# ATTACHMENT 7

## Review of revised Dendrobium Mine Extension Project Prepared by Dr Ian A. Wright

## MSc (by research) Macquarie University PhD Western Sydney University

## **Executive summary**

The revised Dendrobium Extension Project is likely to cause damage to surface waterways from longwall subsidence that causes water quality and ecological impacts, of a greater scale than is predicted in the EIS documents. I am concerned that subsidence damage to stream channels may not be effectively repairable. Previous coal mining activity in Sydney's Drinking Water Catchments have already caused considerable damage. The Dendrobium Extension Project, if approved, is also likely to also generate an increased volume of wastewater that is to be disposed into Allans Creek, flowing to Port Kembla Estuary. Inadequate information on the impact of the waste discharge (current and predicted future) on Allans Creek, and further downstream to the estuary, was provided in the EIS on this discharge. This is a major omission as sediment in Port Kembla Harbour and Allans Creek are known to be historically contaminated with hazardous concentrations of metals.

I have prepared this report in conformance with Part 31 Division 2 and the Expert Witness Code of Conduct in Schedule 7 of the *Uniform Civil Procedure Rules 2005*, and I am willing to be bound by them.

## Scope of this advice

I have been asked to produce an expert report in relation to the Revised Dendrobium Extension Project (Project) that has been exhibited 4 May 2022 to 14 June 2022 to address the following questions:

- 25. We ask that your report addresses the following issues in regard to any impacts arising as a result of the Project:
  - a. In your opinion, is the Project likely to have a significant impact on aquatic ecology (including stygofauna)? Please provide reasoning for your answer.
  - b. The proponent claims that the design of the Project (revised from the earlier refused application) "includes avoidance of key surface features, which would reduce potential impacts to aquatic habitat [and] upland swamp". In your opinion, is this a justifiable and accurate statement?
  - c. Mining subsidence can have deleterious effects on local water resources. In your opinion, have the risks of subsidence been evaluated appropriately as to their potential to harm local water resources—quantity or quality?
  - d. In your opinion, is the impact of water use by the mine as envisaged in its extension fully considered in the environmental impact statement, including the discharge of wastewater from the mine (e.g. discharge into Allans Creek), and the potential proposed pumping station at Cordeaux River?
  - e. The Project proposal indicates that the proponent will continue to conduct aquatic ecology monitoring under 'Mitigation Measures and Monitoring' (see IMC 2020). In your view is the monitoring sufficiently rigorous and robust to meet its aims, track deleterious environmental changes, and inform mitigation activities, if required?
  - f. Provide any further observations or opinions which you consider to be relevant.

In providing this advice I have reviewed the relevant environmental assessment materials provided on the NSW Planning Portal for the revised Dendrobium Mine Extension Project.

## a. In your opinion, is the Project likely to have a significant impact on aquatic ecology?

The EIS Revised Appendix E 'Aquatic Ecology Assessment' (AEA) 30 March 2022

Executive Summary (page iv) makes the following statement:

Subsidence induced fracturing and flow diversion is expected to occur in first and second order drainage lines directly above the proposed longwalls and in first, second and third order watercourses located up to 400 m from the longwalls. Fracturing would also occur in bedrock underlying swamps resulting in reductions in shallow groundwater levels, levels of soil moisture and changes in extent and composition of swamp vegetation communities. These are expected to impact aquatic ecology as follows:

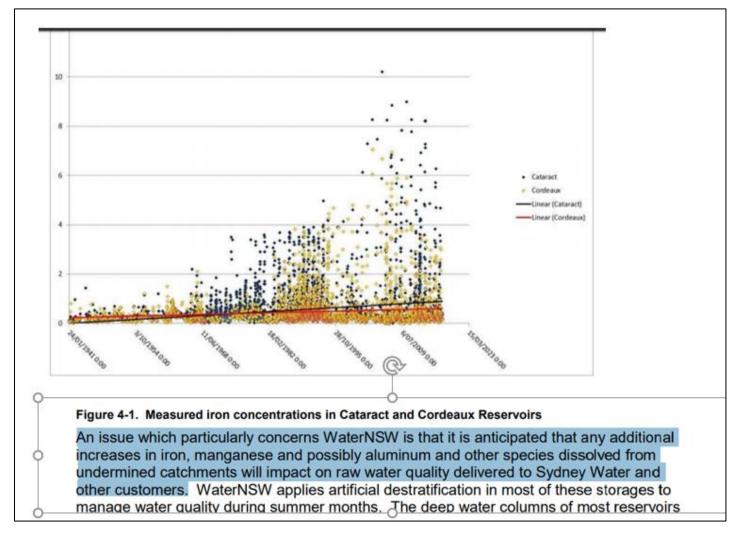
Fracturing and flow diversions in first, second and third order drainage lines are expected to result in localised (i.e. within 400 m of proposed longwalls in Area 5) reductions in aquatic habitat and loss of some biota. This could result in the loss of semi-permanent pool habitat and likely also associated biota (primarily aquatic macroinvertebrates and potentially some non-threatened native fish). However, based on experience of previous mining at Dendrobium Mine, such impacts would be localised and relatively minor compared to the extensive aquatic habitat in the broader region. The cumulative impact to the upper Avon River and Cordeaux River catchments due to loss of such habitat should, however, be considered. No significant reductions in catchment yield and no more than minor, localised and short-term impacts to water quality are predicted. Thus, impacts to downstream aquatic ecology due to reduced water availability are not expected nor are any more than localised and minor impacts to aquatic ecology due to the minor changes in water quality.

I agree that fracturing of bedrock (triggered by longwall subsidence) is likely to modify surface water flows and diminish habitat quality for biota. But my own research on longwall subsidence and impacts to surface water quality and ecology (Wright et al. 2015; Morrison et al., 2018; Morrison et al., 2019) has revealed that subsidence-triggered fracturing of stream channels can also cause changes in water quality and inflict substantial adverse impacts to stream ecosystems that are not 'minor' and 'localised'. My research was conducted in the nearby southern coalfield of the Sydney Basin (impacted Redbank Creek near Picton) in comparable sandstone geology to that of Project. In my opinion, the Aquatic Ecology Assessment produced for the Project understates the potential for adverse impacts, caused by longwall subsidence, that can cause long-term impairment to stream flow, water quality and health of stream ecosystems.

The Aquatic Ecology Assessment (page 35 of AEA) also makes the following statement, which has important implications for impacts on aquatic ecosystems of surface waters:

Potential impacts to water quality as a result of the predicted subsidence effects associated with the Project would be localised and minor (HEC 2022). Although mine subsidence effects can result in isolated, episodic pulses in iron, manganese, aluminium and electrical conductivity, there have been no reports of any measurable effect on water quality in downstream dams in the Southern Coalfield.

This statement is factually incorrect. WaterNSW have made a conflicting statement confirming that metal concentrations in two storages in two 'undermined' catchments have been increasing over several decades (Lake Cordeaux and Lake Cataract). This graph is included in the WaterNSW submission the Independent Expert Panel on Mining in the Catchment (WaterNSW, 2018, page 24).



My own published research on the topic of longwall coal mining subsidence demonstrates that it can cause substantial and complex changes to stream water quality. This is contrary to the statements in the

environmental assessment on the previous page that water quality impacts are likely to the 'isolated, episodic and localised'. I base my opinion on several years of experience and associated peer-reviewed research that I have conducted on water pollution triggered by subsidence and channel fracturing from Tahmoor Colliery on Redbank Creek from 2012 to 2017 (Wright et al. 2015; Morrison et al., 2018; Morrison et al., 2019). This is some of the only peer-reviewed water pollution research done on this topic in Australia. Although based in a different location, it is within the Sydney basin and is likely to be representative of the potential environmental damage to streams and river from the Project as it is a very similar geological and climatic setting, using similar longwall mining technology. It is important to recognise that such independent and peer-reviewed research is currently very difficult to undertake within the Drinking Water Catchment Special Areas, where the Project is located, which has highly restricted access.

I consider it to be inadequate that this assessment has not reported details and data on potential ecological impacts to the aquatic ecology associated with previous similar underground coal mines in the in the southern Sydney basin coal fields. The Tahmoor Colliery and Redbank Creek subsidence is only about 10 to 20 km from the Project. It is my opinion that the proposed long-wall mining activity does risk causing substantial habitat and water quality degradation that is likely to causes much greater harm to aquatic ecosystems than is predicted in the assessment documents.

## b. Subsidence - physical, chemical and biological damage to Redbank Creek

I provide the following information as an example of the type of impacts that can be expected to occur as a consequence of the Project. In my opinion such detail about likely subsidence impacts was not fully considered in the revised Project EIS documents.

The subsidence-induced fracturing of Redbank Creek, Picton NSW ranges from small hair-line fractures, to sections of creek channel that can be described as extensive and high-impact. See Picture 1, the section of creek within this subsidence only flowed after heavy rain, with smaller flows disappearing underground through the fractures.



**Picture 1:** Severe longwall induced subsidence fracturing of Redbank Creek, near Picton. This was upstream of the isolated pool (Picture 1).

Picture 1 shows a section of extreme subsidence fracturing. Whole slabs of sandstone have fractured and been lifted. The Project assessment predicts some occurrence of subsidence and damage to stream channels – damage similar to that observed in Redbank Creek (Picture 1) may potentially result. However, damage from the Project may be hidden from public view as unauthorised public access to the Special Area Water Catchment is prohibited (as it is located within a Special Area with restricted access). Similar fracturing damage to that observed in Redbank Creek, due to long-wall subsidence, has also been observed within Sydney's drinking water catchments. This is detailed in Dr Peter Turner's Open Letter (of which I am a signatory) to the NSW Premier (18 May 2020) [https://ssec.org.au/wp-content/uploads/2020/06/Open\_letter\_to\_Premier\_re\_mining\_in\_the\_Special\_Areas.pdf] and has similar images taken from inspections of waterways within Sydney drinking water catchment special areas.

Downstream of the Redbank Creek 'severe' fracture zone (Picture 1) were a series of isolated pools in low lying sections of the creek channel (Picture 2). I have observed and collected data from pools similar to this over about 1 km of the Redbank Creek channel over the period 2012–2017. These pools all had very poor water quality: high salinity, elevated metals, and typically very low dissolved oxygen levels. Many attributes of water quality were at levels harmful to aquatic biota (ANZECC, 2000) confirmed with severe negative impacts to stream invertebrate communities (Wright et al. 2015). Whilst the revised Project assessment suggests that there may be some water quality impacts, the assessment suggests that the impact is likely to be 'minor and localised'. I disagree that the likely impacts from subsidence would be local and minor. I base this comment on my observations and research on Redbank Creek from 2012 to 2017,

where subsidence has caused impacts that I consider to be of regional impact and substantial in severity of pollution. Given that such impacts from the proposed mining activity associated with the revised Project would occur in a protected drinking water catchment, I consider that any potential negative impact to surface water quality to be unacceptable. The water quality impacts that I consider to be likely from the Project could include physical, chemical and ecological impacts, similar to those that my research documented in Redbank Creek (Wright et al., 2015). This is explained further below.



**Picture 2:** An isolated pool on Redbank Creek, near Picton. This pool was regularly stagnant, with minimal flow due to extensive channel fracturing and loss of flow through bedrock fractures. The mixture of stream water with sections of fracturing rock causes the mobilisation of salts, minerals and metals and combined to create water quality that was hostile to stream biodiversity.



**Picture 3:** Severe subsidence fracturing of Redbank Creek, near Picton. This was the site of a ground water 'vent' where upwelling groundwater flowed into Redbank Creek, through a fresh subsidence fracture. Water quality was extremely poor for the aquatic ecosystem with very low dissolved oxygen, very high salinity, and hazardous concentrations of metals, such as zinc and nickel (Morrison et al. 2018; Morrison et al. 2019).

The pool in Picture 3 was filled by an upwelling of ground water triggered by subsidence fracturing. The upwelling water had very low dissolved oxygen, inadequate for the survival of invertebrates and fish. It also had highly elevated salinity, zinc and nickel that together combined to created highly polluted water that was hazardous to aquatic life (ANZECC, 2000; Morrison et al. 2018; Morrison et al. 2019; Wright et al. 2015).

I consider it to be likely that such subsidence-triggered impacts and damage to stream channels, stream water quality and aquatic ecosystems, similar to Redbank Creek, could occur in the future to water catchment waterways due to the proposed revised Project.



**Picture 4:** This pool in Redbank Creek was affected by subsidence and recorded a very high abundance of mosquito larvae and pupae. It also had very few 'normal' stream invertebrates.

See Picture 4. I sampled this isolated pool on Redbank Creek over a period of more than two years and it provided a rich habitat for mosquitos. More sensitive typical stream invertebrates were missing due to the highly impaired water quality. This pool had depleted dissolved oxygen, very high salinity and elevated zinc and nickel (Wright et al. 2015). This physical subsidence damage to the creek channel, the impaired water quality and adverse impacts to the aquatic ecosystem have all occurred over many years in Redbank Creek. The transformation of a healthy stream ecosystem into one that supports a domination by mosquitos is unusual and is a potential ecological impact. It also has potential human health implications, if the mosquito species are disease vectors.

I am uncertain how quickly and effectively repairs/rehabilitation of such damage to stream channels can be achieved. The damage to Redbank Creek was covered by ABC in September 2018 and Sydney Morning Herald in June 2020 (Hannam, 2020). I am yet to observe major subsidence damage to a creek or river channel effectively repaired. The issue of how effective repairs to channel fracturing (such as polyurethane grouting) is also discussed in the Open Letter by Peter Turner et al. (2019; see page 32). I have personally observed repairs to channel fracturing in the Georges River and also Redbank Creek, but I am yet to see any evidence that repairs are effective.

In my opinion the revised Dendrobium Project environmental assessment fails to provide adequate, detailed information on exactly how minor, moderate and severe damage to stream and river channels is to be repaired. The information provided in Attachment 9 'Rehabilitation Strategy and Mine Closure Addendum' (particularly page A9-23 to 24) lacks detail, and also lacks independent verification that such technology is robust and effective. I remain concerned that subsidence damage from the Project to surface waterways may not be repairable.

In my opinion, the risks of subsidence having deleterious effects on water resources were not fully evaluated appropriately in the EIS. The EIS also fails to refer to published scientific studies that have been conducted on underground-coal-mine subsidence impacts to Redbank Creek at Picton (from Tahmoor Colliery longwall subsidence).

The EIS for the revised Project also failed to mention one of the most detailed studies on the topic of longwall subsidence within a Drinking Water Catchment. The research was done for the Honours thesis by Catherine Cunningham (University of Canberra, 2016) *'The effect of subsidence from long wall coal mining on the ecology and water quality of streams'*. The research by Catherine Cunningham was conducted on two streams (Waratah Rivulet and Eastern Tributary) flowing within the Woronora drinking water catchment. See photo of discolouration of Eastern Tributary, in the Woronora Drinking Water Catchment. Her research detected both water quality impairment and ecological modification (macroinvertebrates and algal diatoms) in the two catchment streams that were affected by underground long wall subsidence.



Picture 5. Discolouration (due to subsidence damage) of Eastern Tributary in Woronora Drinking Water Catchment, 2019. Photo by author.

d. In your opinion, is the impact of water use by the mine as envisaged in its extension fully considered in the environmental impact statement, including the discharge of wastewater from the mine (e.g. discharge into Allans Creek), and the potential proposed pumping station at Cordeaux River?

I am aware that current waste discharges from Dendrobium Colliery, combined with wastes from at least one other mine in South 32's Bulli Seam Operations ('brine'), are released into Allans Creek via LDP5 (under Environment Protection Licence EPL 3241) and are very likely to be causing substantial water quality impairment to the receiving waterways. This is an important issue which is not covered adequately in the revised Project assessment and further information is required. In my opinion, the various assessment documents in the revised Project provided inadequate information on this issue to allow a complete assessment of this issue.

This is a serious issue and I consider the lack of supporting detail on the downstream water quality and ecological impacts of the current and future LDP5 waste discharge to Allans Creek be a major deficiency. I also note that this information is available and should have been included in the EIS as the NSW EPA required a study to be undertaken, as Special Condition E1.1 of EPL 3241, 'Allans Creek Monitoring Program'. See extract from EPL 3241 (page 18). To fully evaluate impacts from the Project such information should be made available.

## 8 Special Conditions

## E1 Allans Creek Monitoring Program

E1.1 The licensee must undertake a water quality monitoring program to determine the effect of the discharge from point 5 on water quality in Allans Creek.

The results of the monitoring must be compared to predicted concentrations and conclusions made about the bioavailability of As, Zn, Ni and Cu in the brine discharge study report (doc ref 2440/1330) titled "Allans Creek licensed discharge water quality and hydrochemical modelling report", EGI, 13 December 2019.

A copy of the monitoring program report must be submitted to the EPA by the due date.

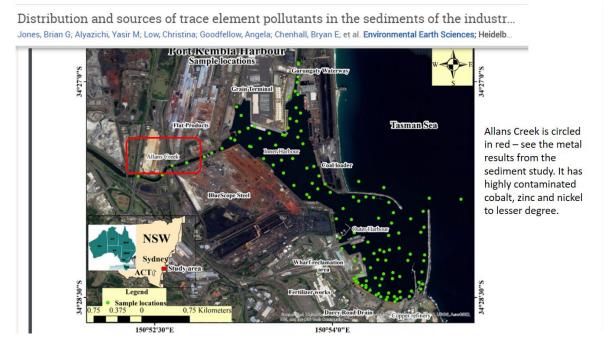
DUE DATE: 6 months following the commencement of operation of the new and expanded water treatment plants required by condition E1.1 of Environment Protection Licence 2504 held by Endeavour Coal Pty Ltd.

Allans Creek forms part of Port Kembla Harbour estuary. Published results indicate that both Port Kembla harbour estuary and Allans Creek has hazardous concentration of several metals that have accumulated in the harbour and creek sediment (Pictures 6,7 and 8; Jones et al. 2019). The data used in the publication by Jones et al. (2019) was published recently but used data collected many years earlier,

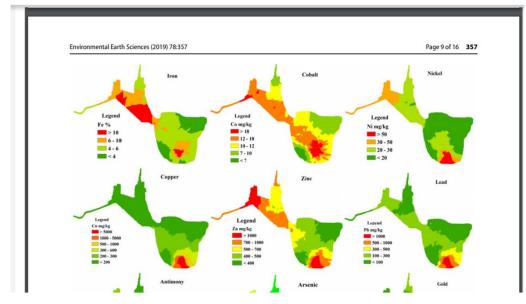
with the majority of samples collected between 1996-1998. In my opinion, further sediment and water sampling should have been undertaken and reported for this EIS, particularly along Allans Creek, above and below the LDP 5 discharge.

I consider that LDP 5 waste discharges are almost certainly contributing to the pollution of Allans Creek and Port Kembla Harbour estuary. The nature and magnitude of the potential pollution are currently unknown, and inadequate information was provided in the EIS documents to understand this likely environmental impact. In my opinion, further information on the current and future waste discharge from LDP5 is urgently required. With the revised Project proposal indicating that increased flow of wastewater would be released from LDP 5, the approval of the revised Dendrobium extension would very likely increase the magnitude of the pollution in Allans Creek. This is potentially serious environmental impact from the current Dendrobium Colliery operation that is inadequately addressed in the revised Dendrobium assessment. The EIS documents for the revised Project claim that the increased flows of wastewater that are anticipated to be generated by the Project will not exceed the EPL 3241 discharge limits. This is not surprising to me. See extract from page 10 of EPL 3241 below. In my opinion these 100 percent concentrations limits are far too large, well above ANZECC (2000) trigger values for protection of aquatic species and probably allow the Project to add to the historic contamination reported in Allans Creek and Port Kembla Harbour by Jones et al.(2019).

Pollutant	Units of Measure	50 percentile concentration limit	90 percentile concentration limit	3DGM concentration limit	100 percentile concentration limit
Arsenic	milligrams per litre				1.3
Copper	milligrams per litre				0.080
Nickel	milligrams per litre				5
Oil and Grease	milligrams per litre				10
рН	pН				6.5-9.0
Total suspended solids	milligrams per litre				30
Zinc	milligrams per litre				0.4



**Picture 6:** Sample locations used by Jones et al. (2019) in their investigation of sediment chemistry in Port Kembla Harbour. Allans Creek is highlighted.



Distribution and sources of trace element pollutants in the sediments of the industr... Jones, Brian G; Alyazichi, Yasir M; Low, Christina; Goodfellow, Angela; Chenhall, Bryan E; et al. Environmental Earth Sciences; Heidelb...

**Picture 7:** Summary results by Jones et al. (2019) in their investigation of sediment chemistry in Port Kembla Harbour. See picture 12 for location of Allans Creek.

Environmental Earth Sciences (2019) 78:357 https://doi.org/10.1007/s12665-019-8358-1

#### **ORIGINAL ARTICLE**



# Distribution and sources of trace element pollutants in the sediments of the industrialised Port Kembla Harbour, New South Wales, Australia

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Received: 20 September 2018 / Accepted: 31 May 2019 / Published online: 8 June 2019 © Springer-Verlag GmbH Germany, part of Springer Nature 2019

#### Abstract

A detailed geochemical analysis of sediment samples from the heavily industrialised Port Kembla Harbour, 70 km south of Sydney in New South Wales, Australia, has delineated areas that are heavily contaminated with trace element pollutants. Local creek delta sand accumulations, basin mud deposits, reclamation deposits and exposed bedrock on the present harbour floor are consistent with the twentieth century Port Kembla industrial land and harbour development history, as well as known maritime shipping practices. The closed electrolytic copper refinery, one of the two major pollutant sources, has provided a very clear signature in the harbour opposite the main drain that discharged from these works. Copper pollution (up to 6070 mg/kg) is strongly correlated with high concentrations of gold, selenium, antimony, arsenic, lead and zinc. These pollutants appear to occur as silt- and sand-sized particulate matter, as well as pollutants that are probably chemically bound to clay, pyrite and organic matter. The second main pollutant source is the BlueScope steelworks that has been responsible for high concentrations of iron (and minor associated cobalt), kish, pyrolytic carbon and fly ash. The steelworks, along with other industrial, urban and automobile sources, supply additional quantities of copper, lead and zinc to the aquatic environment. Coal and coke dust also form important pollutants. The pollutants are currently fairly stable under the prevailing alkaline reducing conditions within the sediment. The proposed future reclamation and dredging within the harbour needs to consider the implications of moving highly contaminated sediment, without altering its alkalinity or oxidation potential, to prevent release of contaminants.

#### Picture 8: Abstract and details of the sediment contamination study by Jones et al. 2019.

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## **Qualifications and experience**

I am an environmental and water scientist with more than 25 years of experience investigating the impact of human activities on waterways of the Sydney basin. I am currently employed as a Senior Lecturer in the School of Science and Health at Western Sydney University. Earlier in my career I was a freshwater scientist, working in various roles at Sydney Water. This included working as a scientific officer in Sydney Water's scientific laboratories at West Ryde. I then worked as catchment officer in Sydney Water's drinking water catchments. After received my PhD, I was awarded a Postdoctoral Research Fellowship in freshwater ecology and water pollution research at Western Sydney University. Before becoming a fulltime lecturer in 2012, I established a consulting business, mainly helping local Government with projects associated with urban water quality and ecology. I am an advocate for sustainable water and catchment management and I strongly support multi-disciplinary projects. I seek to manage industry problems with evidence-based science. I have specialist scientific expertise in freshwater ecology, water chemistry, pollution ecology of waters, freshwater macroinvertebrates as pollution indicators, impact of urban development, sewage effluent, agricultural, and mine waste impacts on streams and rivers. I have expertise in the sampling design of environmental science studies and statistical analysis of environmental data. I have published (as senior or junior co-author) more than 80 peer-reviewed publications. I have provided independent expert testimonies for environmental science matters for the NSW Land & Environment Court. I am an enthusiastic participant in community engagement activities in my field of expertise.

## Qualifications

2006. Doctor of Philosophy, University of Western Sydney.

- 1995. Master of Science (by research), Macquarie University.
- 1988. Bachelor of Applied Science (Agriculture), Hawkesbury Agricultural College.

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#### 1. Education

- 2006 Doctor of Philosophy. Western Sydney University.
- 1995 Master of Science (by Research). Macquarie University.
- 1988 Bachelor of Applied Science (Agriculture) with Merit. Hawkesbury Agricultural College.

#### 2. Employment History

- 2017- Senior Lecturer in School of Science. Western Sydney University (current position)
   2012-6 Lecturer/Associate Lecturer in School of Science. Western Sydney University.
- 2010-12 Casual Lecturer: School of Science and Health. Western Sydney University. Graduate School of the Environment. Macquarie University.
- 2007-9 Post-Doctoral Research Fellow (Aquatic Ecology), Western Sydney University.
- 2006-7 Principal Environmental Scientist, Ku-ring-gai Council.
- 2005-6 Senior Scientist (Collaborative Research), Sydney Catchment Authority.
- 2000-5 Senior Team Leader (Aquatic Ecology), Environmental Policy Analyst, Sydney Water Corporation.
- 1998-0 Environmental Scientist, Sydney Water Corporation.
- 1996-98 Full-time PhD student, Australian National University, Visiting Scientist CSIRO Division of Entomology.
- 1989-96 Scientific Officer, Catchment Protection Officer, Sydney Water Corporation.

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2020. Co-authored 'Heavy rains are great news for Sydney Dams but they come with a big caveat' with Dr Jason Reynolds. Article published February 13 in 'The Conversation'. Available at

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(https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.587.9973&rep=rep1&type=pdf)



25 May 2022

Dr Ian Wright Senior Lecturer, School of Science and Health Western Sydney University

By email: I.Wright@westernsydney.edu.au

## **CONFIDENTIAL AND PRIVILEGED**

Dear Dr Wright,

## Brief to Expert - Dendrobium Mine Extension Project SSI - 33143123

- We act for Protect Our Water Catchment (POWC) in relation to the proposed <u>Dendrobium</u> <u>Mine Extension Project (SSI - 33143123)</u> (Project) by Illawarra Coal Holdings Pty Ltd (Applicant), a subsidiary of South32 Limited.
- 2. The Project is an extension of the Applicant's existing underground coal mine located around 8 km west of Wollongong in the Southern Coalfield of New South Wales (**NSW**). The Applicant is seeking development consent to extract up to 5.2 million tonnes per annum (Mtpa) of ROM coal, through underground mining operations within Area 5 (location of the Project) until approximately 2035, in extending the life of Dendrobium Mine until 2041. The Project is a redesign of the Applicant's previous Significant State Development (**SSD**) application (<u>Dendrobium Extension Project, SSD 8194</u>).
- 3. Our client intends to make a submission on the Project which is currently being publicly exhibited to ensure the decision-maker has independent expert advice on the Project.
- 4. We seek to engage you on behalf of our client to review the environmental impact statement (EIS) for the Project and prepare an independent expert report in relation to your area of expertise, aquatic ecology and mine subsidence, in accordance with the Uniform Civil Procedure Rules 2005 (UCPR) and the Expert Witness Code of Conduct.

## Background

5. On 5 February 2021, the Applicant's SSD application (<u>Dendrobium Extension Project, SSD</u> 8194) for the Project was refused by the Independent Planning Commission (**IPC**). The

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Suite 8.02, Level 8, 6 O'Connell Street Sydney, NSW 2000 ABN: 72002 880 864 Applicant appealed the IPC's decision, which is currently the subject of a judicial review proceedings in the Land and Environment Court of New South Wales.

- 6. On or around December 2021, the Applicant submitted a <u>scoping report</u> for the re-designed SSD Project in support of the application for the Project to be assessed as 'State significant infrastructure' (**SSI**).
- 7. On 2 December 2021, the Project was declared SSI by the Minister for Planning and Public Spaces.
- 8. On 23 December 2021, the Department of Planning, Industry and Environment (now the Department of Planning and Environment) issued the Planning Secretary's environmental assessment requirements (SEARs) for the Project.
- 9. On 4 May 2022, the Project application, EIS and accompanying documents were placed on public exhibition.

## Purpose of your expert report

- 10. We note as a preliminary matter that our primary purpose in briefing you to prepare your report is to provide independent expert advice in your area of expertise. We do not ask you to be an advocate for our client/s. You are requested to prepare an independent report that is clear and well-written.
- 11. In this respect, we draw your attention to Part 31 Division 2 of the Uniform Civil Procedure Rules 2005 (NSW) (UCPR) and the Expert Witness Code of Conduct (Code of Conduct) which govern the use of expert evidence in NSW Courts (attached). The SSI public exhibition process is not a Court proceeding; however, we are of the view that the same Code of Conduct should be adhered to in this instance.
- 12. In particular, clause 2 of the Code of Conduct states that:

"An expert witness is not an advocate for a party and has a paramount duty, overriding any duty to the party to the proceedings or other person retaining the expert witness, to assist the court impartially on matters relevant to the area of expertise of the witness."

- 13. Your expert report must contain an acknowledgment that you have read the Expert Witness Code of Conduct and that you agree to be bound by it.
- 14. Your expert report will be used as evidence in chief of your professional opinion. Information of which you believe the decision maker should be aware must be contained in your expert report.
- 15. In providing your opinion to the decision maker you must set out all the assumptions upon which the opinion is based. This may include, for example, facts observed as a result of fieldwork or 'assumed' facts based on a body of scientific opinion. If the latter, you should provide references which demonstrate the existence of that body of opinion.

16. Your expert report must also set out the process of reasoning which you have undertaken in order to arrive at your conclusions. It is insufficient for an expert report to simply state your opinion or conclusion reached without an explanation as to how this was arrived at. The purpose of providing such assumptions and reasoning is to enable the decision maker and experts engaged by other parties to make an assessment as to the soundness of your opinion.

## **Overview of work requested**

- 17. We request that you undertake the following work:
  - a. review the documents listed below;
  - b. prepare a written expert report that addresses the issues identified below ('Issues to address in your expert report'); and
  - c. ensure that the work is prepared in accordance with independent expert advice as indicated above.

## Documents

- 18. We enclose the Code of Conduct and Part 31 Division 2 of the UCPR.
- 19. If you have previously reviewed relevant EIS documents for the previous SSD application (Dendrobium Extension Project, SSD 8194) you may wish to review your previous expert advice.
- Full Project documentation is available at the following website:
   a) NSW Government Planning Portal: <u>https://www.planningportal.nsw.gov.au/major-projects/projects/dendrobium-mine-extension-project-0</u>
- 21. The following documents relating to the Project are provided for your particular consideration:

## Environmental Impact Statement

#### Executive Summary,

https://majorprojects.planningportal.nsw.gov.au/prweb/PRRestService/mp/01/getContent? AttachRef=SSI-33143123%2120220427T061031.074%20GMT

## Section 1 - Introduction,

https://majorprojects.planningportal.nsw.gov.au/prweb/PRRestService/mp/01/getContent? AttachRef=SSI-33143123%2120220427T061031.900%20GMT

## Section 7 – Environmental Assessment,

https://majorprojects.planningportal.nsw.gov.au/prweb/PRRestService/mp/01/getContent? AttachRef=SSI-33143123%2120220427T061037.452%20GMT, pp. 7-5 to 7-13, 7-37 to 7-64

#### Appendix A – Subsidence Assessment,

https://majorprojects.planningportal.nsw.gov.au/prweb/PRRestService/mp/01/getContent? AttachRef=SSI-33143123%2120220427T061043.537%20GMT

## Appendix E – Aquatic Ecology Assessment,

https://majorprojects.planningportal.nsw.gov.au/prweb/PRRestService/mp/01/getContent? AttachRef=SSI-33143123%2120220427T061047.301%20GMT

- 22. IMC (2020). Illawarra Metallurgical Coal. Dendrobium Area 3B Watercourse Impact Monitoring, Management and Contingency Plan. <u>https://www.south32.net/docs/default-source/illawarra-coal/dendrobium/subsidence-management-plans---longwalls-14-19/attachment-s-dendrobium-area-3-environmental-performance.pdf?sfvrsn=18df35db\_4</u>
- 23. Other documents relating to the SSD application that may be of relevance include:
  - a) Dendrobium Extension Project SSD 8194 Project Area (see attached).
  - b) Catherine Cunningham, Honours Thesis the effect of subsidence from long wall coal mining on the ecology and water quality of streams (see attached).
- 24. You are not limited to the above documents, if there is other material relevant to your expert report, you may wish to refer to this material.

## Issues to address in your expert report

- 25. We ask that your report addresses the following issues in regard to any impacts arising as a result of the Project:
  - a. In your opinion, is the Project likely to have a significant impact on aquatic ecology (including stygofauna)? Please provide reasoning for your answer.
  - b. The proponent claims that the design of the Project (revised from the earlier refused application) "includes avoidance of key surface features, which would reduce potential impacts to aquatic habitat [and] upland swamp". In your opinion, is this a justifiable and accurate statement?
  - c. Mining subsidence can have deleterious effects on local water resources. In your opinion, have the risks of subsidence been evaluated appropriately as to their potential to harm local water resources—quantity or quality?
  - d. In your opinion, is the impact of water use by the mine as envisaged in its extension fully considered in the environmental impact statement, including the discharge of wastewater from the mine (e.g. discharge into Allans Creek), and the potential proposed pumping station at Cordeaux River?
  - e. The Project proposal indicates that the proponent will continue to conduct aquatic ecology monitoring under 'Mitigation Measures and Monitoring' (see IMC 2020). In your view is the monitoring sufficiently rigorous and robust to meet its aims, track deleterious environmental changes, and inform mitigation activities, if required?
  - f. Provide any further observations or opinions which you consider to be relevant.
- 26. We request that you provide us with a draft of your report for review before finalising it. We emphasise that the purpose of this is not to influence the conclusions or recommendations you make but to ensure that the language and expression of the report is clear and complies with the formal legal requirements of an expert report.

## **Key dates**

- 27. The Project application, EIS and accompanying documents are on public exhibition from Wednesday 4 May 2022 until 14 June 2022.
- 28. We kindly request a draft of your expert advice by no later than **Monday 6 June 2022**.
- 29. Please provide your final expert advice by no later than **Thursday 9 June 2022.**

## **Duty of confidentiality**

30. Please treat your work as strictly confidential, unless authorised otherwise by us. Please mark all documents prepared for the purposes of this brief as "Privileged & Confidential".

## **Fees and Terms**

- 31. Thank-you for agreeing to provide your advice in this matter on a pro bono (volunteer) basis. EDO relies on experts such as you to assist in matters with very little financial compensation.
- 32. Please note the following terms:
  - a. your work will only be used by EDO to relation to this matter;
  - b. Either EDO or our client may choose to make your expert advice publicly available. Any public release of your report may result in disclosure of any works in your report over which you may claim copyright;
  - c. EDO will take all reasonable steps to prevent your work being used for purposes other than that mentioned above, but we accept no responsibility for the actions of third parties;
  - d. regardless of the above points, EDO may choose not to use your work; and
  - e. you will not be covered by the EDO's insurance while undertaking the above tasks.
- 33. If you would like to discuss this brief further, please contact Jayme Cooper via email <u>jayme.cooper@edo.org.au</u> (cc: <u>matthew.floro@edo.org.au</u> and <u>edward.butler@edo.org.au</u>).

We are grateful for your assistance in this matter.

Yours sincerely, Environmental Defenders Office

Jayme Cooper Solicitor

Reference number: s 3326



## **Uniform Civil Procedure Rules 2005**

Current version for 1 December 2021 to date (accessed 25 May 2022 at 13:36)

Part 31 > Division 2

## **Division 2 Provisions applicable to expert evidence generally**

#### Note-

The provisions of this Division replace those of former Divisions 2 and 3, as in force immediately before 8 December 2006. The numbering of the individual provisions of this Division varies considerably from that of the provisions of the former Divisions. The following Table identifies the new rules corresponding to former rules 31.17–31.35.

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17		L	е

Former rule	New rule
Rule 31.17	Rule 31.18
Rule 31.18	Rule 31.28
Rule 31.18A	Rule 31.29
Rule 31.19	Rule 31.30
Rule 31.20	Rule 31.31
Rule 31.21	Rule 31.32
Rule 31.22	Rule 31.33
Rule 31.23	Rule 31.27
Rule 31.24	Rule 31.34
Rule 31.25	Rules 31.24 and 31.26
Rule 31.26	Rule 31.35
Rule 31.27	Rule 31.36
Rule 31.28	Rule 31.18
Rule 31.29	Rule 31.46
Rule 31.30	Rule 31.23
Rule 31.31	Rule 31.49
Rule 31.32	Rule 31.51
Rule 31.33	Rule 31.52
Rule 31.34	Rule 31.53
Rule 31.35	Rule 31.54

## **Subdivision 1 Preliminary**

**31.17 Main purposes of Division** (cf Queensland *Uniform Civil Procedure Rules 1999*, rule 423; United Kingdom *Civil Procedure Rules 1998*, rule 35.1)

The main purposes of this Division are as follows-

- (a) to ensure that the court has control over the giving of expert evidence,
- (b) to restrict expert evidence in proceedings to that which is reasonably required to resolve the proceedings,
- (c) to avoid unnecessary costs associated with parties to proceedings retaining different experts,
- (d) if it is practicable to do so without compromising the interests of justice, to enable expert evidence to be given on an issue in proceedings by a single expert engaged by the parties or appointed by the court,
- (e) if it is necessary to do so to ensure a fair trial of proceedings, to allow for more than one expert (but no more than are necessary) to give evidence on an issue in the proceedings,
- (f) to declare the duty of an expert witness in relation to the court and the parties to proceedings.

31.18 Definitions (cf SCR Part 36, rules 13A and 13C; DCR Part 28, rule 8; LCR Part 23, rule 1D)

In this Division-

court-appointed expert means an expert appointed pursuant to rule 31.46.

*expert*, in relation to any issue, means a person who has such knowledge or experience of, or in connection with, that issue, or issues of the character of that issue, that his or her opinion on that issue would be admissible in evidence.

expert witness means an expert engaged or appointed for the purpose of-

- (a) providing an expert's report for use as evidence in proceedings or proposed proceedings, or
- (b) giving opinion evidence in proceedings or proposed proceedings.

*expert's report* means a written statement by an expert (whether or not an expert witness in the proceedings concerned) that sets out the expert's opinion and the facts, and assumptions of fact, on which the opinion is based.

*hospital report* means a written statement concerning a patient, made by or on behalf of a hospital, that the party serving the statement intends to adduce in evidence in chief at the trial.

parties' single expert means an expert engaged pursuant to rule 31.37.

#### Subdivision 2 Expert witnesses generally

#### 31.19 Parties to seek directions before calling expert witnesses

- (1) Any party—
  - (a) intending to adduce expert evidence at trial, or
  - (b) to whom it becomes apparent that he or she, or any other party, may adduce expert evidence at trial,

must promptly seek directions from the court in that regard.

- (2) Directions under this rule may be sought at any directions hearing or case management conference or, if no such hearing or conference has been fixed or is imminent, by notice of motion or pursuant to liberty to restore.
- (3) Unless the court otherwise orders, expert evidence may not be adduced at trial—
  - (a) unless directions have been sought in accordance with this rule, and
  - (b) if any such directions have been given by the court, otherwise than in accordance with those directions.
- (4) This rule does not apply to proceedings with respect to a professional negligence claim.

#### 31.20 Court may give directions regarding expert witnesses

- (1) Without limiting its other powers to give directions, the court may at any time give such directions as it considers appropriate in relation to the use of expert evidence in proceedings.
- (2) Directions under this rule may include any of the following-
  - (a) a direction as to the time for service of experts' reports,
  - (b) a direction that expert evidence may not be adduced on a specified issue,
  - (c) a direction that expert evidence may not be adduced on a specified issue except by leave of the court,
  - (d) a direction that expert evidence may be adduced on specified issues only,
  - (e) a direction limiting the number of expert witnesses who may be called to give evidence on a specified issue,
  - (f) a direction providing for the engagement and instruction of a parties' single expert in relation to a specified issue,
  - (g) a direction providing for the appointment and instruction of a court-appointed expert in relation to a specified issue,
  - (h) a direction requiring experts in relation to the same issue to confer, either before or after preparing experts' reports in relation to a specified issue,
  - (i) any other direction that may assist an expert in the exercise of the expert's functions,
  - (j) a direction that an expert who has prepared more than one expert's report in relation to any proceedings is to prepare a single report that reflects his or her evidence in chief.

#### 31.21 Expert evidence in chief to be given by way of experts' reports

Unless the court otherwise orders, an expert witness's evidence in chief must be given by the tender of one or more expert's reports.

#### 31.22 Expert witness to provide details of contingency fees or deferred payment schemes

- (1) A person who is engaged as an expert witness in relation to any proceedings must include information as to any arrangements under which—
  - (a) the charging of fees or costs by the expert witness is contingent on the outcome of the proceedings, or
  - (b) the payment of any fees or costs to the expert witness is to be deferred,
  - in, or in an annexure to, any report that he or she prepares for the purposes of the proceedings.
- (2) If a report referred to in subrule (1) indicates the existence of any such arrangements, the court may direct disclosure of the terms of the engagement (including as to fees and costs).
- 31.23 Code of conduct (cf SCR Part 39, rule 2; DCR Part 28A, rule 2; LCR Part 38B, rule 2)
  - (1) An expert witness must comply with the code of conduct set out in Schedule 7.
  - (2) As soon as practicable after an expert witness is engaged or appointed—
    - (a) in the case of an expert witness engaged by one or more parties, the engaging parties, or one of them as they may agree, or
    - (b) in the case of an expert witness appointed by the court, such of the affected parties as the court may direct,

must provide the expert witness with a copy of the code of conduct.

- (3) Unless the court otherwise orders, an expert's report may not be admitted in evidence unless the report contains an acknowledgment by the expert witness by whom it was prepared that he or she has read the code of conduct and agrees to be bound by it.
- (4) Unless the court otherwise orders, oral evidence may not be received from an expert witness unless the court is satisfied that the expert witness has acknowledged, whether in an expert's report prepared in relation to the proceedings or otherwise in relation to the proceedings, that he or she has read the code of conduct and agrees to be bound by it.
- **31.24 Conference between expert witnesses** (cf SCR Part 36, rule 13CA; DCR Part 28, rule 9D; LCR Part 23, rule 1E)
  - (1) The court may direct expert witnesses-
    - (a) to confer, either generally or in relation to specified matters, and
    - (b) to endeavour to reach agreement on any matters in issue, and
    - (c) to prepare a joint report, specifying matters agreed and matters not agreed and reasons for any disagreement, and
    - (d) to base any joint report on specified facts or assumptions of fact,

and may do so at any time, whether before or after the expert witnesses have furnished their experts' reports.

