AQUATIC ECOLOGY MONITORING: METROPOLITAN COAL LONGWALLS 20-22

SPRING 2019 SURVEY

Prepared for

METROPOLITAN COAL

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BIO-ANALYSIS Pty Ltd
Marine, Estuarine & Freshwater Ecology
2/1 Botham Close Charmhaven, NSW 2263
(Mobile) 0414 477066, (Email) info@bioanalysis.com.au

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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 20-22 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).

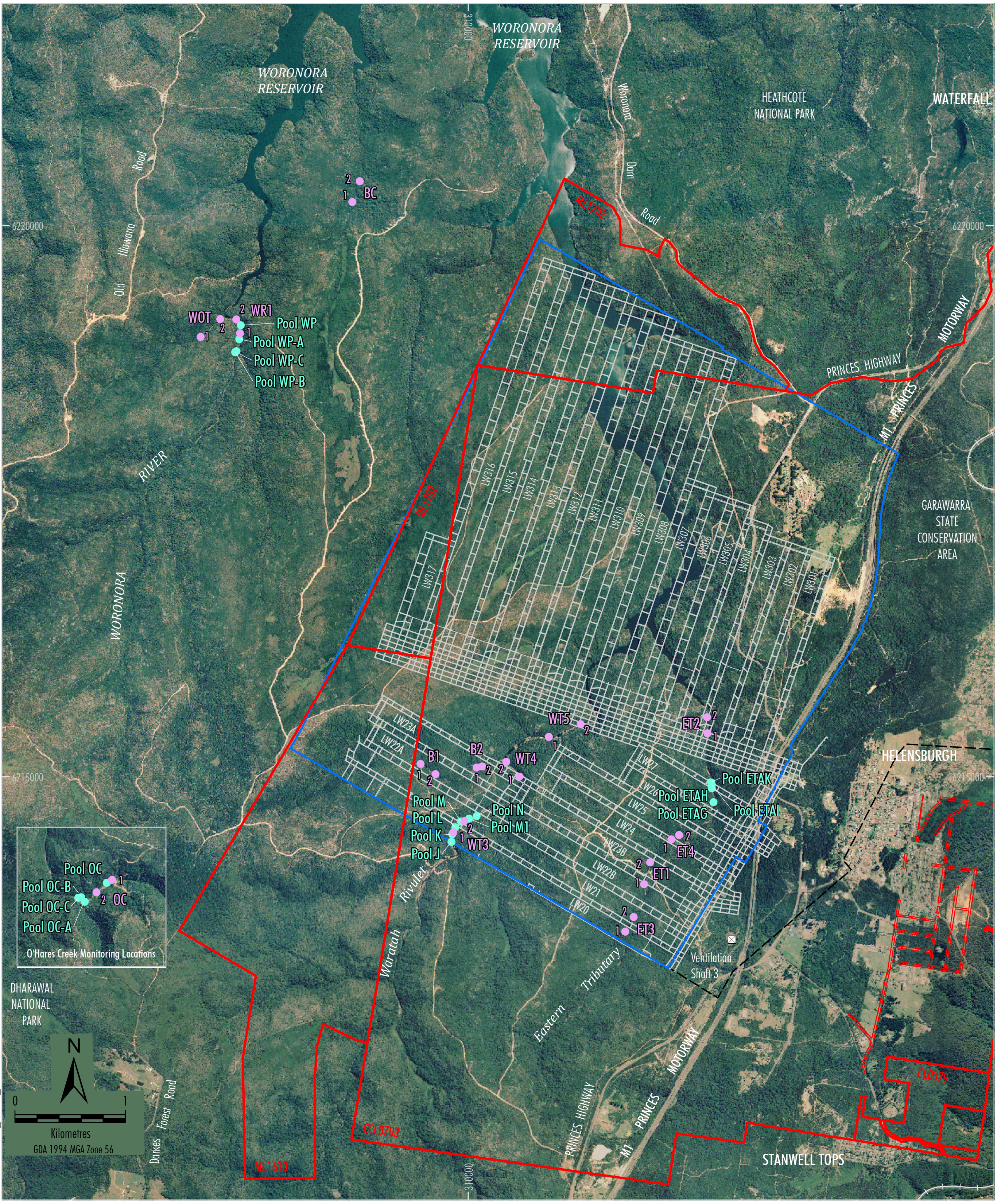
The design of the monitoring programmes uses the current best practice approach for monitoring impacts on aquatic habitats "Beyond BACI" and focused on representative sampling of selected locations within streams in the Longwalls 20-22 mining area and in suitable control streams (i.e. not subject to 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 20-22 Biodiversity Management Plan (2014)*[1] (Metropolitan Coal, 2015).

[1] The *Metropolitan Coal Longwalls 20-22 Biodiversity Management Plan* is superseded by the *Metropolitan Coal Longwall 304 Biodiversity Management Plan.* Mining of Longwall 304 commenced in July 2019.

Mining of the Longwalls 20-22 area commenced in May 2010 and was completed in April 2014. Subsequently, mining of the Longwalls 23-27 area commenced in May 2014 and was completed in March 2017. 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.



LEGEND

- Mining Lease Boundary
- Railway
- Project Underground Mining Area Longwalls 20-27 and 301-317
- Existing Underground Access Drive (Main Drift)

Monitoring

- Pool Aquatic Ecology Sampling Site
- Stream Aquatic Ecology Sampling Site

Source: Land and Property Information (2015); Date of Aerial Photography 1998; Department of Industry (2015); Metropolitan Coal (2020)

Peabody

METROPOLITAN COAL

Aquatic Ecology Monitoring Locations

Figure 1

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 20-22 extend below a tributary of the Woronora Reservoir in the east of the study area (Tributary C/Eastern Tributary), the Waratah Rivulet and an un-named tributary of the Waratah Rivulet (Tributary B) (Figure 1).

The headwaters of Tributary C/Eastern Tributary and the Waratah Rivulet are located in the Darkes Forest area at approximately 300 m AHD and 380 m AHD, respectively. Tributary C/Eastern Tributary and the Waratah Rivulet flow through relatively steep valleys 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). The mid to upper reaches of the Waratah Rivulet traverse the completed Longwalls 14-17 (middle reach) and Longwalls 12 and 13 (upper reach) underground mining areas (Figure 1).

The Woronora River occurs within the WSA but outside the area potentially affected by past and current mining activities at Metropolitan Coal. As such, this system 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 programme has been designed to monitor subsidence-induced impacts on aquatic ecology.

In accordance with the *Metropolitan Coal Longwalls 20-22 Biodiversity Management Plan* (Metropolitan Coal, 2014)[2], the aquatic ecology along Waratah Rivulet and 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 the Longwalls 20-22 program includes sampling two random sites (approximately 100 m in length) at the following stream locations (Appendix 2):

- Locations WT3 on Waratah Rivulet, C1 and C3[3] on Tributary C/Eastern Tributary overlying Longwalls 20-22.
- Locations WT4 and WT5 on Waratah Rivulet and C2[3] on Tributary C/Eastern Tributary, downstream of Longwalls 20-22.
- Control locations: WR1 on Woronora River and OC on O'Hares Creek.

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

Locations WT4 and WT5 on the Waratah Rivulet and Location C2 on Tributary C/Eastern Tributary located downstream of Longwalls 20-27, will assist in determining the spatial extent of any impacts on aquatic habitat and biota over time as mining progresses.

2 The *Metropolitan Coal Longwalls 20-22 Biodiversity Management Plan* is superseded by the *Metropolitan Coal Longwall 304 Biodiversity Management Plan*. The stream monitoring program and monitoring methods remain the same as those implemented during the mining of Longwalls 20-22 and Longwalls 23-27.

3 Locations C1, C2 and C3 are referred to as ET1, ET3 and ET2 in the *Metropolitan Coal Longwalls 301-303 Biodiversity Management Plan* (and previously in the *Metropolitan Coal Longwalls 20-22 Biodiversity Management Plan*).

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).

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 m^2 quadrats, using a stratified sampling technique. This information provided a quantitative measure of aquatic macrophytes within each site at each location.

2.3.3 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 (µS/cm), dissolved oxygen (% Saturation and mg/L), pH, temperature (ºC), 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 reference 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 locations subject to mining (i.e. potential 'impact' sites) in comparison to independent locations that are not subject to mine subsidence (i.e. control streams).

Two to four replicate times (spring 2008 or spring 2009[4] to autumn 2010) have been sampled within the 'Before' commencement of Longwall 20 period and 19 replicate times (spring 2010 to spring 2019) have been sampled within the 'After' period.

4 The sampling of Location C3 on the Eastern Tributary, larger pools J and M1 on Waratah Rivulet, and smaller pools K, L and M on Waratah Rivulet, 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 C3), Waratah Rivulet (Locations WT3, WT4 and WT5), Tributary B (Location B1) and at two control locations (Woronora River, O'Hares Creek) (Figure 1), undertaken between spring 2008 or spring 2009[5] and autumn 2019, is presented below.

<u>Tributary C/Eastern Tributary</u>

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 during dry periods cease to flow and pools may dry up. Three locations were sampled on this tributary (Figure 1).

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).

[5] The sampling of Location C3 on the Eastern Tributary commenced in spring 2009.

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 and 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 2016[6], since sampling commenced in spring 2008. Iron staining/iron flocculent has been observed to cover the stream substratum from the top of Site C2-1 to the bottom of Site C2-2 since spring 2016[7]. 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.

[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.

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 3a).
- Similar to Location C1, submerged aquatic macrophytes have consistently not been present (Appendices 2a&b).

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

Looking upstream

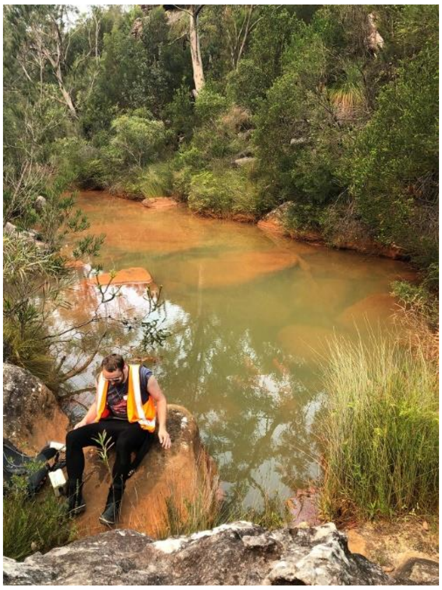


Plate 4: Tributary C – Location C2 (C2-2) (sp-19)

Looking downstream

Location C3

Location C3 is situated within the Longwalls 20-22 mining area and is approximately 300 m upstream of Location C1. In general, the study reach has consisted of pools up to approximately 12 m wide and up to 1.8 m deep, interrupted by a braided channel with large boulders in places. The substratum was predominantly bedrock and sand with large boulders in several places. Submerged aquatic macrophytes have consistently not been present (Appendices 2a&b).

A milky-green tinge to pool water was noted at Location C3 when sampling commenced in spring 2009. In autumn 2013, dieback of riparian vegetation was noted at Site C3-2, thought to be associated with tilting of the stream bank by mine subsidence. Further desiccation and dieback of riparian vegetation was noted at Site C3-2 in autumn 2014.

Cover of the stream substratum by an iron precipitate/micro-organism complex was first noted at Sites C3-1 and C3-2 in autumn 2014. A considerable decline in pool water levels was noted in spring 2015, particularly at Site C3-2. From spring 2015 to autumn 2017 pool water levels appeared to be below pre-mining levels. At the time of the spring 2017 and autumn 2018 surveys, pool water levels appeared similar to pre-mining levels at the upstream site (i.e. Site C3-1) but there was no water at Site C3-2. In spring 2018, pool water level at both sites was similar to pre-mining levels and flow was apparent along both study reaches (Plates 5&6). In autumn 2019, pool water level at Site C3-1 was similar to pre-mining levels but below pre-mining levels at Site C3-2.

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

- Pool water level at Site C3-1 and Site 3-2 was similar to pre-mining levels (Plates 5&6);
- Riparian health at Site C3-2 appears to have continued to improve since obvious signs of desiccation in spring 2018;
- *Empodisma minus*, *Lepidosperma filiforme* and *Gahnia clarkei* were amongst the most abundant components of the riparian emergent macrophyte vegetation (Appendix 3a).

Plate 5: Tributary C – Location C3 (C3-1) (sp-19)

Looking upstream

Plate 6: Tributary C – Location C3 (C3-2) (sp-19)

Looking downstream

Waratah Rivulet

The Waratah Rivulet is some 9 km in length from its headwaters to the point where it flows into the Woronora Reservoir. The Waratah Rivulet starts as a third order stream and becomes a fourth order stream downstream of the Waratah Tributary 1 (also known as Unnamed Tributary and Tributary D in some assessments) confluence (Figure 1).

The Waratah Rivulet flows through a relatively steep valley, sourcing at around 380 m AHD with the full supply level (FSL) of the Woronora Reservoir at about 165 m AHD. The catchment area above the inundation limits (FSL) of the reservoir is about 30 km^2 and the sub-catchment areas vary from 1.6 km^2 to 6.7 km^2 (Gilbert and Associates, 2006). The mean annual flow for the rivulet (based on modeled flows between 1976 and 2006) is 7,300 ML (Gilbert and Associates, 2006). The stream channel in Waratah Rivulet downstream of Flat Rock Crossing is characterised by a gently meandering, relatively shallow, wide channel with a sandstone bed (Gilbert and Associates, 2006). The channel contains a series of in-stream pools that have formed in local depressions in the bedrock and behind rock bars.

Three locations (i.e. WT3, WT4 and WT5) were sampled on Waratah Rivulet (Figure 1).

Location WT3

At the most upstream sampling location (i.e. WT3: approximately 300 m downstream of Flat Rock Crossing and approximately 6 km downstream from its source at 380 m AHD), the rivulet was a broad stream (approximately 4 to 15 m wide) with mostly shallow pools (up to approximately 1.5 m deep in places) interspersed between exposed bedrock shelves (Plates 7&8). Cummins et al. (2007) reported that the dominant riparian vegetation included *Banksia integrifolia, Bauera rubioides*, *Centrolepis strigose*, *Hakea sericea* and *Leptospermum polygalifolium*.

The stream channel has commonly been observed to be covered by an iron precipitate/micro-organism complex since autumn 2008, and the water has had a milky green tinge (Cummins et al., 2008). Evidence of fracturing of the sandstone substratum of the stream channel due to subsidence was first noted at Location WT3 in spring 2013.

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

- Riparian vegetation present in this reach of the Waratah Rivulet appeared healthy and relatively undisturbed;
- The emergent species, *Gleichenia dicarpa* and *Viminaria juncea* have consistently been amongst the most common components of assemblages of aquatic macrophytes (Appendix 3);
- Iron staining continues to be present along the study reach (Plates 7&8). An iron precipitate/micro-organism complex covered up to approximately 95 % of the stream substratum;
- Pool water had a milky-green tinge, as observed on prior sampling occasions (Plates 7&8).

Plate 7: Waratah Rivulet – Location WT3 (WT3-1) (sp-19) Looking upstream

Plate 8: Waratah Rivulet – Location WT3 (WT3-2) (sp-19) Looking upstream

Location WT4

The pool sampled in the upstream section of the study reach at Location WT4 (i.e. Site WT4-1) is approximately 35 m long and 14 m wide. Further downstream (Site WT4-2), pool width varied from approximately 12 to 16 m and 185 m long. The base of both pools was sandstone with several boulders and sand. The submerged aquatic macrophyte, *Triglochin procerum*, has consistently been relatively abundant at this location (Appendices 2a&b). The Charophyte, *Chara/Nitella* spp., has often been present.

