

AQUATIC ECOLOGY MONITORING: METROPOLITAN COAL LONGWALLS 23-27

SPRING 2019 SURVEY



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1.0 INTRODUCTION

Metropolitan Coal is wholly owned by Peabody Energy Australia Pty Ltd and is located approximately 30 kilometres (km) north of Wollongong in NSW. Metropolitan Coal was granted approval for the Metropolitan Coal Project (the Project) under the NSW *Environmental Planning and Assessment Act, 1979* in June 2009.

The Project comprises the continuation, upgrade and extension of underground coal mining operations (Longwalls 20-27 and Longwalls 301-317) and surface facilities at the Metropolitan Coal Mine. Longwalls 305-307 are situated to the west of Longwalls 301-304, and define the next mining sub-domain within the Project underground mining area (Metropolitan Coal, 2019).

BIO-ANALYSIS Pty Ltd (BA) was commissioned by Metropolitan Coal to develop an aquatic ecology monitoring programme for the Longwalls 23-27 Extraction Plan to: i) Monitor subsidence-induced impacts (if any) on aquatic ecology (referred to as stream monitoring); and ii) Monitor the response of aquatic ecosystems to the implementation of stream remediation works (referred to as pool monitoring).

The design of the monitoring programmes uses the current best practice approach for monitoring impacts on aquatic habitats “Beyond BACI” and focused on representative sampling of selected locations (i.e. stream monitoring) and pools (i.e. pool monitoring) within streams in the Longwalls 23-27 mining area and in suitable control streams (i.e. not subject to mine subsidence).

The primary objective of monitoring was to determine whether the extent and nature of observed impacts, primarily subsidence-induced fracturing of bedrock, diversion and loss of aquatic habitat, if any, are consistent with the predictions made in the Extraction Plan and the *Metropolitan Coal Longwalls 23-27 Biodiversity Management Plan (2015)* (Metropolitan Coal, 2015)¹.

¹ The *Metropolitan Coal Longwalls 23-27 Biodiversity Management Plan* is superseded by the *Metropolitan Coal Longwall 304 Biodiversity Management Plan*. Mining of Longwall 304 commenced in July 2019.

Mining of Longwalls 23-27 commenced in May 2014 and was completed in March 2017. Subsequently, mining of Longwalls 301-303 area commenced in April 2017 and was completed in June 2019. Longwall 304 commenced in July 2019.

This report presents the results of the spring 2019 stream monitoring survey (Section 3 – Results) and a temporal analysis of the assemblages of aquatic macrophytes and macroinvertebrates in order to examine patterns in diversity and abundance among sampling events (Section 4 – Temporal Analyses). A reconciliation against the Trigger Action Response Plan in the Metropolitan Coal Longwall 304 Biodiversity Management Plan (Appendix 1) has also been completed in Section 5.3.



2.0 METHODS

2.1 Study Area

The Project underground mining area and surrounds are located within the Woronora Special Area (WSA) (Figure 1). The WSA is largely undeveloped, covered predominantly by native vegetation and public access is restricted and managed by WaterNSW (previously the Sydney Catchment Authority [SCA]). The WSA drains to the Woronora Reservoir, which supplies water to residents in the areas south of the Georges River including Sutherland, Helensburgh, Stanwell Park, Lucas Heights and Bundeena.

Longwalls 23-27 extend below a tributary of the Woronora Reservoir in the east of the study area (Tributary C/Eastern Tributary) and an un-named tributary of the Waratah Rivulet (Tributary B) (Figure 1).

The headwaters of Tributary C/Eastern Tributary are located in the Darkes Forest area at approximately 300 m AHD. Tributary C/Eastern Tributary flows through a relatively steep valley in a northerly direction into the Woronora Reservoir. The upper reaches of Tributary C/Eastern Tributary traverse the completed Longwalls 1-13 underground mining area (Figure 1).

Tributary B is a relatively small stream (approximately 2.75 km long) that flows through a broad valley into Waratah Rivulet (Figure 1). The upper reach of Tributary B traverses the completed Longwalls 14-17 and Longwalls 18-19a underground mining areas and the completed Longwalls 20-22 underground mining area: mining of Longwall 22B was completed in April 2014 (Figure 1).

Bee Creek, the Woronora Tributary and Woronora River occur within the WSA but lie outside the area potentially affected by past and current mining activities at Metropolitan Coal. As such, these systems in addition to O'Hares Creek, which lies outside the mining area in the Dharawal Nature Conservation Area to the south-west of the WSA, represent the control systems for this study.

2.2 Sampling Design

The current best practice approach for monitoring impacts on aquatic habitats is termed “Beyond BACI” (Underwood, 1992; 1993; 1994) and this approach has been used in order to assess potential impacts on aquatic ecology.

The programmes have been designed to monitor:

- (i) subsidence-induced impacts on aquatic ecology; and
- (ii) the response of aquatic ecosystems to the implementation of stream restoration works.

These programmes are described in Section 2.2.1 and Section 2.2.2, respectively.

In accordance with the *Metropolitan Coal Longwall 304 Biodiversity Management Plan*² (Metropolitan Coal, 2019), the aquatic ecology along Tributary C/Eastern Tributary as well as in appropriate control systems (i.e. Woronora River and O’Hares Creek) are monitored annually in spring (September 15 to December 15) and autumn (March 15 to June 15), consistent with the timing required by the Australian River Assessment System (AUSRIVAS) protocol. The control systems lie outside the area potentially affected by past and current Metropolitan Coal mining activities (Figure 1).

The spatial design for Longwalls 23-27 includes sampling two random sites (approximately 100 m in length) at the following stream locations (Appendix 1a)^{3,4}:

- Locations C1 and C4 on Tributary C/Eastern Tributary overlying Longwalls 23-27;
- Location C2 on Tributary C/Eastern Tributary, downstream of Longwalls 23-27;

² The *Metropolitan Coal Longwalls 23-27 Biodiversity Management Plan* is superseded by the *Metropolitan Coal Longwall 304 Biodiversity Management Plan (2019)*. Mining of Longwall 304 commenced in July 2019 and was completed in March 2020.

³ Locations C1, C2 and C4 are referred to as ET1, ET2 and ET4 in the *Metropolitan Coal Longwall 304 Biodiversity Management Plan*.

⁴ Location C1 on Tributary C/Eastern Tributary is situated over Longwalls 22 and 23 and has also been monitored as a component of the Longwalls 20-22 aquatic ecology monitoring program. Location C4 is situated on Tributary C/Eastern Tributary overlying Longwalls 23-27, downstream of mining activities undertaken within the Longwalls 20-22 mining area. Location C2 on Tributary C/Eastern Tributary is situated downstream of Longwalls 23-27 and has also been monitored as a component of the Longwalls 20-22 aquatic ecology monitoring program. Mining of Longwalls 20-22 commenced in May 2010 and was completed in April 2014.

- Control locations: WR1 on Woronora River and OC on O'Hares Creek.

The approximate locations of the sampling sites are shown on Figure 1.

Location C2 on Tributary C/Eastern Tributary is located downstream of Longwalls 23-27 and will assist in determining the spatial extent of any impacts on aquatic habitat and biota over time as mining progresses.

Information on stream characteristics was recorded at each site in accordance with the Australian River Assessment System (AUSRIVAS) protocol (Turak et al., 2004).

Characteristics recorded included a visual assessment of stream width and depth, riparian conditions, signs of disturbance, water quality and percentage cover of the substratum by algae. Samples of assemblages of aquatic macroinvertebrates, aquatic macrophytes and physico-chemical water quality were collected at sites within each of the locations.

2.3 Sampling Techniques

2.3.1 Aquatic Macroinvertebrates

Two methods were used to collect aquatic macroinvertebrates at locations sampled as part of the Stream Monitoring Programme: sampling using the AUSRIVAS protocol and quantitative sampling.

AUSRIVAS

To sample assemblages of macroinvertebrates in accordance with the Rapid Assessment Method (RAM), which is based on the AUSRIVAS protocol (Turak et al., 2004), samples of stream edge habitats were collected using a 250 µm mesh dip net. Edge habitat was defined as areas along stream banks with little or no flow, including alcoves and backwaters, with abundant leaf litter, fine sediment deposits, beds of macrophytes, overhanging banks and areas with trailing vegetation (Turak et al., 2004). Riffle habitat was not sampled, because this habitat was not well represented in the stretches of the streams surveyed.

At each site (approximately 100 m long), samples were collected over a total length of 10 m, usually in 1-2 m sections, ensuring all significant edge sub-habitats within a site (i.e. macrophytes, over-hanging bank and vegetation, leaf-litter, pool rocks, logs) were included in the sample (Turak et al., 2004). The contents of each net sample were placed into a white sorting tray and animals were collected for a minimum period of 30 minutes. Thereafter, removals were carried out in 10-minute periods, up to a total of one hour (Turak et al., 2004). If no new taxa were found within a 10-minute period, removals would cease (Turak et al., 2004). The animals collected were placed inside a labelled container and preserved with 70% alcohol.

Samples were identified using an ISSCO M400 stereomicroscope. Taxa were identified to family level with the exception of Acarina (to order), Chironomidae (to sub-family), Nematoda (to phylum), Nemertea (to phylum), Oligochaeta (to class), Ostracoda (to subclass) and Polychaeta (to class). Some families of Anisoptera (dragonfly larvae) were identified to species, because they could potentially include threatened aquatic species.

Quantitative Sampling

Within each site, three replicate macroinvertebrate samples were collected using timed 1-minute sweeps of all habitats (edge, riffle, pools, etc.) using a 250 µm mesh dip net. For each replicate sample, the contents of the net were placed into white plastic trays filled with fresh water and then placed into pre-labelled plastic sample containers filled with 70 % alcohol. In the laboratory, animals were identified to family level with the exception of some families of Anisoptera (dragonfly larvae), which were identified to species, because they could potentially include threatened aquatic species.

2.3.2 Aquatic Macrophytes

The distribution of floating-attached, submerged and emergent (occurring in-stream and in the riparian zone) macrophytes was estimated along each sampling location by assigning a cover class to each species. The cover classes were: (1) one plant or small patch (i.e. few), (2) not common, growing in a few places (i.e. scattered), and (3) widespread (i.e. common). Cover class information was used to help provide a qualitative assessment of the structure of assemblages of plants found at each location.

Within each site, an assessment of floating-attached, submerged and emergent macrophytes was undertaken by estimating the relative abundance (i.e. percentage cover) of species within five haphazardly placed 0.25 m² quadrats, using a stratified sampling technique. This information provided a quantitative measure of aquatic macrophytes within each site at each location.

2.4.3 Water Quality

A number of water quality variables were measured at each of the sampling sites prior to undertaking the biological sampling. Measurements of physico-chemical water quality were determined using a YEOKAL 611 submersible data logger. Variables included conductivity ($\mu\text{S}/\text{cm}$), dissolved oxygen (% Saturation and mg/L), pH, temperature ($^{\circ}\text{C}$), turbidity (NTU) and oxygen reduction potential (mV).

Two replicate samples of water were also collected to be analysed for alkalinity (mg/L CaCO_3), total nitrogen (mg/L) and total phosphorous (mg/L). Alkalinity was determined in the field using a CHEMetrics' total alkalinity field kit.

For analysis of total nitrogen and total phosphorous, samples were sent to the National Measurement Institute (NMI) laboratory (a NATA accredited laboratory). For the purpose of calculating summary statistics (e.g. mean concentration), any results that were recorded less than the detection limit were assigned a concentration value of half the detection limit, except in instances of zero alkalinity. It should be noted that water quality measurements are intended to provide information relevant to the times of sampling only.

2.4 Data Analyses

AUSRIVAS Model Analysis

Data collected using the AUSRIVAS sampling protocol for the Stream Monitoring Programme were analysed using the New South Wales/Spring/Edge model (see Turak et al., 2004). This predictive model was developed from sampling edge habitat at a number of sites across NSW, which had been determined to be unaffected by human disturbances, between 1994 and 1999 (Turak et al., 2004). Physical and chemical data (see Ransom et al., 2004) are used by the model to determine the predicted (i.e. Expected) composition of macroinvertebrate fauna if the site is undisturbed (Turak et al., 2004). Thus, an AUSRIVAS assessment represents a comparison of the macroinvertebrates collected at a site (i.e. Observed) to those predicted to occur (Expected) if the site is in an undisturbed or 'reference' condition.

The principal outputs of the AUSRIVAS model include:

- Observed to Expected ratio (OE50): the ratio of the number of macroinvertebrate families collected at a site which had a predicted probability of occurrence of greater than 50 % (i.e. Observed) to the sum of the probabilities of all of the families predicted with greater than a 50 % chance of occurrence (i.e. Expected) (Ransom et al., 2004).
- BAND: for each model, the OE50 taxa ratios are divided into bands representing different levels of impairment. Band X represents a more diverse assemblage of macroinvertebrates than control sites; Band A is considered equivalent to reference condition; Band B represents sites below reference condition (i.e. significantly impaired); Band C represents sites well below reference condition (i.e. severely impaired); and Band D represents impoverished sites (i.e. extremely impaired) (Ransom et al., 2004).

Quantitative Analyses

Multivariate and univariate statistical procedures were done using Permutational Multivariate Analyses of Variance (PERMANOVA, Anderson 2001; Anderson et al, 2008) and Plymouth Routines in Multivariate Ecological research (PRIMER, Clarke and Warwick, 1994) software packages to examine temporal and spatial patterns in macroinvertebrates and macrophytes sampled within the study area.

Multivariate methods allow comparisons of two (or more) samples based on the degree to which these samples share particular species, at comparable levels of abundance (Clarke and Warwick, 1994). Multivariate analysis was done on Bray Curtis dissimilarities of the macroinvertebrate and macrophyte assemblage (non-transformed) data. A graphical representation of relationships among samples (i.e. the centroids for each location per time) was produced using Principal Coordinates Analyses (PCoA). The amount of variation “explained” by the principle factors is indicated by each axis and the dissimilarity between data points can be determined from their distance apart on the axes (Anderson et al., 2008). Similarity of percentages (SIMPER) was then used to determine those taxa primarily responsible for the observed similarities (or dissimilarities) (Clarke, 1993).

PERMANOVA analyses on selected univariate estimates (e.g. total number of taxa, total abundance and abundances of the most important taxonomic groups identified from the samples) were done on Euclidean Distances. Each analysis was based on 999 permutations of residuals under a reduced model.

Specifically, PERMANOVA were done to test hypotheses related to differential changes occurring in multivariate and univariate estimates for streams within the Longwalls 23-27 mining area (i.e. potential ‘impact’ sites) in comparison to independent sites that are not subject to mine subsidence (i.e. control streams). These analyses were performed separately (Eastern Tributary [Locations C1, C2 and C4], Woronora River [Location WR] and O’Hares Creek [Location OC]).

To date, for the Longwalls 23-27 aquatic ecology monitoring programme, nine to eleven replicate times (spring 2008 or spring 2009⁵ to spring 2013) have been sampled within the ‘Before’ commencement of Longwall 23 period and twelve replicate times (autumn 2014 to spring 2019) have been sampled within the ‘After-commencement of mining’ period.

A potential impact could be expected to affect the magnitude and/or dispersion of an indicator (e.g. percentage cover). If a statistically significant difference between sample groups is detected that could be attributed to a mining impact, the difference in variance between groups would be explored using the PERMDISP procedure (Anderson et al, 2008). If there is no statistical difference between variances, the statistical difference between groups is most likely due to differences between group means. Pair-wise tests would be used to explore the different possible combinations of the groups of interest, to determine where significant differences occur.

⁵ The sampling of Location C4, larger pool ETAH and smaller pools ETAG, ETAI and ETAK on the Eastern Tributary, WP-A to WP-C on Woronora River and OC-A to OC-C on O’Hares Creek commenced in spring 2009. Sampling at all other sites commenced in spring 2008.