- (2) The court may direct that a conference be held—
  - (a) with or without the attendance of the parties affected or their legal representatives, or
  - (b) with or without the attendance of the parties affected or their legal representatives, at the option of the parties, or
  - (c) with or without the attendance of a facilitator (that is, a person who is independent of the parties and who may or may not be an expert in relation to the matters in issue).
- (3) An expert witness so directed may apply to the court for further directions to assist the expert witness in the performance of his or her functions in any respect.
- (4) Any such application must be made by sending a written request for directions to the court, specifying the matter in relation to which directions are sought.
- (5) An expert witness who makes such an application must send a copy of the request to the other expert witnesses and to the parties affected.
- (6) Unless the parties affected agree, the content of the conference between the expert witnesses must not be referred to at any hearing.

#### 31.25 Instructions to expert witnesses where conference ordered before report furnished

If a direction to confer is given under rule 31.24(1)(a) before the expert witnesses have furnished their reports, the court may give directions as to—

- (a) the issues to be dealt with in a joint report by the expert witnesses, and
- (b) the facts, and assumptions of fact, on which the report is to be based,

including a direction that the parties affected must endeavour to agree on the instructions to be provided to the expert witnesses.

**31.26** Joint report arising from conference between expert witnesses (cf SCR Part 36, rule 13CA; DCR Part 28, rule 9D; LCR Part 23, rule 1E)

- (1) This rule applies if expert witnesses prepare a joint report as referred to in rule 31.24(1)(c).
- (2) The joint report must specify matters agreed and matters not agreed and the reasons for any disagreement.
- (3) The joint report may be tendered at the trial as evidence of any matters agreed.
- (4) In relation to any matters not agreed, the joint report may be used or tendered at the trial only in accordance with the rules of evidence and the practices of the court.
- (5) Except by leave of the court, a party affected may not adduce evidence from any other expert witness on the issues dealt with in the joint report.

#### Subdivision 3 Experts' reports and expert evidence

31.27 Experts' reports (cf SCR Part 36, rule 13C; DCR Part 28, rule 9C; LCR Part 23, rule 1D)

- (1) An expert's report must (in the body of the report or in an annexure to it) include the following-
  - (a) the expert's qualifications as an expert on the issue the subject of the report,
  - (b) the facts, and assumptions of fact, on which the opinions in the report are based (a letter of instructions may be annexed),
  - (c) the expert's reasons for each opinion expressed,
  - (d) if applicable, that a particular issue falls outside the expert's field of expertise,
  - (e) any literature or other materials utilised in support of the opinions,
  - (f) any examinations, tests or other investigations on which the expert has relied, including details of the qualifications of the person who carried them out,
  - (g) in the case of a report that is lengthy or complex, a brief summary of the report (to be located at the beginning of the report).
- (2) If an expert witness who prepares an expert's report believes that it may be incomplete or inaccurate without some qualification, the qualification must be stated in the report.
- (3) If an expert witness considers that his or her opinion is not a concluded opinion because of insufficient research or insufficient data or for any other reason, this must be stated when the opinion is expressed.
- (4) If an expert witness changes his or her opinion on a material matter after providing an expert's report to the party engaging him or her (or that party's legal representative), the expert witness must forthwith provide the engaging party (or that party's legal representative) with a supplementary report to that effect containing such of the information referred to in subrule (1) as is appropriate.
- **31.28 Disclosure of experts' reports and hospital reports** (cf SCR Part 36, rule 13A; DCR Part 28, rule 8; LCR Part 23, rule 3)
  - (1) Each party must serve experts' reports and hospital reports on each other active party—
    - (a) in accordance with any order of the court, or
    - (b) if no such order is in force, in accordance with any relevant practice note, or
    - (c) if no such order or practice note is in force, not later than 28 days before the date of the hearing at which the report is to be used.
  - (2) An application to the court for an order under subrule (1) (other than an order solely for abridgment or extension of time) may be made without serving notice of motion.

- (3) Except by leave of the court, or by consent of the parties—
  - (a) an expert's report or hospital report is not admissible unless it has been served in accordance with this rule, and
  - (b) without limiting paragraph (a), an expert's report or hospital report, when tendered under section 63, 64 or 69 of the *Evidence Act 1995*, is not admissible unless it has been served in accordance with this rule, and
  - (c) the oral expert evidence in chief of any expert is not admissible unless an expert's report or hospital report served in accordance with this rule contains the substance of the matters sought to be adduced in evidence.
- (4) Leave is not to be given as referred to in subrule (3) unless the court is satisfied—
  - (a) that there are exceptional circumstances that warrant the granting of leave, or
  - (b) that the report concerned merely updates an earlier version of a report that has been served in accordance with subrule (1).
- 31.29 Admissibility of expert's report (cf SCR Part 36, rule 13B)
  - (1) If an expert's report is served in accordance with rule 31.28 or in accordance with an order of the court, the report is admissible—
    - (a) as evidence of the expert's opinion, and
    - (b) if the expert's direct oral evidence of a fact on which the opinion was based would be admissible, as evidence of that fact,

without further evidence, oral or otherwise.

- (2) Unless the court otherwise orders, a party may require the attendance for cross-examination of the expert by whom the report was prepared by notice served on the party by whom the report was served.
- (3) Unless the court otherwise orders, such a requirement may not be made later than—
  - (a) in the case of proceedings for which the court has fixed a date for trial, 35 days before the date so fixed, or
  - (b) in any other case, 7 days before the date on which the court fixes a date for trial.
- (4) The parties may not by consent abridge the time fixed by or under subrule (3).
- (5) If the expert's attendance for cross-examination is required under subrule (2), the report may not be tendered under section 63, 64 or 69 of the *Evidence Act 1995* or otherwise used unless the expert attends or is dead or the court grants leave to use it.
- (6) The party using the report may re-examine the expert if the expert attends for cross-examination pursuant to a requirement under subrule (2).
- (7) This rule does not apply to proceedings in the District Court or the Local Court or to proceedings on a trial with a jury.
- 31.30 Admissibility of expert's report in District Court and Local Court (cf DCR Part 28, rule 9; LCR Part 23, rule 2)
  - (1) This rule applies to proceedings in the District Court or the Local Court.
  - (2) If an expert's report is served in accordance with rule 31.28 or in accordance with an order of the court, the report is admissible—
    - (a) as evidence of the expert's opinion, and

#### Uniform Civil Procedure Rules 2005 - NSW Legislation

(b) if the expert's direct oral evidence of a fact on which the opinion was based would be admissible, as evidence of that fact,

without further evidence, oral or otherwise.

- (3) Unless the court orders otherwise—
  - (a) it is the responsibility of the party requiring the attendance for cross-examination of the expert by whom an expert's report has been prepared to procure that attendance, and
  - (b) the party requiring the expert's attendance must notify the expert at least 28 days before the date on which attendance is required.
- (4) Except for the purpose of determining any liability for conduct money or witness expenses, an expert does not become the witness for the party requiring his or her attendance merely because his or her attendance at court has been procured by that party.
- (5) A party who requires the attendance of a person as referred to in subrule (2)—
  - (a) must inform all other parties to the proceedings that the party has done so at least 28 days before the date fixed for hearing, and
  - (b) must pay to the person whose attendance is required (whether before or after the attendance) an amount sufficient to meet the person's reasonable expenses (including any standby fees) in complying with the requirement.
- (6) If the attendance of an expert is required under subrule (2), the report may not be tendered under section 63, 64 or 69 of the *Evidence Act 1995* or otherwise used unless the expert attends or is dead or the court grants leave to use it.
- (7) The party using an expert's report may re-examine an expert who attends for cross-examination under a requirement under subrule (2).
- (8) This rule does not apply to proceedings on a trial with a jury.

#### 31.31 Fees for medical expert for compliance with subpoena (cf SCR Part 36, rule 13BA)

- (1) If a subpoena is served on a medical expert who is to give evidence of medical matters but is not called as a witness, the expert is, unless the court orders otherwise, entitled to be paid, in addition to any other amount payable to the expert, the amount specified in item 2 of Schedule 3.
- (2) The amount payable under subrule (1) must be paid to the expert by the issuing party within 28 days after the date for the expert's attendance.
- (3) A party that requires an expert's attendance under rule 31.29(2), but subsequently revokes it, must pay to the issuing party any amount paid by the issuing party under subrule (2), but otherwise such an amount is not recoverable by the issuing party from any other party unless the court so orders.
- (4) In this rule, *issuing party* means the party at whose request a subpoena is issued.

#### **31.32** Service of subpoena on medical expert (cf SCR Part 36, rule 13BB)

- Service of a subpoena on a medical expert may be effected, at any place at which the expert's practice is carried on, by handing it over to a person who is apparently engaged in the practice (whether as an employee or otherwise) and is apparently of or above the age of 16 years.
- (2) If a person refuses to accept a subpoena when it is handed over, the subpoena may be served by putting it down in the person's presence after he or she has been told of its nature.

- (3) If a subpoend requires a medical expert to attend court on a specified date for the purpose of giving evidence on medical matters, it must be served on the expert not later than 21 days before the date so specified unless the court orders otherwise.
- (4) The parties may not by consent abridge the time fixed by or under subrule (3).

#### 31.33 Subpoena requiring production of medical records (cf SCR Part 36, rule 13BC)

- (1) A subpoena for production may require a medical expert to produce medical records or copies of them.
- (2) A person is not required to comply with a subpoena for production referred to in subrule (1) unless the amount specified in item 3 of Schedule 3 is paid or tendered to the person at the time of service of the subpoena or a reasonable time before the date on which production is required.
- (3) Rule 33.6 (Compliance with subpoena) does not apply to a subpoena to which subrule (1) applies.
- (4) Rule 33.7 (Production otherwise than on attendance) applies to the photocopies in the same way as it applies to the records.
- (5) If, after service of a subpoena for production referred to in subrule (1), the party who requested the issue of the subpoena requires production of the original medical records without the option of producing copies of them, the party must request the issue of, and serve, another subpoena requiring production of the original medical records.
- **31.34** Supplementary reports by expert witness (cf SCR Part 36, rule 13C; DCR Part 28, rule 9C; LCR Part 23, rule 1D)
  - (1) If an expert witness provides a supplementary report to the party by whom he or she has been engaged, neither the engaging party nor any other party having the same interest as the engaging party may use—
    - (a) the supplementary report, or
    - (b) any earlier report affected by the supplementary report,

unless all of those reports have been served on all parties affected.

- (2) For the purposes of this rule, *supplementary report*, in relation to an earlier report provided by an expert witness, includes any report by the expert witness that indicates that he or she has changed his or her opinion on a material matter expressed in the earlier report.
- (3) This rule does not apply to a report prepared by a court-appointed expert.

#### 31.35 Opinion evidence by expert witnesses (cf *Federal Court Rules*, Order 34A, rule 3)

In any proceedings in which two or more parties call expert witnesses to give opinion evidence about the same issue or similar issues, or indicate to the court an intention to call expert witnesses for that purpose, the court may give any one or more of the following directions—

- (a) a direction that, at trial—
  - (i) the expert witnesses give evidence after all factual evidence relevant to the issue or issues concerned, or such evidence as may be specified by the court, has been adduced, or
  - (ii) the expert witnesses give evidence at any stage of the trial, whether before or after the plaintiff has closed his or her case, or
  - (iii) each party intending to call one or more expert witnesses close that party's case in relation to the issue or issues concerned, subject only to adducing evidence of the expert witnesses later in the trial,
- (b) a direction that, after all factual evidence relevant to the issue, or such evidence as may be specified by the court, has been adduced, each expert witness file an affidavit or statement indicating—

- (i) whether the expert witness adheres to any opinion earlier given, or
- (ii) whether, in the light of any such evidence, the expert witness wishes to modify any opinion earlier given,
- (c) a direction that the expert witnesses-
  - (i) be sworn one immediately after another (so as to be capable of making statements, and being examined and cross-examined, in accordance with paragraphs (d), (e), (f), (g) and (h)), and
  - (ii) when giving evidence, occupy a position in the courtroom (not necessarily the witness box) that is appropriate to the giving of evidence,
- (d) a direction that each expert witness give an oral exposition of his or her opinion, or opinions, on the issue or issues concerned,
- (e) a direction that each expert witness give his or her opinion about the opinion or opinions given by another expert witness,
- (f) a direction that each expert witness be cross-examined in a particular manner or sequence,
- (g) a direction that cross-examination or re-examination of the expert witnesses giving evidence in the circumstances referred to in paragraph (c) be conducted—
  - (i) by completing the cross-examination or re-examination of one expert witness before starting the crossexamination or re-examination of another, or
  - (ii) by putting to each expert witness, in turn, each issue relevant to one matter or issue at a time, until the cross-examination or re-examination of all of the expert witnesses is complete,
- (h) a direction that any expert witness giving evidence in the circumstances referred to in paragraph (c) be permitted to ask questions of any other expert witness together with whom he or she is giving evidence as so referred to,
- (i) such other directions as to the giving of evidence in the circumstances referred to in paragraph (c) as the court thinks fit.
- **31.36** Service of experts' reports in professional negligence claims (cf SCR Part 14C, rules 1 and 6; DCR Part 28, rule 9B)
  - (1) Unless the court orders otherwise, a person commencing a professional negligence claim (other than a claim against a legal practitioner) must file and serve, with the statement of claim commencing the professional negligence claim, an expert's report that includes an opinion supporting—
    - (a) the breach of duty of care, or contractual obligation, alleged against each person sued for professional negligence, and
    - (b) the general nature and extent of damage alleged (including death, injury or other loss or harm and prognosis, as the case may require), and
    - (c) the causal relationship alleged between such breach of duty or obligation and the damage alleged.
  - (2) In the case of a professional negligence claim against a legal practitioner, the court may order the plaintiff to file and serve an expert's report or experts' reports supporting the claim.
  - (3) If a party fails to comply with subrule (1) or (2), the court may by order made on the application of a party or of its own motion dismiss the whole or any part of the proceedings, as may be appropriate.
  - (4) Without limiting subrule (1) or (2), the court may, on the application of any of the parties, give directions as to the expert evidence to be adduced at trial.
  - (5) Directions under subrule (4) may be sought at any directions hearing or case management conference or by notice of motion.

- (6) Unless the court otherwise orders, no party may adduce any expert evidence at trial unless the evidence—
  - (a) has been filed and served under subrule (1) or (2), or
  - (b) has been served pursuant to directions given under subrule (4).

## Subdivision 4 Parties' single experts

#### 31.37 Selection and engagement

- (1) If an issue for an expert arises in any proceedings, the court may, at any stage of the proceedings, order that an expert be engaged jointly by the parties affected.
- (2) A parties' single expert is to be selected by agreement between the parties affected or, failing agreement, by, or in accordance with the directions of, the court.
- (3) A person may not be engaged as a parties' single expert unless he or she consents to the engagement.
- (4) If any party affected knows that a person is under consideration for engagement as a parties' single expert—
  - (a) the party affected must not, prior to the engagement, communicate with the person for the purpose of eliciting the person's opinion as to the issue or issues concerned, and
  - (b) if the party affected has previously communicated with the person for that purpose, he or she must notify the other parties affected as to the substance of those communications.

#### 31.38 Instructions to parties' single expert

- (1) The parties affected must endeavour to agree on written instructions to be provided to the parties' single expert concerning the issues arising for the expert's opinion and concerning the facts, and assumptions of fact, on which the report is to be based.
- (2) If the parties affected cannot so agree, they must seek directions from the court.

#### 31.39 Parties' single expert may apply to court for directions

- (1) The parties' single expert may apply to the court for directions to assist the expert in the performance of the expert's functions in any respect.
- (2) Any such application must be made by sending a written request for directions to the court, specifying the matter in relation to which directions are sought.
- (3) A parties' single expert who makes such an application must send a copy of the request to the parties affected.

#### 31.40 Parties' single expert's report to be sent to parties

- (1) The parties' single expert must send a signed copy of his or her report to each of the parties affected.
- (2) Each copy must be sent on the same day and must be endorsed with the date on which it is sent.

#### 31.41 Parties may seek clarification of report

- (1) Within 14 days after the parties' single expert's report is sent to the parties affected, and before the report is tendered in evidence, a party affected may, by notice in writing sent to the expert, seek clarification of any aspect of the report.
- (2) Unless the court orders otherwise, a party affected may send no more than one such notice.
- (3) Unless the court orders otherwise, the notice must be in the form of questions, no more than 10 in number.
- (4) The party sending the notice must, on the same day as it is sent to the parties' single expert, send a copy of it to each of the other parties affected.

- (5) Each notice sent under this rule must be endorsed with the date on which it is sent.
- (6) Within 28 days after the notice is sent, the parties' single expert must send a signed copy of his or her response to the notice to each of the parties affected.

#### 31.42 Tender of reports and of answers to questions

- (1) Subject to rule 31.23(3) and unless the court orders otherwise, the parties' single expert's report may be tendered in evidence by any of the parties affected.
- (2) Unless the court orders otherwise, any or all of the parties' single expert's answers in response to a request for clarification under rule 31.41 may be tendered in evidence by any of the parties affected.

#### 31.43 Cross-examination of parties' single expert

Any party affected may cross-examine a parties' single expert, and the expert must attend court for examination or cross-examination if so requested on reasonable notice by a party affected.

#### 31.44 Prohibition of other expert evidence

Except by leave of the court, a party to proceedings may not adduce evidence of any other expert on any issue arising in proceedings if a parties' single expert has been engaged under this Division in relation to that issue.

#### 31.45 Remuneration of parties' single expert

- (1) The remuneration of a parties' single expert is to be fixed by agreement between the parties affected and the expert or, failing agreement, by, or in accordance with the directions of, the court.
- (2) Subject to subrule (3), the parties affected are jointly and severally liable to a parties' single expert for his or her remuneration.
- (3) The court may direct when and by whom a parties' single expert is to be paid.
- (4) Subrules (2) and (3) do not affect the powers of the court as to costs.

# Subdivision 5 Court-appointed experts

31.46 Selection and appointment (cf SCR Part 39, rule 1; DCR Part 28A, rule 1; LCR Part 38B, rule 1)

- (1) If an issue for an expert arises in any proceedings the court may, at any stage of the proceedings-
  - (a) appoint an expert to inquire into and report on the issue, and
  - (b) authorise the expert to inquire into and report on any facts relevant to the inquiry, and
  - (c) direct the expert to make a further or supplemental report or inquiry and report, and
  - (d) give such instructions (including instructions concerning any examination, inspection, experiment or test) as the court thinks fit relating to any inquiry or report of the expert or give directions concerning the giving of such instructions.
- (2) The court may appoint as a court-appointed expert a person selected by the parties affected, a person selected by the court or a person selected in a manner directed by the court.
- (3) A person must not be appointed as a court-appointed expert unless he or she consents to the appointment.
- (4) If any party affected knows that a person is under consideration for appointment as a court-appointed expert—
  - (a) the party affected must not, prior to the appointment, communicate with the person for the purpose of eliciting the person's opinion as to the issue or issues concerned, and

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(b) if the party affected has previously communicated with the person for that purpose, he or she must notify the court as to the substance of those communications.

#### 31.47 Instructions to court-appointed expert

The court may give directions as to-

- (a) the issues to be dealt with in a report by a court-appointed expert, and
- (b) the facts, and assumptions of fact, on which the report is to be based,

including a direction that the parties affected must endeavour to agree on the instructions to be provided to the expert.

#### 31.48 Court-appointed expert may apply to court for directions

- (1) A court-appointed expert may apply to the court for directions to assist the expert in the performance of the expert's functions in any respect.
- (2) Any such application must be made by sending a written request for directions to the court, specifying the matter in relation to which directions are sought.
- (3) A court-appointed expert who makes such an application must send a copy of the request to the parties affected.
- **31.49** Court-appointed expert's report to be sent to registrar (cf SCR Part 39, rule 3; DCR Part 28A, rule 3; LCR Part 38B, rule 3)
  - (1) The court-appointed expert must send his or her report to the registrar, and a copy of the report to each party affected.
  - (2) Subject to rule 31.23(3) and unless the court orders otherwise, a report that has been received by the registrar is taken to be in evidence in any hearing concerning a matter to which it relates.
  - (3) A court-appointed expert who, after sending a report to the registrar, changes his or her opinion on a material matter must forthwith provide the registrar with a supplementary report to that effect.

#### 31.50 Parties may seek clarification of court-appointed expert's report

Any party affected may apply to the court for leave to seek clarification of any aspect of the court-appointed expert's report.

**31.51** Cross-examination of court-appointed expert (cf SCR Part 39, rule 4; DCR Part 28A, rule 4; LCR Part 38B, rule 4)

Any party affected may cross-examine a court-appointed expert, and the expert must attend court for examination or cross-examination if so requested on reasonable notice by a party affected.

#### 31.52 Prohibition of other expert evidence (cf SCR Part 39, rule 6; DCR Part 28A, rule 6; LCR Part 38B, rule 6)

Except by leave of the court, a party to proceedings may not adduce evidence of any expert on any issue arising in proceedings if a court-appointed expert has been appointed under this Division in relation to that issue.

#### 31.53 Remuneration of court-appointed expert (cf SCR Part 39, rule 5; DCR Part 28A, rule 5; LCR Part 38B, rule 5)

- (1) The remuneration of a court-appointed expert is to be fixed by agreement between the parties affected and the expert or, failing agreement, by, or in accordance with the directions of, the court.
- (2) Subject to subrule (3), the parties affected are jointly and severally liable to a court-appointed witness for his or her remuneration.
- (3) The court may direct when and by whom a court-appointed expert is to be paid.

- (4) Subrules (2) and (3) do not affect the powers of the court as to costs.
- 31.54 Assistance to court by other persons (cf SCR Part 39, rule 7; DCR Part 28A, rule 7; LCR Part 38B, rule 7)
  - (1) In any proceedings, the court may obtain the assistance of any person specially qualified to advise on any matter arising in the proceedings and may act on the adviser's opinion.
  - (2) Rule 31.53 applies to and in respect of a person referred to in subrule (1) in the same way as it applies to and in respect of a court-appointed witness.
  - (3) This rule does not apply to proceedings in the Admiralty List of the Supreme Court or to proceedings that are tried before a jury.



# Uniform Civil Procedure Rules 2005

Current version for 1 December 2021 to date (accessed 25 May 2022 at 13:36) Schedule 7

# Schedule 7 Expert witness code of conduct

(Rule 31.23)

#### 1 Application of code

This code of conduct applies to any expert witness engaged or appointed-

- (a) to provide an expert's report for use as evidence in proceedings or proposed proceedings, or
- (b) to give opinion evidence in proceedings or proposed proceedings.

#### 2 General duties to the Court

An expert witness is not an advocate for a party and has a paramount duty, overriding any duty to the party to the proceedings or other person retaining the expert witness, to assist the court impartially on matters relevant to the area of expertise of the witness.

#### 3 Content of report

Every report prepared by an expert witness for use in court must clearly state the opinion or opinions of the expert and must state, specify or provide—

- (a) the name and address of the expert, and
- (b) an acknowledgement that the expert has read this code and agrees to be bound by it, and
- (c) the qualifications of the expert to prepare the report, and
- (d) the assumptions and material facts on which each opinion expressed in the report is based (a letter of instructions may be annexed), and
- (e) the reasons for and any literature or other materials utilised in support of each such opinion, and
- (f) (if applicable) that a particular question, issue or matter falls outside the expert's field of expertise, and
- (g) any examinations, tests or other investigations on which the expert has relied, identifying the person who carried them out and that person's qualifications, and
- (h) the extent to which any opinion which the expert has expressed involves the acceptance of another person's opinion, the identification of that other person and the opinion expressed by that other person, and
- (i) a declaration that the expert has made all the inquiries which the expert believes are desirable and appropriate (save for any matters identified explicitly in the report), and that no matters of significance which the expert regards as relevant have, to the knowledge of the expert, been withheld from the court, and
- (j) any qualification of an opinion expressed in the report without which the report is or may be incomplete or inaccurate, and

- (k) whether any opinion expressed in the report is not a concluded opinion because of insufficient research or insufficient data or for any other reason, and
- (l) where the report is lengthy or complex, a brief summary of the report at the beginning of the report.

#### 4 Supplementary report following change of opinion

- (1) Where an expert witness has provided to a party (or that party's legal representative) a report for use in court, and the expert thereafter changes his or her opinion on a material matter, the expert must forthwith provide to the party (or that party's legal representative) a supplementary report which must state, specify or provide the information referred to in clause 3(a), (d), (e), (g), (h), (i), (j), (k) and (l), and if applicable, clause 3(f).
- (2) In any subsequent report (whether prepared in accordance with subclause (1) or not), the expert may refer to material contained in the earlier report without repeating it.

#### 5 Duty to comply with the court's directions

If directed to do so by the court, an expert witness must-

- (a) confer with any other expert witness, and
- (b) provide the court with a joint report specifying (as the case requires) matters agreed and matters not agreed and the reasons for the experts not agreeing, and
- (c) abide in a timely way by any direction of the court.

#### 6 Conferences of experts

Each expert witness must—

- (a) exercise his or her independent judgment in relation to every conference in which the expert participates pursuant to a direction of the court and in relation to each report thereafter provided, and must not act on any instruction or request to withhold or avoid agreement, and
- (b) endeavour to reach agreement with the other expert witness (or witnesses) on any issue in dispute between them, or failing agreement, endeavour to identify and clarify the basis of disagreement on the issues which are in dispute.

# The effect of subsidence from long wall coal mining on the ecology and water quality of streams

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A thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Applied Science (Honours) at the University of Canberra

September 2016

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# Abstract

There has been much study as to the environmental effect of pollution from mining on freshwater systems, however, another potential impact of underground mining, subsidence, has been largely ignored from the public literature. Underground long wall coal mining has the potential to impact on surface aquatic environments via subsidence, cracking of underlying rock and increased connectivity between surface and ground water. This would result in reduced surface water, changes in water chemistry (water quality) as a result of water flowing over newly cracked rocks and these changes may ultimately affect biota living in surface streams.

The aim of this study is to investigate changes in macroinvertebrate, diatoms and water quality in streams which overlie long wall coal mining and thus potentially have subsidence. The study was conducted in headwater streams on the Woronora Plateau in eastern New South Wales, Australia where the geology is predominantly sandstone. I measured water depth and water chemistry, and sampled macroinvertebrate and diatom assemblages longitudinally downstream of underground mining areas as well as in reference catchments with no mining. The reference and mined areas were chosen because they were otherwise relatively undisturbed. I attempted to use trace metals as tracers of altered exchange between surface and ground waters as a result of subsidence. These metals are virtually absent from undisturbed streams but are released when groundwater flows through newly cracked sandstone of the study area.

Li was detected in significantly higher concentrations at mined than reference sites. Sites downstream of mining also had significantly higher temperature and electrical conductivity, and concentrations of bicarbonate, barium, calcium, sodium, iron, and chloride. Edge and riffle macroinvertebrate assemblages at both mined and reference sites were dominated by chironomid dipterans. Significantly lower EPT (Ephemoptera, Plecoptera and Trichoptera) taxa richness (3.4 vs 5.5 and 2.4 vs 5.4, in edge and riffle habitat respectively), number of Trichoptera genus (7 vs 19 and 8 vs 22, in edge and riffle habitat respectively) was observed at mined sites relative to reference sites. Diatom communities also differed between mined and non-mined sites with significant reductions in abundance but a greater number of genus observed at mined sites. The differences in water quality and changes in flows following rainfall in streams overlying underground mining and those at reference sites is consistent with greater connection between the ground and surface waters. The changes in the stream macroinvertebrates observed in the current study are consistent with the one previously published study on subsistence. Water quality variables only partially explained these differences in macroinvertebrate and diatom assemblage. While it is impossible to rule out that the streams with mining under them had greater connection with the groundwater prior to mining, the areas were specifically chosen for their similarity. Consequently I conclude that the most likely explanation, is that underground mining has resulted in greater ground-surface water connection and this in turn has resulted in changes to the stream invertebrate and diatom assemblages. This study thus highlights an important poorly considered potential impact from underground long wall coal mining that should be urgently investigated further.

# **Certificate of Authorship of Thesis**

Except as specifically indicated in footnotes and quotations, I certify that I am the sole author of the thesis submitted today entitled *The effect of subsidence from long wall coal mining on the ecology and water quality of streams* in terms of the Statement of Requirements for a thesis issued by the University Research Degrees Committee.

Signature loloundoDate <math>5|9|2016

# Acknowledgements

I would like to thank the following people for assistance with this project: Martin Krogh (Office of Environment & Heritage) for alerting me to the lack of ecological studies associated with subsidence from long wall coal mining; Jerzy Jankowski (Water NSW, formerly Sydney Catchment Authority) for his ideas on the use of trace metals to potentially identify change in connectivity between stream surface water and groundwater; Alec Davie (Water NSW) for the provision of catchment and mining maps, and his invaluable help with initial site inspections and ongoing support with field collection; Henri Wong (Australian Nuclear Science and Technology Organisation) for the modification of current trace metal detection methods and analysing all trace metal samples; Ivor Growns (Department of Primary Industries) for his assistance with reference site selection, quantitative macroinvertebrate field collection techniques, statistical advice and review of diatoms; Sydney Water Monitoring Services laboratories for the loan of field water quality meters and analysing all other water quality variables; Rahana Sudhi (Sydney Water) for diatom identification; and, Colin Besley (Sydney Water) for his initial statistical advice and successive review (at all hours) of the PERMANOVA design.

I am indebted to my two supervisors; Ben Kefford (University of Canberra) and Bruce Chessman (University of New South Wales, University of Canberra) for their expertise and patience, helping to refine and review the study at all stages to what is presented here.

And finally, to my family, for allowing me to be immersed in study again. A special thanks goes to my Mum and Dad for assisting in the field and remaining focussed on the task at hand in the wonderfully biodiverse regions of the restricted access areas of Sydney's drinking water supply catchments. They fulfilled an important role in safely accompanying me into areas that I would otherwise not have been allowed to sample alone.

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# 1. Introduction

#### Long wall coal mining and surface subsidence

Long wall coal mining involves the extraction of large rectangular panels of coal by progressively shaving coal slices from an underground long wall face (IESC, 2014). Vast advances in mining machine technology and the use of temporary hydraulic supports from the 1990's have allowed for large long walls of coal to be extracted, up to 2km long and 300m wide (Holla and Barclay, 2000). At the same time pillar width has reduced, varying from 20-50 m wide, whilst maintaining worker safety. Land subsidence occurs when a sufficiently wide coal seam is mined and the overlying rock strata break and collapse into the extracted area, or goaf, by the influence of gravity and other forces. Above the goaf lies a fractured zone where rock permeability to water is increased to a lesser extent than in the collapse zone (ACARP, 2002). The fractured zone extends to a height above the seam of approximately 20 times the seam thickness, though in weaker strata this can be as high as 30 times the seam thickness (ACARP, 2002). Above this level, the surface strata will crack as a result of bending strains, with the cracks varying in size according to the level of strain, thickness of the rock stratum overlying the goaf and frequency of natural joints or planes of weakness in the strata (Holla and Barclay, 2000). Surface rock fractures associated with subsidence above a goaf have resulted in surface water to groundwater loss at several sensitive features including swamps and creek beds (Krogh, 2007; O'Kane, 2014).

# 1.1 Project aims

This project will look at the impact of surface water lost as a result of subsidence from long wall mining activity in the Woronora catchment in Southern Coalfields of New South Wales, Australia using several hypotheses:

- Firstly, that there is a loss of surface water lost beneath the creek bed and/or greater movement of water between surface and groundwater. This would occur as a result of rock fractures creating a conduit for water to travel into the groundwater. If this were to occur then water flow in stream would be expected to be lower following periods of rain and there would be greater concentrations of dissolved ions as a result of water flowing over newly cracked rock than in areas without mining.
- Secondly, that difference's in stream macroinvertebrate and diatom assemblages can be detected between streams above long wall coal mining and similar nearby streams.

• Finally, that correlating ecological health with water quality data can assist with determining long term impacts of stream surface water loss.

If the first hypothesis is correct then there should be elevated concentrations of four trace metals including molybdenum (Mo), beryllium (Be), chromium (Cr) and lithium (Li) in surface waters in the study region because they are potentially within minerals of local sandstone and have been exposed and released into the water from rock fractures.

# International demand for coal

Many countries around the world rely on the burning of non-renewable coal for the majority of their energy production. Newly industrialised countries, including China and India, are continuing to increase their internal production and import coal to meet their rapidly expanding consumption needs (MCA, 2014). More developed countries including Canada, Australia and the United Kingdom extract coal at rates that exceed their internal needs to meet this export demand. Australia has the fourth-largest share of proven coal reserves in the world and by 2030 is predicted to be the biggest coal exporter in terms of volume (MCA, 2014). Australia currently ranks fourth in national proven coal reserves (8.0%) behind the US (27.6%), Russian Federation (18.2%) and China (13.3%) (BP, 2013). Coal accounts for 29% of the total international primary energy demand, predicted to only drop marginally to 25% by 2035 (MCA, 2014). Its demand currently lags behind oil, but ahead of gas, nuclear, biomass and renewable energy demands (MCA, 2014). At present, predictions on the growth of the renewables sector and reduction in non-renewable energy sources contradict between countries and international convening bodies. The International Energy Agency (IEA) predict renewable energy to meet 25% of global energy demand by 2020, yet the Minerals Council of Australia (MCA, 2014) predict renewables to not reach a total of 5% until 2035. These conflicts make planning and management difficult, but do indicate that the demand for the coal industry may remain strong in Australia and around the world for many decades to come.

In terms of resource life, current estimates of coal resources contributing to Australia's economic prosperity are 110 years for black coal and 510 years for brown coal (lignite) (MCA, 2014). As coal resources around the world are finite, more advanced techniques to extract the maximum possible coal from underground seams are being employed, often exacerbating environmental impacts. An understanding of the cumulative effects of these impacts is a leading theme in current research to help understand the

sustainability of the coal industry. Cumulative impacts may result from complex environmental, economic, social and cultural interactions (Franks *et al* 2010) and a better understanding of management and assessment practices will enhance positive, and avoid and mitigate negative, cumulative impacts.

One of the international concerns raised in more recent decades is the impact of coal mining on headwaters and low order streams, and the implications of these impacts on water quality and ecological health, including biodiversity. These streams are increasingly being recognised for their important role in influencing the character and quality of downstream waters (Meyer *et al*, 2003). Small streams and wetlands play an important role in providing habitat for plant, animal and microbial life, and many species rely on these small water bodies at some stage in their life history (Meyer *et al*, 2003). Land-use changes in the vicinity of headwaters can alter these channels so they are less efficient at re-charging groundwaters, trapping sediments and recycling nutrients (Clarke *et al*, 2008). They are also extremely sensitive to degradation and any loss of water ultimately impacts their use as migration corridors throughout the systems (Meyer *et al*, 2007).

## Coal mining and environmental impacts

There is much literature on the environmental impacts of coal mining. Different areas experience a unique suite of issues associated with the removal of coal, predominantly related to the depth of the coal seams present and the significance of over and under lying geological formations, including aquifers. International literature focusses on the investigation of a vast array of these problems in relation to their impact on the economy and worker safety rather than the environment within particular regions (Zhang, 2005; IESC 2014). Furthermore, as each region has a unique geology, climate and differing anthropogenic activity, predictive models and environmental impact monitoring that may be conducted are often only applicable to the associated area they were conducted in (ACARP 2004, 2011, 2012).

In the Appalachian forests of the USA, the presence of shallow coal seams in mountainous regions has led to the development of the relatively recent mountaintop mining with valley fill operations (MTVF). This technique involves complete removal of the mountain tops using explosives and pushing the debris into adjacent valleys. By 2011, 1.1 million hectares of forest had been reclaimed and converted to surface mines, resulting in the loss of forests, burial of 2,000km of freshwater streams and the downstream transport of sediment and chemical pollutants (Bernhardt & Palmer, 2011). A recent study found sustained ecological damage in terms of water quality and macroinvertebrates in headwater streams draining valley fills (VF) long after reclamation was complete (Pond *et al*, 2014).

In other areas of the US, Europe, South Africa and Australia both surface open-cut and underground long-wall mining techniques are used to extract coal from varying layers of the substratum (generally below 100m). Subsidence, as a result of long wall mining, is increasingly being linked to adverse environmental impacts (Sidle et al, 2000; Krogh, 2007; Jankowski et al, 2008). Again, impacts from subsidence differ in each area and predictions of subsidence impacts are difficult to determine in Australia based on international results (IESC, 2014). One American study in Utah found subsidence during three years of extraction had no impact on stream flow derived from sub-surface flow or near-channel landslides (Sidle et al, 2000). Further analysis of the geological condition of the area determined that mudstones present in the strata swell and act like fillers upon exposure to the atmosphere and thus most impacts from subsidence are short-lived as any apparent cracks in the ground tend to close quite rapidly. In Australia, initial predictions of subsidence were calculated as they were in the UK (IESC, 2014) which proved unsuitable for the sandstone dominated upper strata of the southern coalfields (within which this study was conducted). Cracks that appeared as a result of long wall mining were predicted to close after three to four years but still remain open and exposed decades later (Krogh, 2007).

## Long wall coal mining effects on reduced water flow

Mining induced subsidence that occurs under swamps and creeks increases the interaction between surface and groundwater due to the enlargement of existing, and development of new fractures and fracture zones (Figure 1, Jankowski, 2008).



Figure 1 Evidence of long wall mining induced cracking from subsidence on the left bank (left) and right bank (right) at Waratah Rivulet (WR4), Woronora (April 2015)

A case study in the current study area by Jankowski (2008) drew two main conclusions: firstly, that a loss of surface water to subsurface flow through fractured stream beds causes downstream flow reductions during periods of low-flow; and secondly, changes in the chemical composition of the surface water along the rivulet are attributed to surface water flow into sub-surface aquifers and downstream discharge of the subsurface flow back to the surface. The effect of these two impacts on macroinvertebrate and diatom community assemblages will be further analysed within the aims of this study.

Aquatic ecosystems are highly dependent on the connectivity, quantity and quality of water in streams and groundwater (Boulton *et al*, 2014). Changes in water flow can affect water temperature and salinity, and the connectivity of stream habitats (Bunn *et al*, 2002; Krogh, 2007; Poff *et al* 2010). The effect of changes to this connectivity and flow regime, and subsequent impact on biological health has been researched with respect to river regulation (Bunn *et al*, 2002; Tharme, 2003; Arthington *et al*, 2006 & 2010).

When a permanent flowing stream loses a significant amount of baseflow water to the subsurface, as a result of subsidence induced cracking, the hydrological dynamic Honours Thesis Catherine Cunningham u3140389 changes to that of a ponded stream. In worst cases where all baseflow is lost, it changes to that of a temporary stream. Temporary streams only flow for a period of time after rainfall and are more prone to invasion by non-native species, loss of native species and loss of habitat refuge from predators (Williams, 1996). Bunn *et al* (2002) also found the flow regime is a key driver of aquatic ecosystems and that natural flow regimes assist an ecosystem in maintaining biodiversity and resilience to invasion. The number and diversity of macroinvertebrate species has been shown to be considerably lower in temporary ponds (Collinson *et al*, 1995) and that depth, permanence, flow and altitude were the main drivers of community assemblages (Williams *et al*, 2004).

The increasing number of temporary rivers and high degree of flow intermittence were investigated by Larned *et al* (2010) and are commonly attributed to climate change and water extraction in some areas and, this trend may be increasing over the next century. Long wall mining is further adding to the cumulative impact of changes to the natural water flow but the extent and impact of this and long-term impact on the aquatic ecology has not clearly been established yet.

# Long wall coal mining effects on water quality

A conceptual model of mining induced subsidence on rock fractures, water transport, river ecology and trace metal release and movement is illustrated in Figure 2.

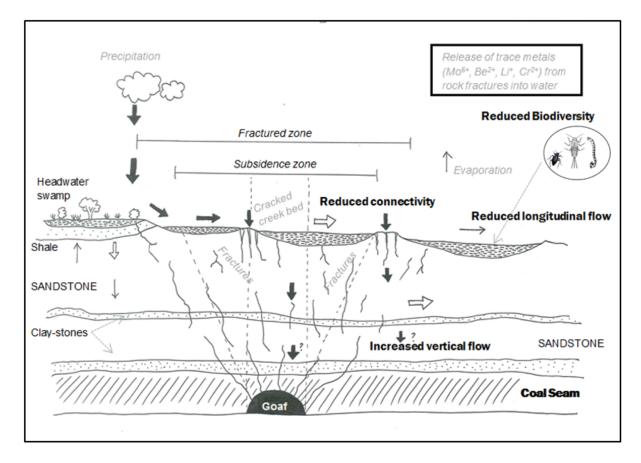


Figure 2 Conceptual model of mining induced subsidence on rock fractures, water transport, stream ecology and trace metal release

Adverse effects of long wall coal mining on water quality have been well documented and understood (Parsons Brinkerhoff, 2007). Increases in salinity, conductivity, heavy metals, iron, manganese, aluminium, sodium, calcium, barium, chloride and sulphate; lowering of pH and dissolved oxygen; and the conversion of carbonates to bicarbonate ions have been commonly reported as a result of the increased mixing of surface and groundwaters because of rock fractures (Weider et al, 1984; Tiwary, 2000; O'Kane, 2014). There are also reductions in depths of pools, gas releases and excessive microbial growth in long wall mined sites (Parsons Brinkerhoff, 2007). The impact of these pollutants, although not necessarily from coal mining, on water quality and biota is a major environmental issue worldwide (Tiwary et al, 1997; Demchak et al, 2004) with long term damage reported (Bhuiyan et al, 2010). The occurrence of iron precipitate and iron-oxidising bacteria is particularly evident in rivers where surface cracking has occurred (Jankowski, 2008). Anaerobic bacteria commonly occur in vast iron mats in Hawkesbury Sandstone areas, where seepage through the rock is often rich in iron compounds (Meyers et al, 1998) and are able to grow in water lacking dissolved oxygen. Where the bacteria grow as thick mats they reduce interstitial habitat, clog streams and reduce available food for higher order organisms (DIPNR 2003). Loss of Honours Thesis Catherine Cunningham u3140389

native plants and animals may occur directly via iron toxicity, or indirectly via smothering.

## Use of trace metals as tracers

The underlying rock in the current study area is sandstone, which consists of a mix of minerals and older rock fragments, of many types, as a result of erosion, and the total variety of rocks is usually represented in the nearby sediments (Pettijohn, 1972). The abundant minerals of sandstone belong to a few major groups and a variety of heavy minerals may be found, most in trace amounts, making up less than one percent of the total composition (Pettijohn *et al*, 2012). Sandstone minerals include tourmaline and zircon, which are resistant to mechanical and chemical attack, and amphiboles and pyroxenes, which show little resistance to decay. These minerals may be lost or modified in three ways: by weathering in the source area; transportation to the site of sedimentation; and, by the alteration of original pore type and geometry due to compaction, cementation and dissolution, otherwise known as diagenesis (Pettijohn *et al*, 2012). These modifications naturally tend to occur over long periods of time.