Pool water at Location WT4 has been commonly observed to have a milky-green tinge.

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

- Pool water levels along the study reach had decreased by approximately 0.3 m since the autumn 2019 survey (Plates 9&10). Reduced pool water levels were noted by the autumn 2019, spring 2018, autumn 2018, spring 2017, autumn 2016 and spring 2015 surveys;
- Flow was apparent into and out of the study reach at each site;
- To date, there has been no evidence of mining related fracturing of the stream substratum;
- Cover of the substratum by iron precipitate was limited on this occasion;
- Pool water had a milky-green tinge, as observed on prior sampling occasions (Plates 9&10);
- The riparian strip was relatively healthy;
- The emergent species, *Lomandra fluviatilis* and *Lepidosperma filiforme* were amongst the most common components of the assemblage of aquatic macrophytes (Appendix 3);
- The weed species, *Isolepis prolifera*, was present at both sites;
- *Triglochin procerum* and patches of *Chara/Nitella* spp. were relatively common (Plates 9&10).

Plate 9: Waratah Rivulet – Location WT4 (WT4-1) (sp-19)
Looking upstream

Plate 10: Waratah Rivulet – Location WT4 (WT4-2) (sp19)
Looking downstream

Location WT5

At Location WT5, a gauging station operated by WaterNSW is situated between the two sampling sites (WT5-1 and WT5-2). The in-stream habitat was predominantly bedrock and sand. Pools have consistently been up to approximately 110 m long, 30 m wide and 2.0 m deep at their deepest point (Plates 11&12). Previously, the dominant riparian vegetation was reported to include *Acacia binervia*, *Acacia floribunda*, *Acacia longifolia*, *Acacia parramattensis*, *Epacris calvertiana*, *Gleichenia dicarpa*, *Leptospermum polygalifolium* and *Tristaniopsis laurina* (Bio-Analysis Pty Ltd, 2008). The submerged aquatic macrophyte, *Triglochin procerum*, has been relatively abundant at this location.

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

- To date, there has been no evidence of mining related fracturing of the stream substratum at this location;
- Pool water levels along the study reach were lower than at the time of the autumn 2019 survey and below levels observed prior to the spring 2017 survey (Plates 11&12). Nevertheless, flow was apparent along the study reach;
- Approximately 95 % of the submerged stream substratum was covered by a diatomaceous/filamentous algal complex;
- Pool water had a milky-green tinge, as observed on prior sampling occasions (Plates 11&12);
- The riparian strip appeared relatively healthy;
- The emergent species, *Gleichenia dicarpa* and *Lepidosperma filiforme* were amongst the most common components of the assemblage of aquatic macrophytes (Appendix 3);
- *Triglochin procerum* and *Chara/Nitella* spp. were observed at both sites.

Plate 11: Waratah Rivulet – Location WT5 (WT5-1) (aut-19[8])
Looking upstream

Plate 12: Waratah Rivulet – Location WT5 (WT5-2) (sp-19)
Looking across stream

<u>Control Locations</u>

Woronora River

Sites were chosen on the Woronora River approximately 7 km from its headwaters (Appendix 2). The river flows through a steep valley into the Woronora Reservoir. The sites sampled were characterised by interconnected pools (up to approximately 1.4 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 13&14) (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*. At the time of this survey, emergent macrophytes included *Lepidosperma filiforme* and *Gleichenia dicarpa* (Appendix 3). No exotic plants or animals were observed (Appendices 2, 3 and 4). 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. Slumping of the left-bank (looking downstream) has been apparent since the autumn 2017 survey.

8 A photo was not available for the spring 2019 survey

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 13&14). Flow has not been apparent along the study reach since the autumn 2018 survey. Visibility of the water was considered fair.

Plate 13: Woronora River (WR1) (sp-19)
Looking upstream

Plate 14: 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 riffle 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 15&16). 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 3a). *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 15: O'Hares Creek (OC1) (sp-19)

Looking upstream

Plate 16: O'Hares Creek (OC2) (sp-19)

Looking downstream

3.1.2 Aquatic Macroinvertebrates

AUSRIVAS Assessment

A total of 37 taxa were collected from the 16 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. *Gambusia* were collected at Location WT3 (5 individuals), WT4 (3 individuals) and WT5 (6 individuals) on the Waratah Rivulet using the AUSRIVAS technique.

For the sites sampled, the OE50 scores ranged between 0.29 (C2-2) and 0.77 (C1-2) (Table 1). Six sites were grouped in Band B (C1-2, C3-1, WT5-1, WR1-2, OC1 and OC2) and ten were grouped in Band C (C1-1, C2-1, C2-2, C3-2, WT3-1, WT3-2, WT4-1, WT4-2, WT5-2 and WR1-1) (Table 1). Thus, fewer families of macroinvertebrates than expected were collected from sites sampled (including reference sites) in spring 2019, compared to reference sites selected by the AUSRIVAS model (Table 1).

Taxon with > 0.86 probability of occurrence but not collected included the water mite family, Acarina, at all sites except C1-2, C2-2, WT4-1, WT5-2, OC1 and OC2. Also expected but not collected was the caddis fly family, Leptoceridae, at C2-1, C2-2, C3-2, WT3-1, WT4-1, WT4-2, WT5-1, WT5-2 and OC1. Gyrinidae (whirligig beetles) were expected but not collected at C1-2, C2-1, C2-2, C3-1, C3-2, WT4-1, WT5-1, WT5-2, WR1-1 and WR1-2.

Table 1. AUSRIVAS outputs for sites sampled in Tributary C/Eastern Tributary (C), Waratah Rivulet (WT), Tributary B (B), Bee Creek (BC), Woronora Tributary (WOT), Woronora River (WR) and O'Hares Creek (OC) (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
	3	1	C3-1	0.57	B
	3	2	C3-2	0.38	C
Waratah Rivulet	3	1	WT3-1	0.44	C
	3	2	WT3-2	0.35	C
	4	1	WT4-1	0.48	C
	4	2	WT4-2	0.46	C
	5	1	WT5-1	0.55	B
	5	2	WT5-2	0.44	C
Woronora River	1	1	WR1-1	0.50	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.

<u>Quantitative Assessment</u>

A total of 1,691 individuals from 40 macroinvertebrate taxon were collected from sites using the quantitative sampling technique (Appendix 5). The most abundant macroinvertebrate taxon was the Leptophlebiidae (986 individuals) followed by the Atyidae (129 individuals), Chironomidae (123 individuals) and the Dytiscidae (107 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 4&5). *Gambusia* were collected at Location WT3 (7 individuals), Location WT4 (1 individuals), Location WT5 (11 individuals) and Site OC1 (2 individuals) on this sampling occasion.

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.

Of the sites sampled, mean diversity was greatest at the O'Hares Creek location followed by Location 4 sampled on the Waratah Rivulet (Figure 2). The total abundance of macroinvertebrates was greatest at the O'Hares Creek location, followed by Location C2 and the Woronora River location (Figure 3).

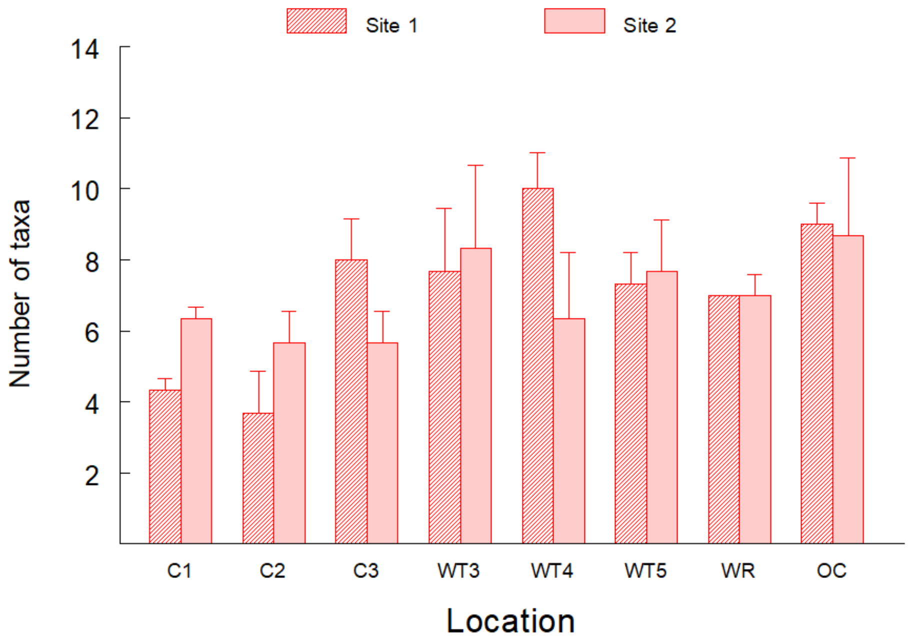


Figure 2. Mean (+SE) diversity of macroinvertebrates at each location (C – Tributary C/Eastern Tributary [C1 – Location 1 etc], WT – Waratah Rivulet [WT3 - Location 3 etc], WR – Woronora River, OC – O'Hares Creek).

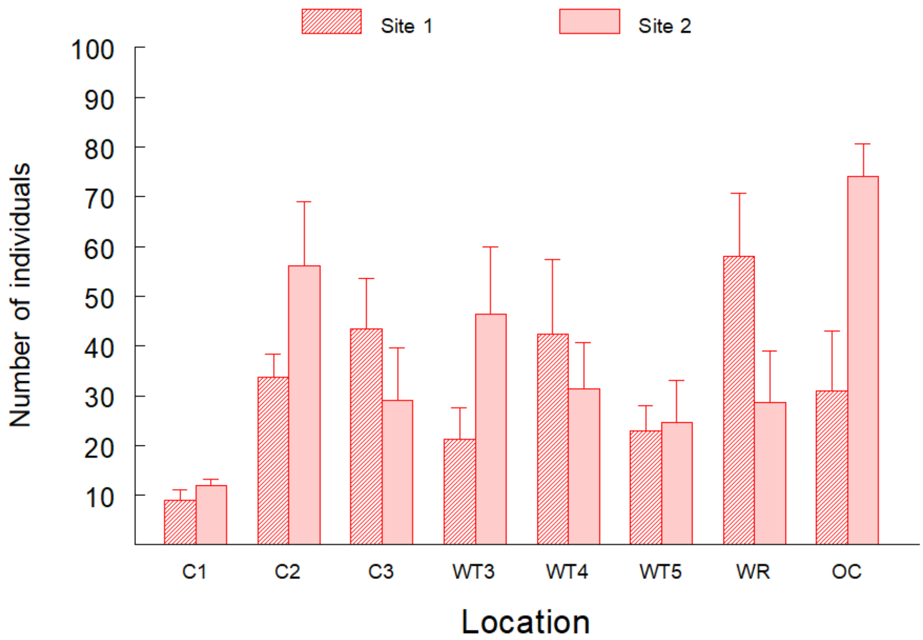


Figure 3. Mean (+SE) abundance of macroinvertebrates at each location (C – Tributary C/Eastern Tributary [C1 – Location 1 etc], WT – Waratah Rivulet [WT3 - Location 3 etc], WR – Woronora River, OC – O'Hares Creek).

3.1.3 Aquatic Macrophytes

A total of 22 aquatic macrophyte species were found in quantitative samples at sites on Tributary C/Eastern Tributary (C1: 6 species, C2: 6 species, C3: 6 species), Waratah Rivulet (WT3: 13 species, WT4: 10 species, WT5: 9 species), Woronora River (10 species) and O'Hares Creek (9 species) (Appendix 3b).

In general, mean percentage cover of macrophytes was greatest at Location WT5 and WT3 situated on the Waratah Rivulet followed by WR situated on the Woronora River (Figure 4). The mean diversity of macrophytes recorded in the 0.25 m^2 quadrats at any one location was generally low (i.e. < 4 species) (Figure 5).

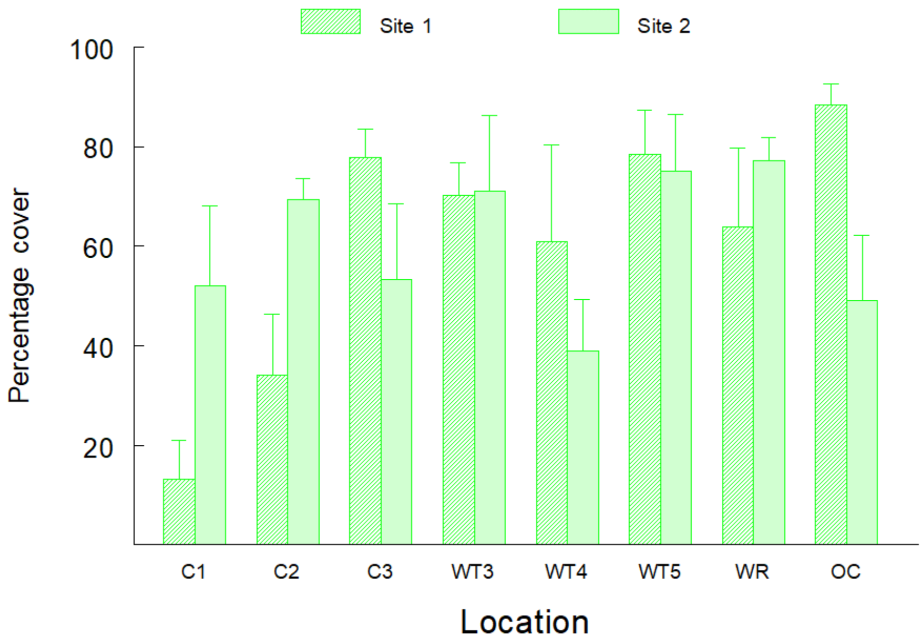


Figure 4. Mean (+SE) percentage cover of macrophytes at each location (C – Tributary C/Eastern Tributary [C1 – Location 1 etc], WT – Waratah Rivulet [WT3 - Location 3 etc], WR – Woronora River, OC – O'Hares Creek).

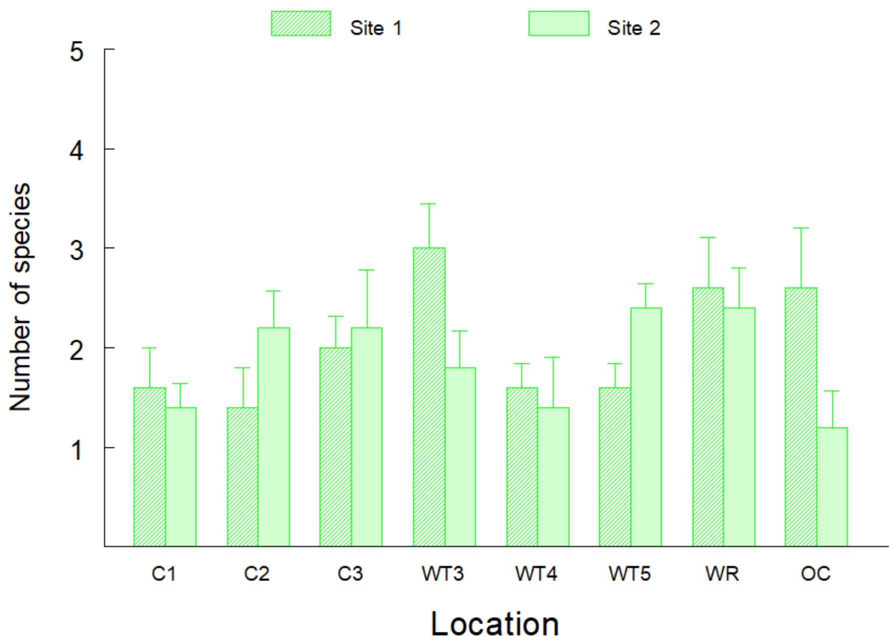


Figure 5. Mean (+SE) diversity of macrophytes at each location (C – Tributary C/Eastern Tributary [C1 – Location 1 etc], WT – Waratah Rivulet [WT3 - Location 3 etc], WR – Woronora River, OC – O'Hares Creek).