3.0 RESULTS

3.1 Stream Monitoring

3.1.1 Stream Characteristics

A summary of findings from surveys of stream characteristics done at stream monitoring locations sampled on Tributary C/Eastern Tributary (Locations C1, C2 and C4 and at two control locations (Woronora River, O'Hares Creek) (Figure 1), undertaken between spring 2008 and spring 2019, is presented below.

Tributary C/Eastern Tributary

Tributary C/Eastern Tributary is a third order stream located in the east of the Project area and is approximately 5.4 km in length (Figure 1). The stream is situated in a moderately steep incised valley with numerous in-stream pools. Observations of sections of Tributary C/Eastern Tributary not subject to mine subsidence indicate that stream sections between pools naturally cease to flow during dry periods and pools may dry up.

Location C1

Sampling location C1 is comprised of pools up to approximately 6 m wide and 1.0 m deep connected by sections of shallow flow over bedrock. The dominant riparian vegetation was reported to include *Gleichenia dicarpa*, *Leptospermum polygalifolium*, *Acacia binervia*, *Acacia floribunda*, *Acacia longifolia*, *Acacia parramattensis*, *Banksia marginata*, *Eucalyptus* sp. and *Grevillea buxifolia* (Bio-Analysis Pty Ltd, 2008).

Pool water at Location C1 has commonly had a green tinge since sampling commenced in spring 2008. Iron staining has commonly been noted at this location since the autumn 2014 survey. Fracturing of the streambed (predominantly bedrock) and a decline in pool water level (by up to 0.8 m) were first noted by the spring 2015 survey, at Site C1-1. Since then, pool water level at Site C1-1 appeared to be below pre-mining levels in autumn 2016, spring 2017, autumn 2018 and autumn 2019. At Site C1-2, which has a predominantly sandy substratum, pool water level appeared lower than pre-mining levels at the time of the autumn 2016 survey but not subsequently.

The main findings from the current survey (spring 2019) are summarised below:

- Whilst lower than pre-mining levels, pool water level at Site C1-1 appeared to have increased considerably since the autumn 2019 survey (Plate 1). At Site C1-2, the water level appeared similar to baseline levels (Plate 2);
- A thick iron precipitate/micro-organism complex was observed at Site C1-2 but not at Site C1-1 (Plate 2);
- The fern, *Gleichenia dicarpa*, continues to appear desiccated in areas immediately adjacent to the stream, particularly Site C1-1 (Plate 1). Reduced pool water levels at Site C1-1 on several occasions are likely to have contributed to observed declines in riparian health;
- *Baumea juncea* and *Viminaria juncea* were relatively abundant at the time of this survey (Appendix 3a).
- Submerged aquatic macrophytes have consistently not been recorded at this location (Appendices 3a&b).



Plate 1: Tributary C – Location C1 (C1-1) (sp-19)

Looking up-stream



Plate 2: Tributary C – Location C1 (C1-2) (sp-19)

Looking downstream

Location C2

Location C2 is situated downstream of the Longwalls 20-22 and the Longwalls 23-27 underground mining areas (Figure 1). Location C2 has commonly consisted of pools up to approximately 8 m wide and 1.3 m deep with a predominantly bedrock and sand substratum (Plates 3&4).

Previously, riparian vegetation was reported to include *Acacia binervia*, *Acacia floribunda*, *Acacia longifolia*, *Acacia parramattensis*, *Bauera rubioides*, *Baumea rubiginosa*, *Chorizandra cymbaria*, *Juncus prismatocarpus* and *Lepidosperma filiforme* (Bio-Analysis Pty Ltd, 2008).

Since the autumn 2013 survey, water in pools at Location C2 has occasionally been noted to have a pale-green milky tinge. Iron staining/iron flocculent was first observed at Location C2 in spring 2016⁶. Iron staining has been observed to cover the stream substratum from the top of Site C2-1 to the bottom of Site C2-2 since spring 2016⁷. In spring 2017, pool water level at Site C2-1 appeared to be ~ 0.5 m below pre-mining levels, but similar to pre-mining levels subsequently.

The main findings from the current survey (spring 2019) are summarised below:

- Heavy iron staining/iron flocculent was observed at Site C2-1 and Site C2-2 and pool water had a pale-green milky appearance (Plates 3&4);
- Pool water levels appeared to be similar to pre-mining levels (Plate 3);
- *Baumea juncea*, *Lepidosperma filiforme* and *Lomandra fluviatilis* were relatively abundant at the time of this survey and in good health (Appendix 2a).
- Similar to Location C1, submerged aquatic macrophytes have consistently not been present (Appendices 3a&b).

⁶ In spring 2016, Location C2 was sampled on 24 October 2016.

⁷ Pools were impacted by the 'Eastern Tributary Incident' (exceedance of the Eastern Tributary performance measure) in spring 2016.



Plate 3: Tributary C – Location C2 (C2-1) (sp-19)

Looking upstream

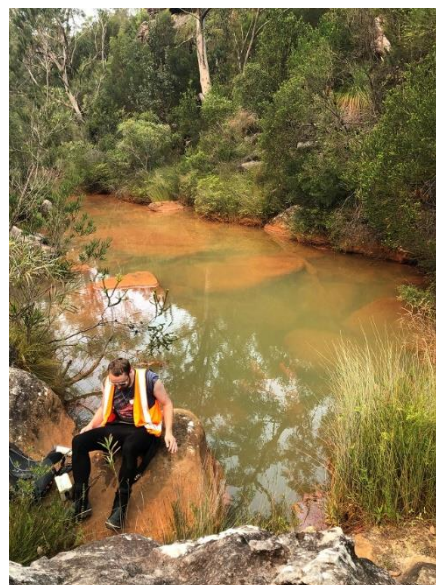


Plate 4: Tributary C – Location C2 (C2-2) (sp-19)

Looking downstream

Location C4

Location C4 is situated approximately 500 m downstream of Location C1, above Longwalls 24-25. The study reach was previously comprised by pools (up to approximately 25 m long, 8 m wide and 1.0 m deep) interrupted by runs and steep (up to approximately 10 m) cascades in places.

Mining related disturbances (i.e. iron staining) were first noted at this location in autumn 2014. At the time of the spring 2015 survey, rock fractures, flow diversions and low water levels were noted at the upstream site (i.e. Site C4-1) and within a relatively steep boulder field between sites C4-1 and C4-2. An iron complex covered up to approximately 95% of the remaining submerged substratum. Desiccation of the riparian assemblage was evident in some places. Water reappeared near the top of the reach at Site C4-2, providing some flow to that section of the stream.

Since spring 2015, large proportions of the study reach at Site C4-1 have commonly been dry but there has been flow along the study reach at Site C4-2.

The main findings from the spring 2019 survey are summarised below:

- The pool at the top of the study reach at Site C4-1 was full but the lower reaches were dry (Plate 5). Surface flow was apparent at Site C4-2;
- An iron complex covered up to approximately 95% of the submerged substratum at Site C4-2 (Plate 6);
- *Gleichenia dicarpa*, *Lepidosperma filiformis* and *Empodisma minus* were amongst the most common emergent macrophytes (Appendix 3a);
- Submerged aquatic macrophytes have consistently been absent (Appendix 3a&2b).



Plate 5: Tributary C – Location 4 (C4-1) (sp-19)

Looking downstream



Plate 6: Tributary C – Location 4 (C4-2) (sp-19)

Looking upstream

Control Locations

Woronora River

Sites were chosen on the Woronora River approximately 7 km from its headwaters (Appendix 1). The river flows through a steep valley into the Woronora Reservoir. The sites sampled were characterised by interconnected pools (up to approximately 1.0 m deep) approximately 1.5 to 11.7 m wide with predominantly sandy bottoms interspersed with large boulders and beds of the native aquatic macrophytes, *Triglochin procerum* and *Myriophyllum pedunculatum* (Plates 7&8) (Appendix 3).

Cummins et al. (2007a) reported that the riparian vegetation was dominated by the species *Acacia parramattensis*, *Banksia integrifolia*, *Juncus prismatocarpus* and *Viminaria juncea*. On this sampling occasion, the most common emergent macrophytes included *Gleichenia dicarpa* and *Lepidosperma filiforme* (Appendix 3).

No exotic plants or animals have been noted (Appendices 3, 4 and 5). With the exception of the gauging station located approximately 300 m downstream, there was no evidence of de-vegetation or bank degradation associated with human activities.

At the time of the spring 2019 survey, pool water levels were similar to levels noted by the spring and autumn 2018 and spring 2017 surveys (Plates 7&8). Flow has not been apparent along the study reach since the autumn 2018 survey. Visibility of the water was considered fair.



Plate 7: Woronora River (WR1) (sp-19)

Looking upstream



Plate 8: Woronora River (WR2) (aut-19)

Looking upstream

O'Hares Creek

The headwaters of this 4th order stream originate in an upland swamp. The creek is set in a sandstone gorge and natural rock-bars and waterfalls are common along the watercourse. The sandstone bedrock provided for short, infrequent riffles separating long reaching pools. The substratum was predominantly bedrock with some boulders and deposits of sand in areas of low flow. The immediate catchment is the Dharawal National Park.

At the sampling location, the creek consisted of a series of relatively large (approximately 4 to 18 m wide), interconnected pools to a depth of up to approximately 1.0 m (Plates 9&10). The substratum was predominantly bedrock with some boulders and deposits of sand in areas of low flow. With the exception of where Fire Road 10C crosses the creek, there was little evidence of disturbance.

At the time of this survey, dominant riparian vegetation included *Gleichenia dicarpa*, *Empodisma minus* and *Baumea* spp. (Appendix 2a). *Triglochin procerum* and *Chara/Nitella* spp. were common at Site OC1, the most upstream site (Appendix 3a). The exotic macrophyte, *Isolepis prolifera*, was observed at Site OC1 on this and many earlier occasions (Appendix 3a). Stream water visibility was excellent.



Plate 9: O'Hares Creek (OC1) (sp-19)

Looking upstream



Plate 10: O'Hares Creek (OC2) (sp-19)

Looking downstream

3.1.2 Aquatic Macroinvertebrates

AUSRIVAS Assessment

A total of 31 taxon were collected from the 10 sites sampled using the AUSRIVAS sampling protocol (Appendix 4). No individuals of the threatened dragonfly species, Adams emerald dragonfly (*Archaeophya adamsi*) (NSW Fisheries, 2002) or Sydney hawk dragonfly (*Austrocordulia leonardi*) (NSW Fisheries, 2007), were found. No individuals of the alien fish, *Gambusia holbrooki*, were collected using the AUSRIVAS technique by the spring 2019 survey.

The OE50 scores ranged between 0.29 (C2-2) and 0.77 (C1-2) (Table 1). Of the ten sites sampled, six were grouped in Band B (C1-2, C4-1, C4-2, WR1-2, OC1 and OC2) and four were grouped in Band C (C1-1, C2-1, C2-2 and WR1-1) (Table 1). Thus, fewer families of macroinvertebrates than expected were collected from the sites sampled in spring 2019 compared to reference sites selected by the AUSRIVAS model (Table 1).

Taxon with > 0.84 probability of occurrence but not collected included the water mite family, Acarina, at all sites except C1-2, C2-2 and OC2, and the caddis fly family, Leptoceridae, at C2-1, C2-2, and OC1. Gyrinidae (whirligig beetles) were expected but not collected at C1-2, C2-1, C2-2, WR1-1 and WR1-2.

Table 1. AUSRIVAS outputs for sites sampled at each location (spring 2019) ($n = 1$).

System	Location	Site	Site Code	OE50	Band
Tributary C	1	1	C1-1	0.48	C
	1	2	C1-2	0.77	B
	2	1	C2-1	0.38	C
	2	2	C2-2	0.29	C
	4	1	C4-1	0.67	B
	4	2	C4-2	0.57	B
Woronora River	1	1	WR1-1	0.5	C
	1	2	WR1-2	0.67	B
O'Hares Creek	1	1	OC1	0.57	B
	1	2	OC2	0.57	B

Sample not collected due to insufficient aquatic habitat.

Quantitative Assessment

A total of 1,065 individuals from 35 macroinvertebrate taxon were collected from 10 sites sampled using the quantitative sampling technique (Appendix 4). In spring 2019, the most abundant macroinvertebrate taxon was the mayfly family, Leptophlebiidae (659 individuals), followed by the Atyidae (110 individuals), Chironomidae (63 individuals) and the Dytiscidae (62 individuals) (Appendix 5). The native freshwater crayfish (*Euastacus* sp.) was not collected by either the AUSRIVAS or quantitative sampling techniques during the survey period (Appendix 5). *Gambusia* was only collected at Site OC1 (2 individuals) using the quantitative technique.

No individuals of the threatened dragonfly species, Adams emerald dragonfly (*Archaeophya adamsi*) (NSW Fisheries, 2002) or Sydney hawk dragonfly (*Austrocordulia leonardi*) (NSW Fisheries, 2007), were found. Confirmation of the presence of the Adams emerald dragonfly or Sydney hawk dragonfly would have triggered a response for further investigations of this species.

Mean abundance of macroinvertebrates was greatest at Site 2 sampled at O'Hares Creek (i.e. Site OC2), mostly due to large numbers of individuals of the mayfly family, Leptophlebiidae (Figure 2). Diversity of macroinvertebrates was greatest at locations sampled along the Woronora River and O'Hares Creek (Figure 3).

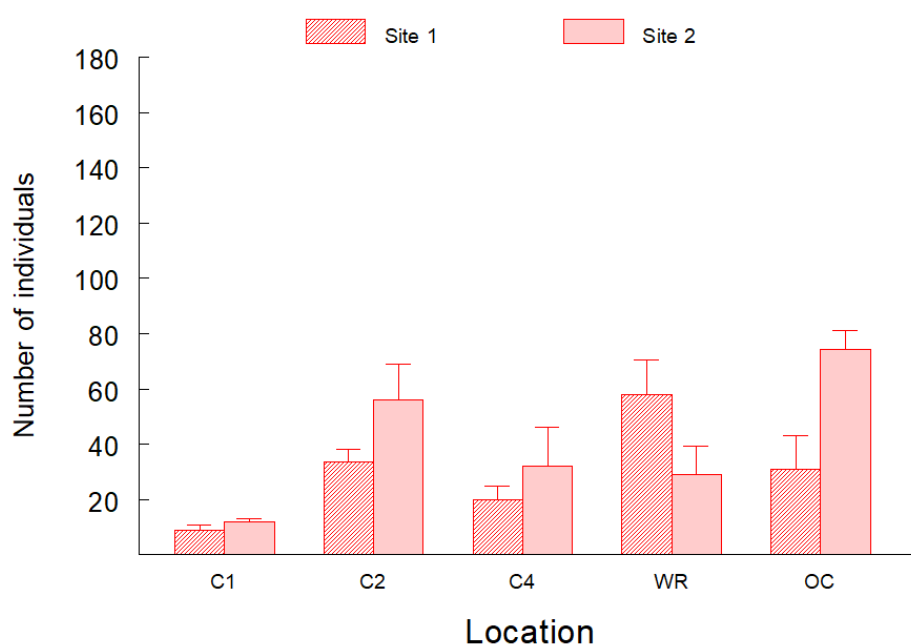


Figure 2. Mean (+SE) abundance of macroinvertebrates at each location (C – Eastern Tributary/Tributary C [C1 – Location 1 etc], WR – Woronora River, OC – O'Hares Creek).

