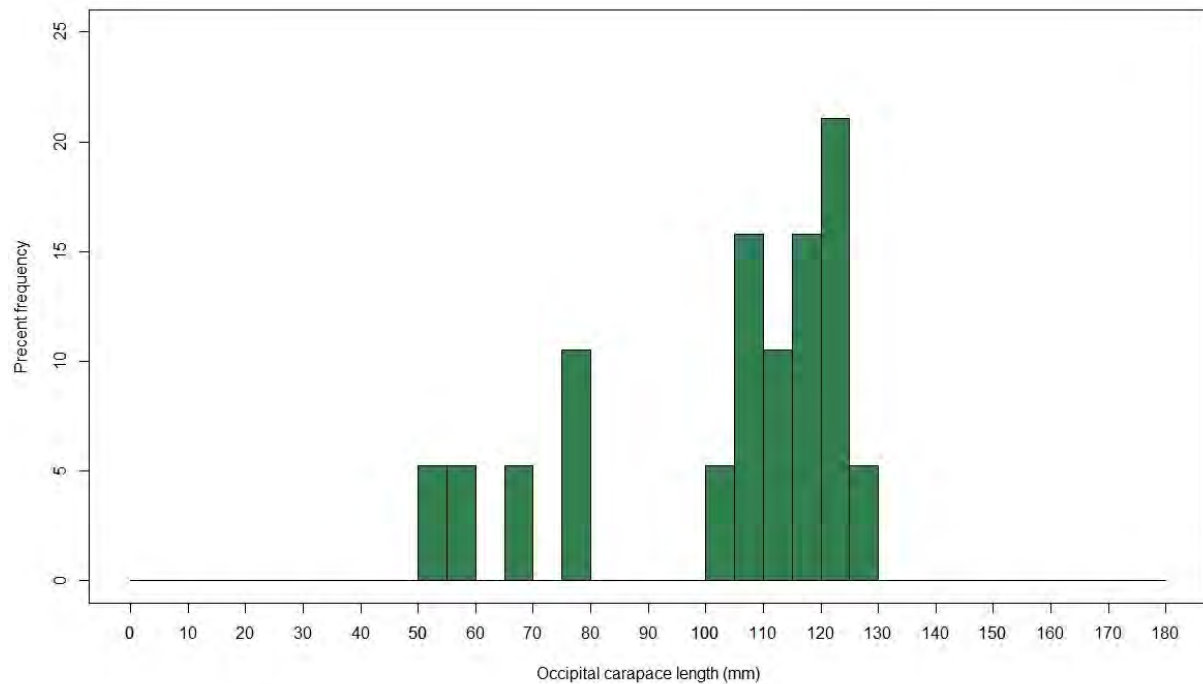


Figure 6. Length structure of Murray Crayfish in Talbingo Reservoir June 2019.

Habitat and methodology descriptors

Table 4. Summary of net type, sampling level and water depth and meso-habitat present where Murray Crayfish were sampled.

Site	Net type	Net no.	Sampling level (mAHD)	Water depth (m)	Meso-habitat	No. of crayfish
1	Hoop	4	532.4	7.8	Irregular (rock)	1
1	Hoop	7	536.4	3.8	Irregular (rock)	1
1	Hoop	9	536.8	3.4	Irregular (snag, rock)	1
2	Hoop	5	533.4	6.8	Irregular (rock)	1
3	Hoop	14	525.1	15.2	Matted bank	1
6	Munyana	2	531.2	7.8	Large wood	1
8	Munyana	1	531.8	7	Irregular (snag)	1
8	Munyana	2	530.5	8.3	Rock	2
9	Hoop	5	531.5	8.4	Irregular (snag, rock)	1
9	Hoop	7	530.4	9.5	Irregular (snag, rock)	1
9	Munyana	2	531.6	8.3	Irregular (snag, rock)	2
9	Munyana	4	530.3	9.6	Irregular (snag, rock)	1
10	Hoop	15	534.2	4.8	Smooth bank	1
11	Hoop	19	533.2	6.3	Irregular (snag, rock)	1
12	Munyana	5	532.5	7	Rock	1
13	Munyana	2	535.8	3.2	Rock	1
14	Munyana	1	530.9	9	Irregular (rock)	1

Nets were deployed at depths between 538.7 to 504.8 mAHD (0.6–35.1 m) but with Murray Crayfish only recorded at depths between 536.8 to 525.1 mAHD (3.2–15.2 m) (Table 4). Of the sampled crayfish, nine were sampled using hoop nets and 10 individuals were detected in Munyana nets (Table 4). Meso-habitat varied amongst sites but an irregular bank with rock and or snags were predominately present where crayfish were captured (Table 4). At the time of sampling, all sites except site two had submerged aquatic vegetation present with Canadian Pondweed *Elodea canadensis* being the dominant species and coverage varying from 20 to 90% between sites (Table 5, Figure 7).