Chemical elements such as major ions, trace elements and metals occurring in the rock matrix are mobilised in freshly exposed rock fractures adding to the chemical composition of surface water (Jankowski, 2008). Subsidence as a result of long wall mining allows fresh rock to undergo chemical interaction with oxygenated surface waters. This can also result in discolouration associated with oxidation of the fresh sandstone and an increased chemical load in surface and ground waters in the immediate area (Pettijohn *et al*, 2012). Such impacts can also occur some distance downstream of the subsidence area and exposes rock to chemical alteration much faster than natural erosion does. Consequently it can be difficult to distinguish between natural and anthropogenic contributions to water chemistry in mined sites.

In aquatic environments, trace metals tend not to deteriorate chemically because of their low solubility and high partition coefficient within neutral pH conditions (Halim *et al*, 2012). Sedimentary dispersal of heavy minerals can be directly correlated to particular source areas undergoing tectonic evolution, or movement, thus making them incredibly useful as tracers (Pettijohn *et al*, 2012). Furthermore their use as geochemical tools for tracing groundwater sources was developed by Johannesson *et al* (1997) and proved especially useful in determining groundwater-rock interactions (Johannesson *et al*, 1997). More specifically beryllium (Be) has been used as a tracer to identify water masses in the Atlantic Ocean (Burton, 1988) and lithium (Li) has been especially useful in helping determine the fate of water through a drainage system (Zellweger, 1990).

To date no research has focussed on the use of four trace metals Mo, Be, Cr and Li but they would be considered useful as tracers for water transport associated with surface and ground water movements over fractured sandstone as a result of subsidence. Previous studies have indicated that elevated trace metal concentrations around mines in a wet climate are principally from non-anthropogenic sources and are readily attenuated by natural processes (Hewlett, *et al.*,2005). This will be further investigated by the collection of samples at six reference sites in the study area to determine natural baseline levels. In this study, their usefulness in determining how mining increases their mobilisation by creating new flow paths will be investigated.

The study is being conducted in the restricted zones within Sydney's drinking water supply because it lies within a near pristine catchment with minimal anthropogenic activity. If the study were conducted elsewhere it would be uncertain if elevated concentrations of the four elements were the result of mining or other human activities. However, in the study catchments any increase in the four selected trace metals, at extremely low detection limits ( $0.01\mu g/L$ ), would be consistent with groundwater travelling through newly cracked sandstone and re-emerging to the surface downstream.

### Long wall mining and macroinvertebrate health

Macroinvertebrates are good indicators of stream health as they are relatively easy to sample, have been extensively researched for their sensitivities and tolerances to varying types of pollution and occupy many areas of in-stream habitats due to their wide range of morphological adaptations across many taxa groups (Rosenberg *et al*, 1993; Lenat, 1993; Bonada *et al*, 2006). By analysing quantitative macroinvertebrate community assemblage across several geo-morphologically similar streams with varying spatial association to long wall coal mining, and comparing to reference streams, conclusions may be drawn on the impact long wall mining is exerting on the aquatic environment.

Despite the fact that long wall coal mining is conducted worldwide and impacts of wastewater discharge on receiving streams is well established (Decker-Hess, 1978; Wright *et al*, 2015), its quantitative effect on the aquatic environment is not well established. A review by DECC (2007) identified a clear lack of published, peer-reviewed studies of the impacts of mining-induced subsidence on riverine ecosystems.

They inferred from observed physical changes to aquatic habitat that no aquatic dependant biota can exist when fluvial systems are cracked as a result of long wall mining and all surface water is lost. When connectivity is restored, aquatic species can recolonise but, they concluded that in areas showing much longer impacts, the cracks do not naturally seal.

No quantitative studies solely linking physical subsidence from long wall mining with changes in species populations and/or community composition have been conducted globally (DECC 2007). Data that does exist as a result of mining monitoring is not publicly available, i.e. is protected by "commercial in confidence", is not collated or published in peer-reviewed journals.

International long wall coal mining research was further reviewed by Krogh (2007) and DECC (2007) and significant impacts (up to 50% loss) to headwater streams were noted in parts of West Virginia, USA (Stout 2004). Stout (2004), while a scientific document, is not published in a peer-reviewed journal, but remains one of the only study of this topic. It concluded:

- 1. Aquatic macroinvertebrate communities in reference streams were ubiquitous across the region, rich in diversity and long-lived
- 2. Long wall mining resulted in a net loss of approximately one-half of all headwater streams in the region
- 3. Streams were particularly impacted near the source and flows re-emerged downstream
- 4. Macroinvertebrate abundance appeared to recover to reference conditions in lower reaches of long wall mined streams
- 5. Macroinvertebrate diversity and longevity did not recover downstream
- 6. There was no indication that the physical, chemical or biological impacts of long wall mined streams recover naturally over time.

Stout (2004) found these effects may still be evident 12 years after mining and resulted in the complete halting of a long wall project due to its threat to water flow in West Virginia (Stout, 2004). A more recent study by Wright *et al* (2015), published during the production of this thesis, found decreased taxa richness, EPT abundance and Ephemeroptera abundance at sites impacted by subsidence from longwall coal mining.

Monitoring reports by mining companies and their contractors within the Southern Coalfields are available on the websites of main contractors engaged to complete the works but generally do not list detailed results and predominantly use rapid assessment (qualitative) family level macroinvertebrate identification data which are analysed with predictive observed/expected models (eg. <u>https://www.south32.net/getattachment/our-</u> operations/Australia/illawarra-coal/Regulatory-Document/Ilawarra-Coal,-

Dendrobium/Dendrobium-Area-3B-Longwall-11-End-of-Panel-Report.pdf). Further research and reports by the Australian Coal and Research Projects (ACARP) are not publicly available and have generally focused on increasing extraction yields, while maintaining safety to workers and protection of groundwater systems. Reports can be purchased from ACARP with varying price depending if purchaser is associated with a research institution or not.

# Long wall mining and diatoms

Diatoms are good indicators of stream health, especially in understanding the ecological effects of flow regulation on streams (Growns & Growns, 2001). They are useful because of their sensitivity to specific environmental disturbances or pollution and they have been shown to correlate more strongly with changes in water quality measurables than changes to physical habitat (Walsh *et al.*, 2001). No data exists on the effect of subsidence from long wall mining on diatom assemblages, rather the literature has focused on diatom response to changes in water quality associated with varying levels of river regulation. Growns & Growns (2001) found that periphytic diatoms differed downstream of flow regulated rivers when compared to upstream sites and sites on unregulated rivers. Recovery downstream of regulation has been shown to occur in diatom assemblages (Cortez *et al.*, 2012), with increased recovery corresponding to decreased regulation. Genus identification was deemed a suitable taxonomic level due to the limited number of species belonging within each genus and similarity of the data when analysed at both species and genus level in previous studies (Growns, 1999).

The potential for increased surface water and groundwater connectivity in streams, as a result of subsidence, to alter hydrology and impact on diatom community assemblages were investigated in this study. This was to help further understand whether changes in water quality or flow regimes were the main drivers of any change which was made possible in the study area because of the lack of additional anthropogenic influence other than the long wall mining.

# 1.2 Project background

The idea for this project arose after an initial enquiry into researching the possible impact of coal seam gas extraction on stream water quality in the restricted areas of Sydney's drinking water catchment, now managed by WaterNSW (formerly Sydney Catchment Authority, SCA). Further investigation revealed that staff at both

Honours Thesis

WaterNSW and Office of Environment & Heritage (OEH) indicated their concern at the complete lack of quantitative studies on the impact of long wall coal mining on aquatic ecosystems. They were of the concern that long term impacts of long wall coal mining on the aquatic health were at present far greater than any yet posed by coal seam gas extraction.

Further review of the literature did indeed reveal a depauperate understanding of quantitative impacts of long wall coal mining and subsequent subsidence on aquatic ecosystems including on macroinvertebrate and diatom community assemblages. Internationally, the impacts of subsidence vary according to climate, geology and mine depth. This thesis studied impact of long wall coal mining occurring at depths of 400 to 800m underground on streams above the Hawkesbury sandstone strata that may have been cracked as a result of subsidence in the Southern Coalfields of NSW.

# 1.3 Study Area

This study took place in the Woronora River catchment and adjoining O'Hares and Cataract River catchments. These catchments are located approximately 50km south of Sydney, New South Wales, Australia (Figure 3). This area was targeted for this research within the restricted access zone of Sydney's drinking water supply catchment. Having sites in this zone ensured that other than long wall coal mining, the area had extremely minimal local anthropogenic activities. While effects due to regional and global anthropogenic activities (e.g. climate change, vegetation clearing, polluted stormwater runoff, transport of toxicants) may have affected reference and test sites equally.

I was granted access into these special areas by Water NSW under approval no. D2015/50283 and D2016/38818.

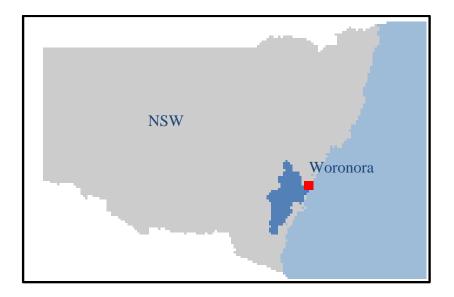


Figure 3 Woronora catchment location south of Sydney, NSW (source: Alec Davie, Water NSW)

Four streams were selected for this study (Figure 5). Two streams potentially impacted by longwall coal mining with a combination of decade old, recent and current mining:

- 1. Waratah Rivulet 10-14 years since mining and recent (2 years) mining activity
- 2. Eastern Tributary 10-14 years since mining, recent and current mining activity

And, two reference streams nearby in which no mining had occurred or was proposed to occur:

- 3. O'Hares Creek
- 4. Loddon River

O'Hares Creek lies within the Dharawal Nature Reserve in the upstream reaches of the Georges River catchment (Figure 5). This catchment lies approximately 4km to the west of the Woronora River catchment and has similar climate, geology and altitude.

The Loddon River drains to the Cataract River, approximately 8km south-west of the Woronora River catchment (Figure 5), also within similar climate, geology and altitude ranges.

On each river, three sites were selected approximately 0.5 to 3.5km apart longitudinally along the streams. On each of the two rivers with long wall mining, all three sites were located downstream of mining activity. Sites on the two non-mined reference rivers were similarly located and these rivers have been included as a reference to compare with the mined streams.



Figure 4 Non-mined O'Hares Creek (left) and mined Waratah Rivulet (right)

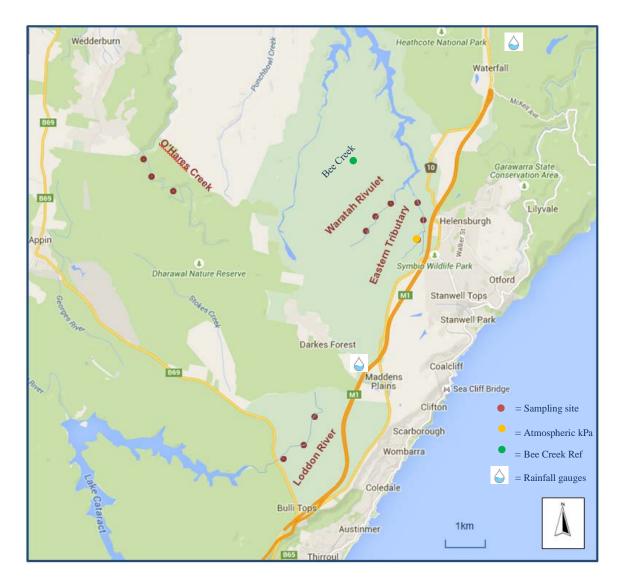


Figure 5 Woronora catchment including Waratah Rivulet and location of reference sampling sites (source: <u>www.Nearmap.com</u>)

Creek	Site Code	Latitude	Longitude	Distance from source
				(km)
Eastern	WR1	-34.196930	150.955541	2.540
Tributary	WR2	-34.185483	150.961761	4.109
(mined)	WR3	-34.183308	150.961740	4.409
	WR4	-34.197197	150.933523	3.169
Waratah Rivulet (mined)	WR5	-34.189370	150.942686	4.558
	WR6	-34.185603	150.943998	5.250
0.11.1	OH1	-34.238956	150.918408	3.832
O'Hares Creek (non-mined)	OH2	-34.234506	150.906461	7.422
	ОНЗ	-34.230061	150.892574	10.063
	LR1	-34.281745	150.916100	2.834
Loddon River (non-mined)	LR2	-34.284690	150.913702	3.649
	LR3	-34.285269	150.910076	4.354

Table 1 Site codes, potential impact, latitude and longitude (obtained from a GPS) and distance from source for all sites

# 1.4 Licenses

### NPWS

A National Parks and Wildlife Service license was required for macroinvertebrate sampling in areas operated by the NSW Office of Environment and Heritage. O'Hares Creek partially falls within this jurisdiction and as such I have included my NPWS licence below.

## License No: SL100638, Catherine Cunningham

### **NSW Fisheries**

A NSW Fisheries license was obtained for the collection of any macroinvertebrates from water courses in NSW (*License No: F93/527-5.0 & OUT15/11731, Catherine Cunningham*). A condition of this license is the assurance that '*no endangered animals will be collected, and if they inherently are sampled in a collection net, that they are safely returned to their habitat as soon as possible*'.

Animals listened on the NSW Threatened Species List likely to occur in study area include:

Sydney Hawk Dragonfly: *Austrocordulia leonardi* Adams Emerald Dragonfly: *Archaeophya adamsi* Spiny Crayfish: *Euasticus dharawalus* 

Animal fact sheets (<u>www.DPI.nsw.gov.au/primefacts</u>) were carried into the field as visual guide for easy identification.

## **Animal Ethics**

An animal ethics approval was not required for this project as macroinvertebrates are exempt from the NSW list of animals requiring ethics approval.

## 2. Materials and Methods

## 2.1 Rainfall

So as to investigate the potential for rain to influence water quality results, I sourced daily rainfall data (mm at 9am for the preceding 24 hours) from April 2015 to February 2016 from the Bureau of Meteorology (BOM) rain gauge at Darkes Forest (gauge no. 68024) and Sydney Water (SW) HYSTRA rain gauge at Yarrawarrah (gauge no. 566056) (Figure 8). These gauges were selected due to their proximity (less than 10km) and similar rainfall patterns to the Woronora catchment. Monthly averages from these gauges for the last 20 years (1995-2015) were also compiled and compared with rainfall over the study period.

## 2.2 Water Level

To enable a better understanding of the upstream and downstream connectivity within each creek, the potential of cease to flow events occurring, and whether this varied between creeks and non-mined and mined sites (non-mined and mined), I used continuously monitored water level data.

To determine this, I used data loggers (HOBO <sup>®</sup> U20L-4) placed inside plastic tubes within upstream and downstream locations of creeks (Figure 5) to collect temperature (°C) and pressure (kPa) data every 30 minutes during the course of the project. An additional data logger was placed at -34.193616, 150.949885 (Figure 5) to measure pressure of the air on top of the water. The Barometric Compensation Assistant (BCA) tool within HOBOware PRO (V2.2 <u>http://www.onsetcomp.com/hoboware-free-</u>download) was used to calculate the water depth based on the following equation:

 $p = h \rho g$ 

where:

p = pressure in fluid (kPa)

h = height of fluid column, or depth in the fluid at which the pressure is measured (m)

 $\rho = \underline{density \ of \ liquid}$  (freshwater is 1000 kg/m<sup>3</sup>)

 $g = \frac{acceleration \ of \ gravity}{(9.81 \ m/s^2)}$ 

Further flow and discharge data were available from Water NSW and Peabody Energy but was deemed unsuitable for this project due to single gauge placement, on only Loddon River and Waratah Rivulet, and location far downstream of study area.

Deployment of four HOBO<sup>®</sup> U20L-4 water level loggers at the upstream and downstream sites on Loddon River and Eastern Tributary sites was not successful. Third party removal of the downstream (WR3) water level logger on the Eastern Tributary, within the first two weeks of deployment, and slow approval from catchment managers to place loggers in the Loddon River, along with numerous restricted access periods due to bushfire and rainfall, resulted in failed deployment at these sites.

Additionally, the first attempt to download data from the remaining in-stream and atmospheric loggers from O'Hares Creek and Waratah Rivulet, after 6 months deployment, also failed due to a low battery error. All battery issues had to be referred to, and subsequent battery replacement, had to be conducted by the manufacturer to avoid warranty issues. The five faulty loggers had to be sent to the Australian distributer in Adelaide, battery replacement had to occur in the United States of America (US) and no replacement HOBO<sup>®</sup> U20L-4 water level loggers were available for deployment. As a result, considerable delay occurred, and data loggers were only redeployed for a 2 month period at upstream and downstream sites on O'Hares Creek and Waratah Rivulet, and the additional logger to compensate for barometric pressure.

## 2.3 Sampling Events

Physicochemical properties of water (water quality) and macroinvertebrates were sampled at all sites on two occasions in August and November, 2015. August sampling proceeded a slight rainfall event, defined as <20 mm in the last 5 days and November proceeded a moderate rainfall event, defined as >20 mm to 70 mm in the last 5 days and as soon as possible once able to gain access to the catchments, which have restricted access following rainfall. I was aiming to collect samples during both wet and dry periods because I hypothesised that the ecological impact of any loss of surface water from long wall mining would be greater during dry periods than during wet periods. Unfortunately, due to restrictions placed on authorised access into the catchment and lack of suitable extended dry periods I was able to sample only following slight and moderate rainfall events, in the winter and spring seasons of 2015. From herein, the two sampling events are referred to as winter (S1) and spring (S2). Algae (diatom) samples were collected only once during the first sampling event in winter 2015 due to time and cost restraints.

## 2.4 Sampling Design

The overall design of the project for statistical comparison was consistent for all chemical and biological variables (Figure 6), except diatoms which were only analysed using the first two levels due to their single sampling event in winter.

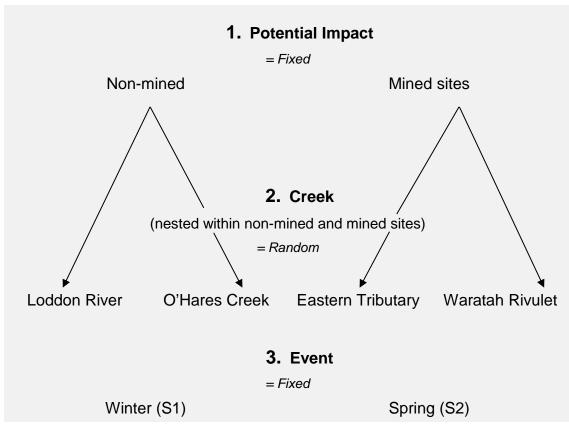


Figure 6 Overall project design and nesting of factors for statistical analysis. See also Figure 5 for a map and Table 1 for site locations. Replicates = water quality x 2, macroinvertebrates x 4 (per habitat) and diatoms x 3.

## 2.5 Water Quality

### Sampling Methodology

Duplicate water samples were collected and subsequently analysed at ANSTO or Sydney Water laboratories (Table 2), and other variables were measured directly in the field (Table 3). Concentrations of Cr, Mo, Li and Be were used as tracers to determine if groundwater to surface water interactions had increased through newly cracked sandstone. Extremely low detection limits of  $0.01\mu g/L$  were required. To avoid contamination aseptic techniques were used (SW,  $2015^1$ ) and sample bottles without preservative were rinsed before filling.

#### Table 2 Water chemistry variables, detection limits and analysis method

Analyte	Detection limit	Method, laboratory, bottle
Part I - Trace metals	(USEPA, 1998)	
Chromium (Cr)	0.01 µg/L	Modified method ANSTO VI 2809, Super acid washed 250ml
Molybdenum (Mo)	0.01 µg/L	Modified method ANSTO VI 2809, Super acid washed 250ml
Lithium (Li)	0.01 µg/L	Modified method ANSTO VI 2809, Super acid washed 250ml
Beryllium (Be)	0.01 µg/L	Modified method ANSTO VI 2809, Super acid washed 250ml
Part II – Major ions (/	APHA, 1998)	
Bicarbonate	0.1 mg/L	WC01TIM, SW, 500 mL PET no preservation
Sulphate	0.1 mg/L	WC0221, SW, 500 mL PET no preservation
Calcium	0.01 mg/L	TM50ML, SW, 250 ml polyethylene pH < 2 with nitric acid
Iron	0.02 mg/L	TM50ML, SW, 250 ml polyethylene pH < 2 with nitric acid
Manganese	0.001 mg/L	TM50ML, SW, 250 ml polyethylene pH < 2 with nitric acid
Aluminium	0.01 mg/L	TM50ML, SW, 250 ml polyethylene pH < 2 with nitric acid
Sodium	0.05 mg/L	TM50ML, SW, 250 ml polyethylene pH < 2 with nitric acid
Barium	0.005 mg/L	TM50ML, SW, 250 ml polyethylene pH < 2 with nitric acid
Chloride	0.1 mg/L	WC0221, SW, Plastic 500 ml no preservation
Carbonates	0.1 mg/L	WC01TIM, SW, Plastic 500 ml no preservation

### Table 3 Field water chemistry variables and instrumentation

Analyte	Instrument
Dissolved Oxygen (%)	WTW Multilinear Universal Meter
Dissolved Oxygen (mg/L)	WTW Multilinear Universal Meter
Conductivity (µS/cm @25°C)	WTW Multilinear Universal Meter
pH (pH units)	WTW Multilinear Universal Meter
Temperature (°C)	WTW Multilinear Universal Meter
Turbidity (NTU)	HACH Turbidimeter

## Laboratory Analysis

## Trace metals

Trace samples (Table 2, Part I) were analysed using a Varians 820-MS inductively coupled plasma mass spectrophotometer (ICP-MS) at the Australian Nuclear Science

and Technology Organisation (ANSTO) laboratories at Lucas Heights, NSW. A modification to their existing standard method, VI 2809 (ANSTO, 2015), was used to determine trace concentrations of Cr, Mo, Li and Be. This modification involved dosing individual samples for each element with a differing ratio of the international standard (IS) as shown in Figure 7b.

The IS used for each element for correction of instrument drift and sample matrix were selected because they were absent from the samples analysed but were very close to each target elements mass. They were:

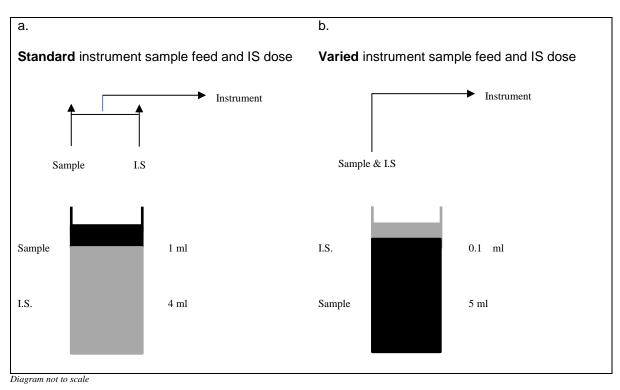
- Isotopic  ${}^{6}\delta$  Lithium for  ${}^{7}\delta$  Li and Be
- Isotopic <sup>103</sup>δ Rhodium for Cr and Mo

The average of three readings was reported for each duplicate sample at each site and sampling occasion. Recovery spikes (using known concentrations of Cr, Mo, Li and Be) were reported separately as concentrations and percentages, respectively. Reliable results within 85%-115% recovery were regarded as reliable. Results outside this range are reported but not considered further in this study.

Care was taken to avoid cross contamination during laboratory analysis to achieve the highest quality analysis with reproducible results. Gloves were worn at all times and, all tubing and cones in the ICP-MS were replaced between samples to avoid any contamination. The ICP-MS machine was also set to high sensitivity mode and tuned between each sample to gain the maximum sensitivity.

As quality assurance, a field blank, filled with ultrapure milli-Q water in the field each season, was analysed to help determine detection limits and sources of possible contaminants from the atmosphere or the sampling process.

Variation to the standard method, VI 2809 (ANSTO, 2015), involved dosing individual samples for each element with a differing ratio of the international standard (IS) as shown in Figure 7b. This modification was possible because the environmental samples were from clean water in a near pristine catchment (Figure 3).



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Figure 7 Existing ANSTO method VI 2809 (a) and variation (b) to achieve new detection limits

## Other variables

All other samples (Table 2, Part II) were delivered to the Sydney Water Analytical Services laboratories within 24 hours of sampling and processed with current methods (APHA, 1998).

# 2.6 Macroinvertebrates

## **Macroinvertebrate Sampling**

Semi-quantitative macroinvertebrate samples were collected to indicate instream aquatic health. Four replicates from both edge and riffle habitats were collected using a 35 by 35cm square opening hand-held dip net with 250µm mesh. Boothroyd & Stark (2000) suggest that between three and six replicates from each habitat should adequately characterise macroinvertebrate communities.

Edge habitats are defined as areas with little or no current. The sampling net was swept rapidly four times within a 1.0m<sup>2</sup> quadrat section of edge from open water towards the stream bank based on method developed by Growns (in draft , 2015). The substratum was then disturbed by foot and a further two sweeps collected. During the process, deposits of silt and detritus on the stream bottom were stirred up so that benthic animals were suspended and caught in the net.

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Riffles were considered a habitat of value with unique fauna to help distinguish community assemblage differences among sites. Riffles at all sites were near 100% bedrock areas with fast flowing water but was usually limited in size and availability. The net was held downstream of four bedrock 1.0 x 0.5m quadrats, clearly marked, which was cleared of all macroinvertebrates and debris using a gloved hand rubbed over the rock surface. Starting at the downstream edge, the net was slowly moved upstream after each small area was cleared, taking special care to clear any crevices. This sampling was modified from Surber (Waters & Knapp, 1961) due to the inability to wedge a Surber sampler into the bedrock substrate.

Macroinvertebrate samples were then tipped into a 250µm mesh sieve, washed to remove fine silt and transferred to 500 ml plastic labelled specimen jars containing 70% un-denatured ethanol as preservative. Clean nets were used for each site and thoroughly washed between field trips to prevent cross-contamination of samples.

### **Macroinvertebrate Processing and Analysis**

Macroinvertebrates were identified and enumerated to the genus level with the aid of Leica M10 stereo and Leica DC1000 compound microscopes. They were identified as per latest Australian published keys (Hawking & MDFRC, 2013) and Sydney Water inhouse keys (SW, 2015<sup>2</sup>), reference specimens and photographs.

Biotic indices calculated on macroinvertebrate results include:

- Total abundance i.e. the total number of individual macroinvertebrates
- Taxa (genus) richness Indeterminate and small immature specimens were removed from data set and life-stages were combined before analysis. Except, where an immature/small specimen was recorded for a family not already represented at a site it was retained in dataset at unique family level.
- EPT (Ephemoptera, Plecoptera and Trichoptera) taxa abundance and richness -. Again, immature and indeterminate specimens were removed from the dataset before analysis.
- SIGNAL-SG score (Sydney, genus) (Chessman and Besley, 2007) to determine whether stream health, as indicated by sensitivity to increasing pollution (1 = tolerant, 10 = sensitive). An abundance weighted calculation (square root) was performed on raw data before analysis. Results were compared to the stream health range in Table 4.

#### Table 4 Signal SG score range

SG score range	Interpretation
>6.5	Indicative of clean water
5.2 - 6.5	Slightly impacted stream health
3.8 - 5.2	Moderately impacted stream health
<3.8	Severely impacted stream health

In addition, abundance data were amalgamated to overall Order (taxa) and functional feeding group (FFG) percent composition. Individual Orders and functional feeding groups that contributed  $\leq 1\%$  of the total abundance across all samples were removed when graphing results.

## 2.7 Diatoms

### **Diatom Sampling**

Diatom sampling was conducted as per Growns and Growns (2001). Three diatom replicate samples were collected from three separate submerged rocks (at least 20cm below surface) within pools. These triplicate samples were collected from upper, mid and lower reaches at each site.

Each diatom sample consisted of scraping from a 2.5 x 2.5cm quadrat on the upper exposed side of the rock using a flat, sharpened stick and placed in a jar containing 20ml water and 7 drops of Lugol's iodine.

### **Diatom Processing and Analysis**

Diatom samples were vortexed for two minutes to break up any clumps and then diluted to 100ml before analysis. Eight 0.5ml<sup>3</sup> volume Lund cell transects were performed from each sample using both 10x and 40x magnification under a compound microscope as per methods (MA51) outlined in Sydney Water (2015<sup>3</sup>). Within each transect only live frustules were identified and enumerated to genus level where possible and dilution factors were applied to the results to calculate whole sample counts.

Indeterminate and immature genera were removed from data set and life-stage combined before analysis.

### 2.8 Statistical Analysis

Individual water quality, macroinvertebrate and diatom (hereafter response variables) were averaged (with +/- 2 standard deviation from the mean) and graphed for each creek by:

- Non-mined and mined sites nested within season, and
- Position on stream (upstream, midstream and downstream) nested within nonmined and mined sites.

Test for statistically significant differences were performed using PERMANOVA (Clarke & Warwick, 2001) as per the nested design in Figure 6. Monte Carlo tests for significance (P(MC) < 0.05) were added to each PERMANOVA due to the reduced size of the data set when >100 permutations could not be run. It also accounted for asymmetry in the design. Diatom replicates were combined for each site and analysed among non-mined and mined sites and stream position only, as sampling occurred only once in winter.

All water quality variables were analysed with principal components analysis (PCA) using Euclidean distance to geometrically best fit combined water quality samples in a 2D plane. A draftsman's plot (PRIMER V6) was first developed to check for highly collinear variables and only one variable was kept where two variables expressed a mutual correlation in excess of 0.95.

Two sample t-Test (two tailed) assuming unequal variance from the mean were used on macroinvertebrate order abundance data only.

Multivariate analysis of weighted individual macroinvertebrate and diatom replicates was performed using PRIMER V6 on square root transformed abundances. Cluster analysis were performed on macroinvertebrate and diatom samples and, further analysed with non-metric multi-dimensional scaling (nMDS) using Bray-Curtis similarity, including visual presentation of 2D and 3D graphics. The water quality PCA was aligned with macroinvertebrate and diatoms nMDS for a BEST (BIOENV) (Clarke & Warwick, 2001) fit correlation.

SIMPER analysis (Clarke and Warwick, 2001) was also performed on biological variables (macroinvertebrates and diatoms) to look at the similarity percentage breakdown by genera.

# 3. Results

# 3.1 Rainfall

The first sampling event followed the month of July where no rain event was greater than 20mm (Figure 8), whereas the second event followed a single rainfall event of greater than 60mm (Figure 8). Both sampling events were both preceded by a period of slightly below-average rainfall (Figure 9).

Three months prior to the first sampling event in April, rainfall was three times higher than its historical average from the last twenty years (436mm and 134mm, Figure 9).

Due to the above-average rainfall experienced in January and early February 2016 (278mm, Appendix 1), further sampling events were halted due to the time constraints around final thesis delivery.

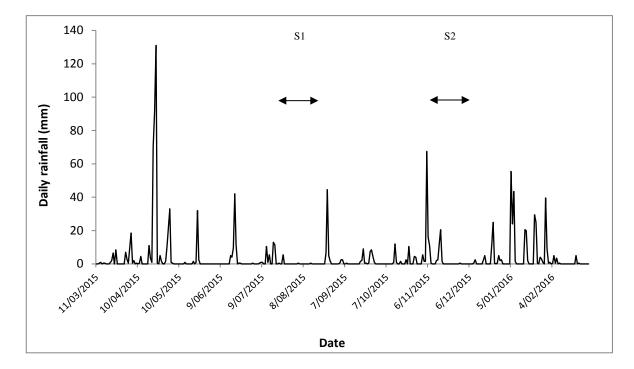


Figure 8 Daily rainfall in mm (Darkes Forest #68024 and Yarrawarrah #566056)

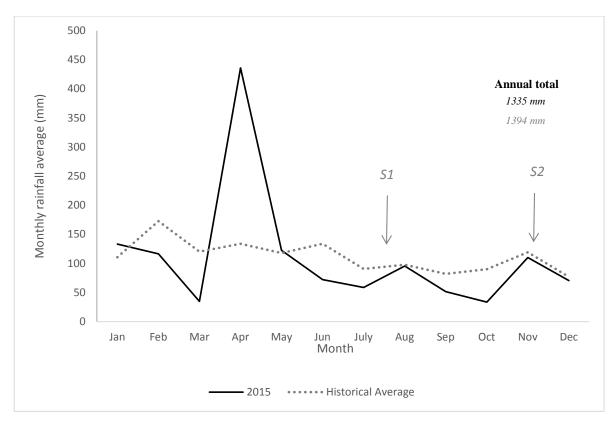


Figure 9 Average monthly rainfall for 2015 and 20 year historical average in mm (Darkes Forest #68024 and Yarrawarrah #566056)

### 3.2 Data Loggers

Pressure (kPa) and temperature (°C) results downloaded from data loggers (Figure 10a, b and Figure 12a, b) indicate a consistently higher daily water temperature fluctuation at the mined upstream Waratah Rivulet site (Figure 12c) compared to other sites. These fluctuations reduced during and for a small period after rainfall, similar to those observed consistently at other sites, including non-mined upstream and downstream sites on O'Hares Creek (Figure 10a, b) and downstream on Waratah Rivulet (Figure 12d).

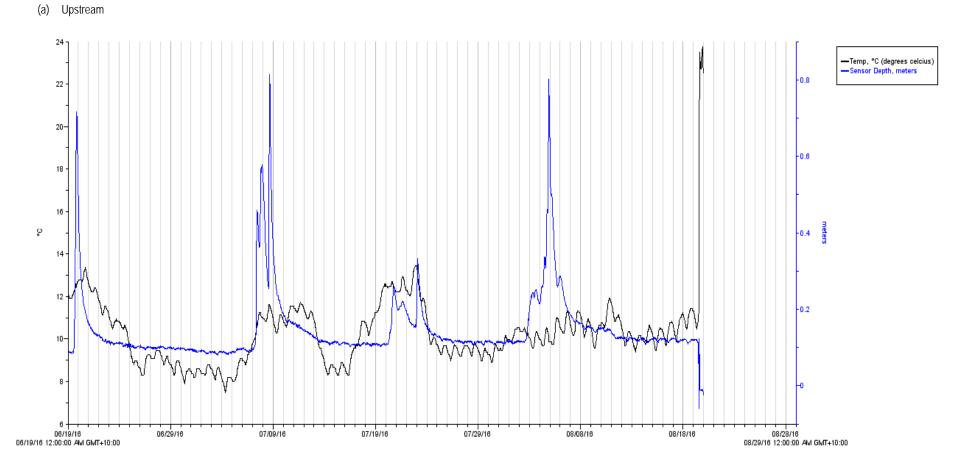


Figure 10a Temperature and water level results from (a) upstream sites on non-mined O'Hares Creek



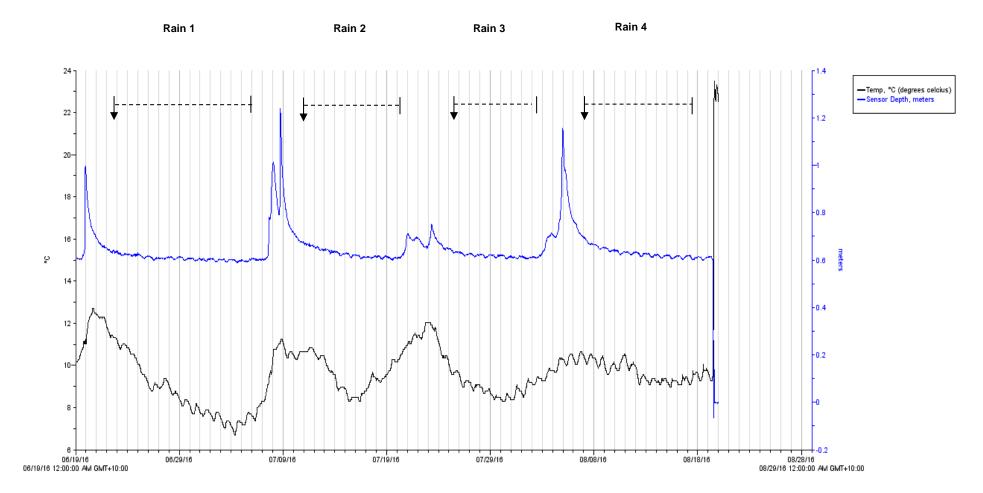


Figure 10b Temperature and water level results from (b) downstream sites on non-mined O'Hares Creek

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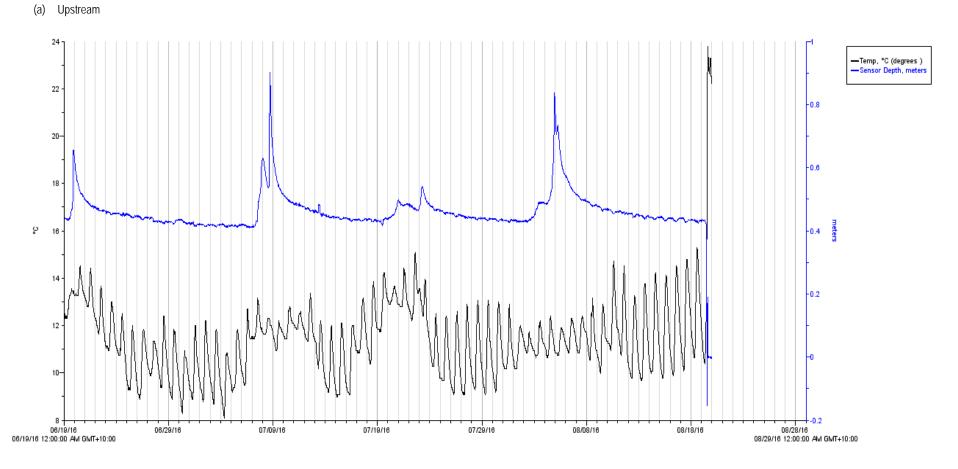


Figure 11 Temperature and water level results from (a) upstream sites on mined Waratah Rivulet

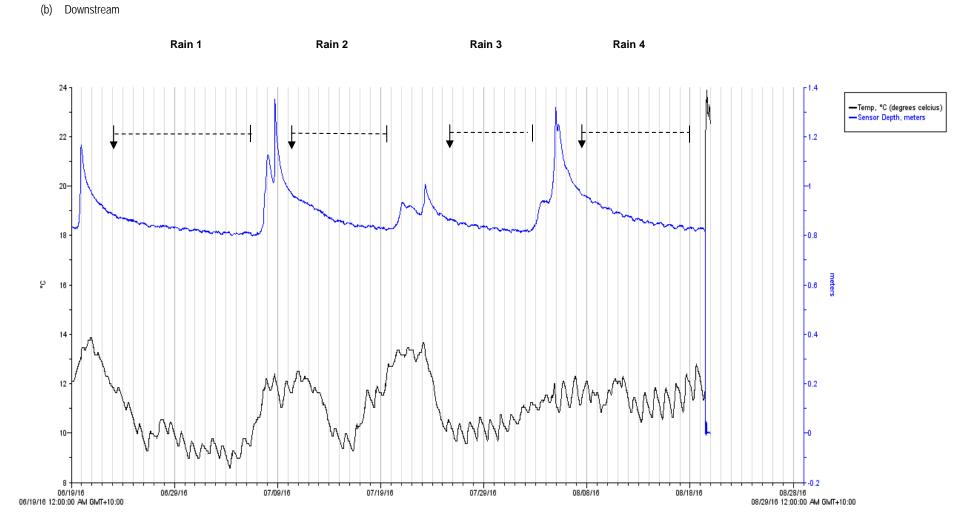


Figure 12 Temperature and water level results from (b) downstream sites on mined Waratah Rivulet

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Water level results after four rain events (Rain 1,2,3 and 4) was extracted three days after each event until one day before the next rain event (black arrows and dashed line, Figure 10b and Figure 12b) to compare water level recovery after rain between nonmined and mined sites as shown in Figure 13a,b,c and d. The non-mined O'Hares Creek sites indicate more constant water level and gradual recovery after rain between upstream and downstream sites (Figure 13a and b). Whereas, the mined Waratah Rivulet, observed a greater water level reduction between upstream and downstream sites as indicated by the steeper trend-lines downstream (Figure 13c and d).

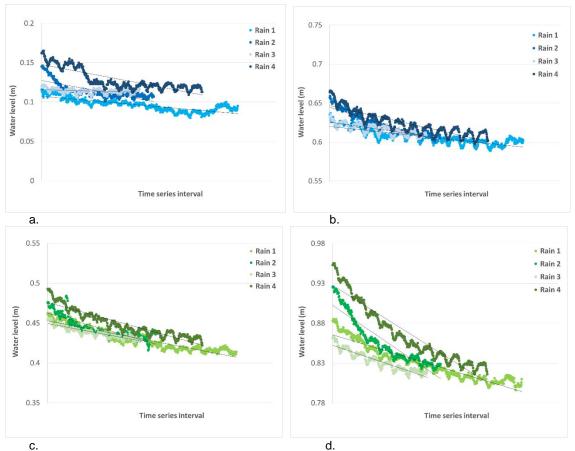


Figure 13 Water level in metres (with overlying trend-lines) three days after rain ceases for four different events at: (a) upstream and (b) downstream of non-mined O'Hares Creek; and, (c) upstream and (d) downstream of mined Waratah Rivulet

These results were consistent with aerial photos on 15<sup>th</sup> December 2015, after several weeks of no rain, supporting evidence of the non-mined O'Hares Creek retaining stream connectivity but the mined Waratah Rivulet indicating increased signs of dewatering and potential habitat fragmentation (Figure 14).



Figure 14 Aerial photos of non-mined O'Hares Creek (left) and mined Waratah Rivulet (right) in December 2015 (www.Nearmap.com)

## 3.3 Water quality

### **Field Variables**

Bee Creek was not considered a suitable reference site due to its pH, 4.6 to 5.0, being well outside that at the other sites (Figure 15) and typical values in Australian freshwaters of 6.5-8.0 (ANZECC, 2000) and this site would likely have different biota to the other sites regardless of mining. Bee Creek pH is likely due to 'humic' water from its close proximity to headland swamps and associated high organic content. For this reason Bee Creek results were not considered further in this study.

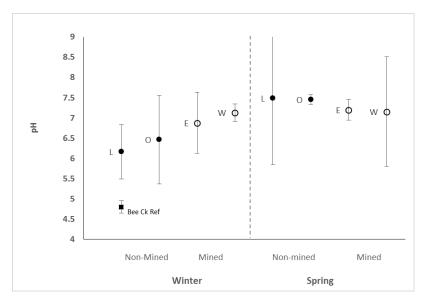


Figure 15 Mean pH of creeks by potential impact and season (error +/- 2 standard deviation from the mean). L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.

Combined field water quality PERMANOVA results from all other sites (Table 5, Appendix 2) indicate significant differences between non-mined and mined sites

(P(MC)=0.0115), between creeks nested within non-mined and mined sites (P=0.0288) and between season (P=0.0307).