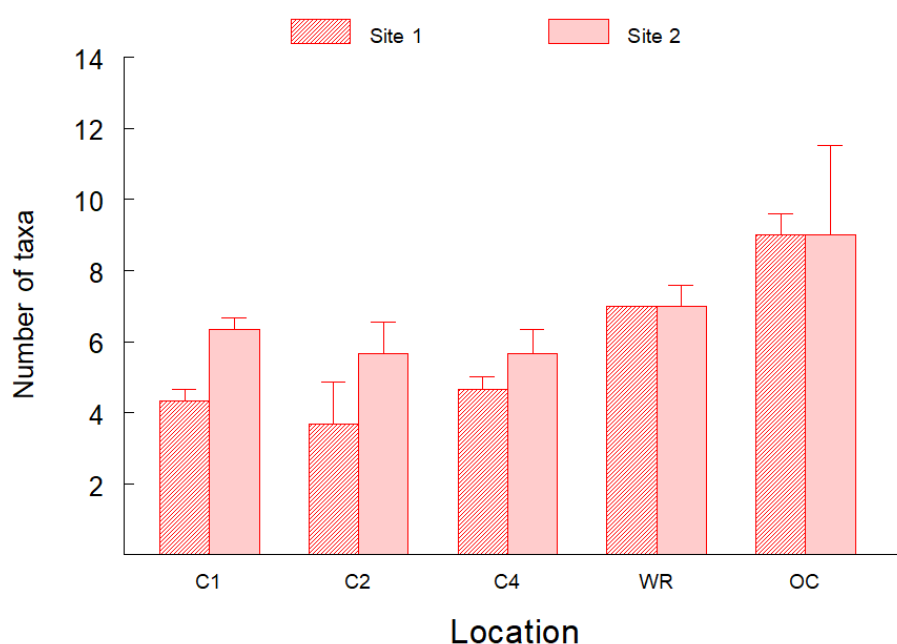


Figure 3. Mean (+SE) diversity of macroinvertebrates at each location (C – Tributary C [C1 – Location 1 etc], WR – Woronora River, OC – O’Hares Creek).

3.1.3 Aquatic Macrophytes

A total of 20 aquatic macrophyte species were identified in quantitative samples at sites on Tributary C/Eastern Tributary (C1: 6 species, C2: 6 species, C4: 10 species), Woronora River (10 species) and O’Hares Creek (9 species) (Appendix 3b).

Mean percentage cover of macrophytes was greatest at the location sampled on the Woronora River compared to all other locations sampled (Figure 4). Mean diversity of macrophytes recorded in the 0.25 m² quadrats at any one location was generally low (i.e. < 4 species) and there were no distinct patterns associated with any location (Figure 5).

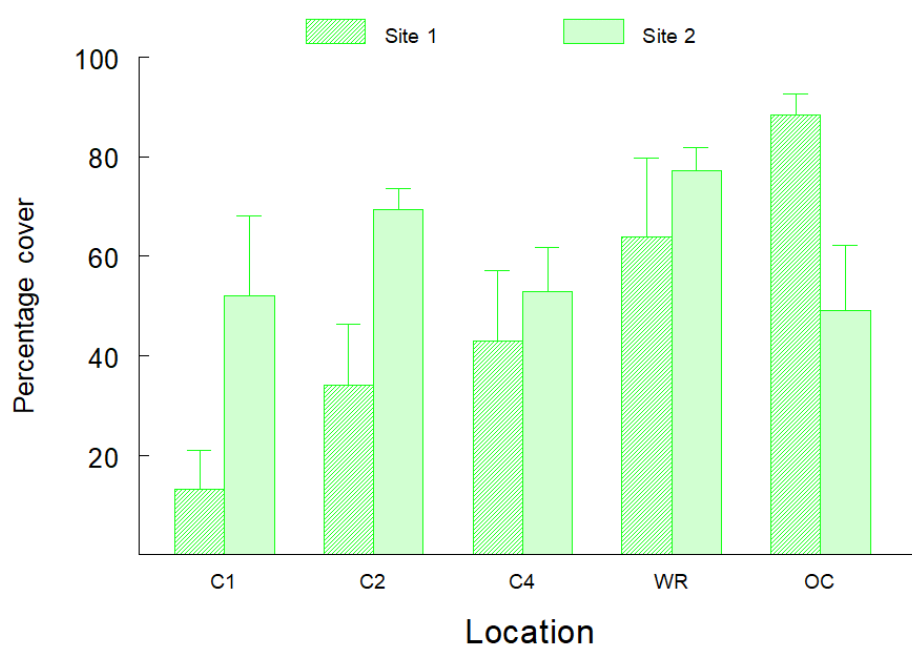


Figure 4. Mean (+SE) percentage cover of macrophytes at each location (C – Tributary C/Eastern Tributary [C1 – Location 1 etc], WR – Woronora River, OC – O’Hares Creek).

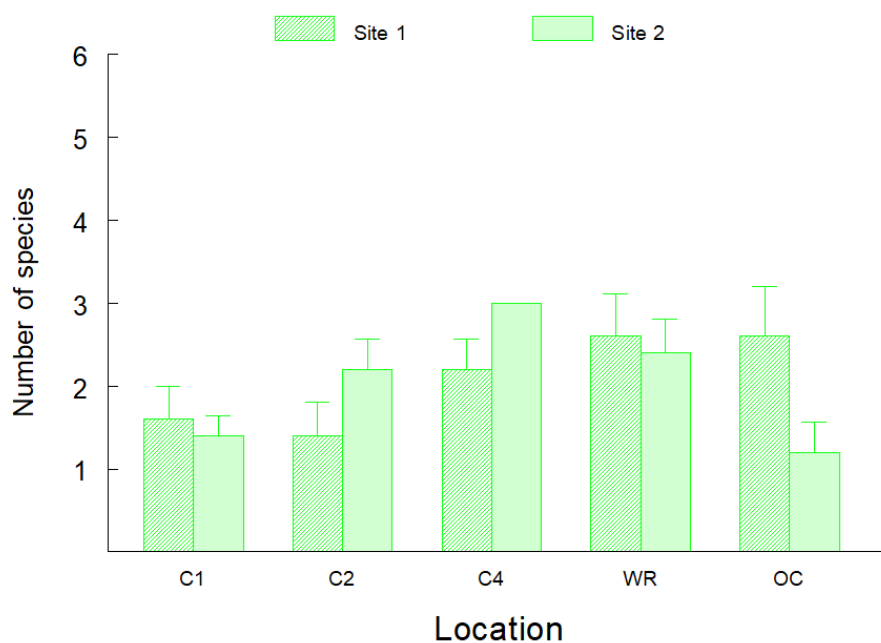


Figure 5. Mean (+SE) diversity of macrophytes at each location (C – Tributary C/Eastern Tributary [C1 – Location 1 etc], WR – Woronora River, OC – O’Hares Creek).

3.1.4 Water Quality

Mean physico-chemical water quality, alkalinity, total phosphorous and total nitrogen measurements are summarised in Table 2. Values highlighted in bold type indicate where results were outside the default trigger values (DTVs) recommended by ANZECC/ARMCANZ (2000) for protection of aquatic ecosystems in upland rivers (i.e. systems at > 150 m altitude) in southeast Australia.

In general, mean water temperature within the six creeks ranged from 14.3 to 23 °C (Table 2). Mean pH (range = 4.4 – 7.0) has commonly been below the lower DTV (i.e. pH 6.5-8.0; ANZECC/ARMCANZ, 2000) for upland rivers at the sites sampled. On this occasion, pH was below the lower DTV at all of the sites sampled except Site B2-2 (Table 2). Relatively low pH values are common in areas with a sandstone substratum.

Mean conductivity levels (range = 111 – 463 µS/cm) were once again above the upper DTV at the sites sampled along Tributary B. At the time of the spring 2019 survey, pool water levels at Site B2-1 appeared similar to pre-mining levels but a large proportion of the study reach at Site B2-2 was dry.

Mean dissolved oxygen levels (range = 28.8 – 89.1 %) were below the lower DTV (range = 90-110% saturation) at all of the sites sampled (Table 2).

Mean turbidity (range = 0.5 – 9.5 NTU) was below the lower DTV recommended by ANZECC/ARMCANZ (2000) (range = 2 – 25 NTU) at Site OC1 and OC2 (Table 2). Mean oxidation-reduction potential at the sites ranged from 493 to 665 mV (Table 2). Mean alkalinity ranged from 0 to 60 mg/L CaCO₃ (Table 2). For nutrients, mean concentrations of total phosphorous (range = <= 0.02 mg/L) were within the DTVs but total nitrogen (range = 0.07 to 0.62 mg/L) was above the upper DTV (0.25 mg/L) at Site C2-1, BC1, BC2, WOT1, WOT2, WR1, WR2, OC1 and OC2 (Table 2). The raw water quality data are provided in Appendix 6.

Table 2. Mean ($\pm$ SE) measurements of water quality variables recorded at each site (spring 2019). Values in bold are outside the default trigger values recommended by ANZECC/ARMCANZ (2000).

	Tributary C			
Location/Site	C1-1	C1-2	C2-1	C2-2
Temperature °C ($n = 3$)	20.6 (0.1)	20.1 (0.1)	23.0 (0.0)	17.5 (0.0)
pH ($n = 3$)	6.3 (0.0)	5.9 (0.0)	6.0 (0.0)	6.3 (0.0)
Conductivity ($\mu\text{S}/\text{cm}$) ($n = 3$)	171.7 (0.3)	187.7 (3.2)	270.3 (1.7)	266.3 (0.3)
DO (% Saturation) ($n = 3$)	76.2 (0.7)	38.0 (0.3)	46.2 (0.1)	34.8 (2.0)
Turbidity (NTU) ($n = 3$)	4.2 (0.0)	4.8 (0.0)	7.8 (0.0)	4.2 (0.1)
ORP (mV) ($n = 3$)	649.0 (0.0)	589.0 (1.2)	632.3 (0.9)	526.3 (7.3)
Alkalinity (mg/L CaCO_3) ($n = 2$)	14	30	50	60
Total phosphorous (mg/L) ($n = 2$)*	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.02 (0.00)
Total nitrogen (mg/L) ($n = 2$)*	0.18 (0.02)	0.16 (0.05)	0.29 (0.10)	0.22 (0.04)
	Tributary C		Woronora River	
Location/Site	C4-1	C4-2	WR1-1	WR1-2
Temperature °C ($n = 3$)	21.7 (0.0)	19.1 (0.0)	20.2 (0.0)	20.7 (0.0)
pH ($n = 3$)	6.4 (0.0)	5.9 (0.0)	5.9 (0.0)	5.8 (0.0)
Conductivity ($\mu\text{S}/\text{cm}$) ($n = 3$)	232.3 (1.7)	204.0 (0.0)	157.0 (2.9)	131.3 (1.3)
DO (% Saturation) ($n = 3$)	85.1 (0.4)	32.5 (0.0)	60.3 (0.2)	89.1 (0.0)
Turbidity (NTU) ($n = 3$)	6.1 (0.2)	2.9 (0.1)	4.7 (0.1)	2.7 (0.0)
ORP (mV) ($n = 3$)	664.7 (0.7)	510.3 (0.9)	642.7 (0.3)	635.0 (0.6)
Alkalinity (mg/L CaCO_3) ($n = 2$)	40	45	5	5
Total phosphorous (mg/L) ($n = 2$)*	0.01 (0.00)	0.02 (0.00)	0.02 (0.01)	0.01 (0.00)
Total nitrogen (mg/L) ($n = 2$)*	0.12 (0.01)	0.07 (0.01)	0.43 (0.01)	0.30 (0.06)
	O'Hares Creek			
Location/Site	OC1	OC2		
Temperature °C ($n = 3$)	22.0 (0.0)	21.5 (0.0)		
pH ($n = 3$)	6.0 (0.0)	6.4 (0.0)		
Conductivity ($\mu\text{S}/\text{cm}$) ($n = 3$)	111.0 (0.0)	118.7 (1.7)		
DO (% Saturation) ($n = 3$)	74.1 (0.2)	86.3 (0.1)		
Turbidity (NTU) ($n = 3$)	0.8 (0.0)	0.5 (0.0)		
ORP (mV) ($n = 3$)	617.7 (0.3)	651.0 (0.0)		
Alkalinity (mg/L CaCO_3) ($n = 2$)	16	18		
Total phosphorous (mg/L) ($n = 2$)*	0.01 (0.00)	0.01 (0.00)		
Total nitrogen (mg/L) ($n = 2$)*	0.30 (0.07)	0.20 (0.01)		

NB: Default trigger values recommended by ANZECC/ARMCANZ (2000) for upland streams: pH (6.5 – 8.0); Conductivity (30 – 350 $\mu\text{S}/\text{cm}$); Turbidity (2 – 25 NTU); Dissolved Oxygen (90–110 % Saturation); Total phosphorous (0.02 mg/L); Total nitrogen (0.25 mg/L). There are no ANZECC/ARMCANZ (2000) guideline values for Temperature, ORP or Alkalinity. *For any site where a value has been recorded as less than the detection limit, a value of half the detection limit was assigned in order to calculate the mean (e.g. <0.02 taken as 0.01). N/R = Not Recorded; I/A = Insufficient Aquatic Habitat.

4.0 TEMPORAL ANALYSES

A temporal comparison of the aquatic macroinvertebrate and macrophyte data was carried out for the Stream Monitoring component of the Longwalls 23-27 Aquatic Ecology Monitoring Programme. The quantitative data from the surveys done between spring 2008⁸, and spring 2019 were analysed using both multivariate and univariate techniques. AUSRIVAS data collected from the stream monitoring locations are also presented.

The objective of the following comparisons of aquatic macroinvertebrates and macrophytes was to determine whether any changes may have occurred at sampling locations in Tributary C/Eastern Tributary following the commencement of mining of the Longwalls 23-27 mining area, in relation to Control locations.

4.1 Tributary C/Eastern Tributary

PERMANOVA was used to test the null hypothesis of no significant change in aquatic macroinvertebrate and macrophyte indicators at Tributary C/Eastern Tributary locations (Locations C1, C2 and C4) Before- vs After- commencement of mining in relation to Control locations (Woronora River and O'Hares Creek). Mining of the Longwalls 23-27 mining area commenced in May 2014 and was completed in March 2017.

Significant differences between groups (e.g. impact versus control) may arise due to differences between group means, differences in dispersion (variance) among groups or a combination of both.

Significant main effects (e.g. Period or Impact) are not indicative of a mining-related impact, and, as such, are not described in detail. The Period x Impact interaction is the scale that would indicate that differences or changes could be attributable to mining.

⁸ Up until autumn 2018, temporal analyses for the Longwalls 23-27 monitoring report examined data for Locations C1 and C2 from spring 2009, to coincide with the commencement of sampling of Locations C4, B2 and Pools ETAH, ETAG, ETAK and ETAI (BIOANALYSIS, 2018). For the spring 2018 survey, data collected at Locations C1 and C2 in spring 2008 and autumn 2009 were also examined to assist in interpretation of results for the Longwalls 20-22 mining area reports and to increase the power of analyses. Similar results were obtained. Consequently, future analyses will include the spring 2008 and autumn 2009 data.

4.1.1 Aquatic Macroinvertebrates

Quantitative Assessment

Location C1 v Controls

Prior Surveys (Spring 2008 – Autumn 2019)

Multivariate analyses have detected a significant before-to-after change in the structure of the assemblage of aquatic macroinvertebrates at Location C1 in relation to the control locations in spring 2016 (T17) and autumn 2019 (T22). SIMPER analyses indicated that mayflies (Leptophlebiidae) and freshwater shrimp (Atyidae) have consistently contributed most to the structure of assemblages of macroinvertebrates at Location C1 and the control locations.

Univariate analyses found mean numbers of Leptophlebiidae at Location C1 have differed significantly before- vs after-commencement of mining in relation to the control locations on the past eight sampling occasions (i.e. since autumn 2015). Analyses have consistently found no significant difference to mean total diversity, total abundance or numbers of Atyidae at Location C1 that would indicate an impact from mining.

Current Survey (Spring 2019)

Similar to the findings of the spring 2016 (T17) survey, multivariate analyses detected a significant before-to-after difference in the structure of the assemblage of aquatic macroinvertebrates at Location C1 in relation to the control locations (Table 3, Figure 4a).