3.1.4 Water Quality

Physico-chemical water quality, alkalinity, total phosphorous and total nitrogen measurements are summarised in Table 2 with values highlighted in bold type indicating where mean values were outside the ANZECC/ARMCANZ (2000) guidelines.

In general, mean water temperature within the seven creeks ranged from 17.3 to 26.5 ° C (Table 2), which is typical for the time of year the samples were collected. Mean pH (range = 5.8 – 7.6) was once again below the lower Default Trigger Values (DTV) (i.e. pH 6.5-8.0; ANZECC/ARMCANZ, 2000) for upland rivers at all of the sites sampled except the rivulet sites (Table 2). Relatively low pH values are common in areas with a sandstone substratum.

Mean conductivity (range = 111 – 270 µS/cm) was within the guideline values at all of the sites sampled (Table 2). Mean dissolved oxygen levels (range = 34.8 – 122 %) were below the lower DTV at all of the sites sampled except Site WT5-1 (Table 2).

Mean turbidity (range = 0.5 – 7.8 NTU) was below the lower DTV recommended by ANZECC/ARMCANZ (2000) at Site WT5-1, WT5-2, OC1 and OC2 while mean oxidation-reduction potential ranged from 503 to 651 mV (Table 2). Mean alkalinity ranged from 5 to 70 mg/L $CaCO_3$ (Table 2). For nutrients, mean concentrations of total phosphorous (0.01 to 0.02 mg/L) were within the DTVs while total nitrogen (range = 0.07 to 0.43 mg/L) was above the upper DTV 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 (+ SE) measurements of water quality variables recorded at each site (spring 2019). Values in bold are outside the guideline values recommended by ANZECC (2000). N/R = not recorded.

Watercourse	**Tributary C/Eastern Tributary**					
Location/Site	**C1-1**	**C1-2**	**C2-1**	**C2-2**	**C3-1**	**C3-2**
Temperature °C (*n* =3)	20.6 (0.1)	20.1 (0.1)	23.0 (0.0)	17.5 (0.0)	17.3 (0.0)	18.1 (0.0)
pH (*n* =3)	**6.3** (0.0)	**5.9** (0.0)	**6.0** (0.0)	**6.3** (0.0)	6.6 (0.0)	7.0 (0.0)
Conductivity (µS/cm) (*n* = 3)	171.7 (0.3)	187.7 (3.2)	270.3 (1.7)	266.3 (0.3)	173.0 (0.0)	204.7 (1.7)
Dissolved Oxygen (% Saturation) (*n* = 3)	**76.2** (0.7)	**38.0** (0.3)	**46.2** (0.1)	**34.8** (2.0)(	**61.1** (0.4)	**82.8** (0.2)
Turbidity (NTU) (*n* = 3)	4.2 (0.0)	4.8 (0.0)	7.8 (0.0)	4.2 (0.1)	3.1 (0.0)	3.3 (0.0)
ORP (mV) (*n* = 3)	649.0 (0.0)	589.0 (1.2)	632.3 (0.9)	526.3 (7.3)	502.7 (0.3)	532.0 (0.6)
Alkalinity (mg/L $CaCO_3$) (*n* = 2)	14	30	50	60	30	30
Total phosphorous (mg/L) (*n* = 2)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.02 (0.00)	0.01 (0.00)	0.01 (0.00)
Total nitrogen (mg/L) (*n* = 2)	0.18 (0.02)	0.16 (0.05)	**0.29** (0.10)	0.22 (0.04)	0.10 (0.00)	0.07 (0.00)
Watercourse	**Waratah Rivulet**					
Location/Site	**WT3-1**	**WT3-2**	**WT4-1**	**WT4-2**	**WT5-1**	**WT5-2**
Temperature °C (*n* =3)	23.2 (0.0)	25.7 (0.0)	18.6 (0.0)	18.5 (0.0)	23.1 (0.0)	26.5 (0.0)
pH (*n* =3)	7.0 (0.0)	7.4 (0.0)	7.0 (0.0)	7.0 (0.0)	7.3 (0.0)	7.6 (0.0)
Conductivity (µS/cm) (*n* =3)	213.7 (1.7)	208.0 (1.5)	195.0 (0.0)	212.0 (0.0)	203.0 (0.0)	245.0 (0.0)
Dissolved Oxygen (% Saturation) (*n* = 3)	92.8 (1.0)	**87.2** (1.2)	**74.9** (0.0)	**82.0** (0.2)	102.8 (0.0)	**122.0** (0.2)
Turbidity (NTU) (*n* =3)	1.8 (0.0)	1.0 (0.0)	0.9 (0.0)	3.1 (0.1)	**1.2** (0.0)	**0.7** (0.0)
ORP (mV) (*n* = 3)	578.0 (0.6)	557.0 (0.0)	508.3 (0.3)	528.7 (0.9)	593.3 (0.3)	592.3 (3.0)
Alkalinity (mg/L $CaCO_3$) (*n* = 2)	36	41	40	45	70	70
Total phosphorous (mg/L) (*n* = 2)	0.01 (0.01)	0.01 (0.00)	0.01 (0.00)	0.01 (0.01)	0.01 (0.00)	0.01 (0.00)
Total nitrogen (mg/L) (*n* = 2)	0.09 (0.09)	0.08 (0.00)	0.09 (0.00)	0.13 (0.01)	0.15 (0.01)	0.14 (0.01)

NB[1]: ANZECC (2000) guideline values for upland streams: pH (6.5 – 8.0); Conductivity (30 – 350 µS/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 (2000) guideline values for Temperature, ORP or Alkalinity.
NB[2]: # = Insufficient aquatic habitat.
NB[3]: *For any site where a value has been recorded as less than the detection limit, it was assigned a value of half the detection limit in order to calculate the mean (e.g. <0.02 taken as 0.01).

Table 2 (Cont'd)

Watercourse	**Woronora River**		**O'Hares Creek**			
Location/Site	**WR1-1**	**WR1-2**	**OC-1**	**OC-2**		
Temperature °C (*n* =3)	20.2 (0.0)	20.7 (0.0)	22.0 (0.0)	21.5 (0.0)		
pH (*n* =3)	**5.9** (0.0)	**5.8** (0.0)	**6.0** (0.0)	**6.4** (0.0)		
Conductivity (µS/cm) (*n* = 3)	157.0 (2.9)	131.3 (1.3)	111.0 (0.0)	118.7 (1.7)		
Dissolved Oxygen (% Saturation) (*n* = 3)	**60.3** (0.2)	**89.1** (0.0)	**74.1** (0.2)	**86.3** (0.1)		
Turbidity (NTU) (*n* = 3)	4.7 (0.1)	2.7 (0.0)	**0.8** (0.0)	**0.5** (0.0)		
ORP (mV) (*n* = 3)	642.7 (0.3)	635.0 (0.6)	617.7 (0.3)	651.0 (0.0)		
Alkalinity (mg/L $CaCO_3$) (*n* = 2)	5	5	16	18		
Total phosphorous (mg/L) (*n* = 2)	0.02 (0.01)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)		
Total nitrogen (mg/L) (*n* = 2)	**0.43** (0.01)	**0.30** (0.06)	**0.30** (0.07)	0.20 (0.01)		

NB[1]: ANZECC (2000) guideline values for upland streams: pH (6.5 – 8.0); Conductivity (30 – 350 µS/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 (2000) guideline values for Temperature, ORP or Alkalinity.
NB[2]: # = Insufficient aquatic habitat.
NB[3]: *For any site where a value has been recorded as less than the detection limit, it was assigned a value of half the detection limit in order to calculate the mean (e.g. <0.02 taken as 0.01).

4.0 TEMPORAL ANALYSES

A temporal comparison of the aquatic macroinvertebrate and macrophyte data was carried out for the Stream Monitoring (Section 4.1) component of the Longwalls 20-22 Aquatic Ecology Monitoring Programme. The quantitative data from the surveys done between spring 2008 or spring 2009 and spring 2019 were analysed using both multivariate and univariate techniques. AUSRIVAS data collected from the stream monitoring locations are also presented.

4.1 Stream Monitoring

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 (Section 4.1.1) and the Waratah Rivulet (Section 4.1.2) following the commencement of mining of the Longwalls 20-22 mining area, in relation to Control locations.

4.1.1 Tributary C/Eastern Tributary

PERMANOVA was used to test the null hypothesis of no significant change in aquatic macroinvertebrate or macrophyte indicators at Tributary C/Eastern Tributary locations (Locations C1, C2 and C3) Before- vs After-commencement of mining within the Longwalls 20-22 mining area in relation to Control locations (Woronora River and O'Hares Creek). Mining of the Longwalls 20-22 mining area commenced in May 2010 and was completed in April 2014.

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.

4.1.1.1 Aquatic Macroinvertebrates

<u>Quantitative Assessment</u>

Location C1 v Controls

Prior Surveys (Spring 2008 – Autumn 2019)

Multivariate and univariate analyses of the Before- vs After-commencement of mining monitoring data indicated that any effect of longwall mining on assemblages of aquatic macroinvertebrates at Location C1 was within the range of natural variability in these assemblages as measured by the control locations.

Current Survey (Spring 2019)

Multivariate analyses did not detect 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 6a). Similar to the findings of the previous survey, with the exception of the factor 'Impact', each of the main factors appeared to have significant effects ($P < 0.05$), particularly 'Location', which contributed 17 % to the components of variation (Table 3).

These results are reflected in the patterns seen in the PCoA, which shows that there is a tendency for samples from the Woronora River location to occur in the upper right of the diagram, compared to those from O'Hares Creek, which occur more in the upper left (Figure 6a). There appears to be greater dispersion among centroids representing the assemblage at Location C1 than for each of the control locations (Figure 6a).

SIMPER analyses indicated that mayflies (Leptophlebiidae) and freshwater shrimp (Atyidae) have consistently contributed to the structure of assemblages of macroinvertebrates at Location C1 and the control locations and to differences over time. Leptophlebiidae increased its contribution to assemblages at Location C1 (Before: 5.1 %; After: 54.0%) and O'Hares Creek (Before: 9 %, After: 35.0 %) between Periods but changed little at the Woronora River Location (Before: 16.1 %, After: 22.0%). Atyid shrimps made a larger contribution to the structure of assemblages at Location C1 within the before- (59.6 %) than after-period (11.8%) but changed little at the Woronora River (Before: 72.8 %; After: 65.4 %) and O'Hares Creek (Before: 4.9 %; After: 5.0 %) locations.

Univariate analyses have found no evidence of significant change in mean diversity or abundance of macroinvertebrates or mean numbers of Leptophlebiidae and Atyidae at Location C1 that may have resulted from mining of Longwalls 20-22 (Table 4, Figure 7a-d). Numbers of Leptophlebiidae appear to have declined since the autumn 2019 survey, to become similar to numbers recorded within the 'before' period (Figure 7c).

In general, macroinvertebrate diversity and abundance have commonly been greatest at the O'Hares Creek location (Figures 7a&b). Macroinvertebrate diversity appears to have increased at Location C1 since the spring 2018 (T21) survey (Figure 7a). Mean abundance at Location C1 appears to have decreased since the autumn 2019 (T22) survey (Figure 7b). Atyidae have not been collected at Location C1 since autumn 2018 (T20) (Figure 7d).

Location C2 v Controls

Prior Surveys (Spring 2008 – Autumn 2019)

Multivariate analyses of the aquatic macroinvertebrate data have consistently found no significant difference between the structure of assemblages at Location C2 and the control locations that would indicate an impact from mining of Longwalls 20-22 (Figure 6b). Macroinvertebrate taxa that contributed most to the structure of assemblages at Location C2 and the Control locations were mayflies (Leptophlebiidae) and freshwater shrimp (Atyidae).

Univariate analyses have consistently found no significant difference to total diversity or abundance of macroinvertebrates or numbers of Leptophlebiidae at Location C2 compared to the controls (Figures 7a-c).

Analyses detected a significant difference in mean numbers of Atyidae at Location C2 within the after-mining period in spring 2015 (T15) and autumn 2017 (T18), but not subsequently (Figure 7d).

Current Survey (Spring 2019)

There were no detectable impacts to assemblages of aquatic macroinvertebrates at Location C2 in relation to the control locations that could be associated with mining (Table 3, Figure 6b). Similar to the findings for Location C1, the 'Location' factor contributed most (19 %) to the components of variation (Table 3, Figure 6b).

Although not significant, the PCoA indicates greater variability in the structure of the assemblage at Location C2 over time than at the control locations (Figure 6b). Notably, the centroid for the most recent survey plots separately from all other times of sampling (Figure 6b). 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 Woronora River Control treatment (Before: 72.8 %; After: 65.4 %). Similar to the findings from the previous survey, Leptophlebiidae contributed most to the structure of the assemblage at Location C2 (93.1 %) (SIMPER).

To date, univariate analyses have found no evidence of significant change in mean diversity, abundance or numbers of individuals of Leptophlebiidae at Location C2 that may have resulted from mining of Longwalls 20-22 (Table 4, Figure 7a-c). Graphically, it can be seen that spikes in the total abundance of aquatic macroinvertebrates and numbers of Leptophlebiidae at Location C2 and at the control locations have been common throughout the study period (Figures 7b&c). Similar numbers of taxa and Leptophlebiidae were collected at Location C2 and the Control treatment in spring 2019 (Figures 7b&c).

Analyses detected a significant difference in mean numbers of Atyidae at Location C2 within the after-mining period in spring 2015 (T15) and autumn 2017 (T18), but not on this sampling occasion (Table 4, Figure 7d). Atyidae were not collected at Location C2 by surveys done between spring 2017 (T19) and autumn 2019 (T22) (Figure 7d). One individual was collected by the spring 2019 (T23) survey (Figure 7d).

Location C3 v Controls

Prior Surveys (Spring 2009 – Autumn 2019)

Multivariate analyses have consistently detected significant small-scale interactions between Time (nested in periods) and Location (nested within type of creek) for Location C3, but not between Period (Before vs After) and Impact (Location C3 vs Control locations) (Figure 6c). A significant interaction between Time and Location indicates that differences in the structure of assemblages of macroinvertebrates between Location C3 and the control locations depended on the time of sampling (Figure 6c).

Univariate analyses have consistently found no significant interactions related to the commencement of mining of the Longwalls 20-22 mining area (Figures 7a-d).

Current Survey (Spring 2019)

Multivariate analyses did not detect a significant change in the structure of the assemblages of aquatic macroinvertebrates at Location C3 in relation to the control locations (Table 3, Figure 6c). Leptophlebiidae contributed most to the structure of the assemblage at Location C3 in spring 2019 (77.2 %), followed by the Dytiscidae (7.4 %) and Chironomidae (5.3 %) (SIMPER).