Source	df	Pseudo-F	P(perm)	P(MC)
Mined/Non-mined	1	8.4231		0.0115
Creek (Mined/Non-mined)	2	2.2965	0.0288	
Season	1	9.7754	0.0307	
Creek (Mined/Non-mined) x Season				

Table 5 PERMANOVA table of results for all field water quality variables

Individual field variable PERMANOVA's further analysed to investigate differences are listed in Table 6. pH (Figure 15) and dissolved oxygen (Figure 16a) differed between seasons (P=0.0306, and 0.0351), temperature and conductivity (Figure 16b and c) were both significantly higher at mined than non-mined sites (P(MC)=0.0249 and P(MC)=0.0275) and, conductivity and turbidity (Figure 16d) significantly differed among creeks within non-mined and mined sites (P=0.0439 and P=0.0011).

Table 6 PERMANOVA table of results for selected field water quality variables

Analyte	Source	df	Pseudo-F	P(perm)	P(MC)
DO	Season	1	24.392	0.0351	
Temp	Mined/Non-mined	1	37.096		0.0249
Cond	Mined/Non-mined	1	34.226		0.0275
Cond	Creek (Mined/Non-mined)	1	3.8279	0.0439	
рН	Season	1	47.89	0.0306	
Turb	Creek (Mined/Non-mined)	2	9.2964	0.0011	

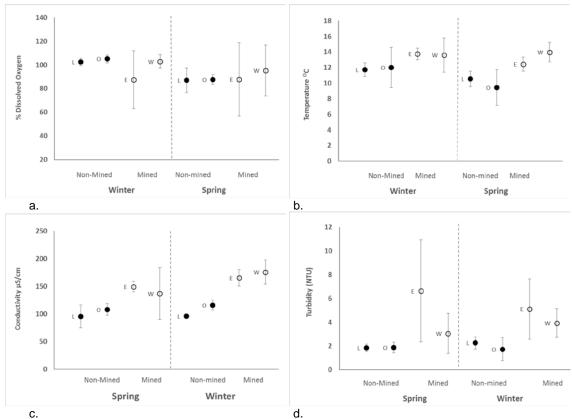


Figure 16 Mean (a) dissolved oxygen, (b) water temperature, (c) electrical conductivity and (d) turbidity of creeks by potential impact and season (error +/- 2 standard deviation from the mean). L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.

### **Trace Metals**

Field blanks for chromium (Table 7) did not return satisfactory results (>0.1 $\mu$ g/L), hence laboratory results are not useful for indicating increased groundwater and surface water connectivity. All other elements including beryllium, lithium and molybdenum, returned acceptable field blanks (<0.01 $\mu$ g/L), indicating no contamination or processing issues.

	N		Trace metal µg/L									
Creek	Non- mined/	Site	E	Be		r	L	i	i Mo			
	Mined		S1	S2	S1	S2	S1	S2	S1	S2		
Field blank	-		<0.01	<0.01	0.1885	0.225	<0.01	<0.01	<0.01	<0.01		
	Non- mined	OH1	0.0185	0.0265	0.1595	0.5605	0.1795	0.261	0.01	0.012		
O'Hares Creek	Non- mined	OH2	0.017	0.0245	0.1445	0.546	0.176	0.2465	0.01	0.012		
	Non- mined	ОНЗ	0.015	0.023	0.1315	0.583	0.1705	0.2285	0.01	0.0155		
	Non- mined	LR1	0.0235	0.031	0.1655	0.6055	0.246	0.296	0.01	0.0175		
Loddon River	Non- mined	LR2	0.029	0.0465	0.136	0.6625	0.252	0.3215	0.01	0.017		
	Non- mined	LR3	0.022	0.0565	0.138	0.6245	0.2575	0.377	0.01	0.015		
	Mined	WR1	0.019	0.0245	0.124	0.4115	0.6035	0.655	0.01	0.0175		
Eastern Tributary	Mined	WR2	0.01	0.0845	0.093	0.922	0.577	0.3805	0.01	0.026		
	Mined	WR3	0.0125	0.0805	0.146	0.8685	0.4935	0.361	0.0135	0.024		
	Mined	WR4	0.0165	0.065	0.0925	0.6755	0.647	0.292	0.0155	0.013		
Waratah Rivulet	Mined	WR5	0.015	0.065	0.089	0.6865	0.6165	0.343	0.023	0.0205		
	Mined	WR6	0.016	0.017	0.1015	0.4355	0.6715	0.7015	0.022	0.052		
Recovery with 0.2 µg/L spike			114.50 %	142.00%	109.50%	60.00%	Not spiked	95.50%	97.75%	125.50%		
Quantificat ion limit			0.01	0.01	0.05	0.1	0.05	0.01	0.01	0.01		

Table 7 Estimated trace metal concentrations

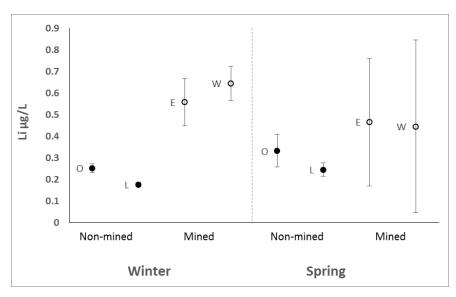
Recovery results from the  $0.2\mu$ g/L spike for trace metals (Table 7) produced varying results. Beryllium and molybdenum were within an acceptable range of 85-115% for winter but were outside the acceptable range for spring. Unfortunately, lithium samples were not spiked in winter but had an acceptable recovery of 95.5% in spring. Results for beryllium, molybdenum and lithium were further analysed using PERMANOVA (Table 8) and implications of the poor part-recovery of beryllium and molybdenum discussed later.

Source	df	Pseudo-F	P(perm)	P(MC)
Mined/Non-mined	1	9.2995		0.0185
Creek (Mined/Non-mined)	2	2.8464	0.0211	
Season	1	25.036	0.0218	

Table 8 Combined trace Be, Mo and Li PERMANOVA table of results

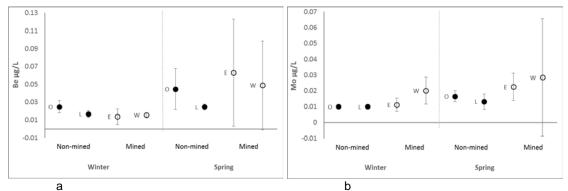
The strongest overall significant difference among trace metal concentrations was found between non-mined and mined sites (P(MC)=0.0185). Differences were also found between creeks nested within non-mined and mined sites (P=0.0211) and between season (P=0.0218).

Further analysis of each individual trace metal PERMONOVA (Table 9) reveal lithium was the only individual trace metal which significantly differed between non-mined and mined sites (Figure 17, P(MC)=0.0246). Strong significant differences within creeks between season (P=0.0366 and P=0.339) were found for beryllium and molybdenum (Figure 18a and b), and for molybdenum between creeks nested within non-mined and mined sites (P=0.0242).



**Figure 17** Mean lithium concentrations in creeks by potential impact and season (error +/- 2 standard deviation from the mean). *L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.* 

Overall the mined Eastern Tributary and Waratah Rivulet sites showed the greatest variability between replicates within seasons, as indicated by the longer error bars.



**Figure 18** Mean beryllium and molybdenum concentrations in creeks by potential impact and season (error +/- 2 standard deviation from the mean). L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.

Further analysis of mean lithium concentrations in winter at mined sites  $(0.60\mu g/L)$  were almost three times the non-mined concentrations  $(0.21\mu g/L)$ , Figure 17). This separation reduced in spring where the mean lithium concentration at mined sites was  $0.46\mu g/L$ , and  $0.28\mu g/L$  at non-mined sites. The spring sampling event was preceded by moderately wet conditions (Figure 2), which may have acted to dilute and hence reduce the lithium concentrations at mined sites.

Metal	Source	df	Pseudo-F	P(perm)	P(MC)
Li	Mined/Non-mined	1	39.81		0.0246
Ве	Season	1	29.94	0.0366	
Мо	Creek (Mined/Non-mined)	2	3.5521	0.0242	
	Season	1	42.785	0.0339	

 Table 9 Selected trace metal PERMANOVA table of results

### **Other Water Chemistry Variables**

Overall PERMANOVA results for all other water quality variables indicate significant difference between non-mined and mined sites (P(MC)=0.0071, Table 10).

 Table 10
 PERMANOVA able of results for all other water quality variables

Source	df	Pseudo-F	P(MC)		
Mined/Non-mined	1	15.152	0.0071		

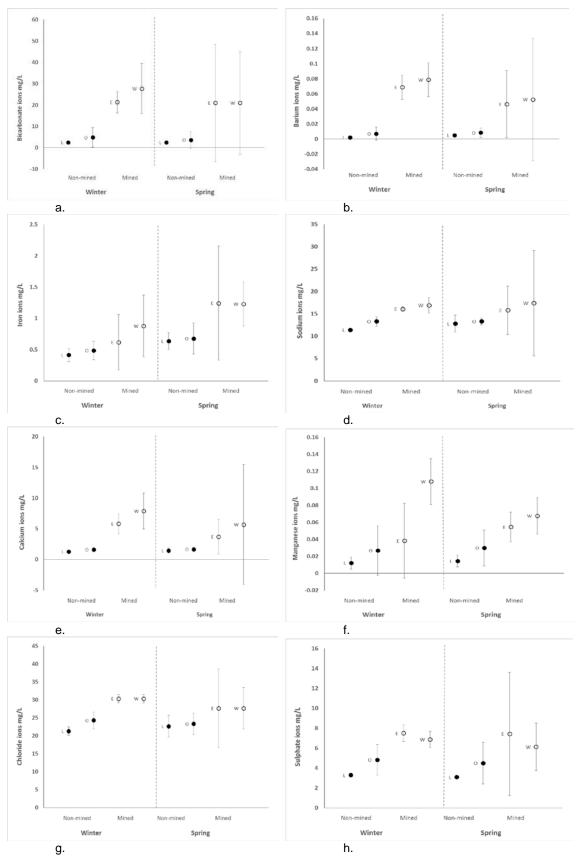
Further analysis of each individual variable using PERMANOVA (Table 11) found several that most contributed to the differences between non-mined and mined sites, including two of the strongest: bicarbonate (Figure 19a, P(MC)=0.0086) and barium (Figure 19b, P(MC)=0.006); followed by calcium, sodium, iron and chloride (P(MC)=0.0236 to 0.0495). Barium and calcium also indicated significant difference within creeks between seasons (Table 11). Compared to their individual non-mined and mined site differences, barium returned a weaker difference (P=0.0385) and calcium a slightly stronger difference (P=0.0304).

Manganese solely contributed to a significant difference between creeks nested within non-mined and mined sites by season (Figure 19f, P=0.0039) but this was not strong enough to be evident in the overall combined PERMANOVA (Appendix 5).

Analyte	Source	df	Pseudo-F	P(perm)	P(MC)
CaCO3	Mined/Non-mined	1	114.71		0.0086
Ва	Mined/Non-mined	1	153.72		0.006
Da	Season	1	104.67	0.0385	
Са	Mined/Non-mined	1	17.59		0.0495
Ga	Season	1			
Na	Mined/Non-mined	1	20.215		0.0465
Fe	Mined/Non-mined	1	41.335		0.0236
Mn	Season	1	18.06	0.0001	
	Creek (Mined/Non-mined) x Season	1	7.4995	0.0039	
CI	Mined/Non-mined	1	44.041		0.0242

### Table 11 PERMANOVA able of results for all other water quality variables

Again, much greater variability within all water quality variables was observed at mined sites compared to non-mined sites, as indicated by longer error bars (Figure 19a to h), except manganese in winter and, chloride and sulphate in spring ((Figure 19).



**Figure 19** Mean water quality variable concentrations in creeks by potential impact and season (error +/- 2 standard deviation from the mean). *L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.* 

### Water Quality PCA

It was necessary to conduct absolute value logarithmic (log(10) transformation of calcium, sodium, aluminium, beryllium and molybdenum concentrations (Appendix 5) due to clumped distribution in correlation with other water quality variables. These transformations resulted in a more even distribution of water quality variables (Figure 20). Barium and calcium were highly inter-correlated in the underlying Bray-Curtis resemblance matrix (0.9653, see Appendix 5), so barium was removed and calcium was retained for the PCA. Chromium was also removed due to the unreliable results from failed spike recovery obtained from ANSTO laboratory analysis (Table 7).

Combined water quality variables using a PCA indicated potential environmental links between samples (Figure 21). The first principal component explained 46.7% of the total variation between samples and was correlated with bicarbonate, calcium, sodium, chloride, sulphate, lithium and conductivity results (Appendix 5). The second principal component explained a further 21.9% of the variation and was correlated with aluminium, iron, beryllium and rainfall. The PCA show a clear separation of the nonmined and mined sites (Figure 21), greater at the non-mined sites while the mined sites were more dispersed (Figure 21).

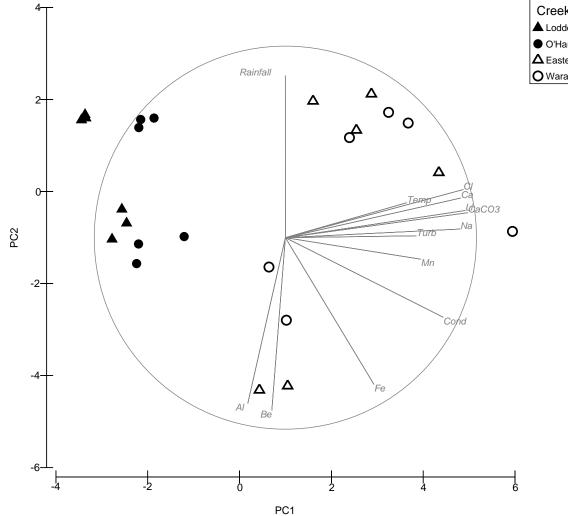
Water quality variables that showed significant difference between mined and nonmined sites were overlaid as vectors onto the PCA (Figure 21) including bicarbonate, calcium (which also represented barium), calcium, sodium, iron, manganese, chloride, lithium, temperature and conductivity. The longest vector length, represented in Figure 21 by chloride and bicarbonate ions, indicate more significant contribution to difference between non-mined and mined sites. Samples in Figure 21 were also coded by creek as PERMANOVA water quality results for conductivity and turbidity (Table 5), molybdenum (Table 8) and manganese (Table 11) indicated significant difference among creek nested within non-mined and mined sites.

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Honours Thesis

Figure 20 Draftsman plot of all water quality variables, including log transformed Ca, Na, Al, Be and Mo

Catherine Cunningham u3140389



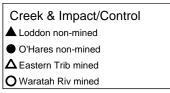


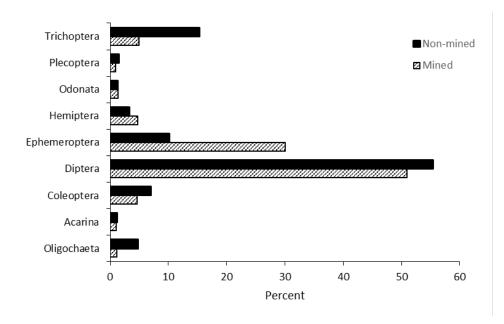
Figure 21 Principal component analysis of rainfall and all water quality variables, with significantly different (*P*<0.05) variables as overlay vectors

## 3.4 Macroinvertebrates

A total of 31,404 macroinvertebrates within 135 genera were identified from edge and riffle habitats across two sampling episodes (Appendix 5).

### **Order Taxa Charts**

Slight differences between percent proportional abundance of orders was evident between edge and riffle habitats (Figure 22 and Figure 23). Dipteran's dominated both habitats, with a similar composition between non-mined and mined sites in edge habitats (55% and 51%, Figure 22), and a slight increase at mined sites compared to non-mined sites in riffle habitats (65% and 55% respectively, Figure 23).



### Edges

#### Figure 22 Proportional abundance of stream macroinvertebrate order in the edge habitat

The next most dominant macroinvertebrate orders in the edge habitat of non-mined and mined sites respectively comprised Ephemoptera (10% and 30%), Trichoptera (15% and 5%) and Coleoptera (7% and 5%), as shown in Figure 22. Ephemoptera were clearly more dominant, but not significantly different (P=0.11), in edges at mined than non-mined sites. The opposite was observed for the order Trichoptera, three times more predominant at the non-mined than mined sites (Figure 22), and strongly significantly different (P=0.00007, Table 12).

Significant differences were also found within percent proportional abundance of Oligochaete and Coleopteran orders between non-mined and mined edge samples (P = 0.0047 and P = 0.0073, Table 12).

Order	Source	df	F	Р	F critical
Oligochaeta	Mined/Non-mined	1	7.49	0.0073	3.94
Coleoptera	Mined/Non-mined	1	8.36	0.0047	3.94
Trichoptera	Mined/Non-mined	1	22.46	0.00007	3.94

 Table 12
 ANOVA of individual order abundance in edges from non-mined and mined sites

### Riffles

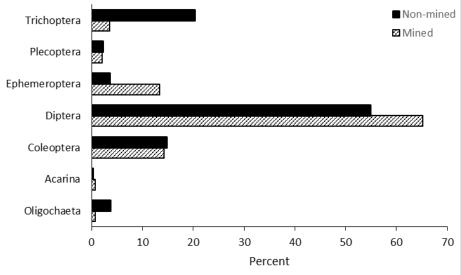


Figure 23 Proportional abundance of stream macroinvertebrate order in the riffle habitat

Following the predominant Diptera in the riffle habitat of non-mined and mined sites were the percent proportional abundance of orders Coleoptera (15% and 14%) Trichoptera (20% and 4%) and Ephemoptera (4% and 13%). Again, Ephemoptera were more predominant, but not significantly different (P=0.39) in riffles between mined and non-mined sites. Trichoptera were over five times more predominant and more strongly significantly different in riffles than in edges (P=0.00043, Table 13) at non-mined than mined sites (Figure 23).

Significant differences were also found within percent proportional abundance of Oligochaete and Dipteran orders between non-mined and mined riffle samples (P=0.0019 and P=0.03, Table 13).

Differences within Trichoptera genus diversity and abundance between non-mined and mined sites was further investigated within EPT Indicator Taxa.

Order	Source	df	F	Р	F critical
Oligochaeta	Mined/Non-mined	1	5.01	0.03	4.09
Diptera	Mined/Non-mined	1	5.63	0.019	3.94
Trichoptera	Mined/Non-mined	1	13.57	0.00043	3.97

### Table 13 ANOVA of individual order abundance in riffles from non-mined and mined sites

## **Taxa Richness**

### Edges

There was a decline in macroinvertebrate genus richness within edge habitats from mined sites for both winter and spring seasons (Figure 24a, Appendix 5) compared to non-mined sites. Mined sites were 5.2 genera lower on average than non-mined sites (Figure 24b). Although PERMANOVA results indicate these differences are not significant (P(MC)=0.3217). A significant difference in average edge genus richness was observed between creeks nested within non-mined and mined sites (P=0.0002,

Table 14 and Appendix 6). The greatest individual difference between edge genus taxa richness occurred between non-mined O'Hares Creek site (OH2) and mined Waratah Rivulet (WR2) with 10 less genera on average at the mined site.

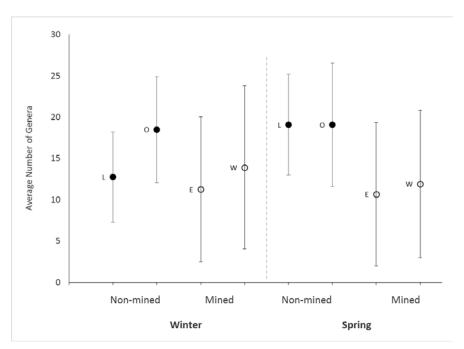


Figure 24 Mean edge genus richness in creeks by potential impact and season (error +/- 2 standard deviation from the mean). L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.

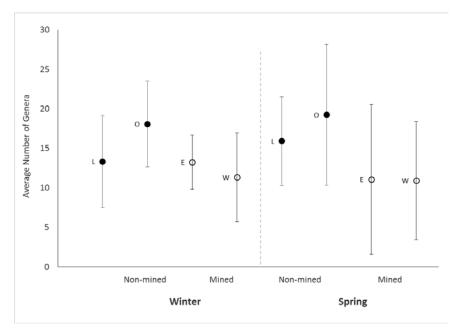
Table 14 PERMANOVA table of results for edge genus richness

Source	df	Pseudo-F	P(perm)
Creek (Mined/Non-mined)		9.3027	0.0002

### Riffles

Genus richness results from riffles indicate a slightly smaller but stronger difference between non-mined and mined sites than edge habitats (Figure 25). On average, 4.6 fewer genera were found at mined sites within riffle habitats than at non-mined sites, and although PERMANOVA results indicate no significant difference (P(MC)>0.0832, Figure 25, Table 15 and Appendix 6) the difference was much stronger than in edges.

A significant difference was also observed among average riffle genus richness between creeks nested within non-mined and mined sites (P=0.0097, Table 15). The largest observed individual difference was observed between the non-mined O'Hares Creek (OH1) and mined Eastern Tributary (WR1), with 11 genera less on average at the mined site.



**Figure 25** Mean riffle genus richness in creeks by potential impact and season (error +/- 2 standard deviation from the mean). L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.

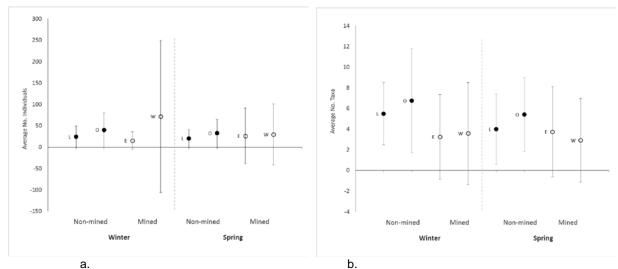
 Table 15
 PERMANOVA table of results for riffle genus richness

Source	df	Pseudo-F	P(perm)
Creek (Mined/Non-mined)		4.7194	0.0097

## **EPT Indicator Taxa**

### Edges

A greater average number of EPT individuals was found within edges at the Waratah Rivulet mined sites in winter than at non-mined sites (Figure 26a) but the individuals were from a smaller number of taxa (Figure 26b). Overall, the average number of EPT individuals in edges was not significantly different between non-mined and mined sites (P(MC)=0.7221), but was significantly different among the average number of EPT taxa between non-mined and mined sites (P(MC)=0.7221), but was significantly different among the average number of EPT taxa between non-mined and mined sites (P(MC)=0.015, Table 17). On average, the mined Eastern Tributary sites in winter had 3.5 less EPT taxa than at the non-mined O'Hares Creek sites (Figure 26b).



**Figure 26** Mean edge EPT total number of (a) individuals and (b) taxa in creeks by potential impact and season (error +/- 2 standard deviation from the mean). L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.

Further analysis of PERMANOVA results (Appendix 6) for average edge EPT total number of individuals found significant differences between creeks nested within non-mined and mined sites (P=0.0009, Table 16, Figure 26a).

Table 16	PERMANOVA table of results for edge EPT total number of individuals
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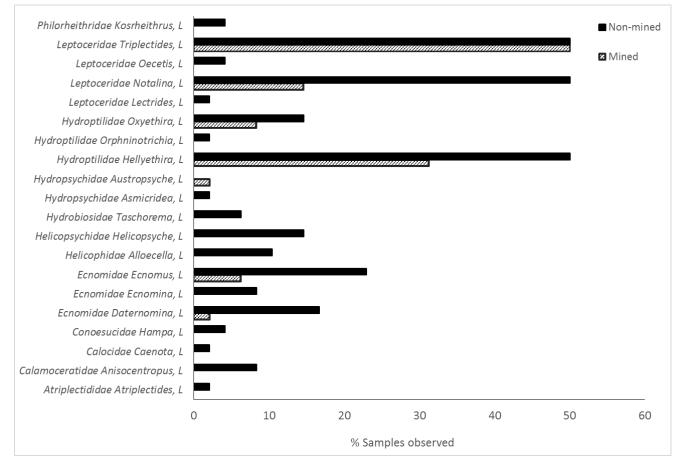
Source	df	Pseudo-F	P(perm)
Creek (Mined/Non-mined)		4.7974	0.0009

### Table 17 PERMANOVA table of results for edge EPT total number of taxa

Source	df	Pseudo-F	P(MC)
Mined/Non-mined	1	6.3174	0.015

The greatest individual difference between average edge EPT taxa occurred between the mined Waratah Rivulet (WR5) site and the non-mined O'Hares Creek site (OH2) with 5.3 less taxa on average at the mined site (Appendix 5).

Significant difference within Trichoptera (P=0.0001, Table 12) and difference within Plecoptera edge order composition were further investigated in Figure 27 and Figure 28.



#### Figure 27 Trichoptera genera present within edges at non-mined and mined sites

Trichopterans were represented by 20 different genera in the edge habitat of non-mined and mined sites (Figure 27). Trichopteran diversity was greatest at non-mined sites, with 19 genera observed in edges at non-mined sites, compared to seven genera from mined sites (Figure 27). Thirteen of the genera were present only at non-mined sites, including the individual most prevalent *Helicopsyche* (Helicopshychidae), observed at 15% of the non-mined edges. Other Trichoptera genera found within more than 5% of non-mined edges only include *Helicophidae* (Alloecella), *Ecnomus* (Ecnomidae), *Anisocentropus* (Calamoceratidae), Taschorema (Hydrobiosidae), Kosrheithrus (Philorheithridae), *Oecetis* (Leptoceridae) and *Hampa* (Conoesucidae). Five further Trichopterans were found within 4% of edges from only non-

mined sites, compared to only one genus, *Austropsyche* (Hydropsychidae) found within 4% of edges at only mined sites (Figure 27).

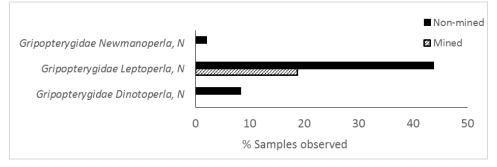
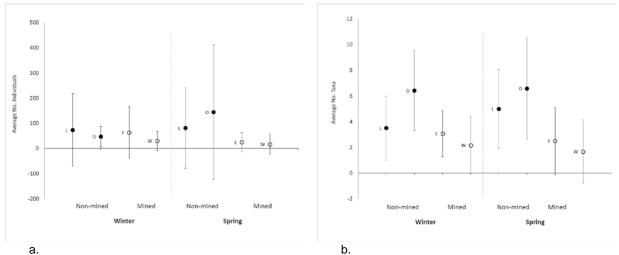


Figure 28 Plecoptera genera present within edges at mined and non-mined sites

Plecopterans were represented by three different genera within edge habitats at non-mined sites (Figure 31). Only one of these, *Leptoperla* (Gripopterygidae), was present at just under half of the number of mined edges compared to non-mined edges. Two Gripopterygidae genera present at non-mined edges, *Newmanoperla and Dinotoperla*, were absent from any of the mined edges.

# Riffles

In contrast to edges, the average number of EPT individuals in riffles were greatest at nonmined sites in spring (Figure 29a). The higher number of individuals also aligned with a higher average number of EPT taxa within the non-mined than mined riffles in winter and spring (Figure 29b). Significant differences were found between both the average EPT number of individuals and taxa, and non-mined and mined sites (P(MC)=0.0106 and 0.0239, Table 18 and Table 19). On average, 3.9 less EPT taxa were found at mined than non-mined riffles in winter and spring (Figure 29b).



**Figure 29** Mean riffle EPT total number of (a) individuals and (b) taxa in creeks by potential impact and season (error +/- 2 standard deviation from the mean). L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.

Further analysis of the riffle PERMANOVA (Table 18 and Table 19, Appendix 6) reveals further significant differences of average number of EPT taxa between creeks within nonmined and mined sites, (P=0.0001, Table 19).

 Table 18
 PERMANOVA table of results for riffle EPT total number of individuals

Source	df	Pseudo-F	P(MC)
Mined/Non-mined	1	10.593	0.0106

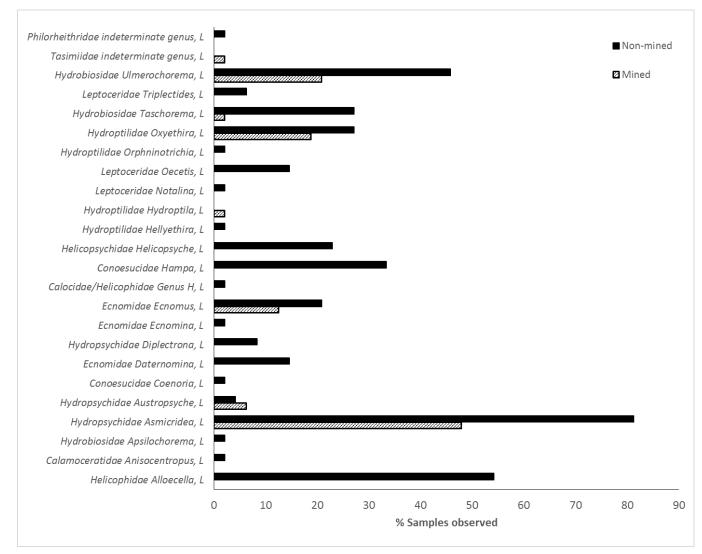
Again, the greatest difference occurred between non-mined O'Hares Creek and mined Waratah Rivulet riffles with an average of 4.2 and 4.9 less taxa in winter and spring (Figure 29b).

Table 19 PERMANOVA table of results for riffle EPT total number of taxa

Source	df	Pseudo-F	P(perm)	P(MC)
Mined/Non-mined	1	5.9512	-	0.0239
Creek (Mined/Non-mined)	2	3.5883	0.0001	-

A similar ratio of missing Trichoptera taxa occurred in mined riffles compared to mined edges (Figure 30). Overall, Trichopterans in riffle habitats were represented by 24 different genera (Figure 30). Trichoptera diversity was greatest at non-mined sites, with 22 genera within riffle habitats observed at non-mined sites, compared to eight genera from mined sites (Figure 30). Sixteen of the genera were present at non-mined sites only, including the three most prevalent, *Alloecella* (Helicophidae), *Helicopsyche* (Helicopshychidae) and *Hampa* (Conoesucidae), observed at 15% of the non-mined riffles. Other Trichoptera genera found within more than 5%

of non-mined riffles include *Oecetis* (Leptoceridae), *Daternomina* (Ecnomidae), *Diplectrona* (Hydropschidae) and *Triplectides* (Leptoceridae). Nine further Trichopeterans were found within less than 5% of non-mined riffles, compared to only two genera found at mined sites only (Figure 30).



#### Figure 30 Trichoptera genera present within riffles at mined and non-mined sites

Plecoptera were represented by three different genera within non-mined riffles (Figure 31). Only one of these, *Dinotoperla* (Gripopterygidae), was present at over half of the number of mined compared to non-mined riffles. Two Gripopterygidae genera, *Leptoperla and Illiesoperla*, were absent from any of the mined sites (Figure 31).

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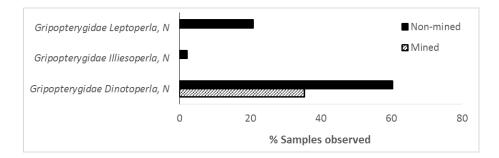


Figure 31 Plecoptera genera present within riffles at mined and non-mined sites

# **Multivariate Analysis**

# Edges

# Cluster analysis and multi-dimensional scaling (MDS)

Cluster analysis of edge macroinvertebrate assemblages indicated separation of non-mined and mined replicates, with several Waratah Rivulet replicates the least similar to all others (Figure 32).

The first split separates one midstream mined edge replicate (WR5) from all others at less than five percent similarity (Figure 32). The following two splits at just under and over 15% similarity separate a further three midstream mined Waratah Rivulet replicates.

The next split, at approximately 20% similarity, separates two further mined replicates from up and downstream Eastern Tributary, one each from winter and spring (WR3 and WR1), and one downstream non-mined replicate from O'Hares Creek in spring (OH3). Following this, at just under 25% similarity, was the separation of all eight (winter and spring) upstream non-mined replicates from O'Hares Creek (OH1) and, at just over 25% similarity, the remaining four midstream mined Waratah Rivulet replicates.

Between 25% to 30% similarity, a separation of an additional non-mined midstream O'Hares Creek (OH2) replicate occurred, along with seven replicates from mined upstream Eastern Tributary sites (WR1 and WR2). No further separation occur between these replicates.

The last few separations under 50% similarity divide the remaining replicates into three main groups:

1. 23 of the 24 non-mined Loddon River replicates from all stream positions separate at approximately 30% similarity, except for one winter downstream replicate

- 2. All remaining mined replicates from all stream positions separate at just over 30% similarity. Several non-mined mid and downstream replicates (OH2 and OH3) from spring also combine with replicates in this similarity group indicating similarity.
- 3. All four non-mined, downstream, winter samples at OH3 separate in one of the final splits at justover 40% similarity.

In summary, the first two sites replicates to separate from all other sites include those from the mined midstream Waratah Rivulet and the non-mined upstream O'Hares Creek (Figure 32).

Group average

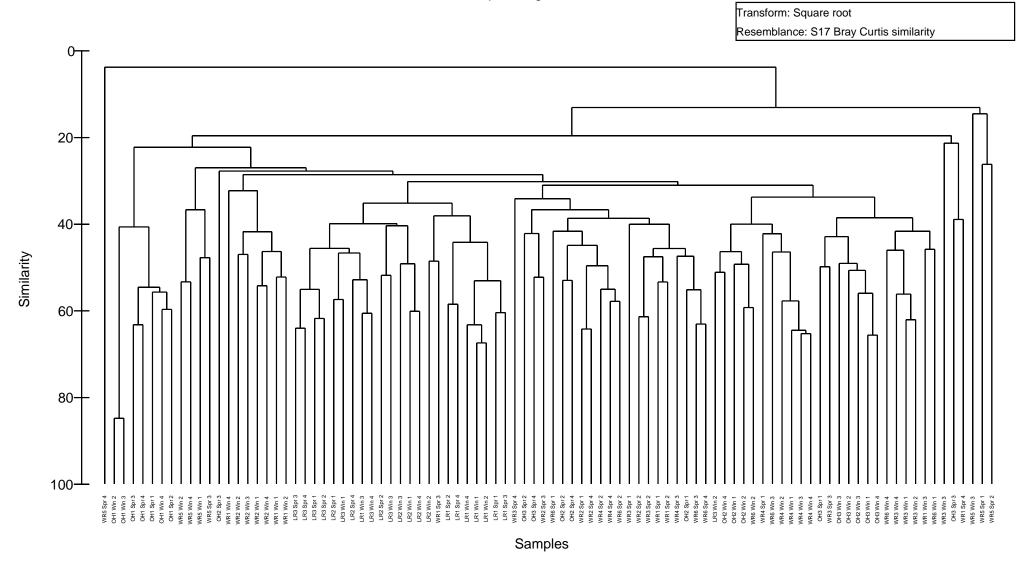


Figure 32 Cluster analysis of macroinvertebrate community composition from edge replicates

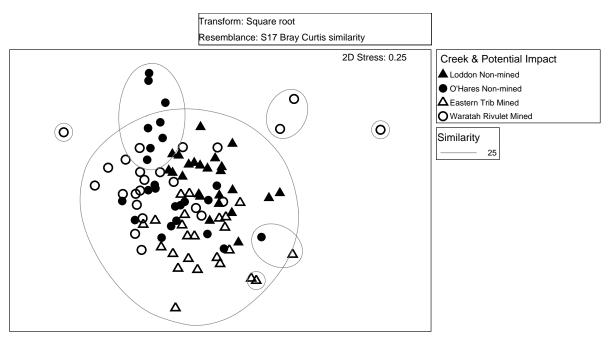


Figure 33 2D Non-MDS of edge macroinvertebrate replicates

The 2D multi-dimensional ordination (Figure 33) is in agreement with the cluster analysis (Figure 32). Separation of four of the midstream mined Waratah Rivulet replicates from all others, and all of the non-mined upstream O'Hares Creek replicates is evident in the 2D ordination (Figure 33). Stress reduced in the 3D ordination (Figure 34) and clearly presented the separation of Waratah Rivulet replicates from each other and from all other replicates.

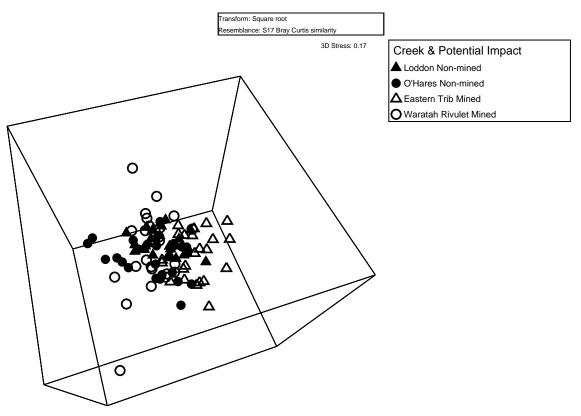


Figure 34 3D Non-MDS of edge macroinvertebrate replicates

### **SIMPER**

Overall similarity of edges at non-mined sites was greater than mined sites (Figure 24). Nonmined Loddon River had the highest similarity between edge replicates (42.79%) of all four creeks, and mined Waratah Rivulet the lowest at 28.43% similarity (Figure 24, Appendix 7). Further investigation of edge macroinvertebrates that dominated over 4% of total genus contributions within SIMPER analysis reveal differences between non-mined and mined creeks (Appendix 7).

The non-mined Loddon River and O'Hares Creek edges were most dominated by three nonbiting midge (Chironomidae) sub-families, including *Chironominae* (15.99%), *Tanypodinae* (12.07%) and *Orthocladiinae* (11.64%), followed by the freshwater shrimp *Paratya* (Atyidae, 11.92%). The next most dominant genera above 4% contribution was the EPT caddis fly *Triplectides* (Leptoceridae, 5.26%).

In contrast, the mined Eastern Tributary and Waratah Rivulet edges were most dominated by the freshwater shrimp *Paratya* (Atyidae, 14.49%) and two chironomids, *Chironominae* (13.97%) *and Tanypodinae* (9.45%). These were followed by two mayflies - immature Baetidae nymphs (8.24%) and, both mature *Koornonga* (Leptophlebiidae, 7.96%) and immature Leptophlebiidae nymphs (7.19%). A further four genera were found contributing to more than 4% of the total including: immature backswimmers (Notonectidae, 4.40%), *Orthocladiinae* (Chironomidae, 4.30%), *Triplectides* (Leptoceridae, 4.30%) and *Centroptilium* (Baetidae, 4.21%) nymphs.

Creek	Non-m (37.4		Mined (30.97)		
Similarity % Loddon River		O'Hares Creek	Eastern Tributary	Waratah Rivulet	
Similarity %	42.79	32.97	33.51	28.43	

 Table 20
 SIMPER analysis of macroinvertebrate similarity within edges

# BEST

The water quality variables analysed using BEST routine (PRIMER V7) found to correlate and potentially contribute to edge macroinvertebrate assemblage include manganese, turbidity and rainfall (Appendix 7). Although, correlations were weak reaching a maximum of 0.285 when including all three variables.

# Riffles

# Cluster analysis and multi-dimensional scaling (MDS)

Cluster analysis of riffle macroinvertebrate assemblages indicated separation of all mined upstream Eastern tributary replicates in spring from all others at approximately 10% similarity (Figure 35).

The second split at just over 30% separates all upstream non-mined Loddon River and O'Hares Creek replicates (LR1 and OH1, Figure 35). Four further splits occurred between approximately 32 to 40% similarity and the groups included:

- 1. All eight spring replicates from the mined upstream and midstream Waratah Rivulet sites (WR4 and WR5)
- 2. All twelve winter mined replicates from midstream and downstream Eastern Tributary and downstream Waratah Rivulet (WR2, WR3 and WR6)
- All winter replicates from mined upstream and midstream Waratah Rivulet and upstream Eastern Tributary (WR4, WR5 and WR1) and, midstream and downstream non-mined O'Hares Creek (OH2 and OH3)
- 4. Contains the highest number of replicates, approximately one third overall, from a mixture of all creeks from both seasons.

In summary, a greater degree of separation between non-mined and mined replicates occurred in riffles than edges, the most dissimilar from upstream Eastern Tributary and upstream and midstream Waratah Rivulet (Figure 35). Group average

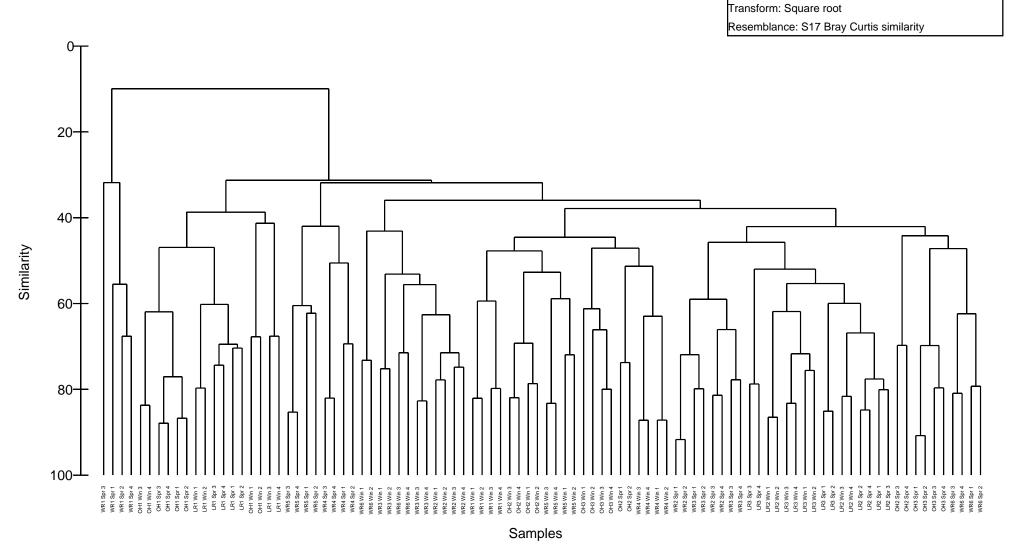
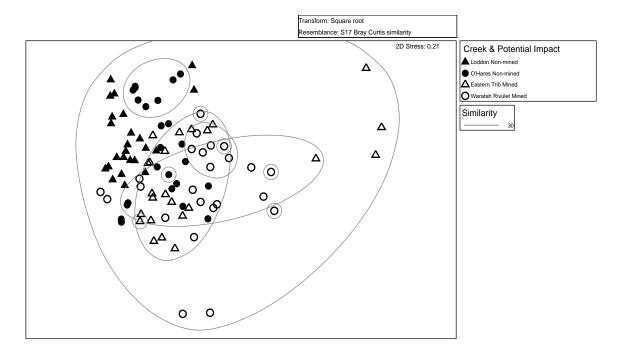
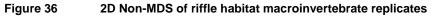
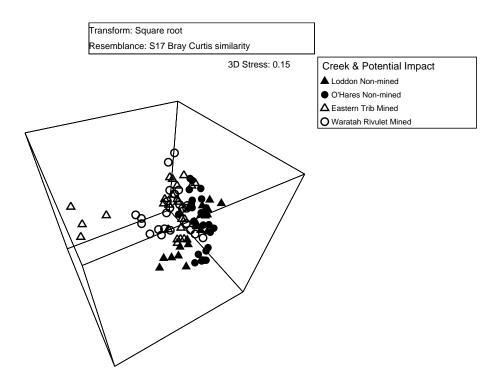


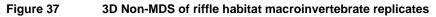
Figure 35 Cluster analysis of macroinvertebrate community composition from riffle replicates





The riffle 2D multi-dimensional ordination (Figure 36) is in agreement, as for edges, with the cluster analysis (Figure 35). Separation of four of the upstream mined Eastern Tributary replicates from all others, and some of the mined upstream and midstream Waratah Rivulet replicates is evident in the 2D ordination (Figure 36). Stress reduced in the 3D ordination (Figure 37) and also clearly indicated the separation of upstream mined Eastern Tributary replicates from all other replicates.