Visual examination of the Principle Co-ordinate Analysis (PCoA) found that there was greater dispersion among centroids representing the assemblage at Location C1 within the ‘after’ period than for each of the control locations, which could have contributed to the relatively low *P*-value (Figure 26a).

Greater dispersion among the C1 centroids coincides with considerable spikes in mean abundance of Leptophlebiidae at Location C1 within the ‘after’ period. For the first time since the autumn 2015 (T14) survey, univariate analyses did not detect a significant Period x Impact interaction for mean abundance of Leptophlebiidae (Table 4, Figure 5c). Numbers of Leptophlebiidae appear to have declined since the autumn 2019 survey, to become similar to numbers recorded within the ‘before’ period (Figure 5c).

Of the other key variables examined, univariate analyses have consistently found no significant difference in mean total diversity and abundance of macroinvertebrates or mean numbers of Atyidae at Location C1 compared to the control locations that would indicate an impact from mining (Table 4, Figures 5a,b&d).

In general, macroinvertebrate diversity appears to have increased at Location C1 since the spring 2018 (T21) survey (Figure 5a). Mean abundance at Location C1 appears to have decreased since the autumn 2019 (T22) survey (Figure 5b). Atyidae have not been collected at Location C1 since autumn 2018 (T20) (Figure 5d).

Location C2 v Controls

Prior Surveys (Spring 2008 – Autumn 2019)

For the first time since sampling commenced at Location C2 (spring 2008), multivariate analysis of the aquatic macroinvertebrate data detected a significant interaction of the factors Period x Impact in autumn 2019 (T22), which indicates an impact from mining at Location C2.

Univariate analyses indicate that mean numbers of Atyidae at Location C2 have differed significantly before- to after-commencement of mining in relation to the control locations since autumn 2016 (T16) (i.e. autumn and spring 2016, autumn and spring 2017, autumn 2018 and spring 2018 and autumn 2019).

A significant Period x Impact change in mean abundance was detected at Location C2 in spring 2018 (T1) and autumn 2019 (T22). Analyses have consistently found no significant change in total diversity or numbers of Leptophlebiidae at Location C2 compared to the controls (Figures 5a-c).

Current Survey (Spring 2019)

Similar to the findings from the autumn 2019 survey, multivariate analysis of the aquatic macroinvertebrate data detected a significant interaction of the factors Period x Impact, which indicates an impact from mining (Table 3, Figure 4b). The Principle Co-ordinate Analysis (PCoA) indicated greater variability in the structure of assemblages at Location C2 than at the control locations within the After-Period (Figure 4b). When PERMDISP was used to formally compare the apparent variability (dispersion), pair-wise tests found that temporal variation (i.e. among surveys) did in fact differ significantly between Periods at Location C2 ($t = 3.62$, $P = 0.005$) but not the Control treatment ($t = 2.32$, $P = 0.048$).

Pair-wise tests examining the composition of assemblages also detected a significant change between Periods at Location C2 ($t = 1.46$, $P = 0.023$) but not the Control treatment ($t = 1.19$, $P = 0.116$). SIMPER indicates that changes in the contribution that Atyidae have made to the assemblage at Location C2 (Before: 71.1 %; After: 34.5 %) have contributed greatly to this result. Atyidae have consistently been ranked highest for the Control treatment (Before: 43.5 %; After: 34.8 %). Similar to the findings from the previous survey, Leptophlebiidae contributed most to the structure of the assemblage at Location C2 (93.1 %) (SIMPER).

Notably, Atyidae were not collected at Location C2 by surveys done between spring 2017 (T19) and autumn 2019 (T22) (Figure 5d). Univariate analyses detected a significant change in mean numbers of Atyidae at Location C2 in relation to the control locations between autumn 2016 (T16) and autumn 2019 (T22), but not on this sampling occasion (Table 4, Figure 5d). One individual was collected by the spring 2019 (T23) survey (Figure 5d).

Similar to the findings from surveys done since spring 2018 (T21), univariate analyses detected a significant Period x Impact change in mean abundance at Location C2 in relation to the Controls (Table 4, Figure 5b). *Post-hoc* tests indicated that this result was mostly due to differences in dispersion ($F = 8.63$; $P = 0.003$), which is indicative of an effect of mining. Overall, there has been a small increase in mean numbers of individuals collected at Location C2 and the Control treatment since the commencement of mining (Figure 5b).

Similar to previous surveys, univariate analyses found no significant difference in mean numbers of species or Leptophlebiidae at Location C2 in relation to the Control (Table 4, Figure 5a&c). Similar numbers of taxa and Leptophlebiidae were collected at Location C2 and the Control treatment in spring 2019 (Figures 5a&c).

Location C4 v Controls

Prior Surveys (Spring 2009 – Autumn 2019)

Multivariate analyses found no evidence of significant change in the structure of the assemblage of macroinvertebrates or in key indicator variables at Location C4 that would indicate an impact from mining of Longwalls 23-27 (Figure 4c).

Analyses have consistently found no significant difference to total abundance of macroinvertebrates or numbers of Leptophlebiidae at Location C4 compared to the control sites. However, a significant ‘before’ to ‘after’ change has been detected for mean diversity at Location C4 in relation to the Control treatment since autumn 2018 (T18). Atyidae differed significantly at Location C4 compared to the Control treatment in autumn 2016 (T14) and spring 2018 (T21).

Current Survey (Spring 2019)

For the first time since sampling commenced at Location C4 (spring 2009), PERMANOVA detected a significant Period x Impact interaction in the structure of assemblages (Table 3). The PCoA and PERMDISP indicated that the structure of the assemblage of macroinvertebrates has become significantly more variable at Location C4 ‘after’ the commencement of mining ($t = 1.64$, $P = 0.003$), but not at the Control locations (PERMDISP: $t = 1.54$, $P = 0.164$) (Figure 4c). Furthermore, the structure of the assemblage at C4 changed significantly from ‘before’- to ‘after’-commencement of mining (Pair-wise test: $t = 1.64$, $P = 0.008$) but not at the Control locations ($t = 1.15$, $P = 0.18$).

SIMPER indicates that changes in the contribution that Atyidae have made to the assemblage at Location C4 (Before: 39.6 %; After: 8.2 %) have contributed greatly to this result. Atyidae have consistently been ranked highest for the Control treatment (Before: 42.4 %; After: 32.9 %). Leptophlebiidae contributed most (39.9 %) to the structure of the assemblage at Location C4 within the ‘after’ period, followed by the family, Chironomidae (31.4 %), and the Dytiscidae (9.8 %) (SIMPER).

There has consistently been no evidence of a significant change in mean abundance of macroinvertebrates or mean numbers of Leptophlebiidae at Location C4 that would indicate an impact from mining of the Longwalls 23-27 area (Table 4, Figure 5a-c). Nevertheless, mean numbers of macroinvertebrates at Location C4 appear more variable since spring 2015 (T15), mostly due to spikes in abundance of Leptophlebiidae (Figures 5b&c). In spring 2019, mean abundance at Location C4 and the controls was similar to the numbers recorded in autumn 2019, whilst Leptophlebiidae increased at Location C4 and the control locations.

Similar to the findings of surveys done since spring 2018 (T19), a significant ‘before’ to ‘after’ change in mean diversity was detected at Location C4 on this occasion (spring 2019) (Table 4, Figure 5a). *Post-hoc* tests indicated that this result was due to a combination of differences in dispersion ($P = 0.002$) and the group means (Location C4: $t = 0.60$, $P = 0.89$; Control: $t = 2.44$, $P = 0.01$).

A significant 'before' to 'after' change in mean numbers of Atyidae was detected at Location C4 on this occasion and in spring 2018 (T19) and autumn 2016 (T16) (Table 4, Figure 5d). Atyidae were not collected at Location C4 in autumn 2017, spring 2018, autumn 2019 or spring 2019 (Figure 5d).

Table 3. PERMANOVA on Bray Curtis dissimilarities of macroinvertebrate assemblage data (non-transformed) to compare locations C1, C2 and C4 sampled on Tributary C/Eastern Tributary with control locations (Woronora River and O'Hares Creek) Before- vs After-commencement of mining. Percentages of Components of Variation (% CV) are shown. Bold numbers indicate significant results at $P < 0.05$.

C1					
Source	df	MS	Pseudo-F	P	% CV
Period = Pe	1	41944	3.51	0.00	7.72
Impact = Im	1	41324	0.31	1.00	0.00
Time (Pe) = Ti(Pe)	21	7074	1.68	0.00	7.69
Location (Im) = Lo(Im)	1	139120	12.58	0.00	17.74
Pe x Im	1	17715	1.90	0.03	6.12
Site (Lo(Im)) = Si(Lo(Im))	3	7072	2.42	0.00	4.47
Pe x Lo(Im)	1	6068	1.13	0.27	2.26
Ti(Pe) x Im	21	5484	1.30	0.02	7.25
Pe x Si(Lo(Im))	3	3706	1.27	0.13	2.75
Ti(Pe) x Lo(Im)	21	4217	1.44	0.00	8.46
Ti(Pe) x Si(Lo(Im))	63	2920	1.63	0.00	11.14
Residual	276	1796			
C2					
Source	df	MS	Pseudo-F	P	% CV
Period = Pe	1	21934	1.60	0.09	6.13
Impact = Im	1	20517	0.30	1.00	0.00
Time (Pe) = Ti(Pe)	21	8373	2.03	0.00	10.36
Location (Im) = Lo(Im)	1	78707	8.82	0.00	19.18
Pe x Im	1	6918	0.88	0.66	0.00
Site (Lo(Im)) = Si(Lo(Im))	3	5093	1.94	0.00	5.02
Pe x Lo(Im)	1	7874	1.66	0.02	6.52
Ti(Pe) x Im	21	4695	1.14	0.19	5.34
Pe x Si(Lo(Im))	3	2201	0.84	0.72	0.00
Ti(Pe) x Lo(Im)	21	4131	1.58	0.00	10.10
Ti(Pe) x Si(Lo(Im))	63	2620	1.55	0.00	11.20
Residual	276	1689			26.15
C4					
Source	df	MS	Pseudo-F	P	% CV
Period = Pe	1	27990	2.82	0.00	7.01
Impact = Im	1	47189	0.40	0.98	0.00
Time (Pe) = Ti(Pe)	19	6666	1.84	0.00	8.68
Location (Im) = Lo(Im)	1	121300	12.46	0.00	19.14
Pe x Im	1	11714	1.75	0.04	5.63
Site (Lo(Im)) = Si(Lo(Im))	3	6328	2.56	0.00	4.98
Pe x Lo(Im)	1	4524	0.91	0.63	0.00
Ti(Pe) x Im	19	4203	1.16	0.13	5.37
Pe x Si(Lo(Im))	3	4058	1.64	0.02	4.51
Ti(Pe) x Lo(Im)	19	3615	1.46	0.00	8.70
Ti(Pe) x Si(Lo(Im))	57	2474	1.49	0.00	10.35
Residual	251	1663			25.64

Table 4. PERMANOVA analysis on Euclidean Distances of four univariate estimates (i.e. non-transformed) (total diversity and abundance and abundances of Leptophlebiidae and Atyidae) of the macroinvertebrate data collected to compare three locations within Tributary C with two control locations Before- vs After-commencement of mining. Bold numbers indicate significant results at $P < 0.05$.

C1		Diversity		Abundance		Leptophlebiidae		Atyidae	
Source	df	MS	Ps-F	MS	Ps-F	MS	Ps-F	MS	Ps-F
Period = Pe	1	253.34	7.29	28216	5.34	9099	5.77	1	0.75
Impact = Im	1	839.35	0.91	29374	1.91	287	0.21	4090	0.24
Time (Pe) = Ti(Pe)	21	37.43	1.67	5562	2.87	1601	8.25	351	1.02
Location (Im) = Lo(Im)	1	934.86	18.81	14145	5.06	1006	3.05	18509	22.26
Pe x Im	1	19.66	2.54	1716	1.59	3827	3.02	165	1.52
Site (Lo(Im)) = Si(Lo(Im))	3	27.91	2.52	1172	0.74	280	0.63	497	2.37
Pe x Lo(Im)	1	0.37	0.44	87	0.49	11	0.58	112	0.77
Ti(Pe) x Im	21	16.19	0.72	2210	1.14	1319	6.80	223	0.65
Pe x Si(Lo(Im))	3	3.85	0.35	1478	0.93	592	1.34	73	0.35
Ti(Pe) x Lo(Im)	21	22.37	2.02	1940	1.22	194	0.44	344	1.64
Ti(Pe) x Si(Lo(Im))	63	11.08	1.27	1589	2.50	442	2.41	210	1.63
Residual	276	8.70		635		184		129	
C2		Diversity		Abundance		Leptophlebiidae		Atyidae	
Source	df	MS	Ps-F	MS	Ps-F	MS	Ps-F	MS	Ps-F
Period = Pe	1	136.89	3.16	6911	2.63	1901	2.16	20	0.49
Impact = Im	1	462.19	0.88	17237	2.99	175	1.28	141	0.05
Time (Pe) = Ti(Pe)	21	50.29	2.25	2660	1.39	554	3.16	511	1.51
Location (Im) = Lo(Im)	1	530.87	15.22	5388	2.18	116	1.15	8792	14.05
Pe x Im	1	0.03	1.29	1227	1.84	17	0.34	533	2.33
Site (Lo(Im)) = Si(Lo(Im))	3	13.11	1.40	1030	1.00	70	0.42	300	1.68
Pe x Lo(Im)	1	0.05	0.36	692	0.80	409	3.06	227	1.10
Ti(Pe) x Im	21	17.28	0.77	1018	0.53	157	0.90	147	0.43
Pe x Si(Lo(Im))	3	3.55	0.38	238	0.23	13	0.08	29	0.16
Ti(Pe) x Lo(Im)	21	22.39	2.39	1912	1.85	175	1.06	339	1.90
Ti(Pe) x Si(Lo(Im))	63	9.37	1.13	1033	2.00	165	2.17	178	1.58
Residual	276	8.32		516		76		113	
C4		Diversity		Abundance		Leptophlebiidae		Atyidae	
Source	df	MS	Ps-F	MS	Ps-F	MS	Ps-F	MS	Ps-F
Period = Pe	1	57.61	2.40	13826	2.96	2008	2.02	237.4	1.62
Impact = Im	1	979.72	1.07	30257	2.02	82	0.14	4513.2	0.27
Time (Pe) = Ti(Pe)	19	32.08	1.36	5382	2.56	982	5.16	358.4	0.99
Location (Im) = Lo(Im)	1	919.81	17.80	14498	4.90	1280	3.73	17991	17.92
Pe x Im	1	103.03	6.54	836	1.96	495	0.92	211.9	4.74
Site (Lo(Im)) = Si(Lo(Im))	3	28.62	3.04	1117	0.88	231	0.81	652.2	3.66
Pe x Lo(Im)	1	1.73	0.29	0	0.40	105	0.63	13.2	0.40
Ti(Pe) x Im	19	17.63	0.75	1498	0.71	642	3.38	108.2	0.30
Pe x Si(Lo(Im))	3	14.19	1.51	1071	0.84	436	1.52	113.6	0.64
Ti(Pe) x Lo(Im)	19	23.59	2.51	2099	1.66	190	0.66	362.4	2.03
Ti(Pe) x Si(Lo(Im))	57	9.40	1.16	1268	1.54	286	1.10	178.4	1.42
Residual	251	8.13		822		260		125.95	