Univariate analyses did not detect a significant difference in mean total diversity and abundance or mean numbers of Leptophlebiidae and Atyidae at Location C3 in spring 2019, in relation to the control locations (Table 4, Figures 7a-d). Graphically, diversity increased at C3 and the control locations between the autumn 2019 (T20) and spring 2019 (T21) surveys (Figure 7a). There was a considerable increase in mean abundance and numbers of Leptophlebiidae at Location C3 between the autumn 2019 (T20) and spring 2019 (T21) surveys (Figure 7b&c). Very few Atyidae have been collected at Location C3 since spring 2016 (T17) (Figure 7d).

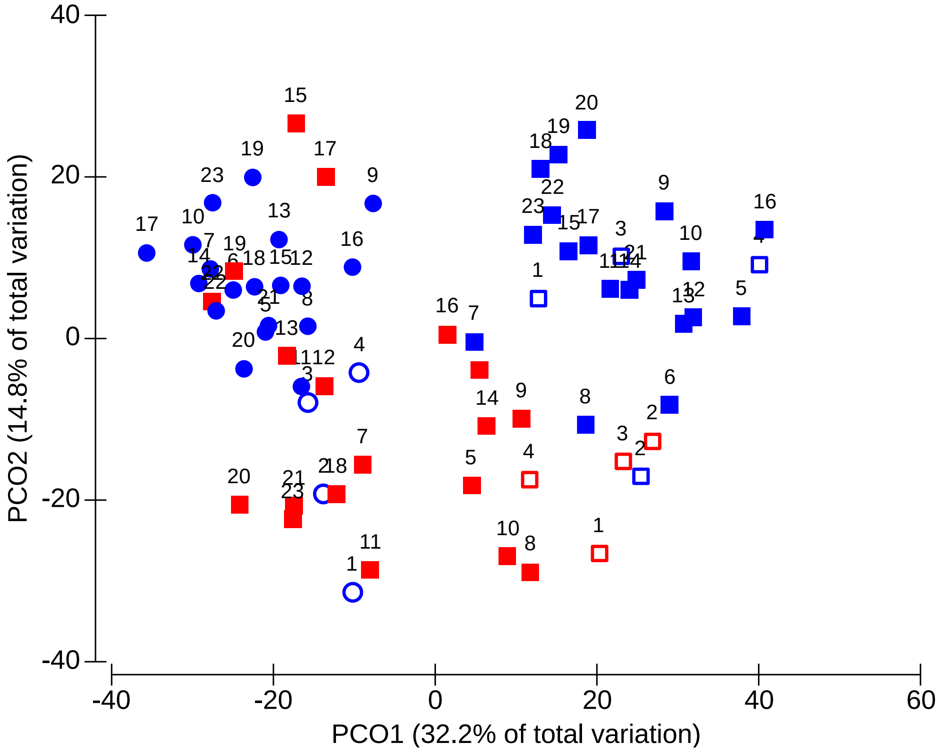
Table 3. PERMANOVA on macroinvertebrate assemblage data to compare locations C1, C2 and C3 sampled on Tributary C/Eastern Tributary with control locations (Woronora River and O'Hares Creek) Before- versus After-mining of Longwalls 20-22. Percentages of Components of Variation (% CV) are shown. Bold numbers indicate significant results at *P* < 0.05.

C1					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	**1**	**33382**	**2.44**	**0.01**	**8.29**
Impact = Im	1	18096	0.26	1.00	0.00
Time (Pe) = Ti(Pe)	**21**	**7482**	**1.81**	**0.00**	**8.29**
Location (Im) = Lo(Im)	**1**	**78699**	**8.99**	**0.00**	**17.32**
Pe x Im	1	12518	1.22	0.26	4.34
Site (Lo(Im)) = Si(Lo(Im))	**3**	**4958**	**1.66**	**0.02**	**4.04**
Pe x Lo(Im)	**1**	**7882**	**1.69**	**0.02**	**6.05**
Ti(Pe) x Im	**21**	**5732**	**1.39**	**0.00**	**8.10**
Pe x Si(Lo(Im))	3	2310	0.77	0.82	0.00
Ti(Pe) x Lo(Im)	**21**	**4130**	**1.38**	**0.00**	**7.91**
Ti(Pe) x Si(Lo(Im))	**63**	**2986**	**1.66**	**0.00**	**11.40**
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	**78705**	**8.82**	**0.00**	**19.18**
Pe x Im	1	6918	0.88	0.67	0.00
Site (Lo(Im)) = Si(Lo(Im))	3	**5092**	**1.94**	**0.00**	**5.02**
Pe x Lo(Im)	1	**7873**	**1.66**	**0.02**	**6.52**
Ti(Pe) x Im	21	4695	1.14	0.18	5.34
Pe x Si(Lo(Im))	3	2200	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
C3					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	9921	1.31	0.22	5.02
Impact = Im	1	15552	0.37	1.00	0.00
Time (Pe) = Ti(Pe)	**19**	**7206**	**1.98**	**0.00**	**10.02**
Location (Im) = Lo(Im)	**1**	**46626**	**6.76**	**0.00**	**20.94**
Pe x Im	1	4152	0.98	0.53	0.00
Site (Lo(Im)) = Si(Lo(Im))	3	3685	1.26	0.18	3.97
Pe x Lo(Im)	1	3130	1.15	0.26	4.10
Ti(Pe) x Im	**19**	**4795**	**1.31**	**0.04**	**8.04**
Pe x Si(Lo(Im))	3	1597	0.55	0.97	0.00
Ti(Pe) x Lo(Im)	**19**	**3649**	**1.25**	**0.02**	**7.38**
Ti(Pe) x Si(Lo(Im))	**57**	**2925**	**2.00**	**0.00**	**14.82**
Residual	252	1465			25.71

Table 4. PERMANOVA analysis on diversity, abundance, and number of Leptophlebiidae and Atyidae of the macroinvertebrate data collected from three locations within Tributary C and at two control locations. 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	30.84	1.11	10181	1.70	4467	2.08	33	0.65
Impact = Im	1	332.03	0.65	13789	2.05	6	0.11	1095	0.16
Time (Pe) = Ti(Pe)	21	**48.07**	**2.14**	**6421**	**3.36**	**1821**	**10.40**	349	1.03
Location (Im) = Lo(Im)	1	**529.60**	**15.32**	5383	2.43	116	2.15	**8792**	**13.74**
Pe x Im	1	35.42	3.74	297	0.74	365	0.29	591	2.17
Site (Lo(Im)) = Si(Lo(Im))	3	12.82	1.15	978	0.59	97	0.21	317	1.49
Pe x Lo(Im)	1	0.03	0.47	690	1.08	**409**	**3.68**	227	1.27
Ti(Pe) x Im	21	15.45	0.69	2277	1.19	**1484**	**8.47**	202	0.60
Pe x Si(Lo(Im))	3	1.53	0.14	244	0.15	63	0.14	7	0.03
Ti(Pe) x Lo(Im)	21	**22.47**	**2.01**	1912	1.16	175	0.38	339	1.59
Ti(Pe) x Si(Lo(Im))	63	11.17	1.29	**1647**	**2.59**	**467**	**2.54**	**213**	**1.65**
Residual	276	8.67		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	3.15	0.06	6908	2.63	1901	2.16	20.4	0.49
Impact = Im	1	0.88	0.56	17233	2.99	175	1.28	140.9	0.05
Time (Pe) = Ti(Pe)	21	**2.24**	**0.02**	2660	1.39	554	3.16	511.1	1.51
Location (Im) = Lo(Im)	1	**15.08**	**0.00**	5383	2.18	116	1.15	**8791.8**	**14.05**
Pe x Im	1	1.30	0.37	1225	1.84	17	0.34	532.7	2.33
Site (Lo(Im)) = Si(Lo(Im))	3	1.42	0.24	1031	1.00	70	0.42	299.7	1.68
Pe x Lo(Im)	1	0.36	0.93	690	0.80	409	3.06	226.6	1.10
Ti(Pe) x Im	21	0.77	0.71	1019	0.53	157	0.90	146.9	0.43
Pe x Si(Lo(Im))	3	0.38	0.76	237	0.23	13	0.08	28.9	0.16
Ti(Pe) x Lo(Im)	21	**2.40**	**0.00**	**1912**	**1.85**	175	1.06	**338.8**	**1.90**
Ti(Pe) x Si(Lo(Im))	63	1.13	0.27	**1033**	**2.00**	**165**	**2.17**	**178.1**	**1.58**
Residual	276	3.15	0.06	516		76		112.9	
C3		**Diversity**		**Abundance**		**Leptophlebiidae**		**Atyidae**	
Source	***df***	**MS**	***Ps-F***	**MS**	***Ps-F***	**MS**	***Ps-F***	**MS**	***Ps-F***
Period = Pe	1	0.2	0.35	814	0.74	1363	1.39	**570**	**7.76**
Impact = Im	1	158.1	0.45	8056	1.82	417	0.84	1127	0.23
Time (Pe) = Ti(Pe)	19	**64.1**	**2.66**	3790	1.82	**993**	**5.32**	119	0.33
Location (Im) = Lo(Im)	1	**389.4**	**6.45**	3943	1.70	141	1.49	**6287**	**9.18**
Pe x Im	1	36.9	2.71	133	1.27	37	0.32	211	1.98
Site (Lo(Im)) = Si(Lo(Im))	3	**38.0**	**3.47**	1036	0.76	206	0.46	340	2.08
Pe x Lo(Im)	1	5.8	0.57	124	0.61	123	2.72	2	0.38
Ti(Pe) x Im	19	16.7	0.69	1620	0.78	**576**	**3.08**	289	0.80
Pe x Si(Lo(Im))	3	5.0	0.46	343	0.25	22	0.05	76	0.47
Ti(Pe) x Lo(Im)	19	**24.1**	**2.21**	2077	1.52	187	0.42	**363**	**2.22**
Ti(Pe) x Si(Lo(Im))	57	10.9	1.29	**1362**	**2.24**	**445**	**3.12**	**163**	**1.76**
Residual	252	8.5		609		143		93	

A



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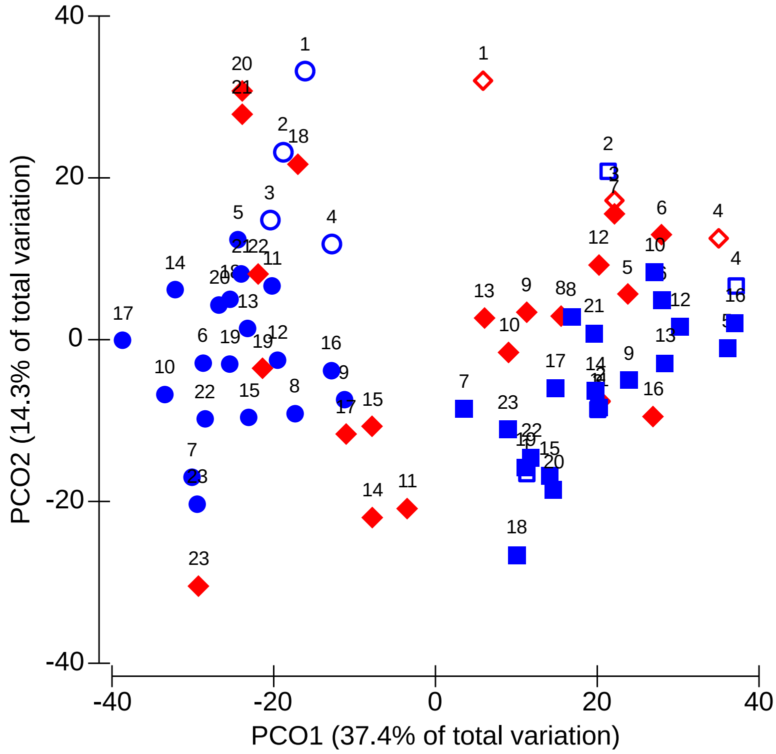


Figure 6. PCoA of centroids for assemblages of aquatic macroinvertebrates sampled at locations a) C1 and b) C2 (red symbols) and two control locations (Woronora River: blue squares; O'Hares Creek (blue circles) between spring 2008 (T1) and spring 2019 (T23). Empty symbols: 'Before-mining'; Filled symbols: 'After-mining Longwalls 20-22.

C

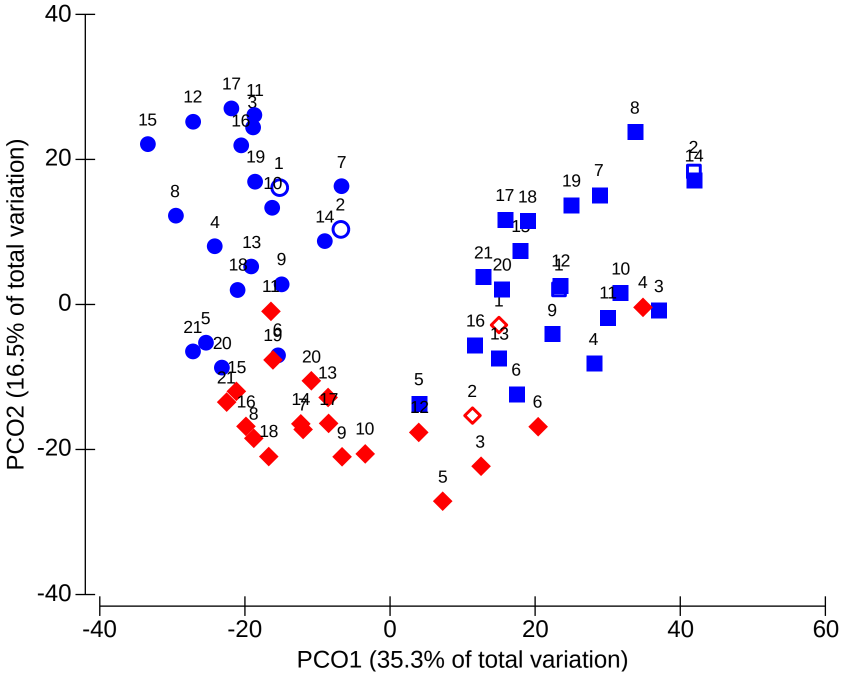


Figure 6 (Cont'd). c) Location C3 and two control locations (Woronora River and O'Hares Creek) for each time of sampling (n = 6). Red triangles: Tributary C; Blue squares: Woronora River; Blue circles: O'Hares Creek. Empty symbols: 'Before' commencement of mining; Filled symbols: 'After' mining. Numbers indicate sampling time. NB Sampling of location C3 commenced in spring 2009.