Figure 7. Prevailing submerged aquatic vegetation, which was predominately introduced Canadian Pondweed (*Elodea canadensis*, bottom right photo: left) but also native *Chara* sp. (bottom right photo: middle) and *Potamogeton* sp. (bottom right photo: right) in Talbingo Reservoir.

Water Quality

Water quality parameters varied across the 14 sites (Table 5). EC was relatively fresh throughout the reservoir and ranged from 25 (Site 4) to 76 μScm^{-1} (Site 13). Water temperature was below 12°C at all sites (4.15–11.82°C) and a slightly acidic pH was found at most sites, (6.35–6.95), except site 9 which was slightly alkaline (7.09). Dissolved oxygen

concentrations (measured during the day) were generally high with all sites having higher than 11 mgL⁻¹.

Table 5. Summary of water quality parameters and percentage submerged aquatic vegetation cover at the 14 sampled sites in Talbingo Reservoir.

Site	Temp (°C)	pH	EC (µScm ⁻¹)	DO (mgL ⁻¹)	Submerged aquatic vegetation cover %
1	10.96	6.45	29	12.85	20
2	11.82	6.81	29	12.02	0
3	11.69	6.35	31	11.76	90
4	8.61	6.71	25	13.29	40
5	10.88	6.77	29	11.17	20
6	9.89	6.79	32	11.56	50
7	9.33	6.63	23	13.61	60
8	10.28	6.71	33	11.87	30
9	11.43	7.09	36	11.78	40
10	11.15	6.90	42	11.88	20
11	11.29	6.89	45	12.74	35
12	9.59	6.95	48	11.33	55
13	4.15	6.69	76	15.38	85
14	10.81	6.91	43	11.4	40

Discussion

The present survey aimed to resolve the status of Murray Crayfish in Talbingo Reservoir. Whilst individuals were recorded at most sites, the majority of sampled Murray Crayfish were found at the sites at the southern end of the reservoir. Although relative abundance was comparatively lower than other areas of its range (see Whiterod and Zukowski 2017; Whiterod *et al.* 2018), it is still evident that viable populations of the species persist in the reservoir. There was a marked reduction in relative abundance across the broader reservoir when comparing the previously sampled sites over time. This rapid decline (e.g. in 2013, relative abundance was similar to that observed over 2008–2010), in less than six years is of considerable concern.

With the declines observed across the reservoir, the importance of the Murray Crayfish population in the existing areas is emphasised. Given the biology and ecology of the species – late maturing (i.e. 8–9 years), long-lived (potentially up to 28 years), likely restricted movement – any environmental disturbances can cause significant risk to present and

future populations. In this context, it is therefore critically important to the long-term viability of the species that the remaining population in Talbingo Reservoir is effectively managed. All but one of Murray Crayfish sampled were found at sampling levels shallower than 530.3 mAHD (water depth <10 m) and most individuals were located in close proximity to bank structure that included wood and rocks. These results are similar to the past findings of Murray Crayfish surveys throughout NSW and may reflect the preferred depth and habitat as guided by environmental parameters such as dissolved oxygen levels, proximity to available burrows, habitat availability and food source availability. Although Murray crayfish generally prefer higher flowing areas to those that are lower flowing such as weir pool impoundments or low flowing dams with low dissolved oxygen concentrations, the dissolved oxygen concentrations in Talbingo Reservoir were consistently high across the reservoir ($>11 \text{ mg L}^{-1}$) and the flows into the reservoir through the various arms provides the fresh flows that the species prefers.

The observed decline in relative abundance at previously sampled sites is of concern and could be directly related to the invasion of submerged aquatic vegetation, namely the introduced Canadian Pondweed, into Talbingo Reservoir. Canadian Pondweed can be introduced by boats, trailers, and birds and can spread quickly and form dense aggregations (Hessen *et al.* 2004). The plant was found to be widespread and locally abundant across the reservoir during this current survey. This plant species was not observed during previous surveys in 2008–2010 (Zukowski *et al.* 2013) or 2013 (NSW DPI, unpublished data); so it has spread rapidly over the past intervening years.