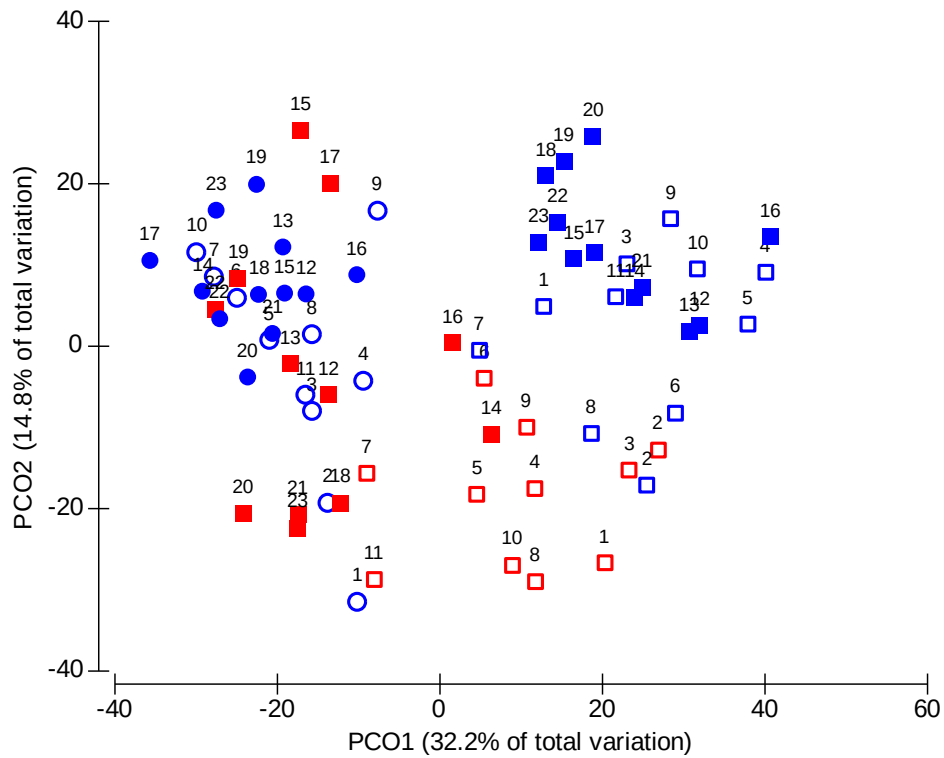
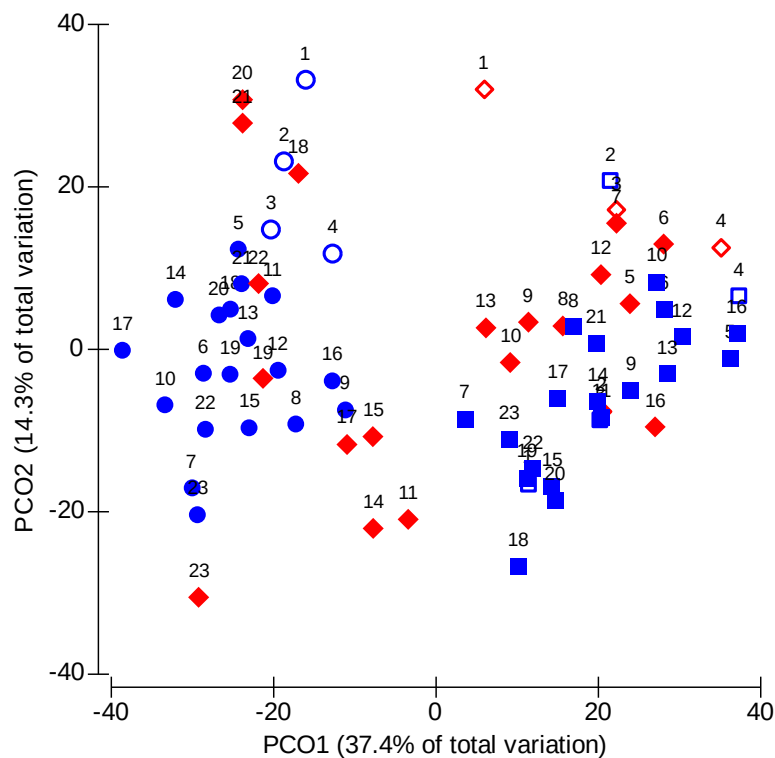
A. C1 v Controls**B. C2 v Controls**

Figure 4. Principle Coordinates ordination (PCoA) of centroids for assemblages of aquatic macroinvertebrates sampled at locations a) C1 (red squares), b) C2 (red diamonds) and two control locations (Woronora River: blue squares; O'Hares Creek (blue circles)) from spring 2008 (T1) to spring 2019 (T23). Empty symbols: 'Before'; Filled symbols: 'After' commencement of mining Longwalls 23-27.

C. C4 v Controls

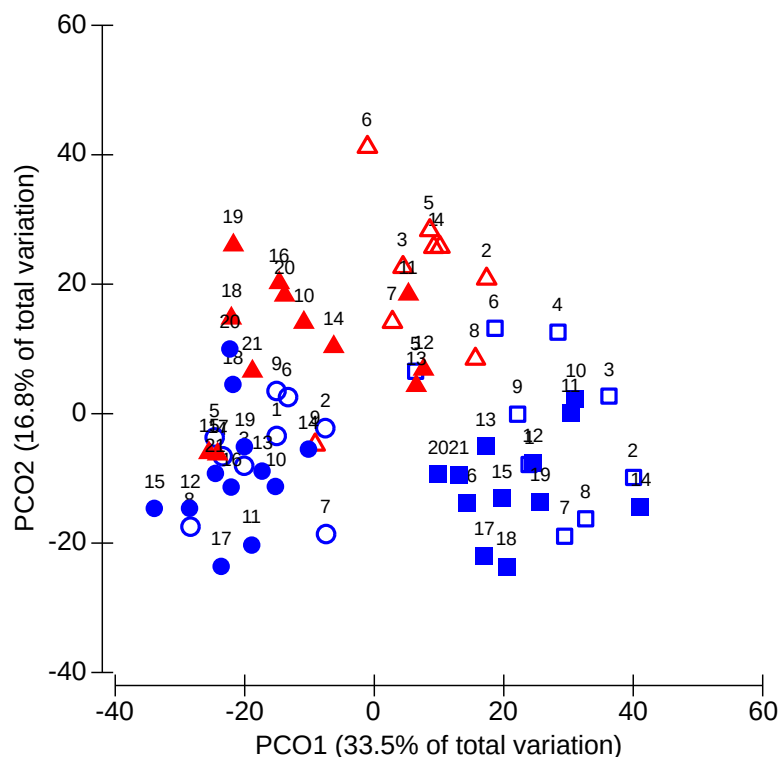


Figure 4 (Cont'd). PCoA of centroids for assemblages of aquatic macroinvertebrates sampled at location c) C4 (red symbols) and two control locations (Woronora River: blue squares; O'Hares Creek (blue circles) between spring 2009 (T1) and spring 2019 (T21). Empty symbols: 'Before-mining'; Filled symbols: 'After-mining Longwalls 23-27'.

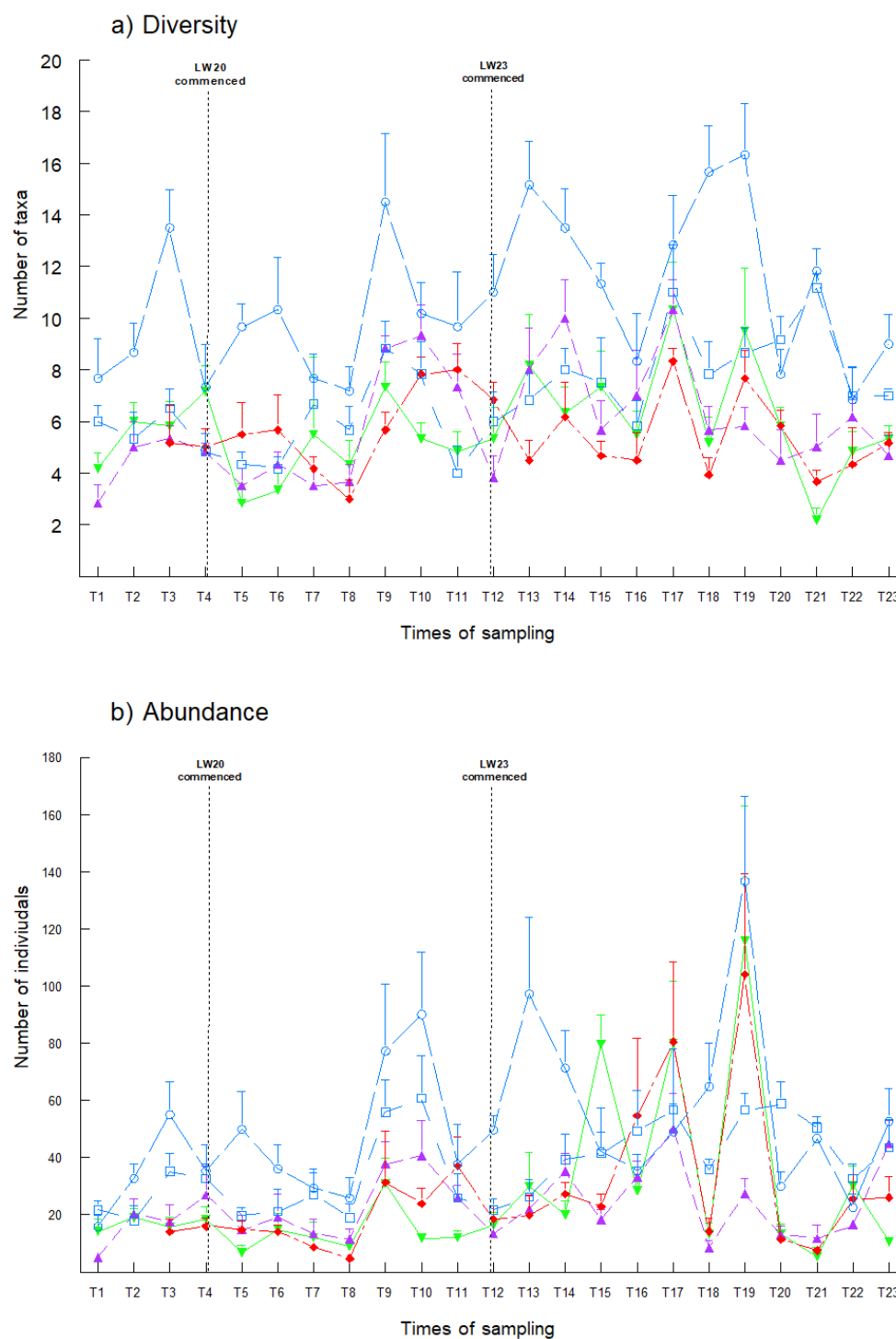


Figure 5. Mean number (+SE) of a) Taxa and b) Individuals of aquatic macroinvertebrates at locations on Tributary C/Eastern Tributary (C1: inverted triangles; C2: triangles; C4: diamonds) and the control locations (Woronora River: squares; O'Hares Creek: circles), between spring 2008 (T1) and spring 2019 (T23) ($n = 6$).

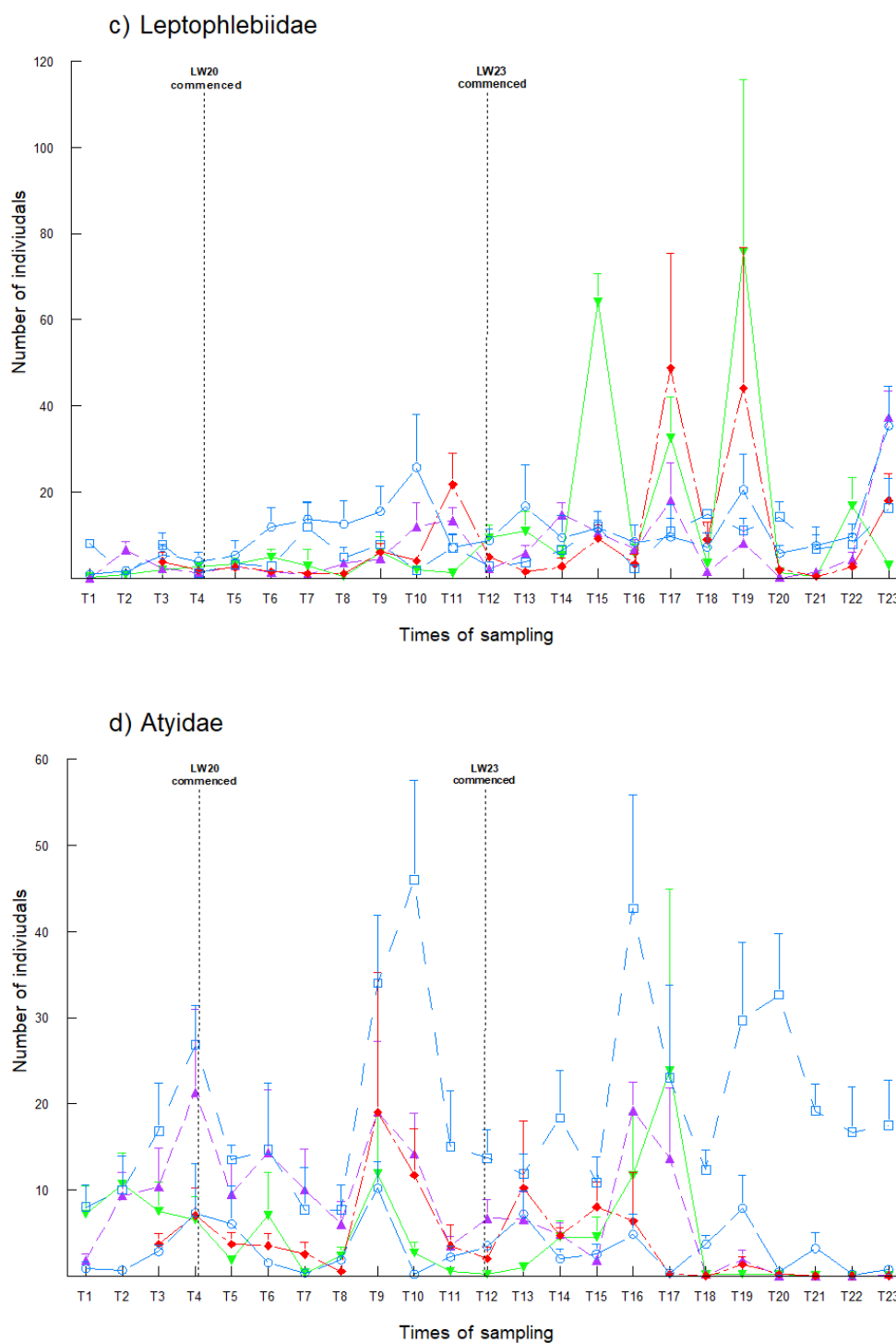


Figure 5 cont'd. Mean number (+SE) of c) Leptophlebiidae and d) Atyidae at locations on Tributary C/Eastern Tributary (C1: inverted triangles; C2: triangles; C4: diamonds) and the control locations (Woronora River: squares; O'Hares Creek: circles) between spring 2008 (T1) and spring 2019 (T23) ($n = 6$).

AUSRIVAS Analyses

For AUSRIVAS surveys done in spring, OE50 scores ranged between 0.10 (C1 in spring 2018) and 0.97 (OC in spring 2014) (Figure 6a). OE50 Taxa Scores for samples collected in autumn ranged from 0.09 (Location C4 in autumn 2016 and autumn 2019) to 0.88 (Location OC in autumn 2017) (Figure 6b). All of the OE50 Taxa scores were below 1.00 (Figure 6a&b), indicating that the number of taxa observed was less on all occasions than would be expected relative to the AUSRIVAS reference watercourses.

Only one location achieved a Band A score (equivalent to AUSRIVAS reference condition) in autumn (i.e. OC in autumn 2017) and two locations in spring (WR in spring 2014 and OC in spring 2014 and spring 2017) (Figure 6a&b). Locations that achieved a Band A score were sampled on control streams (i.e. Woronora River and O'Hares Creek).

Notably, on this sampling occasion, Location C1 and C4 increased to a Band B, possibly due to increased water level at Sites C1-1 and C4-1 (Figure 6a). Location C1 scored a Band D in spring 2018 (Figure 6a).