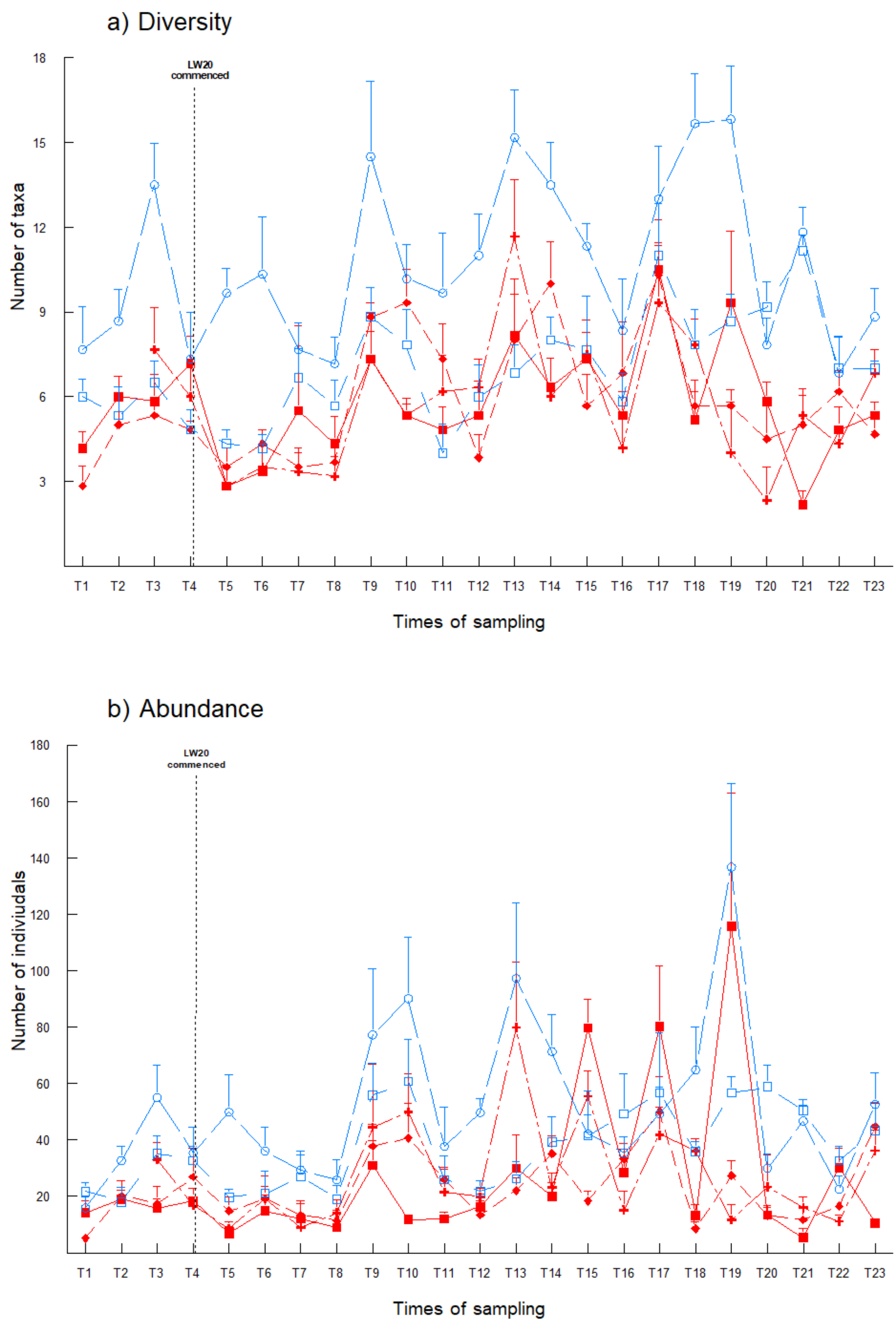
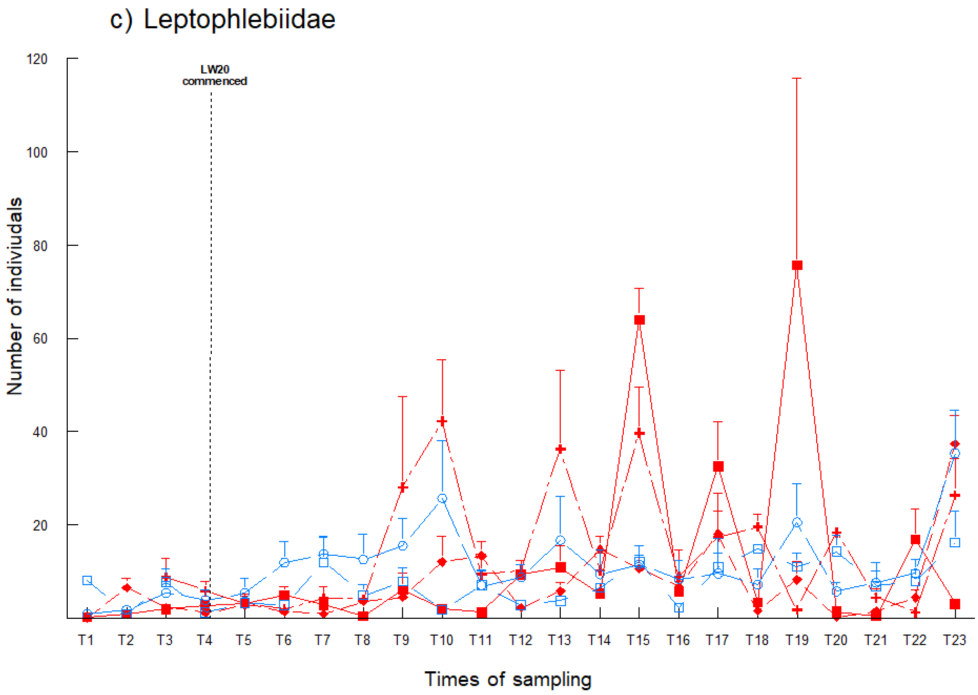


Figure 7. Mean macroinvertebrate (+SE) a) diversity and b) abundance at locations on Tributary C/Eastern Tributary (C1: solid squares; C2: diamonds; C3: plus symbols) and the control locations (Woronora River: empty squares; O'Hares Creek: circles) (*n* = 6). Time 1 = spring 2008, T2 = autumn 2009 etc. NB Sampling of C3 commenced in spring 2009 (T3).



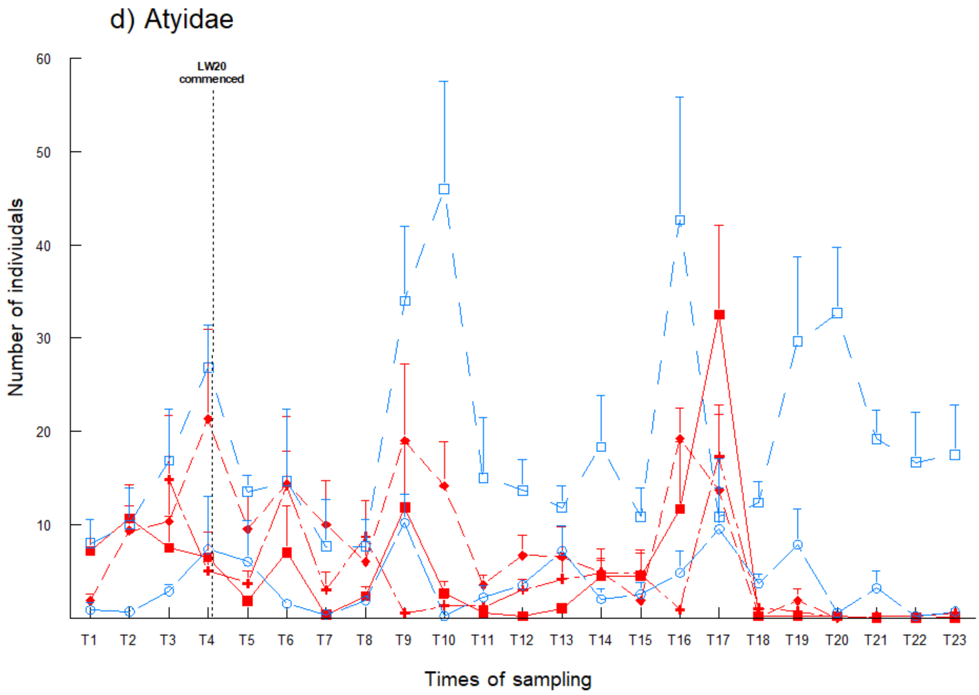


Figure 7 cont'd. Mean number (+SE) of c) Leptophlebiidae and d) Atyidae at locations on Tributary C/Eastern Tributary (C1: solid squares; C2: diamonds; C3: plus symbols) and the control locations (Woronora River: empty squares; O'Hares Creek: circles) (*n* = 6). Time 1 = spring 2008, T2 = autumn 2009 etc. NB Sampling of C3 commenced in spring 2009 (T3).

AUSRIVAS Analyses

For AUSRIVAS surveys done in spring, OE50 scores ranged between 0.10 (Location C1 in spring 2018) and 1.015 (Location C3 in spring 2014) (Figure 8a). OE50 Taxa Scores for samples collected in autumn ranged from 0.30 (Location C3 in autumn 2014) to 0.88 (Location OC in autumn 2018) (Figure 8b). With the exception of one sample (i.e. Location C3 in spring 2014), all of the OE50 Taxa scores were below 1.00 (Figure 8a&b), indicating that the number of taxa observed was less 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 (OC in autumn 2017) (Figure 8a). Four Band A scores have been obtained in spring (Locations C3, WR and OC in spring 2014 and Location OC in spring 2017) (Figure 8a&b).

Notably on this sampling occasion, Location C1 increased from Band D in spring 2018 to Band B in spring 2019, possibly due to increased water level at Location C1-1 (Figure 8a). Location C2 dropped from Band B to Band C between spring 2018 and 2019 (Figure 8a).

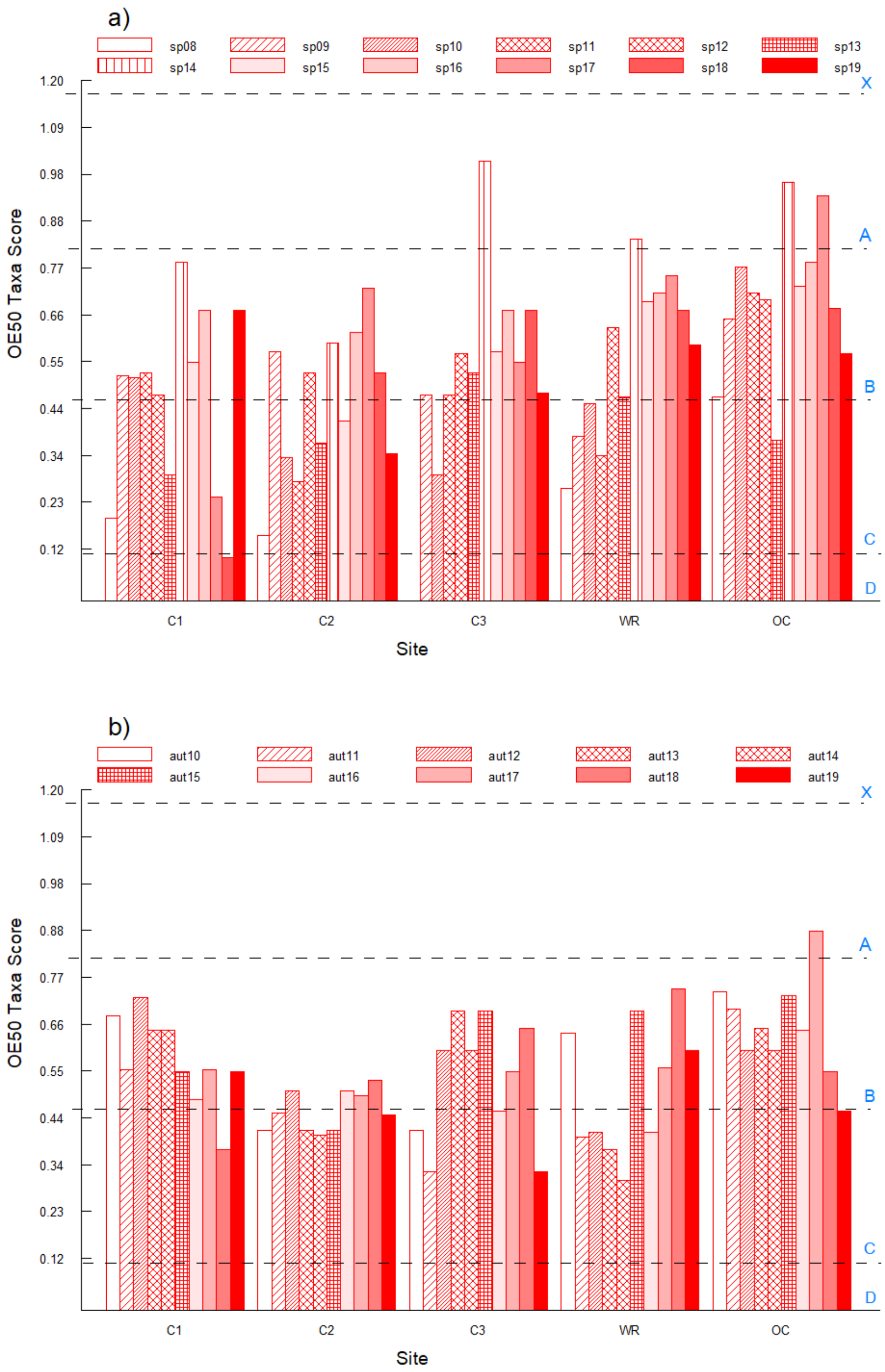


Figure 8. Mean OE50 Taxa Scores and their respective Band Scores (X-D) from AUSRIVAS samples collected from edge habitat at each site in a) spring and b) autumn between 2008 and 2019 (*n* = 2).

4.1.1.2 Aquatic Macrophytes

Location C1 v Controls

Prior Surveys (Spring 2008 – Autumn 2019)

A significant Before-to-After mining change in the structure of the assemblage of aquatic macrophytes was detected at Location C1 in spring 2014 (T13) in relation to the control locations, but not subsequently. This result did not appear to be related to mining activities.

Analyses of univariate estimates of total cover and species diversity at Location C1 have consistently found no significant changes in relation to control locations that would indicate an impact from mining.

Current Survey (Spring 2019)

Results from the multivariate analysis examining patterns in assemblages of macrophytes at Location C1 in relation to the control locations indicated that differences in the structure of assemblages of macrophytes among locations depended on the time of sampling, evidenced by the significant Ti(Pe) x Lo(Im) interaction and Ti(Pe) x Impact results, not mining related activities (Table 5, Figure 9a).

Notable from the PCoA ordination was that assemblages at Location C1 and the control locations (Woronora River and O'Hares Creek) mostly group separately from each other (Figure 9a). The presence of the floating-attached species, *Triglochin procerum,* at the control locations but not at Location C1 contributed greatly to observed differences. In addition, *Baumea juncea* is a relatively abundant component of the riparian strip adjacent to Tributary C but has not been recorded at either of the control locations.

Analyses of univariate estimates of total cover and species diversity of aquatic macrophytes have consistently found no significant differences between the factors of interest (i.e. Pe x Im), indicating that to date, any changes observed throughout the study period are unlikely to be related to mining activities (Table 6, Figures 10a&b).

Graphically it appears that there has been a gradual decrease in mean percentage cover at Location C1 since the spring 2015 (T15) survey (Figure 10b). It is likely that mining related drops in pool water level at Location C1 have contributed to the observed decline in aquatic plants in the riparian zone.

Location C2 v Controls

Prior Surveys (Spring 2008 – Autumn 2019)

Similar to the findings for Location C1, analyses detected a significant before-to-after mining change in the structure of the assemblage of aquatic macrophytes at Location C2 in spring 2014 (T13), in relation to the control locations (Figure 9b). There were no apparent physical changes to the riparian strip that might be associated with mining activities and analyses of data from subsequent surveys have not detected a significant change in relation to assemblages at the control locations.