### SIMPER

Overall similarity of non-mined riffles was greater than mined sites, as for edges, and greater in non-mined riffles than non-mined edges (Table 21). Again, the non-mined Loddon River had the highest similarity between riffles (48.91%) of all four creeks, but the mined Eastern Tributary the lowest at 37.74% similarity (Table 21, Appendix 7). Further investigation of riffle macroinvertebrates that dominated over 4% total genus contributions within SIMPER analysis reveal differences between non-mined and mined creeks (Appendix 7).

The non-mined Loddon River and O'Hares Creek riffles were most dominated by two non-biting midge (Chironomidae) sub-families, including *Orthocladiinae* (19.34%) *and Chironominae* (18.29%), followed by the caddis fly *Asmicridea* (Hydropsychidae, 9.02%). Three additional genera dominant above 4% contribution were two Elmidae riffle beetles *Austrolimnius* (5.85%) and *Kingolus* (4.08%), and another chironomid sub-family, *Tanypodinae* (4.35%).

The mined Eastern Tributary and Waratah Rivulet riffles were also most dominated by the same two chironomid sub-families as non-mined sites, including *Orthocladiinae* (19.67%) *and Chironominae* (19.38%). But were followed by the mayfly, *Offadens* (Baetidae, 8.24%), a crane fly, *Antocha* (Tipulidae 7.20%) and a riffle beetle, *Kingolus* (Elmidae, 5.61%).

Table 21	SIMPER analysis of macroinvertebrate similarity within riffles
----------	--

Creek	<b>Non-m</b> (45.7		Mined (38.20)		
Similarity % Loddon River		O'Hares Creek	Eastern Tributary	Waratah Rivulet	
Similarity %	48.91	42.92	37.74	38.66	

### **BEST (BIOENV)**

The water quality variables analysed using BEST routine (PRIMER V7) found to correlate and potentially contribute to riffle macroinvertebrate assemblage include bicarbonate, sulphate, temperature and rainfall (Appendix 7). Correlations were moderately strong, reaching a maximum of 0.417 when including all four variables and were stronger than any water quality variable correlations within edges.

Further analysis of only the Trichoptera taxa and re-running the BEST routine failed to improve correlations. Correlations were weak at 0.320 and included only two variables, dissolved oxygen and manganese (Appendix 7).

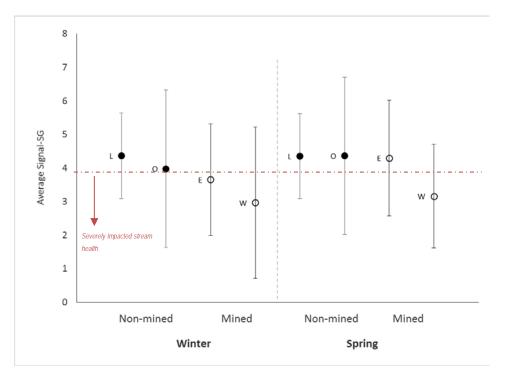
### SIGNAL-SG

# Edges

The only significant difference within signal-SG scores in edges occurred between creek nested within non-mined and mined sites (P=0.0017, Table 22). Average signal-SG scores from all non-mined and one mined edges lay within the moderately impacted stream health range (Table 4), and all other mined average signal-SG scores lay within the severely impacted stream health range (Figure 38).

#### Table 22 PERMANOVA table of results for signal-SG in edges

Source	df	Pseudo-F	P(perm)
Creek (Mined/Non-mined)	2	5.0584	0.0017



**Figure 38** Mean edge Signal-SG scores by non-mined and mined sites and season (error +/-2 standard deviation from the mean). L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.

# Riffles

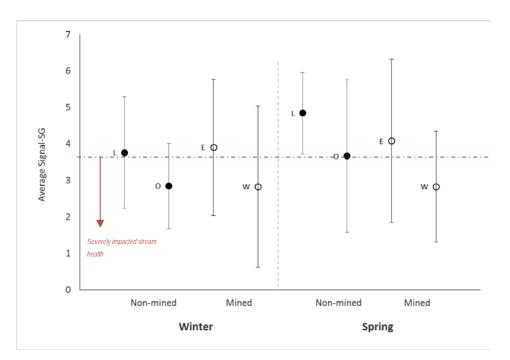
Significant signal-SG differences were also observed in riffles, as for edges, between creeks nested within non-mined and mined sites (P=0.0001, Table 23).

#### Table 23 PERMANOVA table of results for Signal-SG in riffles

Source	df	Pseudo-F	P(perm)
Creek (Mined/Non-mined)	2	14.945	0.0001

Further investigation revealed the greatest average signal-SG score difference of 2.2 and 2.0 between the mined Eastern Tributary and Waratah Rivulet riffles (Figure 39).

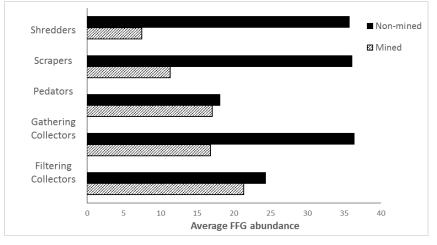
Average signal-SG scores from one non-mined and two mined riffles lay within the severely impacted stream health range (Table 4) and all others lay on the borderline or within the moderately impacted stream health range (Figure 39).

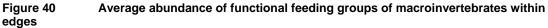


**Figure 39** Mean riffle Signal-SG scores by non-mined and mined sites and season (error +/-2 standard deviation from the mean). *L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.* 

# **Functional Feeding Groups (FFG)**





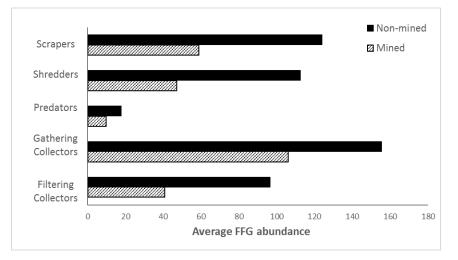


A reduction in abundance of all functional feeding groups within edge habitats occurred within mined compared to non-mined edge habitats, including over 75% reduction in average abundance of scrapers (Sc) (Figure 40). Over 50% reduction was also observed in average abundance for gatherer/collectors (GC) and shredders (S) between mined compared to non-mined edge habitats (Figure 40). Statistically, only shredders and scrapers showed significant differences between mined and non-mined sites (P=0.011 and P=0.0039), Table 24.

Table 24	ANOVA of individual FFE abundance in edges from non-mined and mined sites
----------	---

FFG	Source	df	F	Р	F critical
Shredders	Mined/Non-mined	1	6.65	0.011	3.94
Scrapers	Mined/Non-mined	1	8.79	0.0039	3.94

### Riffles



# Figure 41 Average abundance of functional feeding groups of macroinvertebrates within riffles

As with edges, a reduction in abundance of all functional feeding groups occurred within mined compared to non-mined riffle habitats (Figure 41). The most significant difference between mined and non-mined riffle habitats, in decreasing order of strength, occurred within filtering collectors (P=0.0001), shredders (P=0.0007), predators (P=0.0089) and scrapers (P=0.0013, Table 25 and Figure 41).

#### Table 25 ANOVA of individual FFE abundance in riffles from non-mined and mined sites

FFG	Source	df	F	Р	F critical
Filterer Collectors	Mined/Non-mined	1	16.455	0.0001	3.94
Predators	Mined/Non-mined	1	7.13	0.0089	3.94
Shredders	Mined/Non-mined	1	12.12	0.0007	3.94
Scrapers	Mined/Non-mined	1	12.11	0.0013	3.94

# 3.5 Diatoms

# **Abundance and Taxa Richness**

A total of over 1.2 million epilithic diatoms from fifteen genera were extrapolated from laboratory analysis of samples (Appendix 8). In particular, four genera were found in relatively small numbers at only mined sites: *Navicula, Cocconei, Pinnular* and *Amphora;* and, no genera were unique to non-mined sites (Table 24). *Naviculoid* diatoms were the most abundantly dominant genus, followed by *Gomphonema* which were both more abundant at non-mined than mined sites (Figure 42).

Diatom genera	Non-	mined	Mi	ned	Total collected
	Loddon	O'Hares	Eastern	Waratah	
Gomphonema	√	√	$\checkmark$	$\checkmark$	326,825
Encyonem	√	$\checkmark$	1	√	49,400
Diatomella	√	$\checkmark$	√	√	21,425
Naviculoid	√	$\checkmark$	√	√	774,457
Eunotia	√	$\checkmark$	√	√	21,297
Synedra	√	$\checkmark$	√		6,333
Diatoma			√	√	3,056
Tabellar	√	$\checkmark$	√		21,579
Nitzschi	√	$\checkmark$	√		9,754
Achnantm	√	$\checkmark$	√	√	18,844
Navicular			√	√	351
Cocconei			√		68
Pinnular			√	√	750
Surirell	√	$\checkmark$	√	√	233
Amphora				$\checkmark$	68

### Table 26 Presence/absence of diatom genera at non-mined and mined creeks

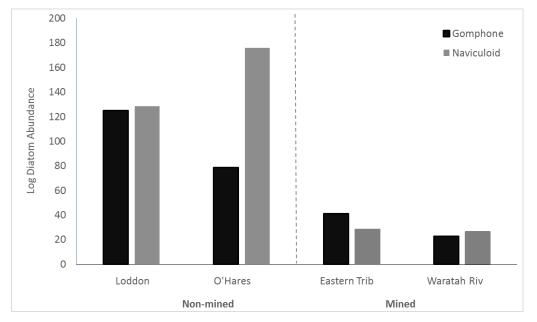
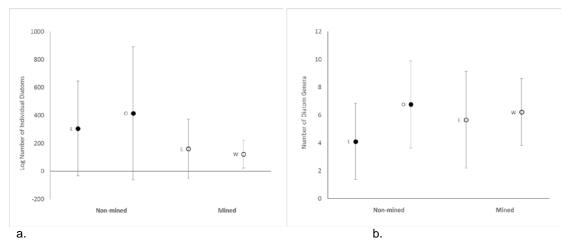


Figure 42 Mean total abundance of two diatom genera within stream by season at nonmined and mined sites



**Figure 43** Mean diatom (a) log abundance and (b) average taxa richness by non-mined and mined sites (error +/- 2 standard deviation from the mean). L=Loddon River, O=O'Hares Creek, E=Eastern Tributary and W=Waratah Rivulet.

Average diatom abundance varied between non-mined and mined sites (Figure 43a), and PERMANOVA analysis indicated this was statistically significant (P(MC)=0.0148, Figure 27). A significant difference was also observed in average diatom taxa between creeks nested within non-mined and mined sites (P=0.0004, Figure 43b and Table 28), indicating more variability between creeks within same potential impact that between them.

#### Table 27 PERMANOVA table of diatom abundance

Source	df	Pseudo-F	P(MC)
Creek (Mined/Non-mined)	2	8.7726	0.0148-

#### Table 28 PERMANOVA table of diatom taxa richness

Source	df	Pseudo-F	P(perm)
Creek (Mined/Non-mined)	2	8.7726	0.0004

#### **Multivariate Analysis**

### Cluster analysis and multi-dimensional scaling (MDS)

Cluster analysis of epilithic diatom community asssemblage indicates clear separation of most of the non-mined from mined replicates (Figure 44). Only one unmined replicate (from a possible total of eighteen) from downstream O'Hares Creek lies within the majority of mined samples, and only three mined replicates from various stream positions lies with the majority of non-mined samples. Separation of these two main non-mined and mined site groups occurred at 30% similarity indicating samples from non-mined sites are more similar than those from mined sites.

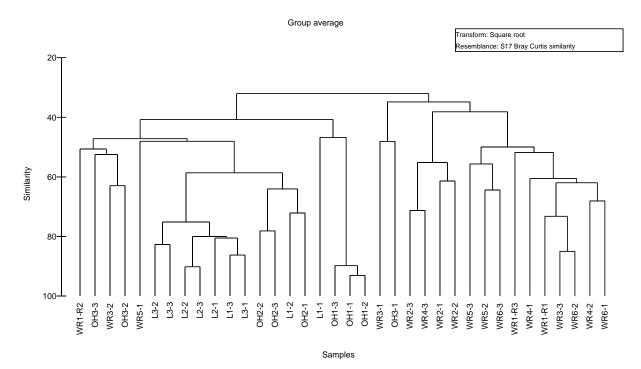


Figure 44 Cluster analysis of epilithic diatom community assemblage from sites

Visual representation of the results 2D non-MDS clearly shows four distinct groups at 40% similarity (Figure 45). The low stress value (0.17) indicates a potentially useful interpretation of the results. The cluster overlay again indicates diatom assemblages from the same non-mined and mined sites are more similar than those between non-mined and mined sites.

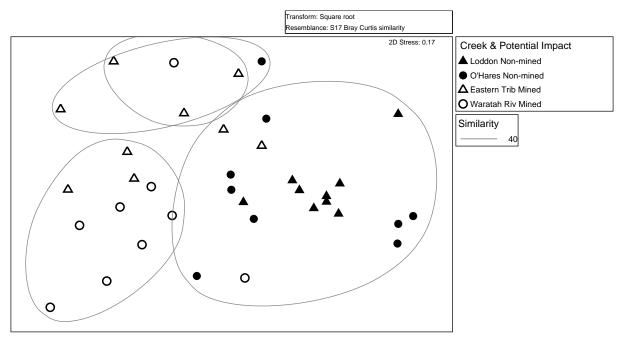


Figure 45 2D Non-MDS of epilithic diatom community replicates

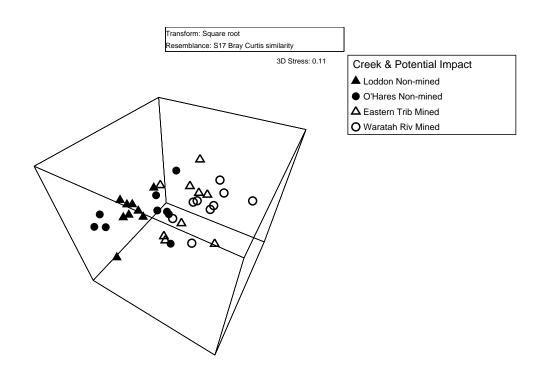


Figure 46 3D Non-MDS of epilithic diatom community replicates

Further analysis of the 3D non-multi-dimensional scale further supports the separation of mined and non-mined replicates from each other, most likely due to the presence of four extra genus of diatoms and loss of abundance of *Gomphone* and *Naviculoid* diatoms at mined sites (Figure 46, Table 24 and Figure 42). A stress of 0.11 supports a good ordination.

Overall PERMANOVA indicates diatom assemblages significantly differed between non-mined and mined sites (P(MC)=0.0251, Table 27) and between creeks nested within non-mined and mined sites (P=0.0001).

#### Table 29 PERMANOVA table of results for diatoms

Source	df	Pseudo-F	P(perm)	P(MC)
Mined/Non-mined	1	3.8582	-	0.0251
Creek (Mined/Non-mined)	2	3.5038	0.0001	-

# SIMPER

Diatom SIMPER analysis (Table 28, Appendix 10) indicate non-mined diatom replicates are more similar (58.40%) than mined replicates (47.22%). Further analysis indicates this is contributed predominantly by the non-mined Loddon River, with diatom similarity between replicates the highest of all four creeks (67.63%, Table 28). Honours Thesis Catherine Cunningham u3140389

The lowest similarity, 44.48%, was observed between diatom replicates from the mined Eastern Tributary.

Creek	<b>Non-m</b> (58.4		Mined (47.22)		
Similarity %	Loddon River	O'Hares Creek	Eastern Tributary	Waratah Rivulet	
Similarity %	67.63	49.17	44.48	49.97	

#### Table 30 SIMPER analysis of diatom similarity within sites

*Gomphone* and *Naviculoid* were the two most predominant diatom genera from all creeks, contributing to between 45 to 94% overall composition, except Eastern Tributary which was dominated by *Gomphone* (46%) and *Diatomella* (19%) (Appendix 10). Diatom composition from Waratah Rivulet were contributed to the lowest by *Naviculoid* and *Gomphone* (23.47% and 21.89%), but were almost equally dominated by two additional genera, *Achnantm* and *Euryonem*, 20.43% and 19.86% - the highest of all other genera across all sites (Appendix 10).

# BEST

The water quality variables analysed using BEST routine (PRIMER V7) found to correlate and potentially contribute to diatom assemblage include bicarbonate, chloride, % dissolved oxygen, pH and turbidity (Appendix 7). Correlations were moderately strong, reaching a maximum of 0.530 when including the latter four variables, stronger than macroinvertebrate correlations.

# 4. Discussion

In order for subsidence from underground long wall coal mining to affect the assemblages of stream biota, it would be necessary for cracking of underling rocks to cause greater downwards flow of surface water below the ground which results in either, or both, of the following: (a) Reduced flow of surface water because cracking of rocks has caused a conduit permitting greater flow of surface water into the groundwater; (b) A change in the water chemistry of the surface water because of return of (some) of this groundwater to the surface after having dissolved minerals while flowing through newly cracked rock. The findings of this thesis are consistent with both of these processes occurring. I will discuss these changes below.

### 4.1 Water level

Water levels in streams overlying underground mining reduced more quickly following rainfall, relative to those in reference sites. This is consistent with a greater amount of surface water being lost underground because of cracking caused by subsidence. There would be increased confidence in this finding with a greater length of water depth record (and the data loggers have been left recording to obtain this). Nevertheless, this finding is consistent with a number of reports (Holla & Barclay, 2000; Krogh, 2007; BHP, 2014) and unpublished studies (Stout, 2004). These studies have found an increase in frequency of dry events in areas impacted by subsidence overlying underground long wall coal mining.

Flow in headwater streams is often critical for maintaining stream health in larger rivers downstream via attenuation of floods, preventing siltation downstream and maintaining water supplies (Alexander *et al*, 2007; Gomi *et al*, 2002; Meyer *et al*, 2007). Degradation and loss of headwaters and their connectivity to ecosystems downstream threaten the integrity of entire river networks. So any changes in the flow of the headwater streams studied could have implications for larger rivers downstream.

Temporal variability of the ecology in ephemeral streams is understudied but Botwe *et al* (2015) suggest sustenance of seasonal flows and maintenance of critical refuge habitats is important for maintaining biological and chemical health of headwater streams. Naturally ephemeral streams may support rich and distinctive biological communities because of their slow formation through long evolutionary periods supporting increased adaptation and resilience to predictable dry periods (Meyer *et al.*,2007). Altered water levels in perennial streams caused by mining subsidence would Honours Thesis Catherine Cunningham u3140389 be expected to be too rapid to allow for similar adaptation and subsequent maintenance of water quality and ecology to mimic those of more intermittent streams.

### 4.2 Water quality

Significant differences were observed between water quality variables between streams overlying underground long wall mining compared to those with no mining. In particular there were significant increases in temperature and electrical conductivity and concentrations of lithium, bicarbonate, barium, calcium, sodium, iron and chloride. These differences are consistent with increased groundwater to surface water connectivity, as groundwaters typically have higher electrical conductivity, pH and concentrations of major, minor and trace elements (Jankowski *et al.*,2008). As groundwater is travelling through newly cracked rock there would be expected to be even greater increases in dissolved ions and thus electrical conductivity and pH. Chemical reactions would increase as water flows over new rock fractures, especially after rainfall, and act to mobilize elements from carbonate rock minerals, which are common in sandstones of the area (Sherwin & Holmes, 1986).

Both mined and one non-mined creek were in drinking water catchments closed to the public with no other anthropogenic activity, and the remaining non-mined creek within national park with minimal anthropogenic activity in the catchment, that could likely contribute to such differences. Wright *et al* (2015) also observed increased electrical conductivity, sodium, chloride, iron and manganese in a small stream disturbed by subsidence from long wall mining.

pH levels between non-mined and mined streams remained similar (between 6.2 to 7.5). This may be due to an increase in carbonate minerals from fractured rock strata in mined streams helping to buffer the dissolution of pyritic material (Stout, 2004).

Of the three trace metals that had acceptable detection limits detecting increased groundwater to surface water connectivity, only lithium was significantly different between streams with and without mining. Relatively little information is available for trace metals in natural rivers (Luck and Othman, 1998). The extremely low levels of lithium (mean  $0.25\mu g/L$ ) in non-mined streams in this study suggest transport from underground sources to surface water is limited by the low permeability of between-lying rock strata. The increased Li concentrations at sites with mining is consistent with groundwater returning to the surface and infiltrating at much faster rates to interact with

newly cracked rock. Lithium has a variety of manufacturing uses (including in heat resistant glass, air conditioners, lithium batteries and grease lubricants). However, due to the extremely limited anthropogenic activities in these catchments it is highly unlikely that lithium was the result of pollution. Lithium is therefore a useful indicator of mining subsidence, but only in streams with very little anthropogenic activity in the catchment.

Beryllium and molybdenum recoveries were within an acceptable range of 85-115% for winter but were outside the acceptable range for spring. This makes it difficult to infer differences that may exist as a result from mining induced subsidence because of possible laboratory interference or contamination. Further development of methods to detect these elements and improve recovery results would be required before their usefulness could be assessed.

Other water quality variables also contribute further to the understanding of surface water quality changes associated with subsidence from long wall coal mining. Significantly elevated bicarbonate, barium, calcium, chloride, sodium, iron and manganese concentrations at mined sites further support evidence of increased chemical reactions from water discharging from subsurface routes to streams. Krogh (2007) indicate increased sub-surface cracks and new fracture networks exposed fresh rock which rapidly reacts with oxygen and flowing groundwater to significantly enhance oxidisation of carbonate rock minerals and release of elements. Jankowski (2008) further determined increased bicarbonate concentrations were a result of increased chemical reactions involving carbonate minerals within the Hawkesbury Sandstone aquifer mix. This thesis is consistent with Jankowski (2008) that increased mobilization of iron, manganese and barium from metal carbonates significantly increased downstream of those creeks where mining subsidence had occurred. Increased availability of iron and manganese in surface waters for iron oxidizing bacteria observed in the mined streams also enable the formation of thick mats of iron/manganeseoxides/hydroxides which further act to reduce in-stream habitat and available food, clog streams and lead to potential loss of biodiversity.

Chloride was significantly elevated at mined compared to non-mined sites. The elevated levels of chloride ions in the surface waters of all streams interfered with the detection of trace levels of chromium (Wilbur *et al*, 2012; Henri Wong pers comm). Chromium is therefore not useful in detecting groundwater to surface water interactions as a result of

subsidence from long wall mining because of the increased chloride produced as a result of cracking.

The greater variability of water chemistry variables at mined compared to non-mined sites and the greater distance between samples when plotted in a 2D space using the principal component analysis suggest a clear shift away from the more stable and consistent water quality results from non-mined sites.

# 4.3 Macroinvertebrates

Overall, EPT individuals in riffles and EPT taxa in edges and riffles, in particular Trichoptera, differed the most between sites with and without mining. The EPT fauna have been shown to be useful indicators of water quality as they are responsive to disturbance (Rosenberg & Resh, 1993). In particular, their long life cycle in the larval/nymph stage, is known to be up to or longer than nine months before emergence as adults (Wallace & Anderson, 1996). The significant reductions in EPT taxa represented by Trichoptera, at mined compared to reference sites (7 vs 19 and 8 vs 22, in edge and riffle habitat respectively) may be linked to longer development stage requirements than other EPT taxa.

Similar results were found within EPT taxa in headwater streams in West Virginia as a result of long wall mining (Stout, 2004) but was difficult to compare to this thesis due to increased occurrence of dry streams and no detailed analysis of any individual EPT order contributing to differences. This thesis was also consistent with Wright *et al* (2015) that found a reduction in number of EPT individuals, but predominantly represented by Ephemoptera, at subsidence impacted mined sites compared to upstream reference sites. Again, it was difficult to directly compare these findings to current results because of the additional influence of urban pollution degrading mined and reference sites.

Further investigation of feeding characteristics of Trichoptera reveal that genera missing from mined but present at non-mined sites were represented by all FFG (scrapers, shredders, filtering collectors, gathering collectors and predators). Schmera (2004) discovered association among Trichoptera belonging to the same FFG and positive coexistence patterns linked to the presence of microhabitats. In effect, even though no particular pattern of dominance or loss emerged within FFG between mined and reference sites within this study, the possibility of increased microhabitats favorably supporting an increased number of Trichoptera taxa within each FFG is evident. This further supports evidence that impaired microhabitat, as a result of cracked rock and loss of surface waters more rapidly at mined than reference sites, is potentially responsible for controlling Trichoptera diversity at sites impacted by mining subsidence, rather than food supply. Additionally, De Moor & Ivanov (2008) described high caddis fly endemism in mountainous regions with high rainfall conditions, similar to the area in which this study was conducted, valuing Trichopterans as extremely important functional components of aquatic ecosystems and thus worthy of environmental protection.

Humpesch (1979) recorded life cycles of particular Baetidae mayflies as short as two and a half to three months in duration and found their emergence was positively correlated with an increase in temperature in laboratory experiments. These findings support the evidence that habitats in this study are possibly retaining water at an optimal interval for development and combined with elevated temperatures may be contributing to the conflicting yet not significantly elevated Ephemoptera abundance in edges and riffles at mined sites.

The significant decrease of overall EPT abundance solely in riffle habitats at mined compared to reference sites (34 vs 86) may be attributed to habitat differences, where riffles in comparison may not retain water as deep or for as long as in edges, and are thus more sensitive to increased surface water loss potentially leading to habitat loss.

The lack of other biological indices, such as taxa richness, to detect significant differences between non-mined and mined sites, could be attributed to the replacement of sensitive taxa with those of another less sensitive or tolerant taxa. This can be common in areas with increasing anthropogenic activity, indicating a loss of sensitive taxa and increase in tolerant taxa (sometimes exceeding sensitive numbers from reference sites), at sites with increasing organic pollution (Lenat, 1983). This was, however, not generally supported by lower Signal-SG scores at mined compared to non-mined sites in this study. Difference among Signal-SG scores between creeks within the same potential impact were stronger than any overall difference between them, indicating other in-stream factors are potentially driving macroinvertebrate sensitivity.

The nMDS and SIMPER results revealed more dissimilar macroinvertebrate assemblages at both edge and riffle habitats within mined compared to reference sites. This was supported by similar results found by Wright *et al* (2015) where

macroinvertebrates were modified to a greater degree in a stream impacted by mining subsidence than by mine waste discharge when compared to reference streams. SIMPER results help further understand the shift in macroinvertebrate structure by highlighting differences within the percent dominance of Trichoptera. They appear within the first five most dominant taxa at both non-mined edge and riffle habitats (5<sup>th</sup> and 3<sup>rd</sup> taxa respectively), but shift to the 9<sup>th</sup> and 11<sup>th</sup> place in mined edges and riffles respectively. No other macroinvertebrate taxa showed a strong pattern of change in dominance between mined and reference sites, highlighting the importance of understanding the physical and chemical environmental requirements of Trichoptera fauna to maintain their populations in streams.

Even though most water quality variables did not reach eco-toxic levels (ANZECC, 2000), the BEST routine attempted to find any water quality variables (collected and collated within this thesis), and physical parameters including rainfall and distance from source, best correlating thus potentially driving macroinvertebrate assemblages. The weak to moderate correlations with edge and riffle macroinvertebrates highlight the difficulty in any one particular water quality variable being associated with the presence or loss of taxa with a wide range of responses. It also indicates the potential for other unmeasured factors to be responsible for controlling assemblages. A re-run of the BEST routine including only Trichoptera fauna actually weakened the correlations for both habitats, further indicating factors other than water chemistry potentially controlling caddis fly assemblages.

Interestingly, rainfall appeared in both edge and riffle BEST routines. As rainfall is directly linked to the permanence of streams, and thus availability and longevity of microhabitats, it would further support evidence in studies by Schmera (2004) and De Moor & Ivanov (2008) that these are two important physical drivers of Trichoptera fauna.

# 4.4 Diatoms

Diatom assemblages indicate a significant decrease in abundance but significant increase in number of taxa (142 vs 361, and 6 vs 5.5 respectively) at mined compared to reference sites. Increased diatom diversity have been positively correlated in published studies (McCormick, 1990) with a reduced number of macroinvertebrate scrapers (grazers). This supports findings in this thesis, as significant reduction in scrapers occurred in edge habitats at mined compared to reference sites, most likely attributed to

the reduction in Trichoptera fauna, largely represented by scrapers. Another possibility is that the greater abundance of a smaller number of taxa at reference sites may be so well-established, they out compete additional diatom taxa from colonizing, but no literature has suggested this in the absence of significant pollution loads favoring tolerant taxa which does not occur in these catchments.

Diatoms have been extensively reported as correlating well with water quality measures (Reid *et al*, 1995; Stevenson & Pan, 1999; Sonnerman *et al*, 2001; Walsh *et al*, 2001). Further evidence supporting this theory was indicated by BEST routine on diatom assemblages which returned the strongest correlation for any biological and physico-chemical data (0.530). Even though the correlation was only moderately strong, it indicates concentrations of bicarbonate, chloride, % dissolved oxygen, pH and turbidity may be influencing diatom communities at sites.

One cannot rule out the possible link between potential loss of surface water and increased habitat fragmentation through mining induced subsidence at mined sites allowing a greater number of taxa to inhabit rocks in relatively small numbers. Their fast recovery and response to any changes in their environment is supported by their short life cycles (Reid *et al*, 1995). Dewatering of the streams may act to clear rocks of diatoms present, increasing chances for recolonization, if an optimal time for rock resubmergence could occur, but further research would need to be conducted before this could be deduced.

# 4.5 Conclusion

The aim of this study was to investigate changes in macroinvertebrate, diatoms and water quality in streams which overlie long wall coal mining and thus potentially have subsidence.

The differences in water quality and changes in flows following rainfall in streams overlying underground mining and those at reference sites is consistent with greater connection between the ground and surface waters. This supports the first hypothesis that:

- there is a loss of surface water lost beneath the creek bed and/or greater movement of water between surface and groundwater
- this is potentially occurring as a result of rock fractures creating a conduit for water to travel into the groundwater

- this is supported by the increased temperature and electrical conductivity, and increased concentrations of lithium, bicarbonate, barium, calcium, sodium, chloride and iron, at mined sites compared to reference sites
- potential faster loss of surface water following periods of rain is partially supported by water level data in mined sites compared to reference sites.

The changes in the stream macroinvertebrates observed in the current study are somewhat consistent with the one previously published study on subsistence. Reduced EPT taxa richness, reduced Trichopteran richness and significantly different diatom abundance and assemblage observed at mined sites relative to reference sites supports the second hypothesis that:

• difference's in stream macroinvertebrate and diatom assemblages can be detected between streams above long wall coal mining and similar nearby streams.

And finally, water quality variables only partially explained these differences in macroinvertebrate and diatom assemblage, so the findings within this thesis only partially support the third hypothesis:

• that correlating ecological health with water quality data can assist with determining long term impacts of stream surface water loss.

While it is impossible to rule out that the streams with mining under them had greater connection with the groundwater prior to mining, the areas were specifically chosen for their similarity.

Consequently I conclude that the most likely explanation, is that underground mining has resulted in greater ground-surface water connection and this in turn has resulted in changes to the stream invertebrate and diatom assemblages.

Further investigation into more accurate flow gauging and water level changes between mined sites compared to non-mined sites would likely improve correlations with biological communities. Especially after rain, and for extended periods after no rain, as this would help accurately determine extent of habitat reduction and possible cease to flow events impacting on macroinvertebrates and diatom communities.

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## APPENDICES

# Appendix 1.

### **Rainfall base table**

### Average monthly rainfall in mm from 1996 to 2015

	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	TOTAL
1996	109.8	91.2	64.4	42.8	232.8	103	62.6	297.8	151.6	50.4	109.8	51.4	1367.6
1997	167.2	185.2	115	5.6	106.6	143.4	55.4	40.7	120.6	81.4	40.2	40.6	1101.9
1998	121.6	58	36	135.4	259.6	142.4	100	744.2	82.2	31.2	139.4	28.2	1878.2
1999	205.6	215.4	79.8	136	49	90	326.6	51	34	334.4	51	215.4	1788.2
2000	0	45.4	360.2	53.8	47	50.2	57.8	26.8	58.8	60.4	219.8	39	1019.2
2001	141.6	243.6	216.6	64.6	123.6	24.4	157.6	70.6	21.8	49.8	79.2	23.2	1216.6
2002	82.6	432.2	245.2	74.4	77.6	34.8	52.8	23.8	10.4	14.4	25.2	97	1170.4
2003	49.6	162.6	0	176.4	417.6	66.2	57.4	49.4	5.8	68.4	111.2	51.8	1216.4
2004	49.2	155	88.2	289.6	13	11	56.6	-	54.6	276.6	85	122.6	1201.4
2005	100.6	99.4	140.6	33.8	42.2	83.6	109.8	4.4	71.2	72.8	161.6	32.8	952.8
2006	146.4	105.6	36.6	4	46	115.8	86.4	49	273.4	18.2	56.4	92.8	1030.6
2007	56	326.4	144.4	198.8	27.4	391.8	60.4	-	51.8	28.6	207.8	103.2	1596.6
2008	114.8	279.2	61.6	165	5.6	204.8	83.2	47.4	117.4	69.8	64	85.8	1298.6
2009	57.6	271.4	62.4	264	180.8	96.6	72.8	5.4	30.6	174.2	69.6	80.2	1365.6
2010	41.6	253.4	162	30.6	173.4	181.8	73.6	46.8	183.2	143.2	156.2	137.8	1583.6
2011	48.6	26.2	312.4	182	133.6	199.4	252.4	89.8	85.6	116	183.8	81.2	1711
2012	242.2	272.4	285.8	111	14.6	165.8	62.2	10.6	24.6	71.4	67.4	39.6	1367.6
2013	187	241	79.4	137.6	167.4	363.2	16	9	132.6	16.2	329.4	69	1747.8
2014	52.4	0	0	-	-	-	12.8	-	-	-	-	-	-
2015	133.2	116.4	34.8	436	122.6	72.2	58.8	95.6	51.6	33.6	110.2	70.6	1335.6
Historical Average	110.9 ata not avail	172.7	120.3	133.8	117.9	133.7	90.8	97.8	82.2	90.1	119.3	77.0	1365.8

- = data not available

## Appendix 2.

### **PERMANOVA** for Trace Metal Results

All TRACE PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem16 Data type: Distance Selection: All Normalise Resemblance: D1 Euclidean distance

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors		
Name	Туре	Levels
Mined/Non-mined	Fixed	2
Creek	Random	4
Season	Fixed	2

Excluded terms Mined/Non-minedxSeason

#### PERMANOVA table of results

	Unique				
Source		SS	MS	Pseudo-F	P(perm)
perms	P(MC)				
Mined/Non-mined	1 40	.349	40.349	9.2995	0.3372
3	0.0185				
Creek(Mined/Non-mined)	2 8.0	6776	4.3388	2.8464	0.0211
9949 `	0.0273				
Season	1 27	.296	27.296	25.036	0.0218
1129	0.0033				
Creek(Mined/Non-mined)xSeason	2 2.	1805	1.0903	0.71525	0.5958
9955 `	0.5877				
Res	41 62	.497	1.5243		
Total	47	141			

Details of the expected mean squares (EMS) for Source	EMS	( <b></b> 1/).
Mined/Non-mined	1*V(Res) + 12*V(Creek(Mined/Non-mined)) + 24*S	(Mined/Non-
mined) Creek(Mined/Non-mined) Season 24*S(Season)	1*V(Res) + 12*V(Creek(Mined/Non-mined)) 1*V(Res) + 6*V(Creek(Mined/Non-mined)xSeason)	+
Creek(Mined/Non-mined)xSeason Res	1*V(Res) + 6*V(Creek(Mined/Non-mined)xSeason) 1*V(Res)	
Construction of Pseudo-F ratio(s) from mean sq	uares	
Source	Numerator	
	Denominator	Num.df
	Den.df	
Mined/Non-mined	1*Mined/Non-mined	
	1*Creek(Mined/Non-mined)	
1	2	
Creek(Mined/Non-mined)	1*Creek(Mined/Non-	
mined)	1*Res	
2	41	
Season	1*Season	
	1*Creek(Mined/Non-mined)xSeason	
1	2	
Creek(Mined/Non-mined)xSeason	1*Creek(Mined/Non-	
mined)xSeason	1*Res	
2	41	

Estimates of components of variation		
Source	Estimate	Sq.root
S(Mined/Non-mined)	1.5004	1.2249
V(Creek(Mined/Non-mined))	0.23454	0.48429
S(Season)	1.0919	1.0449
V(Creek(Mined/Non-mined)xSeason)	-7.2341E-2	-0.26896
V(Res)	1.5243	1.2346

Li PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem2 Data type: Distance Selection: All Normalise Resemblance: D1 Euclidean distance

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors		
Name	Туре	Levels
Mined/Non-mined	Fixed	2
Creek	Random	4
Season	Fixed	2

Excluded terms Mined/Non-minedxSeason

PERMANOVA table of results

	Unique			
Source	df SS	MS	Pseudo-F	P(perm)
perms	P(MC)			
Mined/Non-mined	1 29.152	29.152	39.81	0.3362
3	0.0246			
Creek(Mined/Non-mined)	2 1.4645	0.73227	1.9548	0.1497
9957 `	0.1601			
Season	1 0.47933	0.47933	1.7552	0.3134
1124	0.3142			
Creek(Mined/Non-mined)xSeason	2 0.54619	0.2731	0.72905	0.4856
9949 `	0.4887			
Res	41 15.358	0.37459		
Total	47 47			
10(4)	T/ T/			

Details of the expected mean squares (EMS) for the model Source EMS Mined/Non-mined 1\*V(Res) + 12\*V(Creek(Mined/Non-mined)) + 24\*S(Mined/Nonmined) Creek(Mined/Non-mined) 1\*V(Res) + 12\*V(Creek(Mined/Non-mined)) 1\*V(Res) + 6\*V(Creek(Mined/Non-mined)xSeason) + Season 24\*S(Season) Creek(Mined/Non-mined)xSeason 1\*V(Res) + 6\*V(Creek(Mined/Non-mined)xSeason) 1\*V(Res) Res Construction of Pseudo-F ratio(s) from mean squares Source Numerator Denominator Num.df Den.df Mined/Non-mined 1\*Mined/Non-mined 1\*Creek(Mined/Non-mined) 2 Creek(Mined/Non-mined) 1\*Creek(Mined/Non-1\*Res mined) 41 2 1\*Season Season 1\*Creek(Mined/Non-mined)xSeason 2 1 Creek(Mined/Non-mined)xSeason 1\*Creek(Mined/Non-

mined)xSeason 2	1*Res 41	
<i>Estimates of components of variation</i> Source S(Mined/Non-mined) V(Creek(Mined/Non-mined)) S(Season) V(Creek(Mined/Non-mined)xSeason)	Estimate 1.1841 2.9806E-2 8.5929E-3 -1.6916E-2	Sq.root 1.0882 0.17265 9.2698E-2 -0.13006
V(Res)	0.37459	0.61204