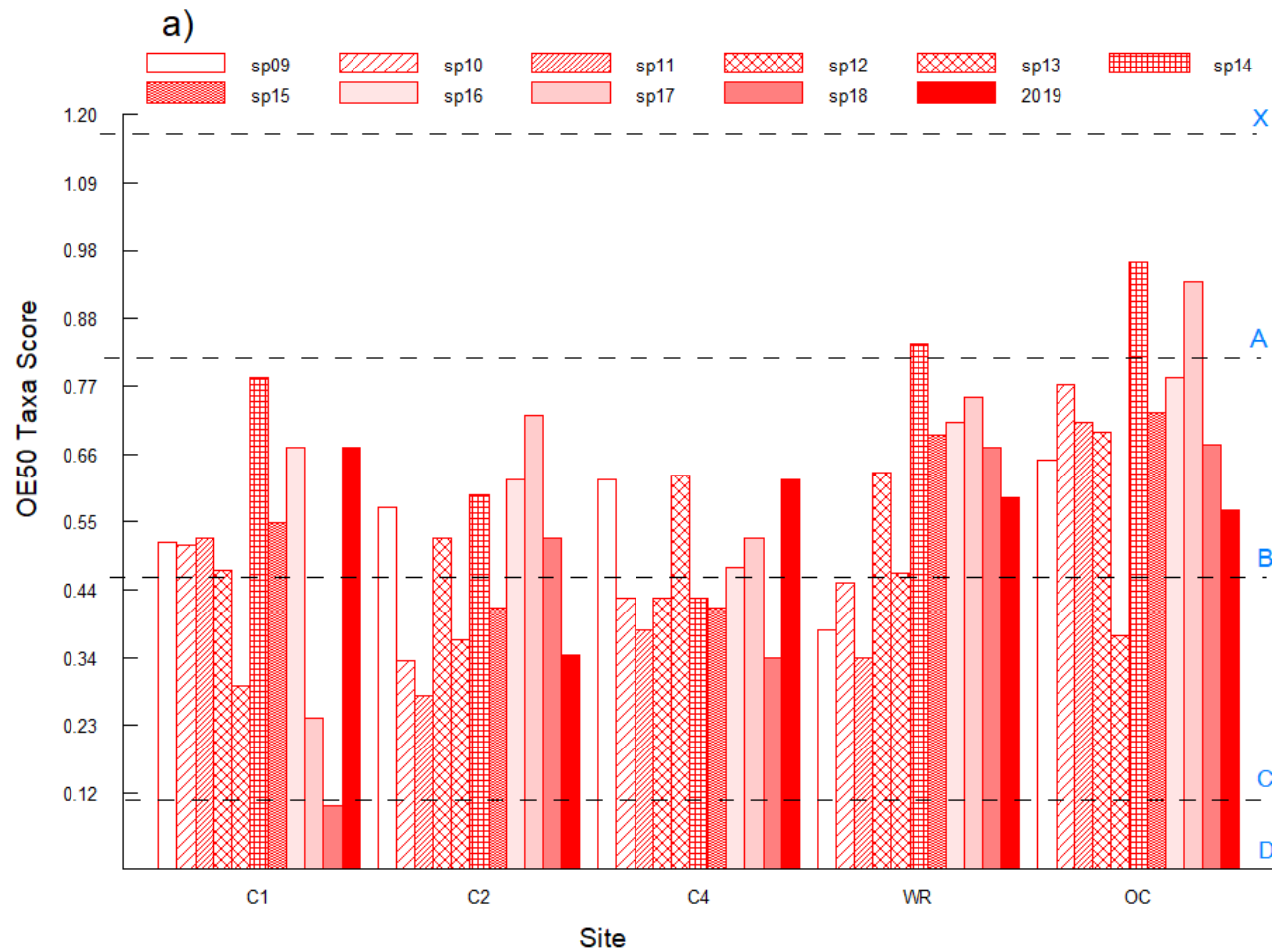


Figure 6a. Mean OE50 Taxa Scores and their respective Band Scores (X-D) from AUSRIVAS samples collected from edge habitat at each site in spring between 2009 and 2019 ($n = 2$).

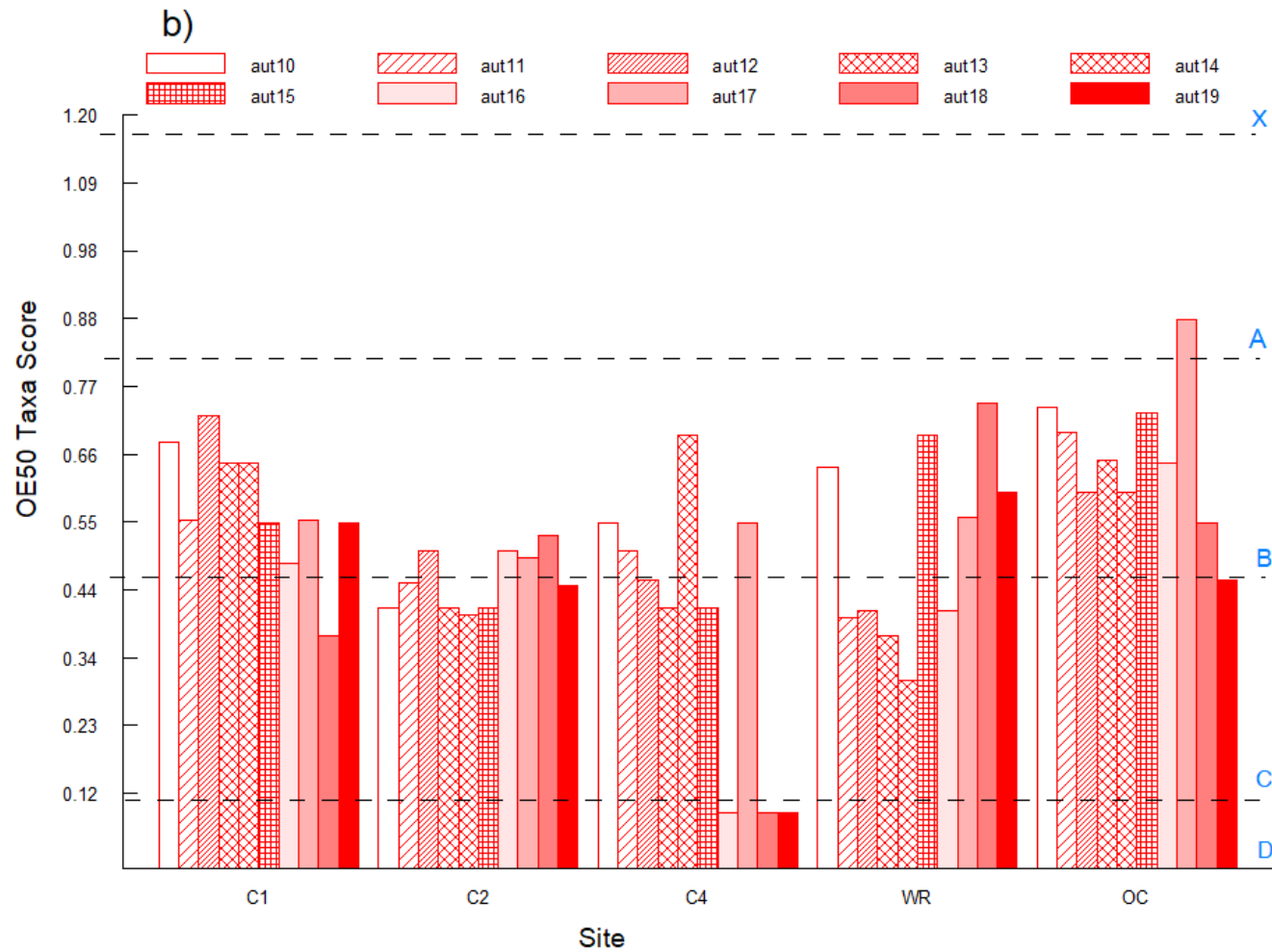


Figure 6b. Mean OE50 Taxa Scores and their respective Band Scores (X-D) from AUSRIVAS samples collected from edge habitat at each site in autumn between 2010 and 2019 ($n = 2$).

4.1.2 Aquatic Macrophytes

Location C1 v Controls

Prior Surveys (Spring 2008 – Autumn 2019)

Multivariate analyses have detected a significant change in the structure of the assemblage of aquatic macrophytes at Location C1 since spring 2017. Total cover and diversity of macrophytes however, have not differed significantly between periods in relation to the control locations.

The presence of the floating-attached species, *Triglochin procerum*, at the control locations but not at Location C1 has contributed greatly to observed differences in the structure of macrophyte assemblages between the treatment groups. In addition, *Baumea juncea* has been common at Location C1 but not at either of the control locations. The submerged species, *Myriophyllum pedunculatum*, has only been recorded at the Woronora River location.

Current Survey (Spring 2019)

Similar to the findings of surveys done since spring 2017 (T19), multivariate analyses detected a significant ‘before’ to ‘after’ change in assemblages of aquatic macrophytes at Location C1 in relation to the control locations (Table 5, Figure 7a). *Post hoc* tests indicated that differences were partly due to the composition of assemblages at Location C1 changing significantly less between periods than at the control locations (Figure 7a).

SIMPER analyses indicate that the fern, *Gleichenia dicarpa*, contributed most to the structure of assemblages at Location C1 within the Before (38.2 %) period but its contribution has declined within the After period (18.8 %). *Triglochin procerum* (29 %), *Lepidosperma filiforme* (25.0 %) and *Gleichenia dicarpa* (15.8 %) contributed most to the control assemblage within the ‘before’ period, whereas *Gleichenia dicarpa* (60.4 %), *Lepidosperma filiforme* (15.5 %) and *Chorizandra cymbaria* (5.1 %) were ranked highest within the ‘after’ period.

Univariate analysis of mean diversity and cover of macrophytes did not detect any changes at Location C1 that might be related to mining of the Longwalls 23-27 area (Table 6, Figures 8a&b). Graphically, it can be seen that total diversity of macrophytes at Location C1 has changed little over time (Figure 8a). Cover at Location C1 however, has declined considerably since the spring 2017 (T19) survey (Figure 8b). Fracturing of the streambed (predominantly bedrock) and a decline in pool water level (by up to 0.8 m) were first noted by the spring 2015 survey, at Site C1-1. Since then, pool water level at Site C1-1 has appeared to be below pre-mining levels.

Location C2 v Controls

Prior Surveys (Spring 2008 – Autumn 2019)

A significant before-to-after change in assemblages was detected at Location C2 in spring 2014 (T13) and between autumn 2016 (T16) and autumn 2019 (T22). A significant increase in species diversity was detected at Location C2 by the autumn 2017 (T18) and spring 2017 (T19) surveys, whilst a significant increase in total cover of macrophytes was detected in spring 2014 (T13).

Changes in the assemblage at Location C2 prior to the autumn 2017 survey did not appear to be related to mining activities given the absence of apparent physical changes to the riparian strip that might be associated with mining activities (e.g. gas release, altered pool-water levels or surface flow). Iron staining and falls in pool water levels below pre-mining levels were, however, noted at Location C2 by the spring 2016 and spring 2017 surveys, respectively.

Current Survey (Spring 2019)

A significant before-to-after change in the structure of the assemblage of aquatic macrophytes was detected at Location C2 in relation to the control locations in spring 2019 (Table 5, Figure 7b). Similar to the findings for Location C1, pairwise tests indicated that differences were mostly due to the composition of assemblages at Location C2 changing significantly less between periods than at the control locations (i.e. Before v After at Location C2: $t = 1.34$, $P = 0.073$; Before v After at the Controls: $t = 1.62$, $P = 0.007$) (Figure 7a).

This result is reflected in the patterns seen in the PCoA, which shows that the centroids representing assemblages at the Woronora River and O'Hares Creek locations within the 'before' period tend to group separately from the centroids within the 'after' period (Figure 7a).

SIMPER analyses indicate that *Baumea juncea* has increased its contribution to assemblages at Location C2 (Before: 13 %; After: 38 %). Contributions made by the fern, *Gleichenia dicarpa*, decreased at Location C2 (Before: 33 %; After: 12 %) but increased at the controls (Before: 14 %; After: 57 %) (SIMPER). In spring 2019, *Baumea juncea* contributed 64 % to the structure of the assemblage at Location C2, whilst *Lepidosperma filiforme* contributed 18 %.

Analyses found no statistically significant interaction in the effects of Period and Impact on variability in mean diversity or percentage cover of aquatic macrophytes (Table 6, Figure 8a&b). Mean cover at Location C2 in spring 2019 (T23) was similar to cover in autumn 2019 (T22) (Figure 8b).

Location C4 v Controls

Prior Surveys (Spring 2009 – Autumn 2019)

Similar to the findings of the autumn 2018 (T18) and spring 2018 (T19) surveys, analyses detected a significant before-to-after change in assemblages at Location C4 in autumn 2019 (T20). Analyses of temporal changes in total cover and species diversity at Location C4 have consistently found no significant changes in relation to control locations that would indicate an impact from mining.

Current Survey (Spring 2019)

Analyses detected a significant before-to-after change in the structure of assemblages of aquatic macrophytes in spring 2019, in relation to the controls (Table 5, Figure 7c). *Post-hoc* tests indicated that this result was due to temporal differences in dispersion ($P = 0.001$) between periods at Location C4 in relation to the control locations as well as changes in the composition of assemblages (C4: $t = 1.96$, $P = 0.002$; Controls: $t = 1.69$, $P = 0.008$) (Figure 7c).

SIMPER indicated that *Lepidosperma filiforme* (37.3 %), *Gleichenia dicarpa* (23.3%), *Lomandra fluviatilis* (20.3 %) contributed most to the structure of the macrophyte assemblage at Location C4 within the ‘before’ period whilst *Lepidosperma filiforme* (31.9 %), *Viminaria juncea* (26.0 %) and *Gleichenia dicarpa* (18.8 %) contributed most within the ‘after’ period.

Analyses of species diversity and total cover of aquatic macrophytes did not detect a significant interaction between the factors of interest (Pe and Im) (Table 6). Overall, species diversity of macrophytes at Location C4 appears to have differed little over time (Figure 8a). Graphically, there appeared to have been a considerable decrease in cover at Location C4 after the spring 2017 (T19) survey (Figure 8b). Large reductions in pool water levels have been noted along the study reach since spring 2015, particularly at the upstream site (Site C4-1). Between the autumn 2019 (T22) and spring 2019 (T23) surveys, mean cover at Location C2 had decreased whilst there was an increase at the control locations (Figure 8b).

Table 5. PERMANOVA on Bray Curtis dissimilarities of macrophyte assemblage data (non-transformed) to compare locations sampled on Tributary C/Eastern Tributary (C1, C2 and C4) with control locations (Woronora River and O'Hares Creek). Percentages of Components of Variation (% CV) are shown.