Analyses of species diversity and total cover of aquatic macrophytes have consistently found no significant differences between the factors of interest (i.e. Pe x Im) (Figure 10a&b).

Current Survey (Spring 2019)

Analyses of temporal changes in the structure of assemblages of aquatic macrophytes (Table 5, Figure 9b) and univariate estimates of total cover and species diversity (Table 6, Figures 10a&b) at Location C2 found no significant changes in relation to control locations that would indicate an impact from mining. In spring 2019, *Baumea juncea* contributed 64 % to the structure of the assemblage at Location C2, whilst *Lepidosperma filiforme* contributed 18 % (SIMPER).

Graphically, it can be seen that mean diversity and percentage cover of macrophytes at Location C2 in spring 2019 (T23) was similar to cover in autumn 2019 (T22) (Figures 10a&b).

Location C3 v Controls

Prior Surveys (Spring 2009 – Autumn 2019)

Analyses of before- (spring 2009 – autumn 2010) vs after- (spring 2010 – autumn 2019) mining changes in the structure of assemblages of aquatic macrophytes (Figure 9c) and univariate estimates of total cover and species diversity (Figures 10a&b) have consistently found no significant changes at Location C3 in relation to control locations that would indicate an impact from mining.

Current Survey (Spring 2019)

Consistent with the findings of the previous surveys, multivariate analyses found a significant interaction between Time nested in Period and Location nested within type of creek (impact or control), indicating that differences in the structure of assemblages of macrophytes between Location C3 and the control locations depended on the time of sampling not mining related activities (Table 5, Figure 9c). In spring 2019, *Lepidosperma filiforme* contributed most to the structure of the assemblage (30 %) at Location C3, followed by *Lomandra fluviatilis* (19 %) and *Gleichenia dicarpa* (19 %) (SIMPER).

Similar to the findings of the previous report, univariate analyses of total diversity found no significant difference at any of the scales examined (Table 6, Figure 10a). Total cover of macrophytes differed significantly between the scales of Time nested in Period and Locations nested within type of Creek (i.e. Ti(Pe) x Lo(Im)), indicating that differences in the structure of assemblages of macrophytes among locations depended on the time of sampling not mining related activities (Table 6, Figure 10b).

Graphically, it can be seen that diversity and cover of macrophytes in the riparian zone at Location C3 was similar between the autumn and spring 2019 surveys (Figures 32a&b). Mean cover increased at each of the control locations (Figure 10b).

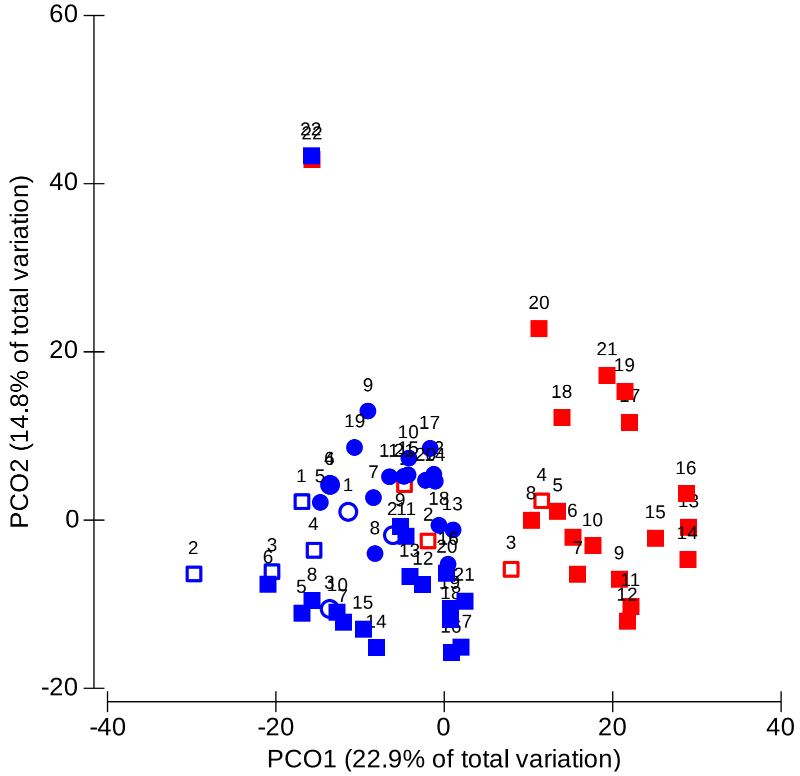
Table 5. PERMANOVA on macrophyte assemblage data to compare locations C1, C2 and C3 sampled on Tributary C/Eastern Tributary with control locations (Woronora River and O'Hares Creek). Percentages of Components of Variation (% CV) are shown. Bold numbers indicate significant results at *P* < 0.05.

C1					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	21720	1.36	0.17	4.09
Impact = Im	1	50215	1.48	0.15	6.52
Time (Pe) = Ti(Pe)	**21**	**6328**	**1.57**	**0.00**	**6.25**
Location (Im) = Lo(Im)	1	31519	1.48	0.06	5.88
Pe x Im	1	12533	0.91	0.62	0.00
Site (Lo(Im)) = Si(Lo(Im))	**3**	**18908**	**6.38**	**0.00**	**10.06**
Pe x Lo(Im)	1	12586	1.33	0.10	4.92
Ti(Pe) x Im	21	**5774**	**1.43**	**0.01**	**7.70**
Pe x Si(Lo(Im))	3	**7296**	**2.51**	**0.00**	**7.55**
Ti(Pe) x Lo(Im)	19	**4059**	**1.45**	**0.00**	**7.42**
Ti(Pe) x Si(Lo(Im))	61	2801	0.80	1.00	0.00
Residual	556	3504			39.61
C2					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	20942	1.24	0.26	3.60
Impact = Im	1	44611	1.37	0.20	6.00
Time (Pe) = Ti(Pe)	21	**5067**	**1.34**	**0.01**	**4.84**
Location (Im) = Lo(Im)	1	**30883**	**1.70**	**0.02**	**7.14**
Pe x Im	1	16029	1.03	0.46	1.77
Site (Lo(Im)) = Si(Lo(Im))	3	**16077**	**5.45**	**0.00**	**9.78**
Pe x Lo(Im)	1	**14906**	**1.72**	**0.02**	**7.38**
Ti(Pe) x Im	21	4324	1.15	0.14	4.47
Pe x Si(Lo(Im))	3	**6613**	**2.24**	**0.00**	**7.31**
Ti(Pe) x Lo(Im)	19	**3771**	**1.28**	**0.01**	**6.30**
Ti(Pe) x Si(Lo(Im))	61	2948	0.83	1.00	0.00
Residual	556	3560			41.41
C3					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	14516	1.47	0.14	5.82
Impact = Im	1	24163	1.34	0.22	6.39
Time (Pe) = Ti(Pe)	19	4565	1.17	0.16	3.74
Location (Im) = Lo(Im)	**1**	**16868**	**1.70**	**0.02**	**7.94**
Pe x Im	1	6758	0.88	0.64	0.00
Site (Lo(Im)) = Si(Lo(Im))	**3**	**7800**	**2.57**	**0.00**	**8.56**
Pe x Lo(Im)	1	7957	1.31	0.12	6.33
Ti(Pe) x Im	19	4104	1.05	0.36	2.96
Pe x Si(Lo(Im))	3	4480	1.48	0.07	6.67
Ti(Pe) x Lo(Im)	**19**	**3894**	**1.29**	**0.02**	**6.93**
Ti(Pe) x Si(Lo(Im))	57	3030	0.84	1.00	0.00
Residual	504	3589			44.66

Table 6. PERMANOVA on estimates of diversity and cover of aquatic macrophytes. Bold numbers indicate significant results at *P* < 0.05.

C1		**Richness**		**Abundance**	
Source	**df**	**MS**	***PseudoF***	**MS**	***PseudoF***
Period = Pe	1	26.84	4.05	719	0.32
Impact = Im	1	0.01	0.58	0	0.36
Time (Pe) = Ti(Pe)	21	4.99	2.24	**3114**	**2.39**
Location (Im) = Lo(Im)	1	0.11	0.44	1484	0.56
Pe x Im	1	1.57	0.68	36	0.25
Site (Lo(Im)) = Si(Lo(Im))	3	1.42	0.96	**2299**	**3.88**
Pe x Lo(Im)	1	1.89	1.14	3357	1.00
Ti(Pe) x Im	21	3.69	1.66	2326	1.79
Pe x Si(Lo(Im))	3	0.60	0.44	**2532**	**4.27**
Ti(Pe) x Lo(Im)	21	2.25	1.53	**1324**	**2.34**
Ti(Pe) x Si(Lo(Im))	63	1.46	1.15	562	0.96
Residual	552	1.27		588	
C2		**Richness**		**Abundance**	
Source	**df**	**MS**	***PseudoF***	**MS**	***PseudoF***
Period = Pe	1	**39.95**	**5.64**	1371	0.95
Impact = Im	1	2.22	1.60	35	0.86
Time (Pe) = Ti(Pe)	21	**6.71**	**3.22**	1492	1.02
Location (Im) = Lo(Im)	1	0.32	0.57	550	0.36
Pe x Im	1	0.82	0.93	77	0.57
Site (Lo(Im)) = Si(Lo(Im))	3	0.81	0.61	2017	2.82
Pe x Lo(Im)	1	0.74	0.80	1497	0.68
Ti(Pe) x Im	21	2.37	1.14	1197	0.82
Pe x Si(Lo(Im))	3	0.49	0.37	1771	2.47
Ti(Pe) x Lo(Im)	21	2.08	1.58	**1461**	**2.04**
Ti(Pe) x Si(Lo(Im))	63	1.32	1.01	**716**	**1.53**
Residual	552	1.31		469	
C3		**Richness**		**Abundance**	
Source	**df**	**MS**	***PseudoF***	**MS**	***PseudoF***
Period = Pe	1	7.17	1.59	19	0.71
Impact = Im	1	0.15	0.67	5	1.95
Time (Pe) = Ti(Pe)	19	3.12	1.53	1584	0.99
Location (Im) = Lo(Im)	1	1.90	0.89	370	0.31
Pe x Im	1	0.00	0.51	661	1.94
Site (Lo(Im)) = Si(Lo(Im))	3	1.66	1.19	1078	2.28
Pe x Lo(Im)	1	2.66	1.76	716	0.38
Ti(Pe) x Im	19	1.39	0.68	455	0.28
Pe x Si(Lo(Im))	3	0.26	0.19	**1512**	**3.20**
Ti(Pe) x Lo(Im)	19	2.04	1.47	**1606**	**3.39**
Ti(Pe) x Si(Lo(Im))	57	1.39	1.02	473	0.93
Residual	504	1.37		508	

A



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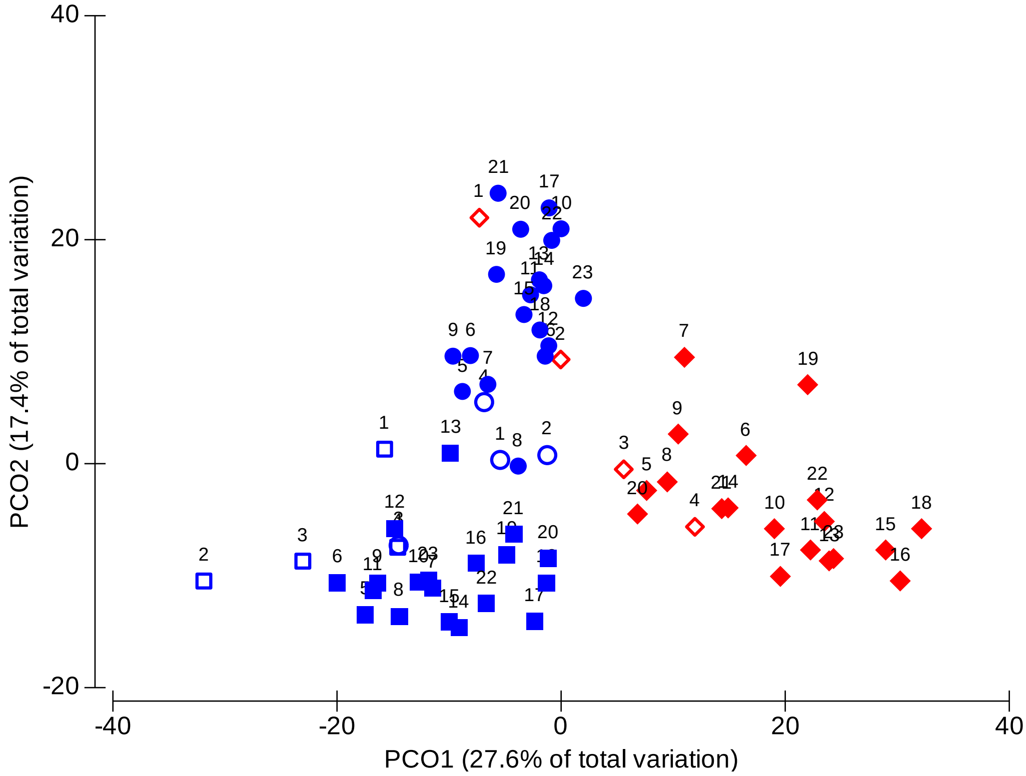


Figure 9. PCoA of centroids for macrophyte data for a) Location C1 and b) Location C2 and two control locations (Woronora River and O'Hares Creek) for each time of sampling (*n*= 10). Red symbols: Potential 'impact' locations; Blue squares: Woronora River; Blue circles: O'Hares Creek. Empty symbols: 'Before' mining; Filled symbols: 'After' mining. Numbers indicate sampling time. NB Sampling of locations C1 and C2 commenced in spring 2008 (T1).

C

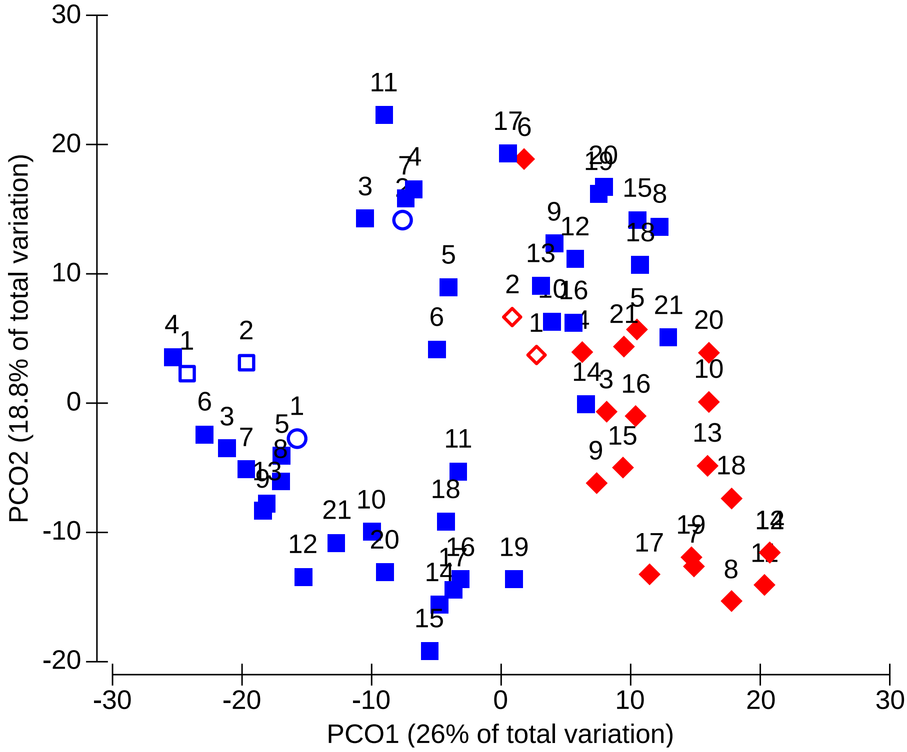


Figure 9 (Cont'd). PCoA of centroids for macrophyte data for c) Location C3. Red triangles: Potential 'impact' location; Blue squares: Woronora River; Blue circles: O'Hares Creek. Empty symbols: 'Before' mining; Filled symbols: 'After' mining. Numbers indicate sampling time. NB sampling of location C3 commenced in spring 2009.