Dense areas of Canadian Pondweed have been shown to directly hinder the movement of freshwater crayfish (Hessen *et al.* 2004). Further, it can cause large fluctuations in dissolved oxygen concentration and pH causing elevated stress levels to crayfish. Hessen *et al.* (2004) specifically demonstrated that, similar to the Talbingo Reservoir, in Lake Steinsfjorden (southeast Norway) as Canadian Pondweed spread over shallow areas it increasingly excluded the Noble Crayfish *Astacus astacus* to the point it was absent from dense vegetation areas. Canadian Pondweed is a threat to crayfish in other areas of southern Australia (Whiterod and Zukowski 2017).

The management of Canadian Pondweed in Talbingo Reservoir should be considered a priority for Murray Crayfish conservation in the reservoir. As Murray Crayfish are very

sensitive to any pollutants or pesticides, these should not be considered in the management of this invasive weed species. If the weed is controlled, future access to the reservoir should include increased signage at the boat ramps to ensure boat users check their boats, trailers and gear to make sure Canadian Pondweed isn't being reintroduced and spread throughout the reservoir.

Monitoring to assess changes associated with proposed Snowy 2.0

The present study has shown the benefit of monitoring across a range of sites in the reservoir including those that have been monitored over time to assess the status of the species. Clearly, it is necessary to implement a future monitoring strategy to allow for regular assessment of the Murray Crayfish population in Talbingo Reservoir if future potential works are planned which may impact the species. The present survey employed robust repeat sampling methodology that detected and assessed the population status (abundance, length structure, evidence of recruitment) of Murray Crayfish as well as assessing the habitat and water quality parameters associated with Murray Crayfish. This methodology should form the basis of the monitoring program to assess changes associated with any proposed activities associated with Snowy 2.0. The monitoring program should focus on before and after works are undertaken (using hoop and munyana netting) at sites assessed in this study for comparative purposes and at any relocation sites to detect the success of the relocation program. Monitoring may not be required post-construction at some of the sites that will be permanently impacted in the Yarrangobilly River arm, as the habitat and conditions will be permanently modified and therefore may no longer provide suitable habitat (Kate Cox EMM Consulting pers. comm.). The spatial scope will provide good coverage across the reservoir and would include repeat sampling of sites where historical data exists, whereas the temporal scope will allow for timely assessment of changes over time.

During any translocation activities, a greater number of Munyana nets could be deployed to sample a greater number of individuals. Equally, a mark-recapture study could be implemented to estimate the population size (see Zukowski *et al.* 2018) with the use of more long-term tagging techniques (e.g. passive integrated transmitter (PIT) and visual implant elastomer (VIS) tags).

Translocations to mitigate risks

Understanding dispersal traits and adaptive potential is critically important when assessing the vulnerability of freshwater species in highly modified ecosystems. Dispersal limitations, coupled with its biological traits, suggest that Murray Crayfish populations in Talbingo Reservoir are vulnerable to environmental disturbance with limited potential for natural recolonisation following population decline. In order to help guide management of Murray Crayfish populations there is a need for information on its population genetic structure to gain insights into the species' life history, and its resilience to environmental disturbance, including opportunities for natural recolonisation following major population declines.

Any translocation program will need to adhere to accepted protocol and guidelines that account for planning (site selection, genetics, modelled predictions,) and implementation (movement of individuals, monitoring) whilst accounting for any risks that may arise (IUCN/SSC 2013; Whiterod and Zukowski 2019; Whiterod *et al.* 2018). In this case, genetic reanalysis following the methods of Whiterod *et al.* (2017) is important to insight into the genetic connection between the reservoir and other areas across the range of the species, but also patterns of gene flow within the reservoir (e.g. are separate sites genetically isolated?). Whiterod and Zukowski (2019), have developed a translocation strategy for the species, which has been successfully implemented in the mid-Murray section of the species range. This strategy could be readily amended and implemented for a translocation program associated with Snowy 2.0.

Conclusions

The present study employed robust repeat sampling methodology to detect the presence of and examine change in status of Murray Crayfish populations in Talbingo Reservoir. Significantly lower numbers of individuals at sites in this survey compared to historical data may be a direct result of the introduced Canadian Pondweed excluding Murray Crayfish from these areas and is of high concern. The presence of Murray Crayfish at most sites is a good indication that Murray Crayfish populations are still strong in Talbingo Reservoir and highlights the importance of the need to a) restore habitat conditions throughout the reservoir (i.e. exclude Canadian Pondweed) to accommodate Murray Crayfish populations throughout the whole reservoir as was the case five years ago, and b) ensure the existing

populations are carefully managed including monitoring and translocations, and ensure that any potential translocation sites are suitable for the species.

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