C1					
Source	df	MS	Pseudo-F	P	% CV
Period = Pe	1	37465	2.38	0.01	5.72
Impact = Im	1	103490	1.83	0.10	8.15
Time (Pe) = Ti(Pe)	21	5582	1.36	0.02	4.78
Location (Im) = Lo(Im)	1	54002	1.06	0.36	2.35
Pe x Im	1	34747	2.35	0.01	7.78
Site (Lo(Im)) = Si(Lo(Im))	3	49396	19.37	0.00	13.02
Pe x Lo(Im)	1	11893	0.91	0.63	0.00
Ti(Pe) x Im	21	4648	1.13	0.21	4.07
Pe x Si(Lo(Im))	3	11711	4.59	0.00	8.14
Ti(Pe) x Lo(Im)	21	4115	1.61	0.00	8.06
Ti(Pe) x Si(Lo(Im))	63	2550	0.74	1.00	0.00
Residual	552	3468			37.94
C2					
Source	df	MS	Pseudo-F	P	% CV
Period = Pe	1	27408	1.87	0.03	4.73
Impact = Im	1	94083	1.50	0.18	7.07
Time (Pe) = Ti(Pe)	21	4759	1.22	0.07	3.87
Location (Im) = Lo(Im)	1	61864	1.35	0.13	5.88
Pe x Im	1	30241	2.18	0.02	7.54
Site (Lo(Im)) = Si(Lo(Im))	3	43911	15.66	0.00	12.99
Pe x Lo(Im)	1	12008	1.09	0.32	2.29
Ti(Pe) x Im	21	3647	0.93	0.70	0.00
Pe x Si(Lo(Im))	3	9628	3.43	0.00	7.48
Ti(Pe) x Lo(Im)	21	3909	1.39	0.00	7.21
Ti(Pe) x Si(Lo(Im))	63	2804	0.79	1.00	0.00
Residual	552	3560			40.94
C4					
Source	df	MS	Pseudo-F	P	% CV
Period = Pe	1	30846	2.83	0.01	6.62
Impact = Im	1	68050	1.13	0.39	4.05
Time (Pe) = Ti(Pe)	19	3560	1.06	0.32	2.04
Location (Im) = Lo(Im)	1	59917	1.44	0.10	7.13
Pe x Im	1	19272	1.92	0.02	6.57
Site (Lo(Im)) = Si(Lo(Im))	3	40011	15.08	0.00	14.06
Pe x Lo(Im)	1	8547	1.22	0.18	3.30
Ti(Pe) x Im	19	3224	0.96	0.62	0.00
Pe x Si(Lo(Im))	3	5788	2.18	0.00	5.76
Ti(Pe) x Lo(Im)	19	3356	1.26	0.01	6.18
Ti(Pe) x Si(Lo(Im))	57	2654	0.74	1.00	0.00
Residual	504	3600			44.28

Table 6. PERMANOVA analysis on Euclidean Distances of non-transformed total diversity and abundance of macrophytes collected from three locations within Tributary C and at two control locations. Bold numbers indicate significant results at $P < 0.05$.

C1		Diversity		Abundance	
Source	df	MS	Pseudo-F	MS	Pseudo-F
Period = Pe	1	39.73	5.55	11453	1.04
Impact = Im	1	1.67	0.82	1171	0.19
Time (Pe) = Ti(Pe)	21	4.42	2.17	2580	2.54
Location (Im) = Lo(Im)	1	0.66	0.60	9087	0.92
Pe x Im	1	3.40	0.78	3280	0.36
Site (Lo(Im)) = Si(Lo(Im))	3	1.27	0.96	9436	17.13
Pe x Lo(Im)	1	3.11	1.12	9424	2.73
Ti(Pe) x Im	21	3.89	1.91	2406	2.37
Pe x Si(Lo(Im))	3	1.92	1.44	2643	4.80
Ti(Pe) x Lo(Im)	21	2.04	1.53	1016	1.84
Ti(Pe) x Si(Lo(Im))	63	1.33	1.03	551	0.96
Residual	552	1.29		576	
Total	689				
C2		Diversity		Abundance	
Source	df	MS	Pseudo-F	MS	Pseudo-F
Period = Pe	1	37.74	4.39	2726	0.36
Impact = Im	1	6.96	4.20	1	0.23
Time (Pe) = Ti(Pe)	21	6.82	3.39	1427	1.30
Location (Im) = Lo(Im)	1	0.00	0.38	3600	0.47
Pe x Im	1	5.82	1.80	316	0.14
Site (Lo(Im)) = Si(Lo(Im))	3	1.38	1.07	8030	12.27
Pe x Lo(Im)	1	2.23	1.04	9092	2.34
Ti(Pe) x Im	21	2.13	1.06	1186	1.08
Pe x Si(Lo(Im))	3	1.35	1.05	3060	4.68
Ti(Pe) x Lo(Im)	21	2.01	1.57	1100	1.68
Ti(Pe) x Si(Lo(Im))	63	1.28	0.98	654	1.39
Residual	552	1.31		469	
Total	689				
C4		Diversity		Abundance	
Source	df	MS	Pseudo-F	MS	Pseudo-F
Period = Pe	1	9.25	1.89	5687	0.73
Impact = Im	1	0.86	2.64	32	0.31
Time (Pe) = Ti(Pe)	19	2.92	1.42	1201	1.01
Location (Im) = Lo(Im)	1	0.16	0.23	3044	0.46
Pe x Im	1	0.54	0.65	15	0.13
Site (Lo(Im)) = Si(Lo(Im))	3	4.28	3.24	6437	15.02
Pe x Lo(Im)	1	3.05	0.78	8180	4.68
Ti(Pe) x Im	19	0.95	0.46	901	0.75
Pe x Si(Lo(Im))	3	3.58	2.70	645	1.51
Ti(Pe) x Lo(Im)	19	2.05	1.55	1195	2.79
Ti(Pe) x Si(Lo(Im))	57	1.32	1.07	429	0.87
Residual	504	1.24		5687	0.73
Total	629				

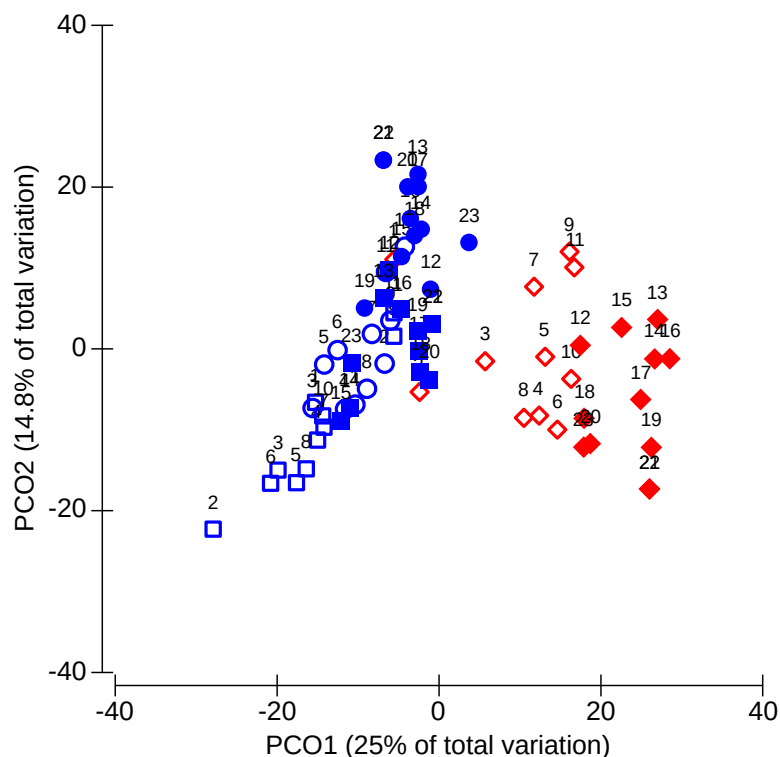
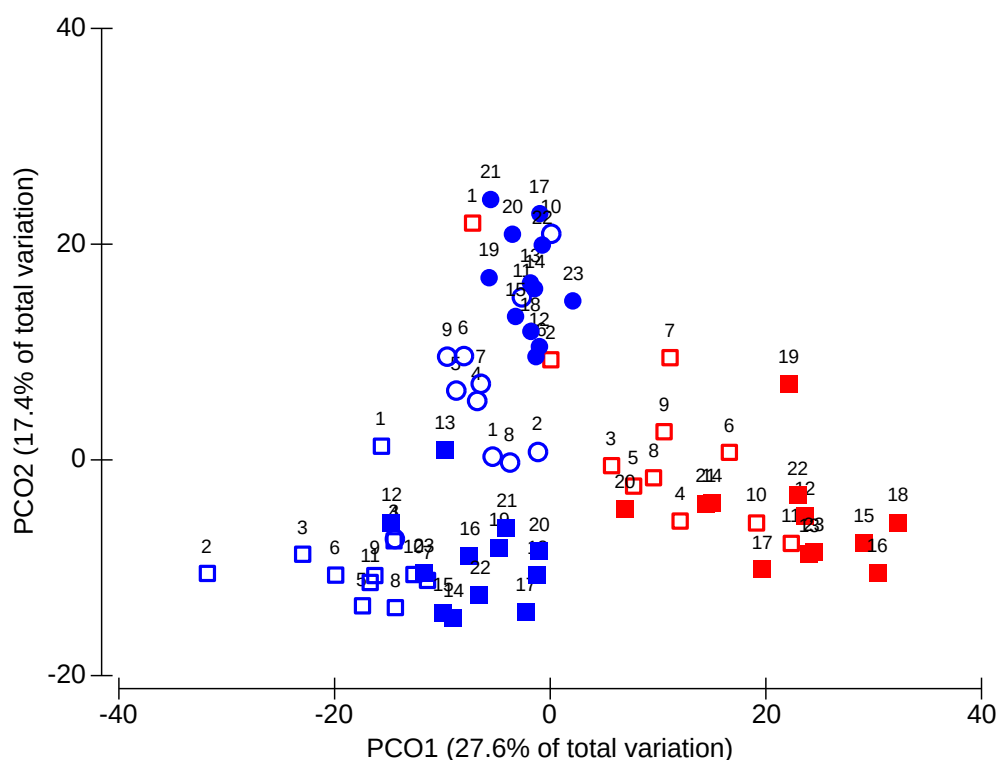
A. C1 v Controls**B. C2 v Controls**

Figure 7. PCoA of centroids for assemblages of macrophytes sampled at locations a) C1 and b) C2 (red symbols) and two control locations (Woronora River: blue squares; O'Hares Creek (blue circles) between spring 2008 (T1) and spring 2019 (T23). Empty symbols: 'Before-mining'; Filled symbols: 'After-mining Longwalls 23-27'.

C. C4 v Controls

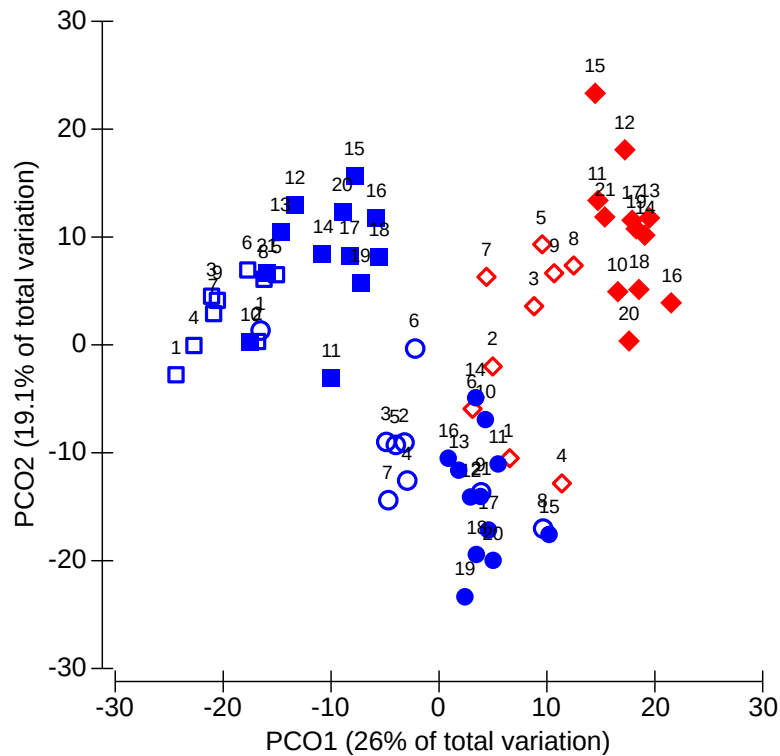


Figure 7 (Cont'd). PCoA of centroids for assemblages of macrophytes sampled at location c) C4 (red symbols) and two control locations (Woronora River: blue squares; O'Hares Creek (blue circles) between spring 2009 (T1) and spring 2019 (T21). Empty symbols: 'Before-mining'; Filled symbols: 'After-mining Longwalls 23-27'.

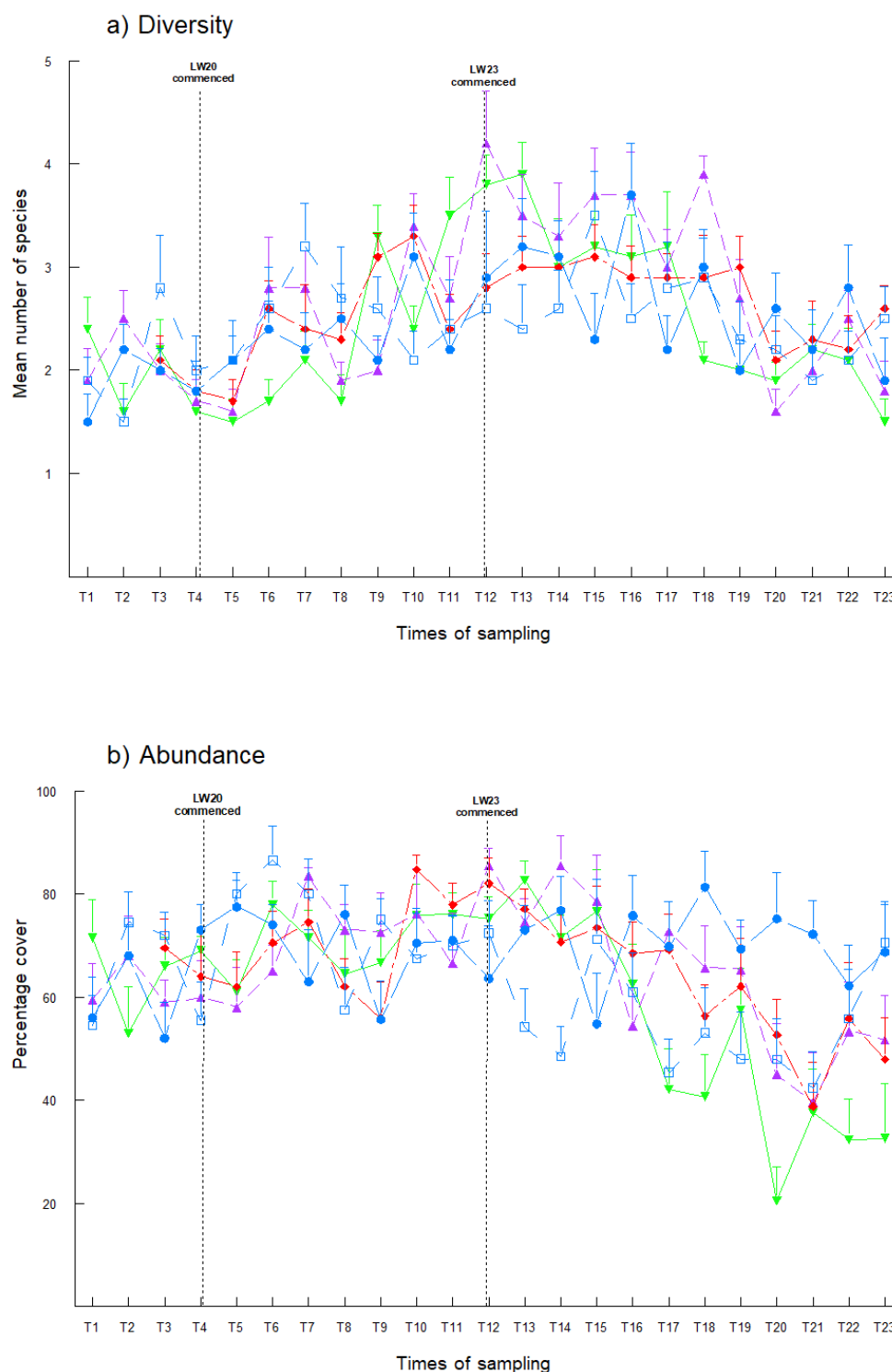


Figure 8. Mean (+SE) a) Number of species/quadrat ($n = 10$) and b) Percentage cover of aquatic macrophytes at locations on Tributary C/Eastern Tributary (C1: inverted triangles; C2: triangles; C4: diamonds) and the control locations (Woronora River: squares; O'Hares Creek: circles) between spring 2008 (T1) and spring 2019 (T23).