# Appendix 3.

# **RAW Water Quality - Laboratory**

				WR1	WR1	WR2	WR2	WR3
				S1	S2	S1	S2	S1
Alkalinity	(Bicarbonate)	mg	CaCO3/L	19	36	24	9	21
Alkalinity	(Carbonate)	mg	CaCO3/L	2.5	<5	2.5	<5	2.5
Alkalinity	(Hydroxide)	mg	CaCO3/L	2.5	<5	2.5	<5	2.5
Alkalinity	(Total)	mg	CaCO3/L	19	36	24	9	21
Total	Barium	mg/L		0.065	0.072	0.078	0.035	0.063
Total	Calcium	mg/L		5.1	5.34	6.65	2.93	5.71
Total	Sodium	mg/L		16.1	18.9	16.3	14.4	15.8
Total	Aluminium	mg/L		0.09	0.09	0.08	0.98	0.09
Total	Iron	mg/L		0.872	0.717	0.509	1.47	0.471
Total	Manganese	mg/L		0.063	0.045	0.000	0.062	0.021
Chloride	Indityariese	mg/L		30	34	31	24	30
Sulphate				7.1	11		5.7	
Sulphate		mg/L		1.1		7.9	5.7	7.5
				WR3	WR4	WR4	WR5	WR5
				S2	S1	S2	S1	S2
Alkalinity	(Bicarbonate)	mg	CaCO3/L	18	21	19	30	10
Alkalinity	(Carbonate)	mg	CaCO3/L	<5	2.5	<5	2.5	<5
Alkalinity	(Hydroxide)	mg	CaCO3/L	<5	2.5	<5	2.5	<5
Alkalinity	(Total)	mg	CaCO3/L	18	21	19	30	10
Total	Barium	mg/L		0.032	0.066	0.024	0.083	0.034
Total	Calcium	mg/L		2.88	6.28	2.63	8.27	3.12
Total	Sodium	mg/L		14.1	15.9	14	17.4	14
Total	Aluminium	mg/L		0.92	0.06	0.43	0.04	0.43
Total	Iron	mg/L		1.54	1.16	1.16	0.763	1.43
Total	Manganese	mg/L		0.057	0.121	0.063	0.094	0.08
Chloride		mg/L		25	30	26	31	26
Sulphate		mg/L		5.6	6.5	5.5	6.8	5.4
				WR6	WR6	OH1	OH1	OH2
				S1	S2	S1	S2	S1
Alkalinity	(Bicarbonate)	mg	CaCO3/L	32	34	2.5	<5	5
Alkalinity	(Carbonate)	mg	CaCO3/L	2.5	<5	2.5	<5	2.5
Alkalinity	(Hydroxide)	mg	CaCO3/L	2.5	<5	2.5	<5	2.5
Alkalinity	(Total)	mg	CaCO3/L	32	34	2.5	<5	5
Total	Barium	mg/L		0.087	0.099	0.002	<0.005	0.01
Total	Calcium	mg/L		9.12	11.3	1.74	1.73	1.44
Total	Sodium	mg/L		17.4	24.2	13.7	13.7	12.7
Total	Aluminium	mg/L		0.04	0.07	0.19	0.29	0.12
Total	Iron	mg/L		0.709	1.1	0.569	0.82	0.466
Total	Manganese	mg/L		0.109	0.06	0.041	0.041	0.028
Chloride		mg/L		30	31	25	25	23
Sulphate		mg/L		7.3	7.5	5.7	5.7	4.3
				OH2	OH3	OH3	LR1	LR1
				S2	S1	S2	S1	S2
Alkalinity	(Bicarbonate)	mg	CaCO3/L	<5	7	6	2.5	<5
Alkalinity	(Carbonate)	mg	CaCO3/L	<5	2.5	<5	2.5	<5
		-	CaCO3/L	<5	2.5	<5	2.5	<5
Alkalinitv	(Hydroxide)	IIIU			1	1		<5
Alkalinity Alkalinity	(Hydroxide) (Total)	mg mg		<5	7	0	2.5	
Alkalinity	(Total)	mg	CaCO3/L	<5 0.009		6 0.011	2.5 0.002	
Alkalinity Total	(Total) Barium	mg mg/L		0.009	0.009	0.011	0.002	<0.005
Alkalinity Total Total	(Total) Barium Calcium	mg mg/L mg/L		0.009	0.009 1.68	0.011	0.002	<0.005 1.53
Alkalinity Total Total Total	(Total) Barium Calcium Sodium	mg mg/L mg/L mg/L		0.009 1.48 13.2	0.009 1.68 13.5	0.011 1.77 13	0.002 1.26 11.5	<0.005 1.53 13.2
Alkalinity Total Total Total Total	(Total) Barium Calcium Sodium Aluminium	mg mg/L mg/L mg/L mg/L		0.009 1.48 13.2 0.24	0.009 1.68 13.5 0.12	0.011 1.77 13 0.29	0.002 1.26 11.5 0.21	<0.005 1.53 13.2 0.34
Alkalinity Total Total Total Total Total	(Total) Barium Calcium Sodium Aluminium Iron	mg mg/L mg/L mg/L mg/L mg/L		0.009 1.48 13.2 0.24 0.594	0.009 1.68 13.5 0.12 0.425	0.011 1.77 13 0.29 0.619	0.002 1.26 11.5 0.21 0.467	<0.005 1.53 13.2 0.34 0.689
Alkalinity Total Total Total Total	(Total) Barium Calcium Sodium Aluminium	mg mg/L mg/L mg/L mg/L		0.009 1.48 13.2 0.24	0.009 1.68 13.5 0.12	0.011 1.77 13 0.29	0.002 1.26 11.5 0.21	<0.005 1.53 13.2 0.34

				LR2	LR2	LR3	LR3
				S1	S2	S1	S2
Alkalinity	(Bicarbonate)	mg	CaCO3/L	2.5	<5	2.5	<5
Alkalinity	(Carbonate)	mg	CaCO3/L	2.5	<5	2.5	<5
Alkalinity	(Hydroxide)	mg	CaCO3/L	2.5	<5	2.5	<5
Alkalinity	(Total)	mg	CaCO3/L	2.5	<5	2.5	<5
Total	Barium	mg/L		0.002	< 0.005	0.002	<0.005
Total	Calcium	mg/L		1.37	1.62	1.26	1.24
Total	Sodium	mg/L		11.3	13.5	11.4	11.7
Total	Aluminium	mg/L		0.21	0.35	0.2	0.3
Total	Iron	mg/L		0.416	0.661	0.36	0.559
Total	Manganese	mg/L		0.012	0.015	0.009	0.011
Chloride		mg/L		21	23	21	21
Sulphate		mg/L		3.3	3.1	3.4	3.1

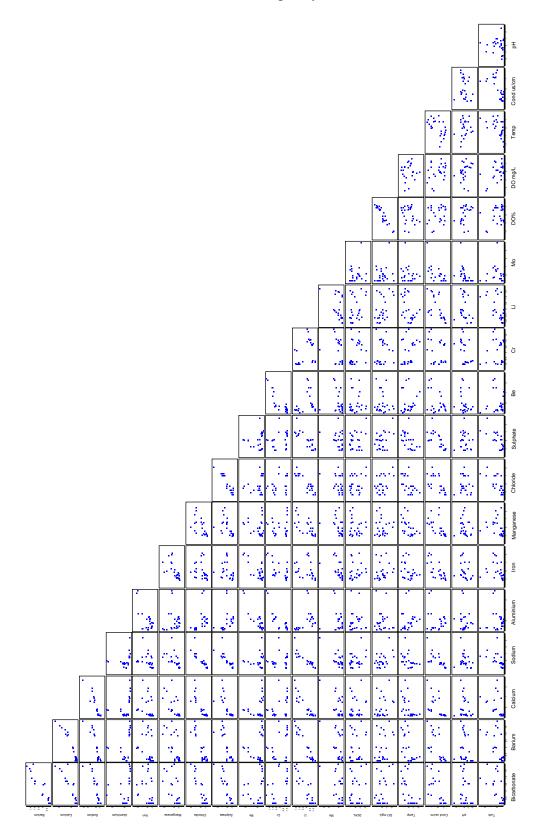
# **RAW Water Quality - Field**

	DO%	DO mg/L	Temp	Cond us/cm	рН	Turb
LR1 Spr u/s	81.9	8.89	10	98	7.14	2.16
LR2 Spr m/s	92.3	9.86	10.7	96	6.9	2.56
LR3 Spr d/s	86.8	9.435	10.95	94	8.43	2.09
OH1 Spr u/s	89.6	9.9	10.6	116	7.4	2.29
OH2 Spr m/s	87.7	9.74	9.4	111	7.45	1.475
OH3 Spr d/s	85.5	9.855	8.3	120	7.52	1.43
LR1 Win u/s	104.3	11.025	11.35	92	6.075	2.02
LR2 Win m/s	100.75	10.44	12.35	91	6.115	1.71
LR3 Win d/s	101.45	10.665	11.7	89	6.63	1.725
OH1 Win u/s	103.45	10.925	11.5	111	5.835	1.975
OH2 Win m/s	105.35	10.56	13.5	102	6.815	1.63
OH3 Win d/s	106.7	11.575	11.05	111	6.745	2.06
WR1 Spr u/s	72.5	7.76	12.2	159	7.065	6.55
WR2 Spr m/s	103.5	11.255	12.15	163.5	7.32	4.14
WR3 Spr d/s	87.2	9.13	12.95	173.5	7.21	4.66
WR4 Spr u/s	100.04	10.5	14.6	164	6.37	4.62
WR5 Spr m/s	102.9	10.145	13.95	178	7.545	3.74
WR6 Spr d/s	83.1	8.53	13.35	185.5	7.545	3.46
WR1 Win u/s	73.65	7.48	13.5	152	6.47	6.84
WR2 Win m/s	96.66	9.625	14.2	152	7.215	8.67
WR3 Win d/s	92.25	9.38	13.6	144	6.945	4.4
WR4 Win u/s	100.04	10.5	13.5	122	7.015	2.14
WR5 Win m/s	102.9	10.145	14.75	124	7.23	3.24
WR6 Win d/s	105.7	11.125	12.55	164	7.13	3.8

## Appendix 4.

## PCA & PERMANOVAS for WQ variables

Untreated Draftsman Plot for all water quality variables



## Draftsman plot correlation for all water quality variables

	Bicarbona	Barium	Calcium	Sodium	Aluminiur	Iron	Manganes	Chloride	Sulphate	Be	Cr	Li	Mo	DO%	DO mg/L	Temp	Cond us/c	рН	Turb
Bicarbona	te																		
Barium	0.949075																		
Calcium	0.906608	0.965301																	
Sodium	0.870524	0.88361	0.913117																
Aluminiur	-0.28942	-0.3256	-0.38935	-0.30209															
Iron	0.303267	0.300182	0.24343	0.313813	0.651725														
Manganes	0.633456	0.667249	0.651731	0.524996	-0.02477	0.635004													
Chloride	0.916879	0.916366	0.849346	0.848049	-0.40145	0.232971	0.607922												
Sulphate	0.874095	0.835684	0.73722	0.785934	-0.25964	0.260952	0.52856	0.935827											
Be	-0.16512	-0.23374	-0.31459	-0.19884	0.875134	0.718217	0.137362	-0.28297	-0.1345										
Cr	-0.21181	-0.27145	-0.30831	-0.10893	0.818919	0.626711	-0.04433	-0.30403	-0.1873	0.850327									
Li	0.914277	0.963891	0.920371	0.875927	-0.35164	0.295136	0.688971	0.918344	0.845055	-0.21738	-0.229								
Mo	0.520908	0.546838	0.645648	0.750465	0.152368	0.545406	0.41653	0.360307	0.348631	0.197821	0.334045	0.511127							
DO%	-0.21864	-0.18546	-0.11363	-0.33004	0.020501	-0.08341	0.150188	-0.26495	-0.28738	0.007544	-0.3042	-0.30903	-0.19599						
DO mg/L	-0.38383	-0.37267	-0.28613	-0.4535	0.121592	-0.12538	0.027855	-0.43879	-0.42529	0.105588	-0.14755	-0.43611	-0.23092	0.934227					
Temp	0.619774	0.627269	0.566158	0.433086	-0.12107	0.353073	0.551969	0.589613	0.53514	-0.08462	-0.31596	0.466938	0.193487	0.253885	-0.06158				
Cond us/c	0.729679	0.708778	0.636817	0.69224	0.243874	0.719152	0.596255	0.654327	0.709624	0.412081	0.300585	0.65949	0.613406	-0.18922	-0.30996	0.541273			
рН	0.148068	0.216441	0.205548	0.226673	0.172332	0.272755	0.113252	0.075446	0.064209	0.219367	0.484965	0.259376	0.466657	-0.33455	-0.27939	-0.16456	0.233512		
Turb	0.652807	0.649335	0.487929	0.490325	0.007578	0.275384	0.262976	0.700228	0.740212	0.01536	-0.03873	0.592679	0.084321	-0.33345	-0.49965	0.562175	0.676845	0.033719	

## PCA

Principal Component Analysis

Data worksheet Name: Data11 Data type: Environmental Sample selection: All Variable selection: All

#### Eigenvalues

PČ	Eigenvalues	%Variation	Cum.%Variation
1	7.94	46.7	46.7
2	3.72	21.9	68.6
3	1.95	11.5	80.1
4	1.3	7.6	87.7
5	0.565	3.3	91.1

#### Eigenvectors

3				
s in the linea	r combinatio	ons of variat	oles making	up PC's)
PC1	PC2	PC3	PC4	PC5
0.339	0.068	-0.011	0.016	-0.016
0.326	0.107	-0.049	-0.233	-0.050
0.325	0.023	-0.174	-0.136	-0.327
-0.070	-0.449	0.211	0.137	-0.074
0.165	-0.398	0.244	-0.052	0.001
0.252	-0.058	0.281	-0.288	0.305
0.330	0.130	-0.031	0.123	0.065
0.316	0.073	-0.016	0.238	-0.003
-0.025	-0.468	0.214	0.105	0.000
0.334	0.074	-0.104	-0.049	0.159
0.217	-0.222	-0.157	-0.433	-0.425
-0.090	0.113	0.529	-0.386	0.106
0.225	0.094	0.413	0.055	0.073
0.293	-0.216	0.130	0.120	-0.119
0.079	-0.251	-0.375	-0.234	0.726
0.243	0.005	0.063	0.571	0.143
0.000	0.439	0.300	-0.052	0.050
	s in the linea PC1 0.339 0.326 0.325 -0.070 0.165 0.252 0.330 0.316 -0.025 0.334 0.217 -0.090 0.225 0.293 0.079 0.243	$ \begin{array}{c cccc} \text{s in the linear combinatio} \\ \hline PC1 & PC2 \\ 0.339 & 0.068 \\ 0.326 & 0.107 \\ 0.325 & 0.023 \\ -0.070 & -0.449 \\ 0.165 & -0.398 \\ 0.252 & -0.058 \\ 0.330 & 0.130 \\ 0.316 & 0.073 \\ -0.025 & -0.468 \\ 0.334 & 0.074 \\ 0.217 & -0.222 \\ -0.090 & 0.113 \\ 0.225 & 0.094 \\ 0.293 & -0.216 \\ 0.079 & -0.251 \\ 0.243 & 0.005 \\ \end{array} $	$ \begin{array}{c ccccc} \text{s in the linear combinations of variab} \\ \hline PC1 & PC2 & PC3 \\ \hline 0.339 & 0.068 & -0.011 \\ \hline 0.326 & 0.107 & -0.049 \\ \hline 0.325 & 0.023 & -0.174 \\ -0.070 & -0.449 & 0.211 \\ \hline 0.165 & -0.398 & 0.244 \\ \hline 0.252 & -0.058 & 0.281 \\ \hline 0.330 & 0.130 & -0.031 \\ \hline 0.316 & 0.073 & -0.016 \\ -0.025 & -0.468 & 0.214 \\ \hline 0.334 & 0.074 & -0.104 \\ \hline 0.217 & -0.222 & -0.157 \\ -0.090 & 0.113 & 0.529 \\ \hline 0.225 & 0.094 & 0.413 \\ \hline 0.293 & -0.216 & 0.130 \\ \hline 0.079 & -0.251 & -0.375 \\ \hline 0.243 & 0.005 & 0.063 \\ \end{array} $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $

#### Principal Component Scores

Principal Compo	nent Scores				
Sample	SCORE1	SCORE2	SCORE3	SCORE4	SCORE5
LR1 Spr u/s	-2.46	-0.685	-1.68	0.33	-6.04E-2
LR2 Spr m/s	-2.56	-0.383	-0.869	0.15	-0.288
LR3 Spr d/s	-2.77	-1.03	-2.2	-0.503	1.56
OH1 Spr u/s	-1.2	-0.976	-1.11	-0.142	0.278
OH2 Spr m/s	-2.2	-1.14	-1.68	-0.424	0.205
OH3 Spr d/s	-2.24	-1.56	-1.98	-0.194	0.226
LR1 Win u/s	-3.34	1.6	0.906	-9.1E-2	-0.812
LR2 Win m/s	-3.36	1.67	0.862	-2.02E-2	-0.811
LR3 Win d/s	-3.44	1.56	0.298	-0.244	-0.208
OH1 Win u/s	-1.86	1.6	1.23	0.107	-1.11
OH2 Win m/s	-2.19	1.39	1.02	-0.477	0.244

OH3 Win d/s	-2.15	1.57	0.275	-0.268	-0.15
WR1 Spr u/s	4.34	0.416	-2.07	2.29	-0.237
WR2 Spr m/s	0.434	-4.31	1.78	-7.25E-2	-0.11
WR3 Spr d/s	1.05	-4.22	1.23	0.938	-0.294
WR4 Spr u/s	0.643	-1.64	2.26	1.05	-0.439
WR5 Spr m/s	1.02	-2.79	1.68	-0.399	0.8
WR6 Spr d/s	5.94	-0.864	-2.23	-2.23	-1.97
WR1 Win u/s	2.55	1.34	-0.106	2.19	-2.03E-2
WR2 Win m/s	2.87	2.12	0.153	1.89	0.968
WR3 Win d/s	1.6	1.97	-0.251	0.823	-1.36E-2
WR4 Win u/s	2.39	1.17	1.14	-1.57	1.04
WR5 Win m/s	3.25	1.72	0.704	-1.6	0.647
WR6 Win d/s	3.67	1.49	0.634	-1.54	0.548

*Outputs* Plot: Graph2

### WQ PERMANOVAS

All Other Variables PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem3 Data type: Distance Selection: All Normalise Resemblance: D1 Euclidean distance

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors

Name	Туре	Levels
Impact/Control	Fixed	2
Creek	Random	4
Position	Random	3
Season	Fixed	2

Excluded terms Position(Creek(Impact/Control))xSeason

PERMANOVA table of results

	Unique			
Source	df SS	MS	Pseudo-F	P(perm)
perms	P(MC)			. ,
Impact/Control	1 107.56	107.56	15.152	0.3369
3	0.01			
Season	1 15.961	15.961	6.5963	0.0868
801	0.041			
Creek(Impact/Control)	2 14.198	7.0991	1.9382	0.1028
8979	0.1356			
Impact/ControlxSeason	1 6.2485	6.2485	2.5824	0.1435
801	0.1706			
Position(Creek(Impact/Control))	8 29.302	3.6628	1.0144	0.4918
9931	0.4863			
Creek(Impact/Control)xSeason	2 4.8394	2.4197	0.67011	0.6156
9947	0.6121			
Res	8 28.887	3.6109		
Total	23 207			

 Details of the expected mean squares (EMS) for the model

 Source
 EMS

 Impact/Control
 1\*V(Res) + 2\*V(Position(Creek(Impact/Control))) +

 6\*V(Creek(Impact/Control)) + 12\*S(Impact/Control)
 1\*V(Res) + 3\*V(Creek(Impact/Control)xSeason) +

12*S(Season) Creek(Impact/Control) 6*V(Creek(Impact/Control)) Impact/ControlxSeason 6*S(Impact/ControlxSeason) Position(Creek(Impact/Control)) Creek(Impact/Control)xSeason Res	1*V(Res) + 2*V(Position(Creek(Impact/Control))) + 1*V(Res) + 3*V(Creek(Impact/Control)xSeason) + 1*V(Res) + 2*V(Position(Creek(Impact/Control))) 1*V(Res) + 3*V(Creek(Impact/Control)xSeason) 1*V(Res)
<i>Construction of Pseudo-F ratio(s) from mean squar</i> Source	Numerator Denominator Num.df
Impact/Control	Den.df 1*Impact/Control 1*Creek(Impact/Control)
1	2
Season	1*Season
1	1*Creek(Impact/Control)xSeason
Creek(Impact/Control)	1*Creek(Impact/Control)
	1*Position(Creek(Impact/Control))
2	8
Impact/ControlxSeason	1*Impact/ControlxSeason 1*Creek(Impact/Control)xSeason
1	2
Position(Creek(Impact/Control))	1*Position(Creek(Impact/Control))
	1*Res
8 Creek(Impact/Control)xSeason	8 1*Creek(Impact/Control)xSeason
Creek(impact/Control)/Season	1*Res
2	8
Estimates of components of variation Source S(Impact/Control) S(Season) V(Creek(Impact/Control)) S(Impact/ControlxSeason) V(Position(Creek(Impact/Control))) V(Creek(Impact/Control)xSeason) V(Res)	EstimateSq.root8.3722.89341.12841.06230.572720.756780.638140.798842.5962E-20.16113-0.39707-0.630133.61091.9002

# Appendix 5.

## RAW macroinvertebrate data

						LR1 Winter	LR1 Winter	LR1 Winter	LR1 Winter
						14313 Edg	14314 Edg	14315 Edg	14316 Edg
Class	Order	Family	SG	Genus	Stage	1	2	3	4
Annelida	Oligochaeta	Oligochaeta indeterminate	_	unassessed genus	Non-Insect	4		5	
Annelida Arthropoda	Oligochaeta Acarina	Opisthopora indeterminate Acarina indeterminate		unassessed genus indeterminate genus	Non-Insect Non-Insect				
Arthropoda	Acarina	Aturidae	10	Axonopsella	Non-Insect				
Arthropoda	Acarina	Hydrodromidae	7	Hydrodroma	Non-Insect				
Arthropoda	Acarina	Hygrobatidae	7	Corticacarus	Non-Insect			1	
Arthropoda	Acarina	Hygrobatidae	8	Caenobates	Non-Insect				
Arthropoda Arthropoda	Acarina Acarina	Hygrobatidae Hygrobatidae	8	Coaustraliobates Australiobates	Non-Insect Non-Insect				
Arthropoda	Acarina	Limnesiidae	7	Limnesia	Non-Insect				
Arthropoda	Acarina	Limnocharidae	9	Limnochares	Non-Insect				
Arthropoda	Acarina	Mideopsidae	7	Gretacarus	Non-Insect				
Arthropoda	Acarina	Momoniidae	9	Momoniella	Non-Insect				
Arthropoda	Acarina	Oxidae Oxidae	7	Oxus	Non-Insect				
Arthropoda Arthropoda	Acarina Acarina	Oxidae	8	Flabellifrontipoda Frontipoda	Non-Insect Non-Insect				
Arthropoda	Acarina	Oxidae		indeterminate genus	Non-Insect				
Arthropoda	Acarina	Torrenticolidae	10	Torrenticola	Non-Insect				
Arthropoda	Acarina	Unionicolidae	6	Unionicola	Non-Insect				
Arthropoda	Acarina	Unionicolidae	7	Koenikea	Non-Insect				2
Arthropoda	Acarina	Unionicolidae	7	Recifella	Non-Insect		2		4
Arthropoda Arthropoda	Acarina Amphipoda	Unionicolidae Chiltoniidae	7	indeterminate genus Austrochiltonia	Non-Insect Non-Insect				
Arthropoda	Amphipoda Amphipoda	Chiltoniidae		indeterminate genus	Non-Insect				
Arthropoda	Coleoptera	Dytiscidae	10	Barretthydrus	Larva				
Arthropoda	Coleoptera	Dytiscidae	5	Chostonectes	Adult				
Arthropoda	Coleoptera	Dytiscidae	5	Chostonectes	Larva				
Arthropoda	Coleoptera	Dytiscidae	5	Rhantus	Larva				
Arthropoda	Coleoptera	Dytiscidae	6	Antiporus	Larva				
Arthropoda Arthropoda	Coleoptera Coleoptera	Dytiscidae Dytiscidae	6	Necterosoma Necterosoma	Adult Larva				
Arthropoda	Coleoptera	Dytiscidae	8	Sternopriscus	Adult				
Arthropoda	Coleoptera	Dytiscidae	8	Sternopriscus	Larva				
Arthropoda	Coleoptera	Dytiscidae		indeterminate genus	Larva				
Arthropoda	Coleoptera	Elmidae	6	Notriolus	Adult				
Arthropoda	Coleoptera	Elmidae	6	Simsonia	Adult				
Arthropoda	Coleoptera	Elmidae	6	Simsonia	Larva			1	
Arthropoda Arthropoda	Coleoptera Coleoptera	Elmidae Elmidae	7	Kingolus Kingolus	Adult Larva			1	
Arthropoda	Coleoptera	Elmidae	8	Austrolimnius	Adult				
Arthropoda	Coleoptera	Elmidae	8	Austrolimnius	Larva			4	
Arthropoda	Coleoptera	Elmidae		indeterminate genus	Larva				
Arthropoda	Coleoptera	Gyrinidae	9	Macrogyrus	Adult				
Arthropoda	Coleoptera	Gyrinidae	9	Macrogyrus	Larva				
Arthropoda Arthropoda	Coleoptera Coleoptera	Hydrochidae Hydrophilidae	5	Hydrochus Paracymus	Adult Adult				
Arthropoda	Coleoptera	Hydrophilidae	7	Berosus	Adult				
Arthropoda	Coleoptera	Hydrophilidae	7	Berosus	Larva				
Arthropoda	Coleoptera	Hydrophilidae		indeterminate genus	Larva				
Arthropoda	Coleoptera	Psephenidae	7	Sclerocyphon	Larva				
Arthropoda	Coleoptera	Scirtidae		shin	Larva			1	
Arthropoda Arthropoda	Decapoda	Atyidae	<u>6</u> 5	Paratya Dacubalaa	Non-Insect	7	7	3	9
Arthropoda Arthropoda	Diptera Diptera	Ceratopogonidae	6	Dasyhelea Bezzia	Larva Larva				1
Arthropoda	Diptera	Ceratopogonidae Ceratopogonidae	7	Nilobezzia	Larva				
Arthropoda	Diptera	Ceratopogonidae		indeterminate genus	Larva				
Arthropoda	Diptera	Chironomidae		indeterminate genus	Larva				
Arthropoda	Diptera	Chironomidae		S.F. Aphroteniiae	Larva	2	1	2	
Arthropoda Arthropoda	Diptera	Chironomidae		S.F. Chironominae	Larva	9	1	4	10
Arthropoda Arthropoda	Diptera Diptera	Chironomidae Chironomidae		S.F. Diamesinae S.F. Orthocladiinae	Larva Larva	2	5	2	7
Arthropoda	Diptera	Chironomidae		S.F. Podonominae	Larva	2	J	2	/
Arthropoda	Diptera	Chironomidae		S.F. Tanypodinae	Larva	7	1	6	1
Arthropoda	Diptera	Culicidae	6	Aedes	Larva				
Arthropoda	Diptera	Culicidae		indeterminate genus	Larva				
Arthropoda	Diptera	Dixidae	9	Dixa	Larva				
Arthropoda Arthropoda	Diptera Diptera	Empididae Muscidae		alpha alpha	Larva Larva				
Arthropoda	Diptera	Muscidae		alpha indeterminate genus	Larva				
Arthropoda	Diptera	Simuliidae	4	Simulium	Larva				
Arthropoda	Diptera	Simuliidae	6	Austrosimulium	Larva				
Arthropoda	Diptera	Simuliidae	6	Austrosimulium	Larva				
Arthropoda	Diptera	Simuliidae	6	Cnephia	Larva				
Arthropoda	Diptera	Simuliidae		indeterminate genus	Larva				
Arthropoda Arthropoda	Diptera Diptera	Thaumaleidae Thaumaleidae		alpha indeterminate genus	Larva Larva				
Arthropoda		Tipulidae	8	Antocha	Larva				1
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Arthropoda	Diptera Diptera			beta					
Arthropoda Arthropoda	Diptera Diptera Diptera	Tipulidae Tipulidae Tipulidae			Larva Larva				

LR1 Spring 169191 Edg 1	LR1 Spring 169195 Edg 2	LR1 Spring 169196 Edg 3	LR1 Spring 169197 Edg 4	LR2 Winter 14321 Edg 1	LR2 Winter 14322 Edg 2	LR2 Winter 14323 Edg 3	LR2 Winter 14324 Edg 4	LR2 Spring 169199 Edg 1	LR2 Spring 169203 Edg 2	LR2 Spring 169204 Edg 3	LR2 Spring 169205 Edg 4	LR3 Winter 14329 Edg 1	LR3 Winter 14330 Edg 2	LR3 Winter 14331 Edg 3
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LR3	LR3	LR3	LR3	LR3	OH1	OH1	OH1	OH1	OH1	OH1	OH1	OH1	OH2	OH2
Winter 14332	Spring 169207	Spring 169211	Spring 169212	Spring 169213	Winter 14289	Winter 14290	Winter 14291	Winter 14292	Spring 169167	OH1 Spring 169171	Spring 169172	Spring 169173	Winter 14297	Winter 14298
Edg 4	Edg 1	Edg 2	Edg 3	Edg 4	Edg 1	Edg 2	Edg 3	Edg 4	Edg 1	Edg 2	Edg 3	Edg 4	Edg 1	Edg 2
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OH2 Winter 14299 Edg 3	OH2 Winter 14300 Edg 4	OH2 Spring 169175 Edg 1	OH2 Spring 169179 Edg 2	OH2 Spring 169180 Edg 3	OH2 Spring 169181 Edg 4	OH3 Winter 14305 Edg 1	OH3 Winter 14306 Edg 2	OH3 Winter 14307 Edg 3	OH3 Winter 14308 Edg 4	OH3 Spring 169183 Edg 1	OH3 Spring 169187 Edg 2	OH3 Spring 169188 Edg 3	OH3 Spring 169189 Edg 4	WR1 Winter 14241 Edg 1
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1          1	WR1 Winter 14242 Edg 2	WR1 Winter 14243 Edg 3	WR1 Winter 14244 Edg 4	WR1 Spring 14530 Edg 1	WR1 Spring 14531 Edg 2	3	WR1 Spring 14533 Edg 4	WR2 Winter 14249 Edg 1	WR2 Winter 14250 Edg 2	WR2 Winter 14251 Edg 3	WR2 Winter 14252 Edg 4	WR2 Spring 14538 Edg 1	WR2 Spring 14539 Edg 2	WR2 Spring 14540 Edg 3	WR2 Spring 14541 Edg 4
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WR4 Spring 14557 Edg 4	WR5 Winter 14273 Edg 1	WR5 Winter 14274 Edg 2	WR5 Winter 14275 Edg 3	WR5 Winter 14276 Edg 4	WR5 Spring 14562 Edg 1	WR5 Spring 14563 Edg 2	WR5 Spring 14564 Edg 3	WR5 Spring 14565 Edg 4	WR6 Winter 14281 Edg 1	WR6 Winter 14282 Edg 2	WR6 Winter 14283 Edg 3	WR6 Winter 14284 Edg 4	WR6 Spring 14570 Edg 1	WR6 Spring 14571 Edg 2
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WR6 Spring 14572	WR6 Spring 14573	WREF Winter 14337	WREF Winter 14338	WREF Winter 14339	WREF Winter 14340	LR1 Winter 14309	LR1 Winter 14310	LR1 Winter 14311	LR1 Winter 14312	LR1 Spring 169190	LR1 Spring 169192	LR1 Spring 169193	LR1 Spring 169194	LR2 Winter 14317
Edg 3	Edg 4	Edg 1	Edg 2	Edg 3	Edg 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1
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LR2 Winter 14318 Riff	LR2 Winter 14319 Riff	LR2 Winter 14320 Riff	LR2 Spring 169198 Riff	LR2 Spring 169200 Riff	LR2 Spring 169201 Riff	LR2 Spring 169202 Riff	LR3 Winter 14325 Riff	LR3 Winter 14326 Riff	LR3 Winter 14327 Riff	LR3 Winter 14328 Riff	LR3 Spring 169206 Riff	LR3 Spring 169208 Riff	LR3 Spring 169209 Riff	LR3 Spring 169210 Riff
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9			1		1	1	1		2 1	3	1		2	1
1	1	32	37	20	25	35	26	27	21	32	25	36	3	1
31 24	42	26	86 2	31 2 2	62 3	50 3	26 3	24 1	22 4 2	30 10 9	24 4	32 1 9	9	10 1 2
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OH1 Winter 14285 Riff	OH1 Winter 14286 Riff	OH1 Winter 14287 Riff	OH1 Winter 14288 Riff	OH1 Spring 169166 Riff	OH1 Spring 169168 Riff	OH1 Spring 169169 Riff	OH1 Spring 169170 Riff	OH2 Winter 14293 Riff	OH2 Winter 14294 Riff	OH2 Winter 14295 Riff	OH2 Winter 14296 Riff	OH2 Spring 169174 Riff 1	OH2 Spring 169176 Riff	OH2 Spring 169177 Riff 3
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126	139	528 4	453	109	111	168	239	111 1	184	374 28	289 25	44 92	42 63	27 7
21	14	5	4	3	2	5	4	1	1	20	25	1		2
1		3	2	6	14	6	6	1		1	1		1	2
		48	31	22	29	122	117					2		1
		40	31	22	27	122	117					2		1
	1	10	5					18	13	23	17		2	4
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OH2 Spring 169178 Riff 4	OH3 Winter 14301 Riff 1	OH3 Winter 14302 Riff 2	OH3 Winter 14303 Riff 3	OH3 Winter 14304 Riff 4	OH3 Spring 169182 Riff 1	OH3 Spring 169184 Riff 2	OH3 Spring 169185 Riff 3	OH3 Spring 169186 Riff 4	WR1 Winter 14237 Riff 1	WR1 Winter 14238 Riff 2	WR1 Winter 14239 Riff 3	WR1 Winter 14240 Riff 4	WR1 Spring 14534 Riff 1	WR1 Spring 14535 Riff 2
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2		1					1		1	1	1	1		
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47	6	21	26	26	9	10	38	1 62	16	19	13	16	1	
40 5 6	26 1	55 2 3	36 3	53 5	4	6	6	18	161	167	51	60		1
			1				1	2		4	1	1		
4		2		3	12	6	1	1	8	10	11	6	12	16
5			3	5	18	22	14	15	1	1				

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WR1 Spring 14536	WR1 Spring 14537	WR2 Winter 14245	WR2 Winter 14246	WR2 Winter 14247	WR2 Winter 14248	WR2 Spring 14542	WR2 Spring 14543	WR2 Spring 14544	WR2 Spring 14545	WR3 Winter 14253	WR3 Winter 14254	WR3 Winter 14255	WR3 Winter 14256	WR3 Spring 14546
Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1
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3	18													
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		108
WR5	WR5	WR5
Vinter	Winter	Winter
4270	14271	14272
Riff	Riff	Riff

WR3 Spring	WR3 Spring	WR3 Spring	WR4 Winter	WR4 Winter	WR4 Winter	WR4 Winter	WR4 Spring	WR4 Spring	WR4 Spring	WR4 Spring	WR5 Winter	WR5 Winter	WR5 Winter	WR5 Winter
14547 Riff 2	14548 Riff 3	14549 Riff 4	14261 Riff 1	14262 Riff 2	14263 Riff 3	14264 Riff 4	14558 Riff 1	14559 Riff 2	14560 Riff 3	14561 Riff 4	14269 Riff 1	14270 Riff 2	14271 Riff 3	14272 Riff 4
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7	24	12	15	14	9	11	11	7	41	27	39	1 23	174	95
129	19 1	16	12 68 3	13 77 5	5 47	5 40	10	5	34 2	34 1	15 87 1	19 73 1	8 222 1	5 138 1
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16	18	13	11	10	2	4	10	1	13	14	1			2

WR5 Spring 14566 Riff 1	WR5 Spring 14567 Riff 2	WR5 Spring 14568 Riff 3	WR5 Spring 14569 Riff 4	WR6 Winter 14277 Riff 1	WR6 Winter 14278 Riff 2	WR6 Winter 14279 Riff 3	WR6 Winter 14280 Riff 4	WR6 Spring 14574 Riff 1	WR6 Spring 14575 Riff 2	WR6 Spring 14576 Riff 3	WR6 Spring 14577 Riff 4
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						Winter 14313	Winter 14314	Winter 14315	Winter 14316
						Edg	Edg	Edg	Edg
Class	Order	Family	SG	Genus	Stage	1	2	3	4
Arthropoda	Diptera	Tipulidae Baetidae	· ·	nu Cloeon	Larva Nymph				
Arthropoda Arthropoda	Ephemeroptera Ephemeroptera	Baetidae	6	Offadens	Nymph				
Arthropoda	Ephemeroptera	Baetidae	9	Bungona	Nymph				
Arthropoda	Ephemeroptera	Baetidae	9	Centroptilum	Nymph	1			
Arthropoda	Ephemeroptera	Baetidae		indeterminate genus	Nymph				
Arthropoda	Ephemeroptera	Caenidae Caenidae	6	Tasmanocoenis indeterminate genus	Nymph				
Arthropoda Arthropoda	Ephemeroptera Ephemeroptera	Leptophlebiidae	10	Koorrnonga	Nymph Nymph	19	8	2	2
Arthropoda	Ephemeroptera	Leptophlebiidae	10	Nousia	Nymph	17	0	2	~
Arthropoda	Ephemeroptera	Leptophlebiidae	10	Ulmerophlebia	Nymph				
Arthropoda	Ephemeroptera	Leptophlebiidae	8	Atalophlebia	Nymph		1		
Arthropoda Arthropoda	Ephemeroptera	Leptophlebiidae	4	indeterminate genus	Nymph	2	2	1	2
Arthropoda	Hemiptera Hemiptera	Corixidae Corixidae	4	Sigara indeterminate genus	Adult Nymph				
Arthropoda	Hemiptera	Gelastocoridae	5	Nerthra	Nymph				
Arthropoda	Hemiptera	Gerridae	7	Tenagogerris	Adult				
Arthropoda	Hemiptera	Gerridae	7	Tenagogerris	Nymph				
Arthropoda	Hemiptera	Gerridae		indeterminate genus	Nymph				
Arthropoda	Hemiptera	Hemiptera indeterminate Hydrometridae	5	indeterminate genus Hydrometra	Nymph Adult				
Arthropoda Arthropoda	Hemiptera Hemiptera	Hydrometridae	5	Hydrometra Hydrometra	Nymph				
Arthropoda	Hemiptera	Micronectidae	6	Micronecta	Adult				1
Arthropoda	Hemiptera	Nepidae	6	Ranatra	Adult				
Arthropoda	Hemiptera	Notonectidae	6	Enithares	Adult				
Arthropoda	Hemiptera	Notonectidae	6	Enithares	Nymph				
Arthropoda Arthropoda	Hemiptera Hemiptera	Notonectidae Notonectidae	7	Anisops Anisops	Adult Nymph				
Arthropoda	Hemiptera	Notonectidae	9	Paranisops	Adult				
Arthropoda	Hemiptera	Notonectidae	9	Paranisops	Nymph				
Arthropoda	Hemiptera	Notonectidae		indeterminate genus	Nymph				
Arthropoda	Hemiptera	Pleidae	4	Paraplea	Adult				
Arthropoda Arthropoda	Hemiptera Hemiptera	Pleidae Veliidae	7	indeterminate genus Microvelia	Nymph Adult				
Arthropoda	Hemiptera	Veliidae		Drepanovelia	Adult				
Arthropoda	Hemiptera	Veliidae		indeterminate genus	Nymph				
Arthropoda	Lepidoptera	Crambidae		indeterminate genus	Larva				
Arthropoda	Megaloptera	Corydalidae	7	Archichauliodes	Larva				
Arthropoda	Odonata	Aeshnidae	6	Adversaeschna	Nymph				
Arthropoda Arthropoda	Odonata Odonata	Aeshnidae Argiolestidae	7	Austroaeschna Austroargiolestes	Nymph Nymph		1		
Arthropoda	Odonata	Austrocorduliidae	9	Austrocordulia	Nymph				
Arthropoda	Odonata	Cordulephyidae	9	Cordulephya	Nymph				
Arthropoda	Odonata	Corduliidae	5	Hemicordulia	Nymph				
Arthropoda	Odonata	Corduliidae		indeterminate genus	Nymph				
Arthropoda Arthropoda	Odonata Odonata	Diphlebiidae Epiproctophora indetermin	8	Diphlebia indeterminate genus	Nymph Nymph				
Arthropoda	Odonata	Gomphidae	6	Austrogomphus	Nymph				
Arthropoda	Odonata	Gomphidae	8	Hemigomphus	Nymph				
Arthropoda	Odonata	Gomphidae		indeterminate genus	Nymph				
Arthropoda	Odonata	Isostictidae	5	Rhadinosticta	Nymph				
Arthropoda	Odonata	Lestidae	6	Austrolestes	Nymph	1			1
Arthropoda Arthropoda	Odonata Odonata	Synlestidae Synlestidae	7	Synlestes indeterminate genus	Nymph Nymph	1			1
Arthropoda	Odonata	Synthemistidae	7	Eusynthemis	Nymph				
Arthropoda	Odonata	Telephlebiidae		indeterminate genus	Nymph				
Arthropoda	Odonata	Zygoptera indeterminate		indeterminate genus	Nymph				
Arthropoda	Plecoptera	Gripopterygidae	7	Dinotoperla	Nymph	-	<u>^</u>	-	-
Arthropoda Arthropoda	Plecoptera Plecoptera	Gripopterygidae Gripopterygidae	8	Leptoperla Newmanoperla	Nymph Nymph	3	2	5	1
Arthropoda	Plecoptera	Gripopterygidae	9	Illiesoperla	Nymph				
Arthropoda	Plecoptera	Gripopterygidae		indeterminate genus	Nymph				-
Arthropoda	Plecoptera	Plecoptera indeterminate		indeterminate genus	Nymph				
Arthropoda	Trichoptera	Atriplectididae	8	Atriplectides	Larva				
Arthropoda	Trichoptera	Calamoceratidae	8	Anisocentropus	Larva				
Arthropoda Arthropoda	Trichoptera Trichoptera	Calocidae Calocidae/Helicophidae	9	Caenota Genus H	Larva Larva				
Arthropoda	Trichoptera	Conoesucidae	8	Coenoria	Larva				
Arthropoda	Trichoptera	Conoesucidae		Hampa	Larva				
Arthropoda	Trichoptera	Conoesucidae		indeterminate genus	Larva				
Arthropoda	Trichoptera	Ecnomidae	6	Ecnomus	Larva				
Arthropoda Arthropoda	Trichoptera Trichoptera	Ecnomidae Ecnomidae	9	Ecnomina Daternomina	Larva Larva		1		
Arthropoda Arthropoda	Trichoptera	Helicophidae	8	Alloecella	Larva				
Arthropoda	Trichoptera	Helicopsychidae	10	Helicopsyche	Larva				1
Arthropoda	Trichoptera	Hydrobiosidae	6	Ulmerochorema	Larva				
Arthropoda	Trichoptera	Hydrobiosidae	8	Taschorema	Larva	1			
Arthropoda	Trichoptera	Hydrobiosidae	9	Apsilochorema	Larva				<u> </u>
Arthropoda Arthropoda	Trichoptera Trichoptera	Hydrobiosidae Hydropsychidae	8	indeterminate genus Asmicridea	Larva				
Anthropoua	Trichoptera	nyuropsychiuae	Ő	Asinichuea	Larva		I	1	L

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LR1 Spring 169191 Edg 1	LR1 Spring 169195 Edg 2	LR1 Spring 169196 Edg 3	LR1 Spring 169197 Edg 4	LR2 Winter 14321 Edg 1	LR2 Winter 14322 Edg 2	LR2 Winter 14323 Edg 3	LR2 Winter 14324 Edg 4	LR2 Spring 169199 Edg 1	LR2 Spring 169203 Edg 2	LR2 Spring 169204 Edg 3	LR2 Spring 169205 Edg 4	LR3 Winter 14329 Edg 1	LR3 Winter 14330 Edg 2	LR3 Winter 14331 Edg 3
			1	4 3		4	63	1	1	1		2 1	5	2
6	3	6		3	5		1	1	1	2	2	2	10	
4	1	2			1				1				1	
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			1											
				1										
1				1									1	
1	1													
5	1	2	4	3		1					2	1 4		1
													1	
				1	1								2	1
												1		

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LR3 Winter 14332 Edg 4	LR3 Spring 169207 Edg 1	LR3 Spring 169211 Edg 2	LR3 Spring 169212 Edg 3	LR3 Spring 169213 Edg 4	OH1 Winter 14289 Edg 1	OH1 Winter 14290 Edg 2	OH1 Winter 14291 Edg 3	OH1 Winter 14292 Edg 4	OH1 Spring 169167 Edg 1	OH1 Spring 169171 Edg 2	OH1 Spring 169172 Edg 3	OH1 Spring 169173 Edg 4	OH2 Winter 14297 Edg 1	OH2 Winter 14298 Edg 2
11	2 1 4	7	2	3	73			2				2	1	30 2
1	6	4	1	1	1			1		1		1	4	2
2	1	2	1	1	1 2			2	2	1		2		2
			2	1				1	2	2		1 2		
														1
					6			1	3	1			1	1
										1	2		4	
										2				
										1				
	1							2	1	2	1			
			1									1		
	1				1			6	3	1		1		
2		7				4	1	1	1		1			3
													1	
						6 1	8							
2	1	2			2 1	1 19	1 1 28	1 1 1	3	1	2 8 1	4		2
		2				1	1	1						