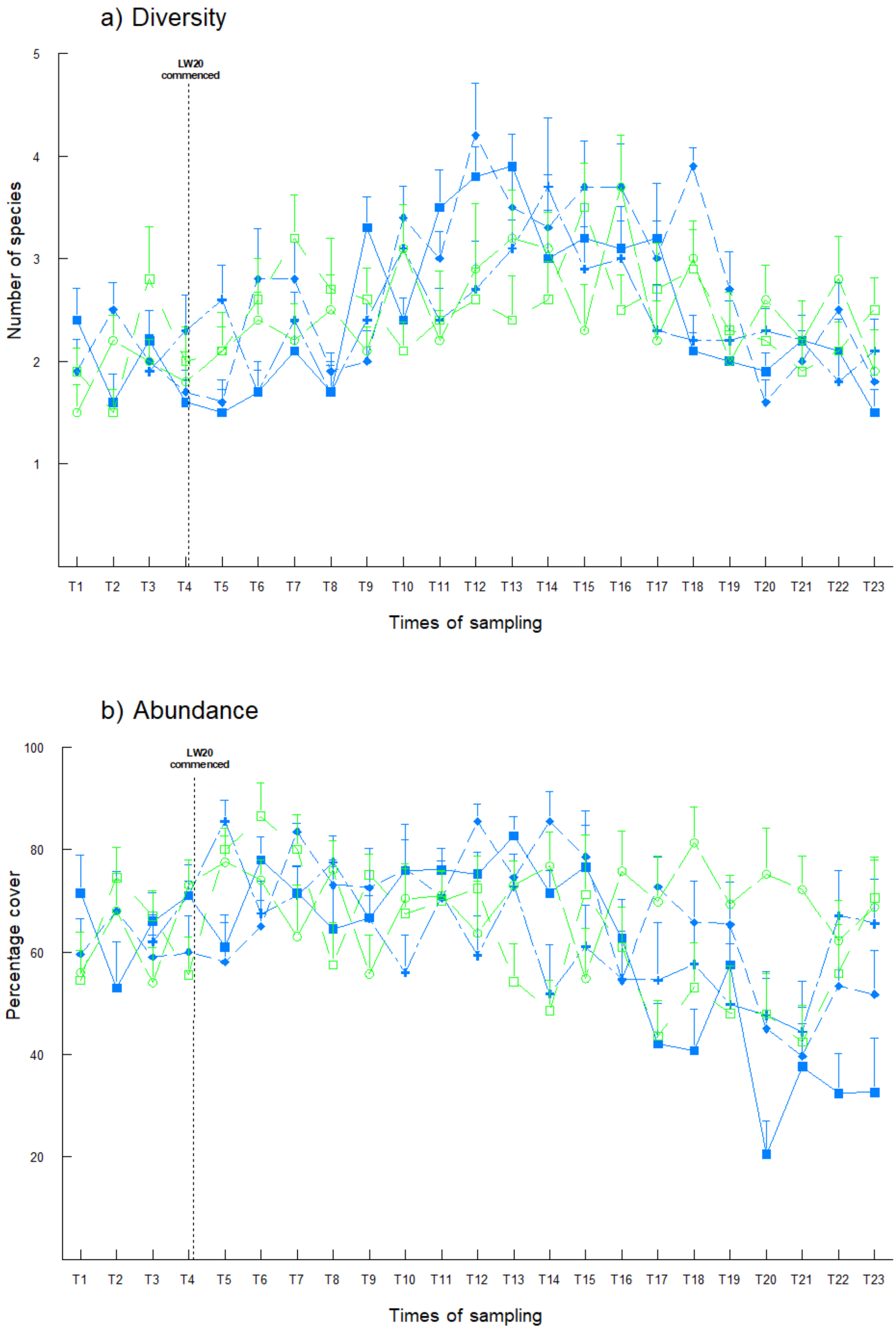


Figure 10. Mean (+SE) a) diversity and b) cover of aquatic macrophytes at locations on Tributary C/Eastern Tributary (C1: solid squares; C2: diamonds; C3: plus symbols) and the reference locations (Woronora River: empty square; O'Hares Creek: empty circle) (*n* = 10). NB Sampling of location C3 commenced in spring 2009.

4.1.2 Waratah Rivulet

PERMANOVA was used to test the null hypothesis of no significant change in aquatic macroinvertebrate or macrophyte indicators at Waratah Rivulet locations (Locations WT3, WT4 and WT5) Before- (spring 2008 to autumn 2010) vs After- (spring 2010 to spring 2019) mining in relation to Control locations (Woronora River and O'Hares Creek). Mining of the Longwalls 20-22 mining area commenced in May 2010 and was completed in April 2014.

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.

4.1.2.1 Aquatic Macroinvertebrates

<u>Quantitative Assessment</u>

Prior Surveys (Spring 2008 – Autumn 2019)
To date, analyses comparing temporal changes in components of assemblages of macroinvertebrates at Locations WT3, WT4 and WT5 in relation to control locations have not detected significant changes from Before- to After-commencement of mining of the Longwalls 20-22 underground mining area.

Univariate analyses detected a significant decrease in mean diversity at Location WT3 within the after-period in spring 2016 (T15), autumn 2018 (T20), spring 2018 (T21) and autumn 2019 (T22).

Current Survey (Spring 2019)

Consistent with previous surveys, there was a significant interaction of the factors Location nested within Impact and Time nested within Period (i.e. Ti(Pe) x Lo(Im)) (Table 7, Figures 11a-c). Most notably, the Principle Component Ordination (PCoA) of centroids for macroinvertebrate data suggests that the assemblage sampled at the Woronora River location consistently differed from the assemblage at the Waratah Rivulet locations and O'Hares Creek (Figures 11a-c: blue square symbols tend to group to the right of the PCoA while blue circle and red 'other' symbols tend to group to the left).

SIMPER analyses indicate that throughout the survey period, temporal differences at the Waratah Rivulet locations in relation to the control locations have been mostly due to differences in abundances of the macroinvertebrate taxa, particularly Atyidae and Leptophlebiidae, rather than the presence or absence of species.

Most notably, Atyidae contributed more to the structure of assemblages at the Waratah Rivulet locations within the before- (WT3: 25.9 %; WT4: 33 %; WT5: 52.8 %) compared to after-mining (WT3: 6.6 %; WT4: 9.5 %; WT5: 15.6 %) period. The contribution that Atyidae made to the assemblage at the control locations differed little between periods (Before: 43.2 %; After: 35.1 %).

Similar to the findings of the spring 2016 (T15), autumn 2018 (T20), spring 2018 (T21) and autumn 2019 (T22) surveys, univariate analyses detected a significant change in mean diversity at Location WT3 within the after-period, in relation to the control locations (Table 8, Figure 12a). When PERMDISP was used to formally compare the apparent variability (dispersion), a non-significant result was obtained ($P = 0.372$). Similarly, pair-wise tests were unable to determine differences among means ($P > 0.05$). Differences appear to be related to diversity at Location WT3 decreasing between periods (Before: 9.5; After: 7.9) whilst there has been an increase at the control locations (Before: 7.8; After: 9.1) (Figure 12a).

Mean diversity did not differ significantly between periods at Location WT4 and Location WT5 in relation to the controls (Table 8, Figure 12a). Graphically, it can be seen that diversity increased at Locations WT3, WT5 and the O'Hares Creek control location between the autumn 2019 (T22) and spring 2019 (T23) surveys (Figure 12a). Diversity changed little between surveys at Location WT4 and the Woronora River location (Figure 12a).

Mean abundance and numbers of Leptophlebiidae and Atyidae collected at Locations WT3, WT4 and WT5 showed no significant interactions at the level of interest (i.e. Period x Impact) (Table 8, Figures 12b-d). Mean abundance of macroinvertebrates and Leptophlebiidae increased at all locations between the autumn 2019 (T22) and spring 2019 (T23) (Figure 12b). Atyidae have consistently been most abundant at the Woronora River location, whilst similar numbers have been collected at the rivulet locations and at O'Hares Creek (Figure 12d).

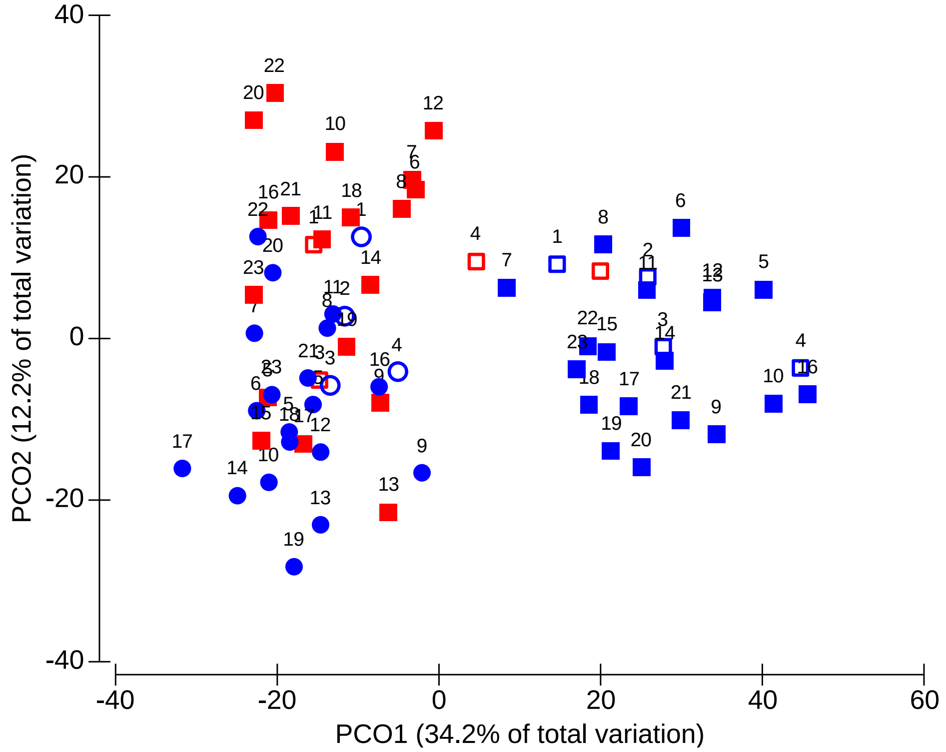
Table 7. PERMANOVA on Bray Curtis dissimilarities of macroinvertebrate assemblage data (non-transformed) to compare locations WT3, WT4 and WT5 sampled on Waratah Rivulet with control locations (Woronora River and O'Hares Creek) Before- vs After-commencement of mining. Percentages of Components of Variation (% CV) are shown. Bold numbers indicate significant results at *P* < 0.05.

WT3					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	14483	1.33	0.15	4.45
Impact = Im	1	23246	0.33	1.00	0.00
Time (Pe) = Ti(Pe)	21	**7331**	**1.50**	**0.00**	**8.12**
Location (Im) = Lo(Im)	1	**78172**	**8.47**	**0.00**	**19.75**
Pe x Im	1	7219	0.91	0.62	0.00
Site (Lo(Im)) = Si(Lo(Im))	3	**5471**	**2.03**	**0.00**	**5.51**
Pe x Lo(Im)	1	**7508**	**1.52**	**0.03**	**6.19**
Ti(Pe) x Im	21	4898	1.20	0.07	6.66
Pe x Si(Lo(Im))	3	2618	0.97	0.51	0.00
Ti(Pe) x Lo(Im)	21	**4079**	**1.52**	**0.00**	**10.01**
Ti(Pe) x Si(Lo(Im))	63	**2692**	**1.48**	**0.00**	**11.26**
Residual	276	1814			
WT4					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	13309	1.20	0.29	3.44
Impact = Im	1	26774	0.39	0.99	0.00
Time (Pe) = Ti(Pe)	**21**	**7278**	**1.89**	**0.00**	**9.73**
Location (Im) = Lo(Im)	**1**	**73071**	**8.57**	**0.00**	**19.30**
Pe x Im	1	6837	0.92	0.61	0.00
Site (Lo(Im)) = Si(Lo(Im))	**3**	**4986**	**1.92**	**0.00**	**5.15**
Pe x Lo(Im)	1	7047	1.46	0.06	5.82
Ti(Pe) x Im	21	4581	1.19	0.09	6.37
Pe x Si(Lo(Im))	3	2758	1.06	0.37	1.86
Ti(Pe) x Lo(Im)	**21**	**3848**	**1.48**	**0.00**	**9.58**
Ti(Pe) x Si(Lo(Im))	**63**	**2602**	**1.62**	**0.00**	**12.13**
Residual	276	1603			26.62
WT5					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	17736	1.48	0.13	5.25
Impact = Im	1	21886	0.31	1.00	0.00
Time (Pe) = Ti(Pe)	21	**7239**	**1.77**	**0.00**	**9.02**
Location (Im) = Lo(Im)	1	**78172**	**8.11**	**0.00**	**19.17**
Pe x Im	1	6847	0.90	0.63	0.00
Site (Lo(Im)) = Si(Lo(Im))	3	**5887**	**2.27**	**0.00**	**5.84**
Pe x Lo(Im)	1	7508	1.48	0.05	5.84
Ti(Pe) x Im	21	4634	1.14	0.15	5.34
Pe x Si(Lo(Im))	3	2747	1.06	0.37	1.76
Ti(Pe) x Lo(Im)	21	**4079**	**1.57**	**0.00**	**5.25**
Ti(Pe) x Si(Lo(Im))	63	**2597**	**1.44**	**0.00**	**0.00**
Residual	276	1805			27.26

Table 8. 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 Waratah Rivulet with two control locations Before- vs After-commencement of mining. Bold numbers indicate significant results at *P* < 0.05.