5.0 CONCLUSIONS

Sampling of aquatic indicators for the Longwalls 23-27 Aquatic Ecology Monitoring program was undertaken at potential impact places (i.e. stream locations and pools) sampled along Tributary C/Eastern Tributary and at appropriate control places. To date, nine to eleven monitoring surveys have been carried out prior to commencement of mining of Longwall 23 (i.e. from spring 2008 or spring 2009 to spring 2013) and twelve surveys within the ‘After-mining’ (i.e. autumn 2014 to spring 2019) monitoring phase.

The objective of this monitoring program is to assess whether significant changes in aquatic macroinvertebrate and/or aquatic macrophyte indicators have occurred at the potential impact places sampled in Tributary C/Eastern Tributary following the commencement of mining of Longwall 23, in relation to the control places.

A summary of the main findings to date are presented below.

5.1 Tributary C/Eastern Tributary (Locations C1, C2, C4)

Locations C1 and C4 overly the Longwalls 23-27 underground mining area while Location C2 is downstream of the Longwalls 23-27 underground mining area.

Since autumn 2014, subsidence associated with extraction of Longwalls 23-27 appears to have reduced the quality and availability of aquatic habitat at Locations C1 and C4. Impacts include iron staining, cracking of the stream substratum and occasional falls in pool water levels. At Location C2, iron staining and a fall in pool water levels below pre-mining levels were first noted by the spring 2016 and spring 2017 surveys, respectively.

The condition of the aquatic macroinvertebrate fauna at Location C1 has ranged from Band B (below AUSRIVAS reference condition) in spring 2014 to Band D (extremely impaired) in spring 2018.

Multivariate and univariate analyses of the monitoring data were used to test whether there was evidence of significant change in aquatic macroinvertebrate and macrophyte indicators at Tributary C/Eastern Tributary locations before- versus after-commencement of mining the Longwalls 23-27 area (mining commenced in May 2014), in relation to Control locations.

To date, multivariate analyses have detected a significant before-to-after change in the structure of the assemblage of aquatic macroinvertebrates at Location C1 in relation to the control locations in spring 2016, autumn 2019 and spring 2019, which would indicate an impact from mining. Mean numbers of the mayfly family, Leptophlebiidae, were significantly more variable within the after-period at Location C1 between autumn 2015 and autumn 2019, but not in spring 2019. Assemblages of macrophytes at Location C1 appear to have experienced a degree of environmental stress (desiccation) as a result of mining activities since spring 2017.

At Location C2, the AUSRIVAS condition of the fauna at Location C2 ranged from an OE50 score of 0.15 (Band C) in spring 2008 to 0.72 (Band B) in spring 2017. Multivariate analysis of the aquatic macroinvertebrate data detected a significant Period x Impact interaction in autumn 2019 and spring 2019). Changes appear to coincide with mining of the Longwalls 301-303 area. Analyses have detected a significant change in mean abundance at Location C2 in spring 2018, autumn 2019 and spring 2019. A significant decrease in mean numbers of the freshwater shrimp family, Atyidae, at Location C2 since autumn 2016, but not in spring 2019. Notably, up until the spring 2019 survey, reduced numbers of Atyidae had persisted at Location C2 for three consecutive years.

The structure of the macrophyte assemblage at Location C2 appears to have changed significantly within the after-period for Longwalls 23-27 (i.e. spring 2014, autumn 2016 to spring 2019) although changes prior to the spring 2017 survey did not appear to be related to mining activities.

At Location C4, the AUSRIVAS condition of the fauna ranged from an OE50 score of 0.09 (Band D) in autumn 2016, 2018 and 2019 to 0.63 (Band B) in spring 2013. For the first time since sampling commenced at Location C4 (spring 2009), analyses detected a significant Period x Impact change in the structure of assemblages in spring 2019, which indicates an impact from mining. Temporal patterns in diversity of macroinvertebrates at Location C4 have differed significantly between periods since autumn 2018, in relation to the control locations. A significant decrease in Atyidae has been detected at Location C4 within the after-period in autumn 2016, spring 2018 and spring 2019. Analyses have detected a significant before-to-after mining change in the structure of assemblages of aquatic macrophytes at Location C4 since autumn 2018.

It is noted that Locations C1 and C2 are also sampled to assess potential impacts associated with mining of the Longwalls 20-22 area. To date, the main findings from analyses that include data collected for the spring 2019 survey include:

- No significant changes to aquatic macroinvertebrate or macrophyte assemblages that might be related to mining of the Longwalls 20-22 area;
- A significant difference in patterns of change in mean numbers of the freshwater shrimp family, Atyidae, was detected at Location C2 in relation to the control locations within the after-mining period in spring 2015 and autumn 2017 (BIOANALYSIS, 2020).

5.2 Metropolitan Coal Longwall 304 Biodiversity Management Plan Trigger Action Response Plan

A reconciliation against the Trigger Action Response Plan in the Metropolitan Coal Longwall 304 Biodiversity Management Plan (Appendix 1) has been completed (Table 7).

Table 7. Assessment of Environmental Performance – Underground Mining Area and Surrounds (spring 2019).

Performance Measure	Performance Indicator	Monitoring Site(s) being Assessed	Parameters	Highest Significance Level/Trigger Recorded		Comments	Subsidence Impact Performance Indicator Exceeded?	Subsidence Impact Performance Measure Exceeded?
Monitoring of Aquatic Biota, Stream Monitoring								
Negligible impact on Threatened Species, Populations, or Ecological Communities	The aquatic macroinvertebrate and macrophyte assemblages in streams are not expected to experience long-term impacts as a result of mine subsidence	Two sampling sites (approximately 100 m in length) at the following locations: - Locations ET1 and ET4 on the Eastern Tributary overlying Longwalls (LW) 20-27. - Location ET2 on the Eastern Tributary, downstream of LW20-27. - Control Locations: WR1 on Woronora River; and OC on O’Hares Creek.	Aquatic macroinvertebrates Aquatic macrophytes	Level 1	Data analysis indicates no significant changes in relation to control places pre-mining ⁶ compared to post-extraction ⁷ occur in the aquatic macroinvertebrate and/or macrophyte assemblages at Locations ET1, ET2 or ET4 on the Eastern Tributary during the mining of LW304.	Locations ET1, ET2, ET4 (all parameters excluding those noted in Levels 2 and 3 below).	No	No
				Level 2	Data analysis indicates significant (not long-term), changes in relation to control places pre-mining ⁶ compared to post-extraction ⁷ occur in the aquatic macroinvertebrate and/or macrophyte assemblages at Locations ET1, ET2 or ET4 on the Eastern Tributary during the mining of LW304.	Location ET1 (significant change in assemblage of macroinvertebrates recorded in spring 2016, autumn 2019 and spring 2019; altered macrophyte assemblage within the after-period however, changes prior to spring 2017 do not appear to be related to mining). Location ET2 (significant change in assemblage of macroinvertebrates in autumn and spring 2019; and abundance of macroinvertebrates in spring 2018, autumn and spring 2019). Location ET4 (significant change in assemblage of macroinvertebrates in spring 2019; altered patterns of diversity of macroinvertebrates since autumn 2018; decreased numbers of Atyidae in autumn 2016, spring 2018 and spring 2019; altered macrophyte assemblage within the after-period in autumn 2018, spring 2018 and spring 2019).	No	No
				Level 3	Data analysis indicates significant long-term changes ⁸ in relation to control places pre-mining ⁶ compared to post-extraction ⁷ occur in the aquatic macroinvertebrate and/or macrophyte assemblages at Locations ET1, ET2 or ET4 on the Eastern Tributary during the mining of LW304.	Location ET1 (increased variability in numbers of Leptophlebiidae between autumn 2015 and autumn 2019 but not in spring 2019) Location ET2 (altered numbers of Atyidae between autumn 2016 and autumn 2019 but not in spring 2019; altered assemblage of aquatic plants within the after-period since autumn 2016).	Yes Yes	Assessment against the performance measure was triggered in autumn 2018. Consider the need for management measures, in accordance with Sections 8 and 9.

¹ Multivariate Analysis: comparisons of two (or more) samples based on the degree to which these samples share particular species, at comparable levels of abundance.

² Univariate Analysis: comparison of individual variables (e.g. total number of taxa, total abundance, abundances of individual taxa).

³ Cummins, S. P., Roberts, D. E. (2009a; 2009b; 2010a; 2010b). Aquatic Ecology Monitoring: Metropolitan Coal Longwalls 20-22 Spring 2008 to Autumn 2010 Survey Reports. Prepared for Metropolitan Coal Pty Ltd. BIO-ANALYSIS: Marine, Estuarine & Freshwater Ecology.

⁴ Cummins, S. P., Roberts, D. E. (2010a; 2010b; 2011; 2012a; 2012b; 2012c; 2013a; 2013b, 2014). Aquatic Ecology Monitoring: Metropolitan Coal Longwalls 23-27 Spring 2009 to Spring 2013 Survey Reports. Prepared for Metropolitan Coal Pty Ltd. BIO-ANALYSIS: Marine, Estuarine & Freshwater Ecology.

⁶ Pre-mining data is as follows: sites WT3 and ET1 (spring 2008 to autumn 2010); site ET3 (spring 2009 to autumn 2010); site ET4 (spring 2009 to spring 2013); site ET2 (will be assessed for two periods: spring 2008 to autumn 2010 [i.e. pre-mining of Longwalls 20-22] and spring 2009 to spring 2013 [i.e. pre-mining of Longwalls 23-27]).

⁷ Post-extraction data is represented as follows: sites WT3 and ET1 (from spring 2010 on); site ET3 (from spring 2010 on); site ET4 (from autumn 2014 on); site ET2 (will be assessed for two periods: spring 2010 on [Longwalls 20-22] and autumn 2014 on [Longwalls 23-27]).

⁸ Long-term changes to the macroinvertebrate and macrophyte assemblages are considered to be significant changes that are persistent (over time) and resulting from mining.

⁹ Threatened species, populations and ecological communities include those listed under the BC Act, EPBC Act or Fisheries Management Act at the time of Project Approval (i.e. the lists current as at 22 June 2009).

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APPENDICES

Appendix 1. Longwall 304 Trigger Action Response Plan – Monitoring of Aquatic Biota, Stream Monitoring

Performance Measure	Performance Indicator	Monitoring Sites	Parameters	Frequency/ Sample Size	Analysis Methodology	Error Types	Baseline	Highest Significance Level/Trigger Recorded		Action/Response
Negligible impact on Threatened Species, Populations, or Ecological Communities	The aquatic macroinvertebrate and macrophyte assemblages in streams are not expected to experience long-term impacts as a result of mine subsidence.	Two sampling sites (approximately 100 m in length) at the following locations: - Location WT3 on Waratah Rivulet and Locations ET1, ET3 and ET4 on the Eastern Tributary overlying Longwalls (LW) 20-27. - Location WT4 on Waratah Rivulet adjacent to LW20-27. - Location WT5 on the Waratah Rivulet and Location ET2 on the Eastern Tributary, downstream of LW20-27. - Control Locations: WR1 on Woronora River; and OC on O'Hares Creek.	- Aquatic macroinvertebrates. - Aquatic macrophytes.	Biannually, in autumn and spring.	Analysis of macroinvertebrate and macrophyte multivariate ¹ and univariate ² data using PERMANOVA to test the null hypothesis of no significant change in relation to control places, bi-annually following completion of survey.	Statistical significance levels. Significant = $P < 0.05$	- LW20-22 stream sites, as detailed in the LW20-22 aquatic ecology monitoring reports for the spring 2008 to autumn 2010 surveys³. - LW23-27 stream sites, as detailed in the LW23-27 aquatic ecology monitoring reports for the spring 2009 to spring 2013 surveys⁴.	Level 1	Data analysis indicates no significant changes in relation to control places pre-mining ⁶ compared to post-extraction ⁷ occur in the aquatic macroinvertebrate and/or macrophyte assemblages at Locations WT3, WT4 or WT5 on the Waratah Rivulet or Locations ET1, ET2, ET3 or ET4 on the Eastern Tributary during the mining of LW304.	Continue monitoring. Six monthly reporting.
								Level 2	Data analysis indicates significant (not long-term ⁸), changes in relation to control places pre-mining ⁶ compared to post-extraction ⁷ occur in the aquatic macroinvertebrate and/or macrophyte assemblages at Locations WT3, WT4 or WT5 on the Waratah Rivulet or Locations ET1, ET2, ET3 or ET4 on the Eastern Tributary during the mining of LW304.	Consider recent stream features mapping results and pool water level monitoring data. Consider status/progress of stream remediation activities. Six monthly reporting.
								Level 3	Data analysis indicates significant long-term changes ⁸ in relation to control places pre-mining ⁶ compared to post-extraction ⁷ occur in the aquatic macroinvertebrate and/or macrophyte assemblages at Locations WT3, WT4 or WT5 on the Waratah Rivulet or Locations ET1, ET2, ET3 or ET4 on the Eastern Tributary during the mining of LW304.	Initiate assessment against the performance measure ⁹ . Consider the need for management measures, in accordance with Sections 8 and 9.

¹ Multivariate Analysis: comparisons of two (or more) samples based on the degree to which these samples share particular species, at comparable levels of abundance.

² Univariate Analysis: comparison of individual variables (e.g. total number of taxa, total abundance, abundances of individual taxa).

³ Cummins, S. P., Roberts, D. E. (2009a; 2009b; 2010a; 2010b). Aquatic Ecology Monitoring: Metropolitan Coal Longwalls 20-22 Spring 2008 to Autumn 2010 Survey Reports. Prepared for Metropolitan Coal Pty Ltd. BIO-ANALYSIS: Marine, Estuarine & Freshwater Ecology.

⁴ Cummins, S. P., Roberts, D. E. (2010a; 2010b; 2011; 2012a; 2012b; 2012c; 2013a; 2013b, 2014). Aquatic Ecology Monitoring: Metropolitan Coal Longwalls 23-27 Spring 2009 to Spring 2013 Survey Reports. Prepared for Metropolitan Coal Pty Ltd. BIO-ANALYSIS: Marine, Estuarine & Freshwater Ecology.

⁶ Pre-mining data is as follows: sites WT3 and ET1 (spring 2008 to autumn 2010); site ET3 (spring 2009 to autumn 2010); site ET4 (spring 2009 to spring 2013); site ET2 (will be assessed for two periods: spring 2008 to autumn 2010 [i.e. pre-mining of Longwalls 20-22] and spring 2009 to spring 2013 [i.e. pre-mining of Longwalls 23-27]).

⁷ Post-extraction data is represented as follows: sites WT3 and ET1 (from spring 2010 on); site ET3 (from spring 2010 on); site ET4 (from autumn 2014 on); site ET2 (will be assessed for two periods: spring 2010 on [Longwalls 20-22] and autumn 2014 on [Longwalls 23-27]).

⁸ Long-term changes to the macroinvertebrate and macrophyte assemblages are considered to be significant changes that are persistent (over time) and resulting from mining.

⁹ Threatened species, populations and ecological communities include those listed under the BC Act, EPBC Act or Fisheries Management Act at the time of Project Approval (i.e. the lists current as at 22 June 2009).

Appendix 2. GPS positions (UTMs) for stream monitoring sample locations on the Tributary C, Woronora River and O'Hares Creek (spring 2019).

System	Location	Site	Easting	Northing
Tributary C	1	1	0311588	6214118
	1	2	0311642	6214197
	2	1	0312169	6215404
	2	2	0312157	6215559
	4	1	0311805	6214447
	4	2	0312032	6214497
Woronora River	1	1	0307964	6219031
	1	2	0307943	6219054
O'Hares Creek	1	1	0303812	6213711
	1	2	0303659	6213601