OH2 Winter 14299	OH2 Winter 14300	OH2 Spring 169175	OH2 Spring	OH2 Spring 169180	OH2 Spring 169181 Edg	OH3 Winter 14305 Edg	OH3 Winter 14306	OH3 Winter 14307 Edg	OH3 Winter 14308 Edg	OH3 Spring 169183	OH3 Spring 169187	OH3 Spring 169188	OH3 Spring 169189	WR1 Winter 14241
Edg 3	Edg 4	Edg 1	Spring 169179 Edg 2	Edg 3	Edg 4	Edg 1	Edg 2	Edg 3	Edg 4	Edg 1	Edg 2	Edg 3	Edg 4	Edg 1
								11	6	11		4	1	
8	23	4 2		2		4	1	4		1	2		1	
18 3	11 8	1	7	7	1	1 26	1 12	2 26	2	20	1 1 2			1
4	0	1	4	3	7	20	3	20	1	20	2			
2	1 3	2			3	6	1	2	3	1 1				1 2
									1					
		2 7		1	29 4					51		1	1	
2					1	1		1	3			1		1
	2	2	1		1		1			1 13	2	1		
		1 13	10		17		1			1		2 11	1	
		13	10		17					1			3	
				1										
		1								2			1	
						1								
3			2		2		1							
	2										1			
2		1		1	1			3						
1		1	2	2	1 1	1	2			1	1	1		
		1	2	2						5	1	1		
1								2						
			3		4	2		1		3		1	2	
						1								
						1								
1			1	1		1								
	1		1	2		1		7	5		1			
	1		1					1	5		1			
												1		

WR1 Winter 14242 Edg	WR1 Winter 14243 Edg	WR1 Winter 14244 Edg	WR1 Spring 14530 Edg	WR1 Spring 14531 Edg	WR1 Spring 14532 Edg	WR1 Spring 14533 Edg	WR2 Winter 14249 Edg	WR2 Winter 14250 Edg 2	WR2 Winter 14251 Edg	WR2 Winter 14252 Edg	WR2 Spring 14538 Edg	WR2 Spring 14539 Edg	WR2 Spring 14540 Edg	WR2 Spring 14541 Edg
2 2	3	<u>Eug</u> 4	£ug 1	2 2	Eug 3	4 2	 1	2 2	3	<u>Eug</u> 4	Uy 1	2 2	Bug 3	<u>Eug</u> 4
							1							
1											1 3		4	4
1	2			1			6 2	5		6				
1 2	4	1 2	3	13	2		1	2 1	1	1	7	2	6	2
								3	1		2			4
4	5	1	1	1	1		1	1 12	1	2	3 1	1		1
				1										
				1					1				2	
			2								1		2	
				1										
							1		3		1	2	1	2
										1				
			19	8	1	6					9	7	2	1
	1			1										
				4									2	
	1													
							3	1					1	
									1					
								3						
							5			3				
							3			3				
	1	1	1										1	
						1							4	
				3								1		

WR3 Winter 14257	WR3 Winter 14258	WR3 Winter 14259	WR3 Winter 14260	WR3 Spring 14550	WR3 Spring 14551	WR3 Spring 14552	WR3 Spring 14553	WR4 Winter 14265	WR4 Winter 14266	WR4 Winter 14267	WR4 Winter 14268	WR4 Spring 14554	WR4 Spring 14555	WR4 Spring 14556
Edg 1	Edg 2	Edg 3	Edg 4	Edg 1	Edg 2	Edg 3	Edg 4	Edg 1	Edg 2	Edg 3	Edg 4	Edg 1	Edg 2	Edg 3
1														
								54	205	53	44	74	29	2
11 2	3 2		1	2			3	68 7	62 6	20 8	55	35	13	1
1	2		4	3	12	32	37	2	13	2	1	3		8
				2			1	10	9	3	3		1	
2 12	1 9	1	7	2				1 5	1 15	11	3	1 1	1	3
							1	1				1		1 2
												5		1
														1
						1		1		2				
	1			1	2	1 2	1			7		4 8	1	2
1				-	5	15	2					9		
						15				1	1	,		
					4							6	7	57
												1		1
													1	
										1				
2														
6 1	2	1	1	5					4	1	2		1	6
			1											
			1						1					
	1					6	10	1	1				2	
	1					0							2	
1			1						1					
2				1										

1	1	6
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WR4 Spring 14557 Edg 4	WR5 Winter 14273 Edg 1	WR5 Winter 14274 Edg 2	WR5 Winter 14275 Edg 3	WR5 Winter 14276 Edg 4	WR5 Spring 14562 Edg 1	WR5 Spring 14563 Edg 2	WR5 Spring 14564 Edg 3	WR5 Spring 14565 Edg 4	WR6 Winter 14281 Edg 1	WR6 Winter 14282 Edg 2	WR6 Winter 14283 Edg 3	WR6 Winter 14284 Edg 4	WR6 Spring 14570 Edg 1	WR6 Spring 14571 Edg 2
18 5	1 2	16 25	1	11		1	1 1 3		1 1 3 7 1	29 6 5	1 4 28 4	1	3	24 7
2									5		1	1	4	1
	1	1		3					4	1		2 12	2 2 1	1
2				1	1	2								1
			1				1			1		1		
									1			1	1	1
18			1		1	15	3	2					11	1 17
			1			1 3		1					1	
									1			2 4		
												5		
										1	2	1	2	1
													1	
										1	1			

WR6 Spring 14572	WR6 Spring 14573	WREF Winter 14337	WREF Winter 14338	WREF Winter 14339	WREF Winter 14340	LR1 Winter 14309	LR1 Winter 14310	LR1 Winter 14311	LR1 Winter 14312	LR1 Spring 169190	LR1 Spring 169192	LR1 Spring 169193	LR1 Spring 169194	LR2 Winter 14317
Edg 3	Edg 4	Edg 1	Edg 2	Edg 3	Edg 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1
3	4						1		1					
4								1	1					
				2	1					2	2	2	2	
2	1	1		2		2	1	2	3	2				
					6									
1	4	10										1		
		1										1		
1	3													
			1											
28	10													
1	2													
	2												1	
	1												1	
													1	
1														
	2										1			
1	2					10	6	3	8	3	6	3	2	13
						5	1		3		1	1	2	2
					1						1 1			
						1	2					1	1	2
										1 1	3	1 2		
						1 1	1 2	1		1	1	7	8	23

LR2 Winter 14318 Riff 2	LR2 Winter 14319 Riff 3	LR2 Winter 14320 Riff 4	LR2 Spring 169198 Riff 1	LR2 Spring 169200 Riff 2	LR2 Spring 169201 Riff 3	LR2 Spring 169202 Riff 4	LR3 Winter 14325 Riff 1	LR3 Winter 14326 Riff 2	LR3 Winter 14327 Riff 3	LR3 Winter 14328 Riff 4	LR3 Spring 169206 Riff 1	LR3 Spring 169208 Riff 2	LR3 Spring 169209 Riff 3	LR3 Spring 169210 Riff 4
											6	1		
1														
1														
9	8	11	1	2	2	5	22	29	17	24	6	8	6	4
2							2							
				1					1					
1	2	1	2	10	4	7	1				1	6	3	
		1	1	1		6			1	2		1		1
1														
42	180	1 190	7 105	1 188	68	2 229	56	2 39	5 16	5 28	3 51	51	20	19

OH1 Winter 14285	OH1 Winter 14286	OH1 Winter 14287	OH1 Winter 14288	OH1 Spring 169166	OH1 Spring 169168	OH1 Spring 169169	OH1 Spring 169170	OH2 Winter 14293	Winter 14294	OH2 Winter 14295	OH2 Winter 14296	OH2 Spring 169174	169176	Spring 169177
Riff 1	Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3
								3	4	3		8	16	17
55	9 1	1	2										1	4
1														7
	1													1
			2			1		1						1
	1													
	8													
		10	11					14	14	11	17	1		
1	1													
										1	1			
														1
		7	2		2	3	5	1	4	2			1	
5	3	3	2	6 3	6 3	11 3	8							4
		14	13	6	6	12	26	2	1 2 4	5	3	1	2	1 2
	1	3	1	4	11 1	10	6	1 5	5		8	1	1	1
		1						10	7	6	3	8	1	5

OH2 Spring 169178 Riff	OH3 Winter 14301 Riff	OH3 Winter 14302 Riff	OH3 Winter 14303 Riff	OH3 Winter 14304 Riff	OH3 Spring 169182 Riff	OH3 Spring 169184 Riff	OH3 Spring 169185 Riff	OH3 Spring 169186 Riff	WR1 Winter 14237 Riff	WR1 Winter 14238 Riff	WR1 Winter 14239 Riff	WR1 Winter 14240 Riff	WR1 Spring 14534 Riff	WR1 Spring 14535 Riff
4	1	2	3	4	1	2	3	4	1	2	3	4	1	2
19	12	44	27	36	75	39	25	38		1	1	2		
	3	8		2		1	2	4						
													1	
7							1							
5		2					1							
1								1						
·														
	1	1	2	1			1	1	1	5		4		
1	1	2					1			1				
	2													
	3	5	18	22	42	49	6	21						
											1			
	1			1				2						
2	4	11 1	2	10 1	1	3 2	1	1						
							2							
3	1	4	10	2 8	198	200	129	4 177	1					

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WR1 Spring 14536 Riff	WR1 Spring 14537 Riff	WR2 Winter 14245 Riff	WR2 Winter 14246 Riff	WR2 Winter 14247 Riff	WR2 Winter 14248 Riff	WR2 Spring 14542 Riff	WR2 Spring 14543 Riff	WR2 Spring 14544 Riff	WR2 Spring 14545 Riff	WR3 Winter 14253 Riff	WR3 Winter 14254 Riff	WR3 Winter 14255 Riff	WR3 Winter 14256 Riff	WR3 Spring 14546 Riff
3	4	1	2	3	4	1	2	3	4	1	2	3	4	1
		93	84	42	48	19	20	30	36	41	42	54	66	10
	2	3	14	6	2					4	2		2	18
1														1
1	1	2	3	6	9					5	9	13	17	
1		1	2	3	7						1		1	
						2	2							2
			1					1				1		
										1		2	1	
		3	3	1	11	20	19	9	9	1		2	29	

														122
WR3 Spring 14547	WR3 Spring 14548 Riff	Spring 14549	Winter 14261	Winter 14262	Winter 14263	Winter 14264	WR4 Spring 14558 Riff	14559	WR4 Spring 14560 Riff	Spring 14561	Winter 14269	14270	WR5 Winter 14271	WR5 Winter 14272 Riff
Riff 2	3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	кііі 1	Riff 2	3	Riff 4	Riff 1	Riff 2	Riff 3	4
14	9	7	18	18	7	9		1	1		2	3	4	5
9	2	2	1					1 2	7					
9	3	2	1			2	2	2	7	6			2	
														<u> </u>
					22							4		
			3		32	22						1		

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		3		32	22			1
		2		7	4			3
1								
	21	3	3					

1

WR5 Spring 14566 Riff 1	WR5 Spring 14567 Riff 2	WR5 Spring 14568 Riff 3	WR5 Spring 14569 Riff 4	WR6 Winter 14277 Riff 1	WR6 Winter 14278 Riff 2	WR6 Winter 14279 Riff 3	WR6 Winter 14280 Riff 4	WR6 Spring 14574 Riff 1	WR6 Spring 14575 Riff 2	WR6 Spring 14576 Riff 3	WR6 Spring 14577 Riff 4
4		4	2	30	37	43	60	1		9	2
							2		1		
				1			1				
									1		
								2	1		2
			1				3			1	
			1				3	5	7	10	3

						LR1	LR1	LR1	LR1
						Winter	Winter	Winter	Winte
						14313	14314	14315	1431
						Edg	Edg	Edg	Edg
Class	Order	Family	SG	Genus	Stage	1	2	3	4
Arthropoda	Trichoptera	Hydropsychidae	8	Diplectrona	Larva				
Arthropoda	Trichoptera	Hydropsychidae		Austropsyche	Larva				
Arthropoda	Trichoptera	Hydropsychidae		indeterminate genus	Larva				
Arthropoda	Trichoptera	Hydroptilidae	4	Hydroptila	Larva				
Arthropoda	Trichoptera	Hydroptilidae	4	Orphninotrichia	Larva				
Arthropoda	Trichoptera	Hydroptilidae	6	Hellyethira	Larva	11	8	1	3
Arthropoda	Trichoptera	Hydroptilidae	8	Oxyethira	Larva				
Arthropoda	Trichoptera	Hydroptilidae		indeterminate genus	Larva				
Arthropoda	Trichoptera	Leptoceridae	6	Notalina	Larva	6	18	4	18
Arthropoda	Trichoptera	Leptoceridae	7	Oecetis	Larva				
Arthropoda	Trichoptera	Leptoceridae	8	Triplectides	Larva			4	
Arthropoda	Trichoptera	Leptoceridae	9	Lectrides	Larva				
Arthropoda	Trichoptera	Leptoceridae		indeterminate genus	Larva				
Arthropoda	Trichoptera	Philorheithridae	7	Kosrheithrus	Larva				
Arthropoda	Trichoptera	Philorheithridae		indeterminate genus	Larva				
Arthropoda	Trichoptera	Tasimiidae		indeterminate genus	Larva				
Arthropoda	Trichoptera	Trichoptera indeterminate		indeterminate genus	Larva	4	8		
Arthropoda	Trombidioidea	Johnstonianidae		alpha	Non-Insect				
Mollusca	Hygrophila	Planorbidae		indeterminate genus	Non-Insect				
Mollusca	Veneroida	Sphaeriidae	5	Musculium	Non-Insect				
Mollusca	Veneroida	Sphaeriidae		indeterminate genus	Non-Insect				
Vematoda	Indeterminate	Nematoda indeterminate		unassessed genus	Non-Insect				
Vemertea	Indeterminate	Nemertea indeterminate		alpha	Non-Insect				
Platyhelminthes	Tricladidia	Dugesiidae	4	Cura	Non-Insect				
Porifera	Indeterminate	Spongillidae		alpha	Non-Insect				

LR1	LR1	LR1	LR1	LR2	LR3	LR3	LR3							
Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter
169191	169195	169196	169197	14321	14322	14323	14324	169199	169203	169204	169205	14329	14330	14331
Edg														
1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
							1							
		2		1		1	5	2	1		1	2		1
		2					5	2				2		
6	5		2	4	4	15	19	6				2		
0	5		2			15	17	0				2		
1	3	4												
	5													
											1			

LR3	LR3	LR3	LR3	LR3	OH1	OH2	OH2							
Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter
14332	169207	169211	169212	169213	14289	14290	14291	14292	169167	169171	169172	169173	14297	14298
Edg														
4	1	2	3	4	1	2	3	4	1	2	3	4	1	2
1				1										
	11	94	3	4										
		1	1						1					
1								4		8		4	2	5
														1
										1	1			
			1	1					2		2	19		
						1	2					1		
							2							

OH2	OH2	OH2	OH2	OH2	OH2	OH3	WR1							
Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter
14299	14300	169175	169179	169180	169181	14305	14306	14307	14308	169183	169187	169188	169189	14241
Edg														
3	4	1	2	3	4	1	2	3	4	1	2	3	4	1
				ĺ								ĺ		
		3		3	1	1		2	2	2	1		5	
						1			1		9			
3			1			5		1	1	4	1		2	
		1											1	
9	4	49	10	3	16	10	15	3	10	10	31	34	7	3
								1						
								1						
		2	3	2		1				1			5	
						1								
							1							
							3	1						
1														

WR1	WR2													
Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring
14242	14243	14244	14530	14531	14532	14533	14249	14250	14251	14252	14538	14539	14540	14541
Edg														
2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1														
											1	1	3	1
											1			1
									2			2	1	
7	1		2			3	2			1	3	4	2	
							1	1	1					
											1			
			1	1			1				2	3	4	3
				2										

WR3	WR3	WR3	WR3	WR3	WR3	WR3	WR3	WR4	WR4	WR4	WR4	WR4	WR4	WR4
Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring
14257 Edg	14258	14259 Edg	14260	14550	14551 Edg	14552	14553 Edg	14265	14266 Edg	14267	14268	14554	14555	14556
Edg 1	Edg 2	<u>Eug</u>	Edg 4	Edg 1	2 Edg	Edg 3	4 Edg	Edg 1	Edy 2	Edg 3	Edg 4	Edg 1	Edg 2	Edg 3
	2	3	4		2	J	4		2		4		2	<u> </u>
	1													
	2				2	1		2					1	
										1				
2			11			2				1				
2						2				1				
1	1		2		2	4	6	2		4			15	3
	1													
				5	1		66						20	1
				5	1								20	
										4	5			
			1											
							1							
											1			

1	2	6
I	4	υ

WR4	WR5	WR6	WR6	WR6	WR6	WR6	WR6							
Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring
14557	14273	14274	14275	14276	14562	14563	14564	14565	14281	14282	14283	14284	14570	14571
Edg														
4	1	2	3	4	1	2	3	4	1	2	3	4	1	2
1										1	1		2	2
							1				1			
2												2	8	
1	1						1						10	2
1							1						10	2
1		2									3			
		2									3			
		1		2										
				2										

WR6	WR6	WREF	WREF	WREF	WREF	LR1	LR2							
Spring	Spring	Winter	Spring	Spring	Spring	Spring	Winter							
14572	14573	14337	14338	14339	14340	14309	14310	14311	14312	169190	169192	169193	169194	14317
Edg	Edg	Edg	Edg	Edg	Edg	Riff								
3	4	1	2	3	4	1	2	3	4	1	2	3	4	1
4				1										
									2	3	4		2	13
											_			
											1			
5					1					1				
				1										
4	1					3	5					1		3
4	1					3	5					1		3
							1							

LR2	LR3													
Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring
14318	14319	14320	169198	169200	169201	169202	14325	14326	14327	14328	169206	169208	169209	169210
Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4	Riff 1	Riff 2	Riff 3	Riff 4
2	3	4		2	3	4		2	3	4		2	3	4
														2
	19	25	2	2	3	9	1				14	12		2
	17	23	2	2	5	7	1				14	12		2
9							1			1	14	16		
1														
							1							
														<u> </u>
														<u> </u>

OH1	OH2												
Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring
14286	14287	14288	169166	169168	169169	169170	14293	14294	14295	14296	169174	169176	169177
Riff													
2	3	4	1	2	3	4	1	2	3	4	1	2	3
			3		1	1							
	1	5											
					1		2	1		1			27
								1					
										1			2
											1		2
				1									
	1	1	4	10	15	11			1		24	11	19

OH2	OH3	WR1	WR1	WR1	WR1	WR1	WR1							
Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring
169178	14301	14302	14303	14304	169182	169184	169185	169186	14237	14238	14239	14240	14534	14535
Riff														
4	1	2	3	4	1	2	3	4	1	2	3	4	1	2
									1		1	5		
31														
	1													
			1		1		1							
								0						
	1				1	1	4	2						
23					20	21	106	102						
23					20	21	100	102						

WR1	WR1	WR2	WR3	WR3	WR3	WR3	WR3							
Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring
14536	14537	14245	14246	14247	14248	14542	14543	14544	14545	14253	14254	14255	14256	14546
Riff														
3	4	1	2	3	4	1	2	3	4	1	2	3	4	1
			4		5							32	24	1
						1								
		2	1					1	1		1			
														1
1	1					3	2	2	1	1		5	1	1
						5	2	2		1		J	- 1	

WR3	WR3	WR3	WR4	WR5	WR5	WR5	WR5							
Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter
14547	14548	14549	14261	14262	14263	14264	14558	14559	14560	14561	14269	14270	14271	14272
Riff														
2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
													4	4
1									1	1				
	2	2								2	1	2		
						1					1	2	1	1

WR5	WR5	WR5	WR5	WR6							
Spring	Spring	Spring	Spring	Winter	Winter	Winter	Winter	Spring	Spring	Spring	Spring
14566	14567	14568	14569	14277	14278	14279	14280	14574	14575	14576	14577
Riff											
1	2	3	4	1	2	3	4	1	2	3	4
						2	2				
											4
		3	1					15	24	43	30

### Appendix 6.

### **Macroinvertebrate PERMANOVAS**

Edge Taxa Richness PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem2 Data type: Similarity Selection: All Transform: Square root Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors		
Name	Туре	Levels
Impact/Control	Fixed	2
Creeks	Random	4
Position	Random	3
Season	Fixed	2

#### PERMANOVA table of results

	Unique		
Source	df SS	MS	Pseudo-F
	P(perm)	perms	P(MC)
Impact/Control	1 1318.4	1318.4	1.6186
0.6732	3 0.323		
Season	1 83.384	83.384	3.6882
0.1854	801	0.1742	
Creeks(Impact/Control)	2 1629.1	814.53	1.9222
0.2074	8963	0.1979	
Impact/ControlxSeason	1 19.894	19.894	0.87995
0.4463	799	0.4413	
Position(Creeks(Impact/Control))	8 3390	423.75	7.697
0.0001	9936	0.0001	
Creeks(Impact/Control)xSeason	2 45.217	22.608	0.43174
0.6959	9953	0.6868	
Position(Creeks(Impact/Control))xSeason	8 418.93	52.366	0.95118
0.4919	9944	0.4786	
Res	72 3963.9	55.054	
Total	95 10869		

Details of the expected mean squares (EMS) for the model	
Source	EMS
Impact/Control	1*V(Res) +
8*V(Position(Creeks(Impact/Control))) + 24*V(Creeks(Impac	t/Control)) + 48*S(Impact/Control)
Season	1*V(Res) +
4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creek	(Impact/Control)xSeason) + 48*S(Season)
Creeks(Impact/Control)	1*V(Res) +
8*V(Position(Creeks(Impact/Control))) + 24*V(Creeks(Impac	t/Control))
Impact/ControlxSeason	1*V(Res) +
4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creek	(s(Impact/Control)xSeason) +
24*S(Impact/ControlxSeason)	
Position(Creeks(Impact/Control))	1*V(Res) +
8*V(Position(Creeks(Impact/Control)))	
Creeks(Impact/Control)xSeason	1*V(Res) +
4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creek	(Impact/Control)xSeason)
Position(Creeks(Impact/Control))xSeason	1*V(Res) +
4*V(Position(Creeks(Impact/Control))xSeason)	
Res	1*V(Res)
	· ·
Construction of Pseudo-F ratio(s) from mean squares	
Source	Numerator

Source

Numerator Denominator

-0.33628

6.7887

-1.5747 -0.81976

7.4199

-0.11308 46.087

-2.4798

-0.672

55.054

Impact/Control	Num.df Den.df 1*Impact/Control 1*Creeks(Impact/Control)
1 Season	2 1*Season 1*Creeks(Impact/Control)xSeason
1 Creeks(Impact/Control)	2 1*Creeks(Impact/Control) 1*Position(Creeks(Impact/Control))
2 Impact/ControlxSeason	8 1*Impact/ControlxSeason 1*Creeks(Impact/Control)xSeason
1 Position(Creeks(Impact/Control))	2 1*Position(Creeks(Impact/Control)) 1*Res
8 Creeks(Impact/Control)xSeason	72 1*Creeks(Impact/Control)xSeason 1*Position(Creeks(Impact/Control))xSeason
2 Position(Creeks(Impact/Control))xSeason	8 1*Position(Creeks(Impact/Control))xSeason 1*Res
8	72
Estimates of components of variation Source S(Impact/Control) S(Season) V(Creeks(Impact/Control))	Estimate Sq.root 10.498 3.24 1.2662 1.1252 16.283 4.0352

S(Impact/Control)) S(Creeks(Impact/Control)) S(Impact/ControlxSeason) V(Position(Creeks(Impact/Control))) V(Creeks(Impact/Control)xSeason) V(Position(Creeks(Impact/Control))xSeason) V(Res)

# Edge EPT Individual PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem3 Data type: Similarity Selection: All Transform: Square root Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors		
Name	Туре	Levels
Impact/Control	Fixed	2
Creeks	Random	4
Position	Random	3
Season	Fixed	2

PERMANOVA table of results

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	Unique		
Source	df SS	MS	Pseudo-F
	P(perm)	perms	P(MC)
Impact/Control	1 1452.3	1452.3	0.4565
0.6603	3 0.7358		
Season	1 1003.8	1003.8	1.3326
0.3571	799	0.3393	
Creeks(Impact/Control)	2 6362.6	3181.3	1.4942

0.2433 Impact/ControlxSeason 0.4672 Position(Creeks(Impact/Control))	8947 0.2262 1 679.14 679.14 0.90155 800 0.4953 8 17033 2129.1 4.3956
0.0001	9896 0.0001
Creeks(Impact/Control)xSeason	2 1506.6 753.31 0.93703
0.4857 Position(Creeks(Impact/Control))xSeason	9948 0.4868 8 6431.4 803.93 1.6597
0.0252	9883 0.0372
Res	72 34875 484.37
Total	95 69344
Details of the expected mean squares (EMS) for the model	
Source	EMS
Impact/Control	1*V(Res) +
8*V(Position(Creeks(Impact/Control))) + 24*V(Creeks(Impact/Co	
Season 4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creeks(In	1*V(Res) + mpact/Control)xSeason) + 48*S(Season)
Creeks(Impact/Control)	1*V(Res) +
8*V(Position(Creeks(Impact/Control))) + 24*V(Creeks(Impact/Co	
Impact/ControlxSeason 4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creeks(In	1*V(Res) + mpact/Control)xSeason) +
24*S(Impact/ControlxSeason)	mpace control x ceasony +
Position(Creeks(Impact/Control))	1*V(Res) +
8*V(Position(Creeks(Impact/Control)))	1*\/(Baa) +
Creeks(Impact/Control)xSeason 4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creeks(In	1*V(Res) + mpact/Control)xSeason)
Position(Creeks(Impact/Control))xSeason	1*V(Res) +
4*V(Position(Creeks(Impact/Control))xSeason)	
Res	1*V(Res)
Construction of Pseudo-F ratio(s) from mean squares	
Source	Numerator
	Denominator Num.df
	Den.df
Impact/Control	1*Impact/Control
	1*Creeks(Impact/Control)
1	2
Season	1*Season
	1*Creeks(Impact/Control)xSeason
1	2
Creeks(Impact/Control)	1*Creeks(Impact/Control)
	1*Position(Creeks(Impact/Control))
2	8
Impact/ControlxSeason	1*Impact/ControlxSeason
·	1*Creeks(Impact/Control)xSeason
1	2
Position(Creeks(Impact/Control))	1*Position(Creeks(Impact/Control))
	1*Res
0	72
8 Creeks(Impact/Control)xSeason	1 <sup>2</sup> 1*Creeks(Impact/Control)xSeason
	1*Position(Creeks(Impact/Control))xSeason
	0
2 Position(Creeks(Impact/Control))xSeason	8 1*Position(Creeks(Impact/Control))xSeason
	1*Res
0	70
8	72
Estimates of components of variation	
Source	Estimate Sq.root
S(Impact/Control)	-36.022 -6.0018 5.2191 2.2845
S(Season) V(Creeks(Impact/Control))	5.2191 2.2845 43.841 6.6213
S(Impact/ControlxSeason)	-3.0903 -1.7579
V(Position(Creeks(Impact/Control)))	205.59 14.339
V(Creeks(Impact/Control)xSeason)	-4.2188 -2.054

79.889	8.9381
484.37	22.009

**Edge** EPT Taxa PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem4 Data type: Similarity Selection: All Transform: Square root Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors		
Name	Туре	Levels
Impact/Control	Fixed	2
Creeks	Random	4
Position	Random	3
Season	Fixed	2

PERMANOVA table of results

Impact/Control

	Unique		
Source	df SS	MS	Pseudo-F
	P(perm)	perms	P(MC)
Impact/Control	1 4123.8	4123.8	6.3174
0.3312	3 0.0154		
Season	1 524.6	524.6	1.2915
0.3572	801	0.3445	
Creeks(Impact/Control)	2 1305.6	652.78	0.45714
0.8845	8948	0.8644	
Impact/ControlxSeason	1 493.97	493.97	1.2161
0.3636	800	0.3742	
Position(Creeks(Impact/Control))	8 11424	1427.9	3.0867
0.0001	9860	0.0001	
Creeks(Impact/Control)xSeason	2 812.39	406.2	0.72671
0.6281	9948	0.6592	
Position(Creeks(Impact/Control))xSeason	8 4471.6	558.95	1.2082
0.1691	9861	0.2136	
Res	72 33308	462.61	
Total	95 56464		

Unique

1\*Impact/Control

Details of the expected mean squares (EMS) for the model EMS Source 1\*V(Res) + Impact/Control 8\*V(Position(Creeks(Impact/Control))) + 24\*V(Creeks(Impact/Control)) + 48\*S(Impact/Control) 1\*V(Res) + Season 4\*V(Position(Creeks(Impact/Control))xSeason) + 12\*V(Creeks(Impact/Control)xSeason) + 48\*S(Season) Creeks(Impact/Control) 1\*V(Res) + 8\*V(Position(Creeks(Impact/Control))) + 24\*V(Creeks(Impact/Control)) Impact/ControlxSeason 1\*V(Res) + 4\*V(Position(Creeks(Impact/Control))xSeason) + 12\*V(Creeks(Impact/Control)xSeason) + 24\*S(Impact/ControlxSeason) Position(Creeks(Impact/Control)) 8\*V(Position(Creeks(Impact/Control))) 1\*V(Res) + Creeks(Impact/Control)xSeason 1\*V(Res) + 4\*V(Position(Creeks(Impact/Control))xSeason) + 12\*V(Creeks(Impact/Control)xSeason) Position(Creeks(Impact/Control))xSeason 1\*V(Res) + 4\*V(Position(Creeks(Impact/Control))xSeason) Res 1\*V(Res) Construction of Pseudo-F ratio(s) from mean squares Source Numerator Denominator Num.df Den.df

#### 1\*Creeks(Impact/Control)

2 1\*Season 1\*Creeks(Impact/Control)xSeason

2 1\*Creeks(Impact/Control) 1\*Position(Creeks(Impact/Control))

8 1\*Impact/ControlxSeason 1\*Creeks(Impact/Control)xSeason

2 1\*Position(Creeks(Impact/Control)) 1\*Res

72 1\*Creeks(Impact/Control)xSeason 1\*Position(Creeks(Impact/Control))xSeason

8 1\*Position(Creeks(Impact/Control))xSeason 1\*Res

72

Estimates of components of variation		
Source	Estimate	Sq.root
S(Impact/Control)	72.314	8.5038
S(Season)	2.4668	1.5706
V(Creeks(Impact/Control))	-32.299	-5.6832
S(Impact/ControlxSeason)	3.6573	1.9124
V(Position(Creeks(Impact/Control)))	120.67	10.985
V(Creeks(Impact/Control)xSeason)	-12.729	-3.5678
V(Position(Creeks(Impact/Control))xSeason)	24.084	4.9075
V(Res)	462.61	21.508

#### Riffle Taxa Richness PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem1 Data type: Similarity Selection: All Transform: Square root Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors		
Name	Туре	Levels
Impact/Control	Fixed	2
Creeks	Random	4
Position	Random	3
Season	Fixed	2

PERMANOVA table of results

<u>.</u>	Unique	MO	
Source	df SS	MS	Pseudo-F
	P(perm)	perms	P(MC)
Impact/Control	1 2087.4	2087.4	8.3133
0.3337	3 0.0864		
Season	1 37.946	37.946	0.73748
0.4964	801	0.4893	
Creeks(Impact/Control)	2 502.17	251.09	1.7549

#### 1 Season

1 Creeks(Impact/Control)

## Impact/ControlxSeason

Position(Creeks(Impact/Control))

#### 8

Creeks(Impact/Control)xSeason

### 2

Position(Creeks(Impact/Control))xSeason

#### 8

:5)

2

0.2244 Impact/ControlxSeason 0.1296 Position(Creeks(Impact/Control)) 0.0001 Creeks(Impact/Control)xSeason 0.7542 Position(Creeks(Impact/Control))xSeason 0.0001 Res Total	8987       0.2265         1       315.46       315.46       6.1308         801       0.1011       143.08       5.3704         9938       0.0001       2       102.91       51.454       0.30341         9945       0.7789       8       1356.7       169.59       6.3654         9932       0.0001       72       1918.2       26.642         95       7465.4       7465.4       7465.4       7465.4
Details of the expected mean squares (EMS) for the model Source Impact/Control 8*V(Position(Creeks(Impact/Control))) + 24*V(Creeks(Impact/Co Season 4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creeks(Im Creeks(Impact/Control) 8*V(Position(Creeks(Impact/Control))) + 24*V(Creeks(Impact/Co Impact/ControlxSeason 4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creeks(Im 24*S(Impact/ControlxSeason) Position(Creeks(Impact/Control)) 8*V(Position(Creeks(Impact/Control)) 8*V(Position(Creeks(Impact/Control)) 8*V(Position(Creeks(Impact/Control)) Creeks(Impact/Control)xSeason 4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creeks(Im Position(Creeks(Impact/Control))xSeason) 4*V(Position(Creeks(Impact/Control))xSeason) 8*V(Position(Creeks(Impact/Control))xSeason) 4*V(Position(Creeks(Impact/Control))xSeason) 8*V(Position(Creeks(Impact/Control))xSeason) 8*V(Position(Creeks(Impact/Control))xSeason)	1*V(Res) + mpact/Control)xSeason) + 48*S(Season) 1*V(Res) + ontrol)) 1*V(Res) + mpact/Control)xSeason) + 1*V(Res) + 1*V(Res) +
<i>Construction of Pseudo-F ratio(s) from mean squares</i> Source	Numerator Denominator
Impact/Control	Num.df Den.df 1*Impact/Control 1*Creeks(Impact/Control)
1 Season	2 1*Season 1*Creeks(Impact/Control)xSeason
1 Creeks(Impact/Control)	2 1*Creeks(Impact/Control) 1*Position(Creeks(Impact/Control))
2 Impact/ControlxSeason	8 1*Impact/ControlxSeason 1*Creeks(Impact/Control)xSeason
1 Position(Creeks(Impact/Control))	2 1*Position(Creeks(Impact/Control)) 1*Res
8 Creeks(Impact/Control)xSeason	72 1*Creeks(Impact/Control)xSeason 1*Position(Creeks(Impact/Control))xSeason
2 Position(Creeks(Impact/Control))xSeason	8 1*Position(Creeks(Impact/Control))xSeason 1*Res
8	72
Estimates of components of variation Source S(Impact/Control) S(Season) V(Creeks(Impact/Control)) S(Impact/ControlxSeason) V(Position(Creeks(Impact/Control))) V(Creeks(Impact/Control)xSeason)	EstimateSq.root38.2566.1851-0.28141-0.530484.50032.1214113.316614.5553.815-9.8445-3.1376

V(Position(Creeks(Impact/Control))xSeason) V(Res)

**Riffle** EPT Individual PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem5 Data type: Similarity Selection: All Transform: Square root Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors		
Name	Туре	Levels
Impact/Control	Fixed	2
Creeks	Random	4
Position	Random	3
Season	Fixed	2

PERMANOVA table of results

	Unique		
Source	df SS	MS	Pseudo-F
	P(perm)	perms	P(MC)
Impact/Control	1 9474.3	9474.3	10.593
0.3373	3 0.0061		
Season	1 1271.8	1271.8	1.6277
0.2715	798	0.2661	
Creeks(Impact/Control)	2 1788.8	894.42	0.2122
0.9275	8880	0.9705	
Impact/ControlxSeason	1 2708.1	2708.1	3.4658
0.122	800	0.0768	
Position(Creeks(Impact/Control))	8 33720	4215.1	12.342
0.0001	9905	0.0001	
Creeks(Impact/Control)xSeason	2 1562.8	781.38	0.93645
0.472	9950	0.4794	
Position(Creeks(Impact/Control))xSeason	8 6675.3	834.41	2.4432
0.0001	9885	0.0007	
Res	72 24589	341.52	
Total	95 81791		

Details of the expected mean squares (EMS) for the model Source EMS Impact/Control 1\*V(Res) + 8\*V(Position(Creeks(Impact/Control))) + 24\*V(Creeks(Impact/Control)) + 48\*S(Impact/Control) 1\*V(Res) + Season 4\*V(Position(Creeks(Impact/Control))xSeason) + 12\*V(Creeks(Impact/Control)xSeason) + 48\*S(Season) Creeks(Impact/Control) 1\*V(Res) + 8\*V(Position(Creeks(Impact/Control))) + 24\*V(Creeks(Impact/Control)) Impact/ControlxSeason 1\*V(Res) + 4\*V(Position(Creeks(Impact/Control))xSeason) + 12\*V(Creeks(Impact/Control)xSeason) + 24\*S(Impact/ControlxSeason) Position(Creeks(Impact/Control)) 1\*V(Res) + 8\*V(Position(Creeks(Impact/Control))) Creeks(Impact/Control)xSeason 1\*V(Res) + 4\*V(Position(Creeks(Impact/Control))xSeason) + 12\*V(Creeks(Impact/Control)xSeason) Position(Creeks(Impact/Control))xSeason 1\*V(Res) + 4\*V(Position(Creeks(Impact/Control))xSeason) Res 1\*V(Res) Construction of Pseudo-F ratio(s) from mean squares Source Numerator Denominator Num.df Den.df Impact/Control 1\*Impact/Control

1\*Creeks(Impact/Control)

1 Season	2 1*Season 1*Creeks(Impact/Control)xSeason
1 Creeks(Impact/Control)	2 1*Creeks(Impact/Control) 1*Position(Creeks(Impact/Control))
2 Impact/ControlxSeason	8 1*Impact/ControlxSeason 1*Creeks(Impact/Control)xSeason
1 Position(Creeks(Impact/Control))	2 1*Position(Creeks(Impact/Control)) 1*Res
8 Creeks(Impact/Control)xSeason	72 1*Creeks(Impact/Control)xSeason 1*Position(Creeks(Impact/Control))xSeason
2 Position(Creeks(Impact/Control))xSeason	8 1*Position(Creeks(Impact/Control))xSeason 1*Res
8	72
Estimates of components of variation	

Estimates of components of variation		
Source	Estimate	Sq.root
S(Impact/Control)	178.75	13.37
S(Season)	10.217	3.1965
V(Creeks(Impact/Control))	-138.36	-11.763
S(Impact/ControlxSeason)	80.28	8.9599
V(Position(Creeks(Impact/Control)))	484.19	22.004
V(Creeks(Impact/Control)xSeason)	-4.4191	-2.1022
V(Position(Creeks(Impact/Control))xSeason)	123.22	11.101
V(Res)	341.52	18.48

Riffle EPT Taxa PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem6 Data type: Similarity Selection: All Transform: Square root Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors		
Name	Туре	Levels
Impact/Control	Fixed	2
Creeks	Random	4
Position	Random	3
Season	Fixed	2

PERMANOVA table of results

	Unique		
Source	df SS	MS	Pseudo-F
	P(perm)	perms	P(MC)
Impact/Control	1 8559.5	8559.5	5.9512
0.3227	3 0.0241		
Season	1 753.41	753.41	2.2068
0.1944	797	0.1871	
Creeks(Impact/Control)	2 2876.6	1438.3	3.3143
0.0228	8901	0.0147	
Impact/ControlxSeason	1 880.67	880.67	2.5796
0.1487	800	0.1371	
Position(Creeks(Impact/Control))	8 3471.7	433.96	1.1691

0.2114	9885 0.2655
Creeks(Impact/Control)xSeason 0.7243	2 682.8 341.4 0.59424 9943 0.7397
Position(Creeks(Impact/Control))xSeason	8 4596.1 574.51 1.5478
0.0233	9884 0.055
Res	72 26725 371.18
<b>T</b>	05 40540
Total	95 48546
Details of the expected mean squares (EMS) for the model	
Source	
Impact/Control 8*V(Position(Creeks(Impact/Control))) + 24*V(Creeks(Impact/Co	1*V(Res) +
Season	1*V(Res) +
4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creeks(I	mpact/Control)xSeason) + 48*S(Season)
Creeks(Impact/Control)	1*V(Res) +
8*V(Position(Creeks(Impact/Control))) + 24*V(Creeks(Impact/Co Impact/ControlxSeason	ontrol)) 1*V(Res) +
4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creeks(I	
24*S(Impact/ControlxSeason)	
Position(Creeks(Impact/Control))	1*V(Res) +
8*V(Position(Creeks(Impact/Control))) Creeks(Impact/Control)xSeason	1*V(Res) +
4*V(Position(Creeks(Impact/Control))xSeason) + 12*V(Creeks(I	I v(Res) + mpact/Control)xSeason)
Position(Creeks(Impact/Control))xSeason	1*V(Res) +
4*V(Position(Creeks(Impact/Control))xSeason)	
Res	1*V(Res)
Construction of Pseudo-F ratio(s) from mean squares	
Source	Numerator
	Denominator
	Num.df
leane et/Control	Den.df
Impact/Control	1*Impact/Control 1*Creeks(Impact/Control)
1	2
Season	1*Season
	1*Creeks(Impact/Control)xSeason
1	2
Creeks(Impact/Control)	1*Creeks(Impact/Control)
	1*Position(Creeks(Impact/Control))
2	8
_ Impact/ControlxSeason	1*Impact/ControlxSeason
	1*Creeks(Impact/Control)xSeason
1	2
1 Position(Creeks(Impact/Control))	2 1*Position(Creeks(Impact/Control))
	1*Res
8 Cracks(Import(Control))/Concern	72
Creeks(Impact/Control)xSeason	1*Creeks(Impact/Control)xSeason 1*Position(Creeks(Impact/Control))xSeason
2	8
Position(Creeks(Impact/Control))xSeason	1*Position(Creeks(Impact/Control))xSeason
	1*Res
8	72
-	
Estimates of components of variation	
Source	Estimate Sq.root
S(Impact/Control) S(Season)	148.36 12.18 8.5835 2.9298
V(Creeks(Impact/Control))	41.847 6.4689
S(Impact/ControlxSeason)	22.47 4.7402
V(Position(Creeks(Impact/Control)))	7.848 2.8014
V(Creeks(Impact/Control)xSeason)	-19.426 -4.4075
V(Position(Creeks(Impact/Control))xSeason) V(Res)	50.834 7.1298 371.18 19.266
	571.10 13.200

## Appendix 7.

Edge SIMPER Similarity Percentages - species contributions

Two-Way Analysis

Data worksheet Name: Edge SqRt Data type: Abundance Sample selection: All Variable selection: All

Parameters Resemblance: S17 Bray Curtis similarity Cut off for low contributions: 90.00%

<b>F</b> . 0			
Factor G		0	
Sample			
LR1	Non-min		Loddon
LR2	Non-min		Loddon
LR3	Non-min		Loddon
OH1	Non-min		O'Hares
OH1	Non-min		O'Hares O'Hares
OH1 OH2	Non-min Non-min		O Hares O'Hares
OH2 OH2	Non-min		O'Hares
OH2 OH3	Non-min		O'Hares
OH3	Non-min		O'Hares
WR1	Mined	Eastern	
WR2	Mined	Eastern	
			-

WR2	Mined	Eastern Trib
WR2	Mined	Eastern Trib
WR3	Mined	Eastern Trib
WR4	Mined	Waratah Rivulet
WR5	Mined	Waratah Rivulet
WR6	Mined	Waratah Rivulet
Evami	ines Imnact	arouns

Examines Impact groups (across all Creek groups) Group Non-mined Average similarity: 37.44

Species Av.AbundAv.SimSim/SDDiptera Chironomidae S.F. Chironominae LDiptera Chironomidae S.F. Tanypodinae LDiptera Chironomidae S.F. Orthocladiinae LDecapoda Atyidae Paratya X1.74Trichoptera Leptoceridae Triplectides LEphemeroptera Baetidae Centroptilum NTrichoptera Leptoceridae Notalina L1.00	Contrib% 3.12 2.20 3.01 3.88 1.45 1.08 1.33	5.99 4.52 4.36 0.88 1.97 1.36 0.42	1.72 1.65 1.18 10.36 0.67 0.56 3.55	15.99 12.07 11.64 50.06 5.26 3.63 62.50	15.99 28.06 39.71 55.32 58.95		
Oligochaeta Oligochaeta indeterminate unasse Ephemeroptera Leptophlebiidae Koorrnonga N Coleoptera Elmidae Austrolimnius L 1.06	ssed genu	us X 1.25 0.51	1.36 0.55 3.27	1.26 3.34 72.46	0.56 69.19	3.36	65.85
Trichoptera Hydroptilidae Hellyethira L Ephemeroptera Leptophlebiidae indeterminate Plecoptera Gripopterygidae Leptoperla N Ephemeroptera Caenidae Tasmanocoenis N Coleoptera Scirtidae shin L 0.59 0.73 Diptera Ceratopogonidae Bezzia L 0.65		1.20 0.77 0.80 0.79 1.96 0.55	0.58 0.84 0.43 0.48 84.09 1.87	3.20 0.53 2.14 2.10 85.96	75.65 2.23 80.03 82.13	77.89	
Diptera Chironomidae S.F. Aphroteniiae L Ephemeroptera Leptophlebiidae Ulmerophlebia Coleoptera Elmidae Austrolimnius A 0.66 Amphipoda Chiltoniidae Austrochiltonia X	0.66 N 0.42 0.65	0.60 0.54 0.34 0.41	0.41 0.43 1.13 0.31	1.61 0.36 89.85 1.09	87.57 1.15 90.94	88.72	
Group Mined Average similarity: 30.97							
Species Av.AbundAv.SimSim/SDDecapoda Atyidae Paratya X1.74Diptera Chironomidae S.F. Chironominae LDiptera Chironomidae S.F. Tanypodinae LEphemeroptera Baetidae indeterminate genus I	4.49 2.55 1.66	Cum.% 0.80 4.33 2.93 1.85	14.49 1.25 1.17 2.55	14.49 13.97 9.45 0.71	28.46 37.91 8.24	46.15	

Ephemeroptera Leptophlebiidae Koorrnonga N1.282.46Ephemeroptera Leptophlebiidae indeterminate genus N1.21Hemiptera Notonectidae indeterminate genus N1.281.36Diptera Chironomidae S.F. Orthocladiinae L0.961.33Trichoptera Leptoceridae Triplectides L0.881.33Ephemeroptera Baetidae Centroptilum N1.631.30Diptera Chironomidae S.F. Aphroteniiae L1.051.07Coleoptera Scirtidae shin L0.640.590.401.89Trichoptera Trichoptera indeterminate indeterminate genus LAmphipoda Chiltoniidae Austrochiltonia X0.730.45Oligochaeta Oligochaeta indeterminate unassessed genus XEphemeroptera Caenidae Tasmanocoenis N0.520.31Trichoptera Hydroptilidae Hellyethira L0.390.27	0.79 2.23 0.43 0.63 0.55 0.38 0.49 83.85 0.78 0.24 0.37 0.29 0.30	7.96 0.88 4.40 4.30 4.21 3.45 0.56 1.44 0.39 1.02 0.87	54.11 7.19 65.69 70.00 74.30 78.51 81.96 0.39 87.11 0.27 89.40 90.27	61.29 1.82 1.27	85.67 88.39
Groups Non-mined & Mined No pairs of groups with samples					
Examines Creek groups (across all Impact groups) Group Loddon Average similarity: 42.79					
Species Av.AbundAv.SimSim/SDContrib% Cum.%Decapoda Atyidae Paratya X2.507.451.91Diptera Chironomidae S.F. Chironominae L2.647.11Diptera Chironomidae S.F. Orthocladiinae L2.075.79Diptera Chironomidae S.F. Tanypodinae L1.874.83Trichoptera Leptoceridae Notalina L1.522.540.60Trichoptera Hydroptilidae Hellyethira L1.022.19Ephemeroptera Baetidae Centroptilum N1.042.06Coleoptera Elmidae Austrolimnius L1.242.040.66Plecoptera Gripopterygidae Leptoperla N0.921.51Ephemeroptera Leptophlebiidae indeterminate genus N0.640.62Ephemeroptera Leptophlebiidae Koorrnonga N0.870.92Oligochaeta Oligochaeta indeterminate unassessed genus X0.870.92	17.41 1.84 2.19 1.45 5.93 0.86 0.73 4.77 0.61 0.96 0.53 0.38 0.76	17.41 16.61 13.54 11.28 64.78 5.11 4.81 79.47 3.53 0.54 2.15 2.15 0.82	34.03 47.57 58.85 69.89 74.70 83.00 2.24 87.39 89.54 0.41	85.24	91.47
Group O'Hares Average similarity: 32.97					
Species Av.AbundAv.SimSim/SDContrib% Cum.%Diptera Chironomidae S.F. Chironominae L3.555.05Diptera Chironomidae S.F. Tanypodinae L2.504.26Trichoptera Leptoceridae Triplectides L2.523.52Diptera Chironomidae S.F. Orthocladiinae L3.873.16Oligochaeta Oligochaeta indeterminate unassessed genus XEphemeroptera Leptophlebiidae Koorrnonga N1.581.53Coleoptera Scirtidae shin L1.101.350.794.08	1.78 2.03 1.10 0.78 1.91 0.71 62.13	15.32 12.93 10.67 9.58 1.62 4.63	15.32 28.25 38.92 48.50 0.69 58.04	4.91	53.41
Diptera Ceratopogonidae Bezzia L1.051.210.82Decapoda Atyidae Paratya X1.040.890.52Ephemeroptera Baetidae Centroptilum N1.110.77Amphipoda Chiltoniidae Austrochiltonia X1.250.75Ephemeroptera Leptophlebiidae indeterminate genus N0.89Ephemeroptera Caenidae Tasmanocoenis N0.850.68Odonata Synlestidae Synlestes N0.630.580.54Coleoptera Elmidae Austrolimnius L0.910.540.47	3.67 2.69 0.43 0.43 0.73 0.44 1.75 1.63	65.79 68.48 2.34 2.27 0.54 2.05 79.11 80.74	70.82 73.09 2.22 77.36	75.31	
Diptera Chironomidae S.F. Aphroteniiae L0.680.49Trichoptera Trichoptera indeterminate indeterminate genus LTrichoptera Hydroptilidae Hellyethira L0.540.37Hemiptera Notonectidae indeterminate genus N0.760.350.35Hemiptera Notonectidae Enithares A0.480.340.38Coleoptera Elmidae Austrolimnius A0.770.340.34Trichoptera Leptoceridae Notalina L0.520.320.38	0.43 0.67 0.37 0.25 1.04 1.02 0.96	1.48 0.37 1.12 1.07 86.59 87.61 88.57	82.22 0.37 84.47 85.55	1.14	83.36
Ephemeroptera Leptophlebiidae Ulmerophlebia N       0.56         Ephemeroptera Baetidae indeterminate genus N       0.46         Croup Factora Trib       0.46	0.32 0.31	0.31 0.33	0.96 0.93	89.53 90.46	
Group Eastern Trib Average similarity: 33.51					
Species Av.AbundAv.SimSim/SDContrib% Cum.%Decapoda Atyidae Paratya X2.548.521.60Ephemeroptera Leptophlebiidae Koorrnonga N1.904.44Ephemeroptera Leptophlebiidae indeterminate genus N1.29Diptera Chironomidae S.F. Tanypodinae L1.483.11Diptera Chironomidae S.F. Chironominae L1.502.66	25.41 1.37 3.12 1.16 0.89	25.41 13.24 1.12 9.29 7.94	38.65 9.31 57.25 65.19	47.96	

Trichoptera Leptoceridae Triplectides L1.062.29Diptera Chironomidae S.F. Aphroteniiae L0.921.34Hemiptera Notonectidae indeterminate genus N0.861.00Ephemeroptera Baetidae indeterminate genus N0.73Amphipoda Chiltoniidae Austrochiltonia X1.040.74Trichoptera Trichoptera indeterminate indeterminate genus LDiptera Chironomidae S.F. Orthocladiinae L0.570.58Coleoptera Scirtidae shin L0.450.550.381.65Odonata Epiproctophora indetermin indeterminate genus NGroup Waratah Rivulet0.00	0.78 0.56 0.34 0.75 0.29 0.88 0.43 89.03 0.49	6.85 4.01 3.00 0.42 2.20 0.72 1.75 0.41	72.03 76.04 79.03 2.23 83.47 0.42 87.38 0.32	81.27 2.16 1.23	85.63 90.26
Average similarity: 28.43Species Av.AbundAv.Sim Sim/SD Contrib% Cum.%Diptera Chironomidae S.F. Chironominae L3.605.99Ephemeroptera Baetidae indeterminate genus N2.98Diptera Chironomidae S.F. Tanypodinae L1.842.74Ephemeroptera Baetidae Centroptilum N3.062.56Diptera Chironomidae S.F. Orthocladiinae L1.352.08Hemiptera Notonectidae indeterminate genus N1.701.72Ephemeroptera Leptophlebiidae indeterminate genus N1.13Diptera Chironomidae S.F. Aphroteniiae L1.170.79Oligochaeta Oligochaeta indeterminate unassessed genus XColeoptera Scirtidae shin L0.820.620.422.17Ephemeroptera Leptophlebiidae Koorrnonga N0.670.49Ephemeroptera Caenidae Tasmanocoenis N0.720.480.20Decapoda Atyidae Paratya X0.950.460.30Trichoptera Trichoptera indeterminate indeterminate genus LTrichoptera Leptoceridae Triplectides L0.700.37Diptera Ceratopogonidae Bezzia L0.390.290.320.32	$\begin{array}{c} 1.94 \\ 4.35 \\ 1.20 \\ 0.57 \\ 0.86 \\ 0.52 \\ 1.33 \\ 0.41 \\ 0.66 \\ 80.77 \\ 0.42 \\ 0.37 \\ 1.63 \\ 0.67 \\ 0.33 \\ 1.00 \end{array}$	21.08 1.09 9.64 9.02 7.32 6.06 0.71 2.79 0.77 1.74 1.70 85.83 0.40 1.30 89.55	21.08 15.31 46.03 55.04 62.37 68.42 4.69 75.90 0.39 82.51 84.21 0.37 88.55	36.39 73.11 2.69 1.41	78.59 87.25
Hemiptera Notonectidae Enithares A       0.42       0.26       0.27         Groups Loddon & O'Hares       Average dissimilarity = 70.25         Edge BEST       Biota and/or Environment matching         Data worksheet       Name: Data12         Data type: Environmental       Sample selection: All         Variable selection: All       Variable selection: All         Parameters       Rank correlation method: Spearman         Method: BVSTEP       Termination criteria:         rho > 0.95       delta rho < 0.001	0.92	90.47			
9 Sulphate 10 Log Be 11 Li 12 Log Mo					

13 DO% 14 DO mg/L 15 Temp 16 Cond us/cm 17 pH 18 Turb 19 Rainfall

Steps for global test: No.Vars Corr. Selections 1 0.246 7 2 0.268 7,18

- 3 0.285 7,18,19

Global Test Sample statistic (Rho): 0.285 Significance level of sample statistic: 1% Number of permutations: 99 (Random sample) Number of permuted statistics greater than or equal to Rho: 0

#### Best results

Multiple No.Vars Corr. Selections 1 3 0.285 7,18,19

## Outputs

Plot: Graph8

### Riffle SIMPER

Similarity Percentages - species contributions

**Two-Way Analysis** 

Data worksheet Name: Riffle SqRt Data type: Abundance Sample selection: All Variable selection: All

#### Parameters

Resemblance: S17 Bray Curtis similarity Cut off for low contributions: 90.00%

#### Factor Groups

Faciol G		
Sample	Impact Creek	
LR1	Non-mined	Loddon
LR2	Non-mined	Loddon
LR3	Non-mined	Loddon
OH1	Non-mined	O'Hares

OH1	Non-mine	a d	O'Hares
-			
OH2	Non-mine		O'Hares
OH2	Non-mine	ed	O'Hares
OH2	Non-mine	ha	O'Hares
-			
OH2	Non-mine	ea	O'Hares
OH2	Non-mine	ed	O'Hares
OH2	Non-mine	od.	O'Hares
OH2	Non-mine	ed	O'Hares
OH2	Non-mine	ed	O'Hares
OH3	Non-mine	ha	O'Hares
OH3	Non-mine		O'Hares
OH3	Non-mine	ed	O'Hares
OH3	Non-mine	he	O'Hares
OH3	Non-mine		O'Hares
OH3	Non-mine	ed	O'Hares
OH3	Non-mine	he	O'Hares
OH3	Non-mine		O'Hares
WR1	Mined	Eastern 7	Trib
WR1	Mined	Eastern <sup>-</sup>	Trih
WR1	Mined	Eastern <sup>-</sup>	
WR1	Mined	Eastern <sup>-</sup>	Γrib
WR1	Mined	Eastern <sup>-</sup>	Trib
WR1	Mined	Eastern <sup>-</sup>	lrid
WR1	Mined	Eastern <sup>-</sup>	Trib
WR1	Mined	Eastern <sup>-</sup>	Trib
WR2	Mined	Eastern 7	Irid
WR2	Mined	Eastern 7	Γrib
WR2	Mined	Eastern <sup>-</sup>	Trib
WR2	Mined	Eastern 7	
WR2	Mined	Eastern <sup>-</sup>	Trib
WR2	Mined	Eastern <sup>-</sup>	Trih
WR2	Mined	Eastern <sup>-</sup>	
WR2	Mined	Eastern 7	Γrib
WR3	Mined	Eastern <sup>-</sup>	Frih
WR3	Mined	Eastern 7	
WR3	Mined	Eastern <sup>-</sup>	Γrib
WR3	Mined	Eastern <sup>-</sup>	Trih
WR3	Mined	Eastern 7	
WR3	Mined	Eastern 7	Γrib
WR3	Mined	Eastern <sup>-</sup>	Frih
WR3			
	Mined	Eastern 7	
WR4	Mined	Waratah	Rivulet
WR4	Mined	Waratah	Rivulet
WR4	Mined	Waratah	
WR4	Mined	Waratah	Rivulet
WR4	Mined	Waratah	Rivulet
WR4	Mined	Waratah	Divulot
WR4	Mined	Waratah	Rivulet
WR4	Mined	Waratah	Rivulet
WR5	Mined	Waratah	
WR5	Mined	Waratah	
WR5	Mined	Waratah	Rivulet
WR5	Mined	Waratah	
WR5	Mined	Waratah	
WR5	Mined	Waratah	Rivulet
WR5	Mined	Waratah	
WR5	Mined	Waratah	
WR6	Mined	Waratah	Rivulet
WR6	Mined	Waratah	Rivulet
WR6	Mined	Waratah	
WR6	Mined	Waratah	
WR6	Mined	Waratah	Rivulet
WR6	Mined	Waratah	
WR6	Mined	Waratah	
WR6	Mined	Waratah	Rivulet
-			-

Examines Impact groups (across all Creek groups) Group Non-mined Average similarity: 45.79

Species Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%			
Diptera Chironomidae S.F.	Orthoclac	iinae L	7.30	8.86	1.81	19.34	19.34
Diptera Chironomidae S.F.	Chironom	inae L	5.87	8.37	2.74	18.29	37.63
Trichoptera Hydropsychida	e Asmicrio	lea L	4.56	4.13	0.78	9.02	46.65

Coleoptera Elmidae Austrolimnius L2.832.680.95Diptera Chironomidae S.F. Tanypodinae L1.841.99Coleoptera Elmidae Kingolus A1.981.870.78Coleoptera Elmidae Kingolus L1.761.700.72Oligochaeta Oligochaeta indeterminate unassessed genus XPlecoptera Gripopterygidae Dinotoperla N1.531.28Diptera Ceratopogonidae Bezzia L1.371.250.75Ephemeroptera Baetidae Offadens N1.521.230.45Coleoptera Elmidae Austrolimnius A2.101.210.51Diptera Tipulidae Antocha L1.601.200.632.63Diptera Simuliidae Austrosimulium L1.770.970.58Trichoptera Conoesucidae Hampa L1.010.690.45Diptera Empidiae alpha L0.970.670.581.46Trichoptera Helicophidae Alloecella L1.040.660.58Trichoptera Hydrobiosidae indeterminate genus L0.800.80	5.85 0.87 4.08 3.71 2.04 0.59 2.72 2.69 2.65 81.54 2.13 1.49 1.52 88.45 1.45 0.55	52.49 4.35 60.92 64.63 1.57 2.80 73.57 76.26 78.91 83.67 0.83 86.99 89.90 0.54	56.85 0.61 70.85 0.42 1.20	3.42 1.81 91.10	68.06 85.48
Group Mined Average similarity: 38.20					
Species Av.AbundAv.SimSim/SDContrib% Cum.%Diptera Chironomidae S.F. Orthocladiinae L4.727.51Diptera Chironomidae S.F. Chironominae L4.217.40Ephemeroptera Baetidae Offadens N3.274.910.90Diptera Tipulidae Antocha L2.012.750.827.20Coleoptera Elmidae Kingolus L1.852.150.64	1.17 1.37 12.86 59.11 5.61	19.67 19.38 51.91 64.73	19.67 39.05		
Coleoptera Elmidae Kingolus L1.652.150.64Coleoptera Elmidae Kingolus A1.331.430.66Diptera Simuliidae Austrosimulium L0.991.120.44Diptera Chironomidae S.F. Podonominae L0.871.12Diptera Chironomidae S.F. Diamesinae L0.760.94Diptera Ceratopogonidae Dasyhelea L1.200.90Trichoptera Hydropsychidae Asmicridea L1.110.82Ephemeroptera Baetidae indeterminate genus N0.84Plecoptera Gripopterygidae Dinotoperla N0.870.76Diptera Simuliidae indeterminate genus L0.560.69Trichoptera Trichoptera indeterminate indeterminate genus LColeoptera Elmidae Austrolimnius A0.690.580.39	5.61 3.74 2.94 0.60 0.34 0.48 0.46 0.79 0.38 0.20 0.87 1.53	64.73 68.47 71.41 2.93 2.47 2.36 2.15 0.45 1.98 1.80 0.61 90.30	74.34 76.81 79.17 81.32 2.06 85.36 87.16 0.40	83.38 1.61	88.77
Groups Non-mined & Mined No pairs of groups with samples					
Examines Creek groups (across all Impact groups) Group Loddon					
Average similarity: 48.91					
	2.98 1.97 1.08 7.42 1.19 6.03 6.01 3.99 0.72 2.74 2.03 1.86 89.24 90.65	19.36 14.57 13.42 54.77 6.36 67.17 73.18 77.17 3.95 83.86 85.89 87.76	19.36 33.93 47.35 61.14 81.12		
Average similarity: 48.91Species Av.AbundAv.SimSim/SDContrib% Cum.%Diptera Chironomidae S.F. Chironominae L5.669.47Diptera Chironomidae S.F. Orthocladiinae L4.537.12Trichoptera Hydropsychidae Asmicridea L5.846.57Coleoptera Elmidae Austrolimnius L3.013.631.05Diptera Chironomidae S.F. Tanypodinae L2.383.11Coleoptera Elmidae Kingolus L2.702.951.05Coleoptera Elmidae Kingolus A2.582.941.03Coleoptera Elmidae Austrolimnius A2.691.950.64Plecoptera Gripopterygidae Dinotoperla N1.871.93Diptera Ceratopogonidae Bezzia L1.461.340.64Trichoptera Helicophidae Alloecella L1.030.990.78Diptera Simuliidae Austrosimulium L1.060.910.61Diptera Tipulidae Antocha L1.120.730.481.49	$1.97 \\ 1.08 \\ 7.42 \\ 1.19 \\ 6.03 \\ 6.01 \\ 3.99 \\ 0.72 \\ 2.74 \\ 2.03 \\ 1.86 \\ 89.24$	14.57 13.42 54.77 6.36 67.17 73.18 77.17 3.95 83.86 85.89	33.93 47.35 61.14		
Average similarity: 48.91Species Av.AbundAv.SimSim/SDContrib% Cum.%Diptera Chironomidae S.F. Chironominae L5.669.47Diptera Chironomidae S.F. Orthocladiinae L4.537.12Trichoptera Hydropsychidae Asmicridea L5.846.57Coleoptera Elmidae Austrolimnius L3.013.631.05Diptera Chironomidae S.F. Tanypodinae L2.383.11Coleoptera Elmidae Kingolus L2.702.951.05Coleoptera Elmidae Kingolus A2.582.941.03Coleoptera Elmidae Austrolimnius A2.691.950.64Plecoptera Gripopterygidae Dinotoperla N1.871.93Diptera Ceratopogonidae Bezzia L1.461.340.64Trichoptera Helicophidae Alloecella L1.030.990.78Diptera Simulidae Austrosimulium L1.060.910.61Diptera Empididae alpha L0.940.690.491.40Group O'Hares0.940.690.491.40	$1.97 \\ 1.08 \\ 7.42 \\ 1.19 \\ 6.03 \\ 6.01 \\ 3.99 \\ 0.72 \\ 2.74 \\ 2.03 \\ 1.86 \\ 89.24$	14.57 13.42 54.77 6.36 67.17 73.18 77.17 3.95 83.86 85.89	33.93 47.35 61.14	6.03	47.53

Coleoptera Elmidae Kingolus A1.400.8Diptera Chironomidae S.F. Podonominae L1.Plecoptera Gripopterygidae Dinotoperla N1.Diptera Empididae alpha L1.000.650.7Trichoptera Hydrobiosidae Ulmerochorema L0.Coleoptera Elmidae Kingolus L0.860.5Trichoptera Hydrobiosidae indeterminate genus LGroup Eastern Trib	03       0.5         .32       0.9         .38       0.7         .75       0.8         .20       0.6         75       1         .73       0.5         55       0.6	56       2.         96       0.7         72       2.         36       0.4         58       0.5         .52       86.5         56       0.6	06 81.43 3 2.01 3 1.59 5 1 1.30 29 89.15	79.37 83.44 85.03 87.86 1.27	90.42	
Diptera Chironomidae S.F. Orthocladiinae L4.Ephemeroptera Baetidae Offadens N4.04Diptera Chironomidae S.F. Chironominae L2.Coleoptera Elmidae Kingolus L2.82Diptera Tipulidae Antocha L2.08Coleoptera Elmidae Kingolus A1.46Diptera Tipulidae Antocha L2.08Coleoptera Elmidae Kingolus A1.46Plecoptera Gripopterygidae Dinotoperla N1.Diptera Ceratopogonidae Dasyhelea L1.Trichoptera Hydropsychidae Asmicridea L1.Coleoptera Elmidae Austrolimnius A1.171.1Ephemeroptera Baetidae indeterminate genus NDiptera Simuliidae Austrosimulium L1.05Los0.9Diptera Simuliidae indeterminate genus L0.9	.76 4.6 36 1.0 33 6 29 0.9 .20 1.3 .32 1.3 .52 1.2 11 0.5 1 04 0.5	06         0.9           02         14           01         1.3           01         10           .87         59.8           .88         6.           .87         0.55           .86         0.6           .20         0.55           .68         2.           .08         1.0           .51         2.           .38         2.           .72         0.1	.44         30.49           6         12.21           .23         52.93           0         0           07         65.88           8         3.64           5         3.61           3         3.18           94         79.26           5         0.54           75         84.78           43         87.21           6         1.90		82.03	
Diptera Chironomidae S.F. Chironominae L5.Diptera Chironomidae S.F. Orthocladiinae L4.Ephemeroptera Baetidae Offadens N2.51Diptera Tipulidae Antocha L1.95Diptera Chironomidae S.F. Diamesinae L1.Diptera Chironomidae S.F. Podonominae L0.Diptera Simuliidae Austrosimulium L0.921.2	31       7         .51       1.8         .88       1.5         21       0.4         .50       0.6         45       1         9 genus L       57       0.3         55       0.4       1         9 genus X       1       1	20         1.8           97         1.5           91         11           .53         68.4           38         0.5           55         0.7           40         3.           36         0.3           .69         83.8           1.         1.           .35         1.           .40         1.	2 23.19 .32 60.89 2 4.87 8 4.00 13 80.42 9 1.71 2 14 0.58 47 86.78 43 88.21 74 0.54		1.49 1.39 90.96	85.32 89.60

Riffle BEST Biota and/or Environment matching

Data worksheet Name: Data12 Data type: Environmental Sample selection: All Variable selection: All

Resemblance worksheet Name: Resem3 Data type: Similarity Selection: All

Parameters Rank correlation method: Spearman Method: BVSTEP Termination criteria: rho > 0.95 delta rho < 0.001 Use fixed starting variables Starting selection: Resemblance: Analyse between: Samples Resemblance measure: D1 Euclidean distance

Variables 1 DFS 2 Bicarbonate 3 Log Ca 4 Log Na 5 Log Al 6 Iron 7 Manganese 8 Chloride 9 Sulphate 10 Log Be 11 Li 12 Log Mo 13 DO% 14 DO mg/L 15 Temp 16 Cond us/cm 17 pH 18 Turb 19 Rainfall

*Steps for global test:* No.Vars Corr. Selections 1 0.361 2 2 0.395 2,9 3 0.403 2,9,19 4 0.417 2,9,15,19

Global Test Sample statistic (Rho): 0.417 Significance level of sample statistic: 1% Number of permutations: 99 (Random sample) Number of permuted statistics greater than or equal to Rho: 0

Best results Multiple No.Vars Corr. Selections 1 4 0.417 2,9,15,19

Outputs Plot: Graph9

Riffle Trichoptera BEST Biota and/or Environment matching

Data worksheet Name: Data12 Data type: Environmental Sample selection: All Variable selection: All

Resemblance worksheet Name: Trichoptera for BEST Data type: Similarity Selection: All

Parameters Rank correlation method: Spearman Method: BVSTEP Termination criteria: rho > 0.95 delta rho < 0.001 Use fixed starting variables Starting selection: Resemblance: Analyse between: Samples Resemblance measure: D1 Euclidean distance

Variables 1 DFS 2 Bicarbonate 3 Log Ca

4 Log Na 5 Log Al 6 Iron 7 Manganese 8 Chloride 9 Sulphate 10 Log Be 11 Li 12 Log Mo 13 DO% 14 DO mg/L 14 DO mg/L 15 Temp 16 Cond us/cm 17 pH 18 Turb 19 Rainfall

Steps for global test: No.Vars Corr. Selections 1 0.288 7 2 0.320 7,14

Global Test Sample statistic (Rho): 0.32 Significance level of sample statistic: 1% Number of permutations: 99 (Random sample) Number of permuted statistics greater than or equal to Rho: 0

Best results Multiple No.Vars Corr. Selections 1 2 0.320 7,14

**Outputs** Plot: Graph8

# Appendix 8.

### **RAW Diatom data**

HS ref-3	HSref-2	HSref-1	OH3-3	OH3-2	OH3-1	OH2-3	OH2-2	OH2-1	OH1-3	OH1-2	OH1-1	L3-3	L3-2	L3-1	L2-3	12-2	12-1	L1-3	L1-2	5	WR6-3	WR6-2	WR6-1	WRr-3	WRr-2	WRr-1	WR5-3	WR5-2	WR5-1	WR4-3	WR4-2	WR4-1	WR3-3	WR3-2	WR3-1	WR2-3	WR2-2	WR2-1	WR1-R3	WR1-R2	WR1-R1	
												10283														296												3992				
				3704	8	927	855	71	372	395		541	271	198				148		7828	445	296	672				70	271	423	2922	410		68	1502		4490	20862	1426			148	;;
			348	3260	9567	214			372											373		74											203	2322	888		148	214	71	2852	519	
3		522	2299	4149		1996	3421	3564	224063	171874	177208	8840	16778	25485	21336	25287	14965	20447	2593	22739	815	667	1194		489	222	210	204	7078		410	212	406	12155			741	143	214	2445	519	
		136	488	652	508	214	356	1140	2973	3260	2845			198	790	3260	2223	1778	74		148					74		136		ម							148		71			
	18		2020	1830			356				198							296			222	74	68				280	68		71		71	17	656		71			អ			
																					18						335							2131	68	71	148	285				
			634	985		71		998	5797	5532	4267									2609	45		68				70	68										æ				
				667			143							18															8636	71		71					148					
			418	6668	1185	1639	2274	71	372		395						445				74	570	821				210	136	339		683	565	338	819	751	71						
																															137					214						
																																			68							
																												204						546								
18				73			18										36	18		36											17			35								
																							68																			

### Appendix 9.

### PEMANOVA, SIMPER and BEST Diatom data

**Diatom** PERMANOVA Permutational MANOVA

Resemblance worksheet Name: Resem2 Data type: Similarity Selection: All Transform: Square root Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial) Fixed effects sum to zero for mixed terms Permutation method: Permutation of residuals under a reduced model Number of permutations: 9999

Factors		
Name	Туре	Levels
Impact	Fixed	2
Creek	Random	4
Position'	Random	3

PERMANOVA table of results

PERMANOVA table of results						Unique				
Source	df	SS	MS	Pseudo-F	P(perm)	perms				
P(MC) Impact	1	4095.2	4095.2	32.75	0.3299	3				
impact	-	168	4033.2	52.75	0.5255	5				
Creek(Impact)	2	250.09	125.05	0.51749	0.7051	8883				
Position'(Creek(Impact))	0.6 8	527 1933.1	241.64	1.9525	0.0716	9925				
	-	806	241.04	1.5525	0.0710	5525				
Res	24	2970.2	123.76							
Total	35	9248.6								
Details of the expected mean squares	EMS	) for the mo	del							
Source	EM	S								
mpact 1*V(Res) + 3*V(Position'(Creek(Impact))) + 9*V(Creek(Impact)) +										
Creek(Impact)	8*S(Impact) reek(Impact) 1*V(Res) + 3*V(Position'(Creek(Impact))) + 9*V(Creek(Impact))									
Position'(Creek(Impact))				Creek(Impact)))		(inipact))				
Res		(Res)	· · ·							
Construction of Pseudo-F ratio(s) from	mean	sauares								
Source		nerator			Denomina	ator				
		n.df			Den.df					
Impact		npact 1			1*Creek(li 2	mpact)				
Creek(Impact)		reek(Impac	ct)		_	n'(Creek(Impact))				
		2	,		8	(/)				
Position'(Creek(Impact))		osition'(Cre 8	ek(Impact))		1*Res 24					
		0			24					
Estimates of components of variation										
Source		Estimate	Sq.roo							
S(Impact) V(Creek(Impact))		220.57 -12.955	14.85 -3.599							
V(Position'(Creek(Impact)))		39.292	6.268							
V(Res)		123.76	11.12							

150

**Diatom SIMPER** Similarity Percentages - species contributions

Two-Way Analysis

Data worksheet Name: Data1 Data type: Abundance Sample selection: All Variable selection: All

Parameters

Resemblance: S17 Bray Curtis similarity Cut off for low contributions: 100.00%

Factor Gro	oups
Sample	İmpa

Factor Gro	Factor Groups									
Sample	Impact	Creek								
WR1-R1	Mined	Eastern Trib								
WR1-R2	Mined	Eastern Trib								
WR1-R3	Mined	Eastern Trib								
WR2-1	Mined	Eastern Trib								
WR2-2	Mined	Eastern Trib								
WR2-3	Mined	Eastern Trib								
WR3-1	Mined	Eastern Trib								
WR3-2	Mined	Eastern Trib								
WR3-3	Mined	Eastern Trib								
WR4-1	Mined	Waratah Riv								
WR4-2	Mined	Waratah Riv								
WR4-3	Mined	Waratah Riv								
WR5-1	Mined	Waratah Riv								
WR5-2	Mined	Waratah Riv								
WR5-3	Mined	Waratah Riv								
WR6-1	Mined	Waratah Riv								
WR6-2	Mined	Waratah Riv								
WR6-3	Mined	Waratah Riv								
L1-1	Non-mined	Loddon								
L1-2	Non-mined	Loddon								
L1-3	Non-mined	Loddon								
L2-1	Non-mined	Loddon								
L2-2	Non-mined	Loddon								
L2-3	Non-mined	Loddon								
L3-1	Non-mined	Loddon								
L3-2	Non-mined	Loddon								
L3-3	Non-mined	Loddon								
OH1-1	Non-mined	O'Hares								
OH1-2	Non-mined	O'Hares								
OH1-3	Non-mined	O'Hares								
OH2-1	Non-mined	O'Hares								
OH2-2	Non-mined	O'Hares								
OH2-3	Non-mined	O'Hares								
OH3-1	Non-mined	O'Hares								
OH3-2	Non-mined	O'Hares								
OH3-3	Non-mined	O'Hares								

Examines Impact groups (across all Creek groups) Group Mined Average similarity: 47.22

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
GOMPHONE_CCNT	31.86	15.67	1.83	33.19	33.19
NAVICULOID_CCNT	27.39	9.43	1.26	19.96	53.15
ENCYONEM_CCNT	27.35	7.44	1.00	15.75	68.90
ACHNANTM_CCNT	13.25	5.87	0.78	12.44	81.34
DIATOMELLA_CCNT	11.79	4.16	0.66	8.81	90.14
SYNEDRA_CCNT	6.54	2.68	0.71	5.68	95.83
DIATOMA_CCNT	6.36	0.74	0.40	1.57	97.40
TABELLAR_CCNT	2.88	0.64	0.30	1.34	98.74
EUNOTIA_CCNT	2.80	0.34	0.23	0.72	99.46
NITZSCHI_CCNT	6.77	0.25	0.20	0.54	100.00
Group Non-mined					

Av.Sim

Sim/SD

Contrib%

Cum.%

Av.Abund

Average similarity: 58.40

S	pecies	

NAVICULOID CCNT	152.19	25.85	1.50	44.27	44.27
GOMPHONE_CCNT	101.87	20.45	2.21	35.02	79.28
EUNOTIA_CONT	27.75	4.61	1.19	7.90	87.18
ACHNANTM_CCNT	16.30	2.46	0.61	4.21	91.39
ENCYONEM_CCNT	18.43	1.80	0.71	3.08	94.47
TABELLAR_CCNT	20.19	1.78	0.55	3.04	97.51
DIATOMELLA_CCNT	12.60	0.85	0.33	1.46	98.97
SYNEDRA_CCNT	7.66	0.49	0.25	0.85	99.81
SURIRELL_CCNT	1.61	0.06	0.24	0.11	99.92
NITZSCHI_CCNT	2.33	0.05	0.12	0.08	100.00

*Groups Mined & Non-mined* No pairs of groups with samples

*Examines Creek groups* (across all Impact groups) Group Eastern Trib Average similarity: 44.48

Species GOMPHONE_CCNT DIATOMELLA_CCNT NAVICULOID_CCNT ENCYONEM_CCNT ACHNANTM_CCNT DIATOMA_CCNT SYNEDRA_CCNT EUNOTIA_CCNT TABELLAR_CCNT NITZSCHI_CCNT NAVICULA_CCNT COCCONEI_CCNT PINNULAR_CCNT SURIRELL_CCNT AMPHORA_CCNT	Av. Abund 41.10 22.63 28.49 34.26 9.20 10.21 4.90 2.29 0.66 1.35 1.63 0.92 2.60 0.66 0.00	Av.Sim 20.41 8.32 7.13 4.95 1.54 1.38 0.63 0.13 0.00 0.00 0.00 0.00 0.00 0.00 0.0	Sim/SD 3.37 1.24 0.98 0.64 0.38 0.40 0.17 ####### ####### ####### ######## ######	Contrib% 45.88 18.71 16.03 11.12 3.45 3.09 1.42 0.30 0.00 0.00 0.00 0.00 0.00 0.00 0.0	Cum.% 45.88 64.58 80.61 91.74 95.19 98.28 99.70 100.00 100.00 100.00 100.00 100.00 100.00 100.00 100.00
<i>Group Waratah Riv</i> Average similarity: 49.97					
Species NAVICULOID_CCNT GOMPHONE_CCNT ACHNANTM_CCNT ENCYONEM_CCNT SYNEDRA_CCNT TABELLAR_CCNT EUNOTIA_CCNT NITZSCHI_CCNT DIATOMA_CCNT	Av. Abund 26.29 22.61 17.29 20.44 8.18 5.11 3.30 12.20 2.51	Av.Sim 11.73 10.94 10.21 9.92 4.74 1.27 0.55 0.51 0.11	Sim/SD 1.67 1.34 1.31 1.57 1.11 0.44 0.28 0.29 0.17	Contrib% 23.47 21.89 20.43 19.86 9.48 2.54 1.10 1.01 0.21	Cum.% 23.47 45.36 65.79 85.65 95.13 97.68 98.78 99.79 100.00
<i>Group Loddon</i> Average similarity: 67.63					
Species NAVICULOID_CCNT GOMPHONE_CCNT EUNOTIA_CCNT ENCYONEM_CCNT SURIRELL_CCNT	Av.Abund 128.37 124.96 21.91 17.16 1.80	Av.Sim 36.81 26.37 3.15 1.22 0.09	Sim/SD 3.24 3.78 0.67 0.57 0.29	Contrib% 54.42 38.99 4.65 1.80 0.14	Cum.% 54.42 93.41 98.07 99.86 100.00
<i>Group O'Hares</i> Average similarity: 49.17					
Species NAVICULOID_CCNT GOMPHONE_CCNT EUNOTIA_CCNT ACHNANTM_CCNT TABELLAR_CCNT ENCYONEM_CCNT DIATOMELLA_CCNT SYNEDRA_CCNT NITZSCHI_CCNT SURIRELL_CCNT	Av.Abund 176.01 78.79 33.60 30.25 34.71 19.71 23.05 13.41 4.20 1.42	Av.Sim 14.90 14.53 6.08 4.92 3.55 2.38 1.70 0.99 0.09 0.03	Sim/SD 0.99 2.01 2.92 1.08 0.93 0.85 0.50 0.36 0.17 0.17	Contrib% 30.30 29.55 12.36 10.00 7.23 4.84 3.46 2.01 0.19 0.07	Cum.% 30.30 59.85 72.21 89.43 94.27 97.73 99.74 99.93 100.00

BEST Biota and/or Environment matching

Data worksheet Name: Data15 Data type: Environmental Sample selection: All Variable selection: All

Resemblance worksheet Name: Resem5 Data type: Similarity Selection: All

Parameters Rank correlation method: Spearman Method: BVSTEP Termination criteria: rho > 0.95 delta rho < 0.001 Use fixed starting variables Starting selection: Resemblance: Analyse between: Samples Resemblance measure: D1 Euclidean distance

Variables

1 Bicarbonate 2 Barium 3 Log Ca 4 Log Na 5 Log Al 6 Iron 7 Manganese 8 Chloride 9 Sulphate 10 Log Be 11 Li 12 Log Mo 13 DO% 14 DO mg/L 15 Temp 16 Cond us/cm 17 pH 18 Turb 19 Rainfall

*Steps for global test:* No.Vars Corr. Selections

1 0.406 1 2 0.456 1,13 3 0.497 1,13,17 4 0.518 1,13,17,18 5 0.522 1,8,13,17,18 4 0.530 8,13,17,18

#### Global Test

Sample statistic (Rho): 0.53 Significance level of sample statistic: 1% Number of permutations: 99 (Random sample) Number of permuted statistics greater than or equal to Rho: 0

Best results Multiple No.Vars Corr. Selections 1 4 0.530 8,13,17,18

Outputs Plot: Graph7

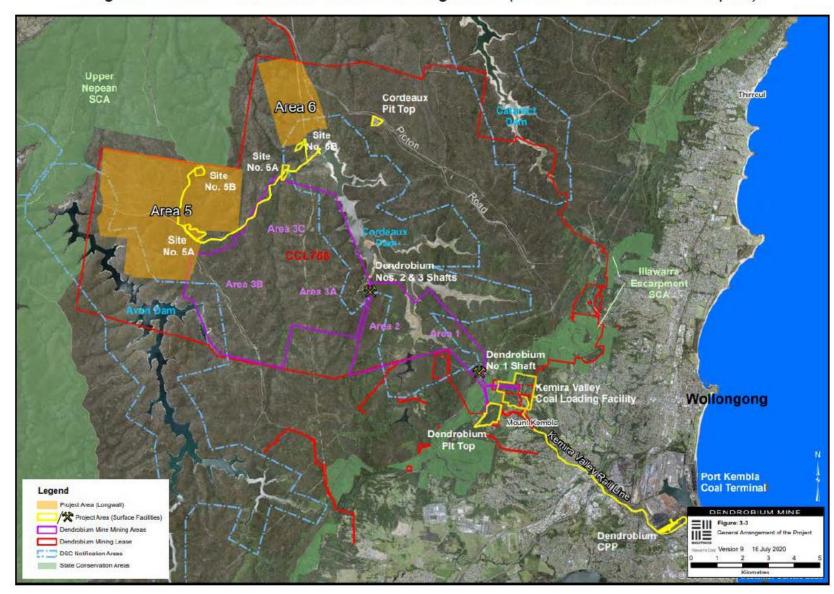


Figure 2 - Local Context & General Arrangement (Source: Amendment Report)

This map shows the spatial extent (Areas 5 & 6) of the Applicant's previous Significant State Development (**SSD**) application (<u>Dendrobium Extension Project, SSD 8194</u>), refused by the Independent Planning Commission.