WT3		**Diversity**		**Abundance**		**Leptophlebiidae**		**Atyidae**	
Source	***df***	**MS**	***Ps-F***	**MS**	***Ps-F***	**MS**	***Ps-F***	**MS**	***Ps-F***
Period = Pe	1	0.69	0.11	1473	0.32	270.79	0.41	41	0.26
Impact = Im	1	2.70	0.05	1787	0.50	316.67	1.14	1916	0.25
Time (Pe) = Ti(Pe)	21	76.60	3.97	**4275**	**2.41**	456.79	1.45	190	1.12
Location (Im) = Lo(Im)	1	**556.96**	**15.64**	5595	2.25	116.02	1.36	**8792**	**14.00**
Pe x Im	1	**109.75**	**6.89**	5934	3.25	978.76	1.60	624	2.43
Site (Lo(Im)) = Si(Lo(Im))	3	12.93	1.29	1048	0.98	81.28	0.35	301	1.86
Pe x Lo(Im)	1	0.00	0.28	642	0.53	408.71	0.79	227	1.11
Ti(Pe) x Im	21	19.31	0.83	1775	0.93	314.61	1.80	170	0.50
Pe x Si(Lo(Im))	3	12.51	1.25	1291	1.20	634.96	2.73	11	0.07
Ti(Pe) x Lo(Im)	21	**23.32**	**2.34**	**1918**	**1.79**	175.15	0.75	**339**	**2.09**
Ti(Pe) x Si(Lo(Im))	63	9.99	1.06	**1072**	**1.68**	**232.42**	**1.78**	**162**	**1.89**
Residual	276	9.46		636		130.75		86	
WT4		**Diversity**		**Abundance**		**Leptophlebiidae**		**Atyidae**	
Source	***df***	**MS**	***Ps-F***	**MS**	***Ps-F***	**MS**	***Ps-F***	**MS**	***Ps-F***
Period = Pe	1	103.17	2.21	9680	2.06	721	0.72	252	0.83
Impact = Im	1	95.86	0.20	2956	0.63	1600	2.32	1953	0.25
Time (Pe) = Ti(Pe)	21	**57.16**	**2.45**	**4998**	**2.61**	**832**	**4.75**	484	1.43
Location (Im) = Lo(Im)	1	**556.96**	**14.67**	5595	2.33	116	1.25	**8792**	**12.85**
Pe x Im	1	0.26	0.95	290	0.79	437	0.58	7	0.64
Site (Lo(Im)) = Si(Lo(Im))	3	15.35	1.45	965	0.85	75	0.38	366	1.42
Pe x Lo(Im)	1	0.00	0.43	642	0.62	409	2.41	227	1.15
Ti(Pe) x Im	21	24.74	1.06	2148	1.12	**650**	**3.71**	317	0.94
Pe x Si(Lo(Im))	3	1.05	0.10	956	0.84	76	0.39	82	0.32
Ti(Pe) x Lo(Im)	21	**23.32**	**2.21**	1918	1.69	175	0.89	339	1.31
Ti(Pe) x Si(Lo(Im))	63	10.56	1.18	**1133**	**1.73**	197	1.38	**258**	**2.04**
Residual	276	8.94		654		143		127	
WT5		**Diversity**		**Abundance**		**Leptophlebiidae**		**Atyidae**	
Source	***df***	**MS**	***Ps-F***	**MS**	***Ps-F***	**MS**	***Ps-F***	**MS**	***Ps-F***
Period = Pe	1	86.57	2.06	11794	3.03	2207	2.90	14	0.51
Impact = Im	1	162.93	0.32	9921	1.58	230	0.93	798	0.13
Time (Pe) = Ti(Pe)	21	**53.47**	**2.29**	3890	2.03	412	2.35	465	1.37
Location (Im) = Lo(Im)	1	**556.96**	**15.13**	5595	2.26	116	0.97	**8792**	**13.02**
Pe x Im	1	0.12	1.14	46	0.78	1	0.24	499	1.83
Site (Lo(Im)) = Si(Lo(Im))	3	14.15	1.42	1030	0.97	109	0.68	349	2.13
Pe x Lo(Im)	1	0.00	0.41	642	0.79	409	2.91	227	1.09
Ti(Pe) x Im	21	20.48	0.88	1875	0.98	319	1.82	232	0.69
Pe x Si(Lo(Im))	3	1.15	0.11	240	0.23	20	0.13	18	0.11
Ti(Pe) x Lo(Im)	21	**23.32**	**2.33**	**1918**	**1.81**	175	1.10	**339**	**2.07**
Ti(Pe) x Si(Lo(Im))	63	10.00	1.03	**1063**	**1.67**	**159**	**1.73**	**164**	**1.67**
Residual	276	9.68		635		92		98	

A



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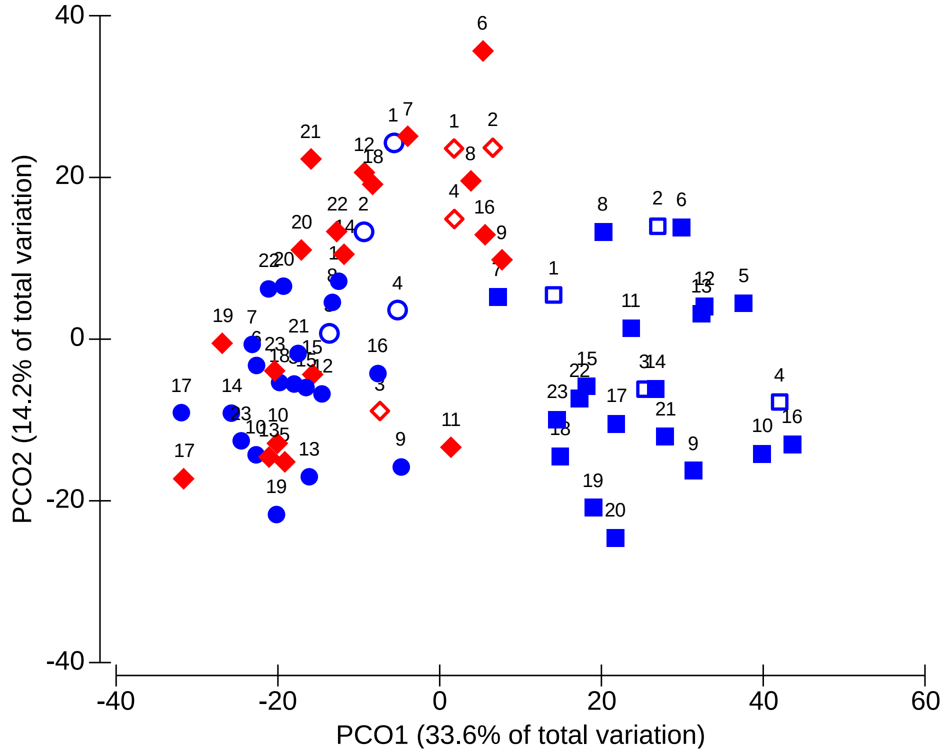


Figure 11. PCoA of centroids for macroinvertebrate data for locations a) WT3 and b) WT4 and two control locations (Woronora River and O'Hares Creek) for each time of sampling (*n* = 6). Red symbols: Waratah Rivulet; Blue squares: Woronora River; Blue circles: O'Hares Creek. Empty symbols: 'Before' commencement of mining; Filled symbols: 'After' mining. Numbers indicate sampling time. Time 1 = spring 2008, T2 = autumn 2009 etc.

C

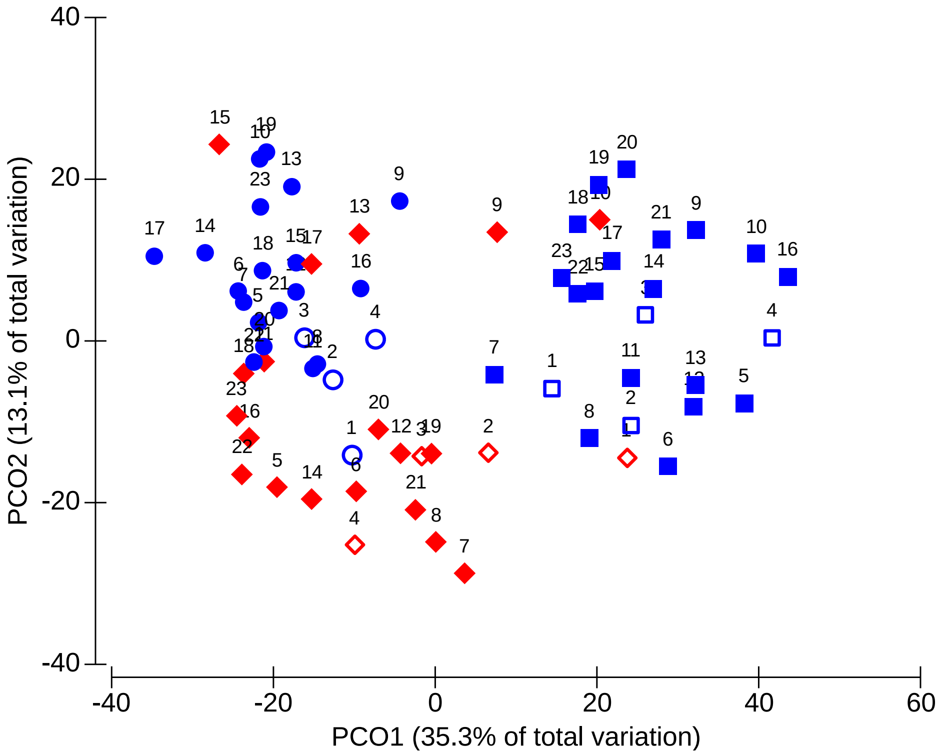


Figure 11 (Cont'd). PCoA of centroids for macroinvertebrate data for c) Location WT5 (Red triangles) and two control locations (Woronora River and O'Hares Creek) for each time of sampling (n = 6); Blue squares: Woronora River; Blue circles: O'Hares Creek. Empty symbols: 'Before' commencement of mining; Filled symbols: 'After' mining. Numbers indicate sampling time. Time 1 = spring 2008, T2 = autumn 2009 etc.

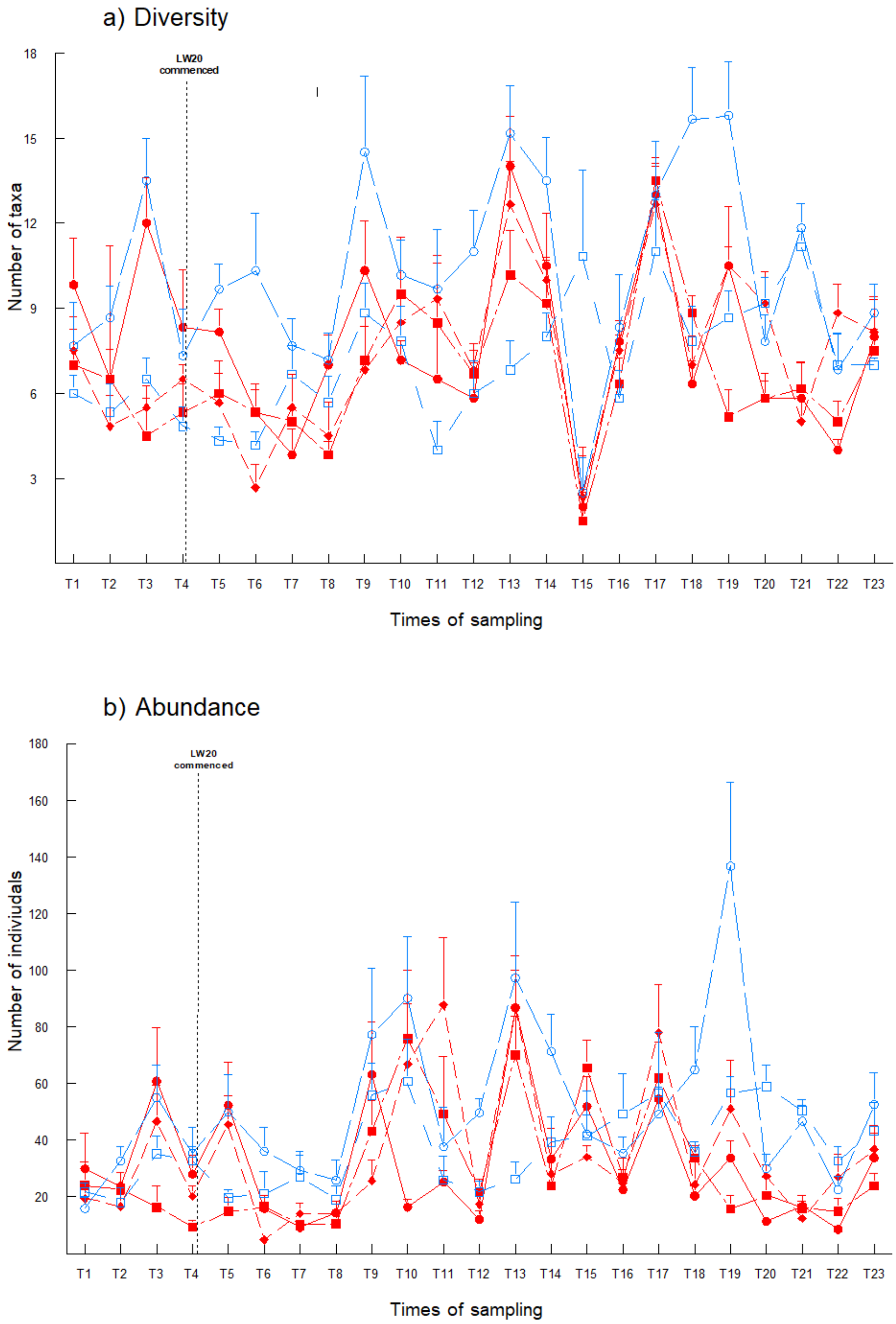
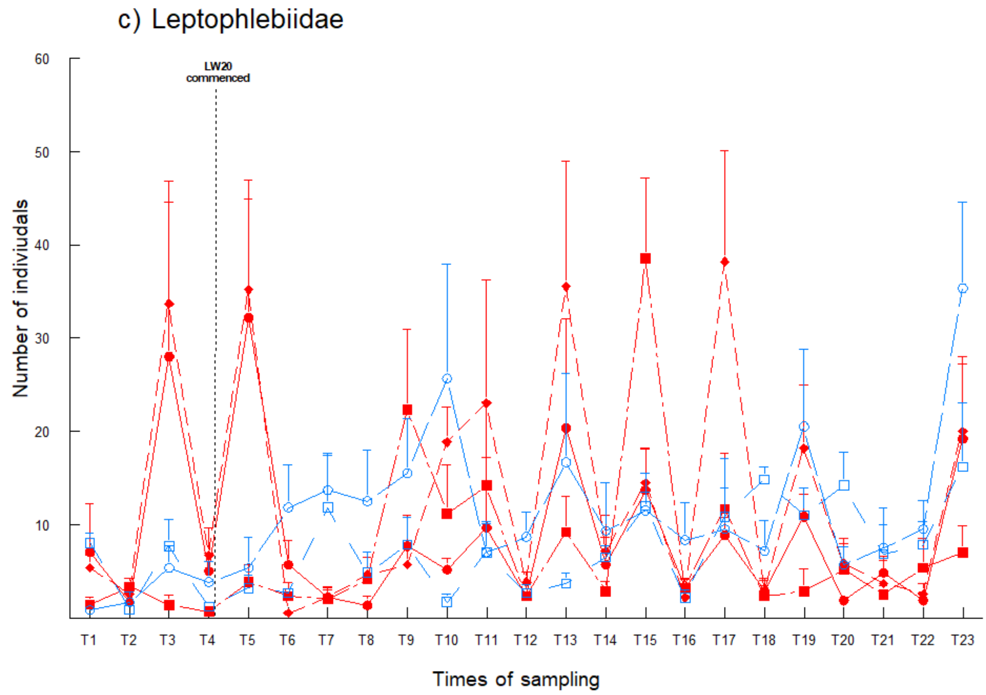


Figure 12. Mean macroinvertebrate (+SE) a) diversity and b) abundance at locations on Waratah Rivulet (WT3: solid circle; WT4: diamond; WT5: solid square) and the reference locations (Woronora River: empty square; O'Hares Creek: empty circle) (*n* = 6). Time 1 = spring 2008, T2 = autumn 2009 etc.



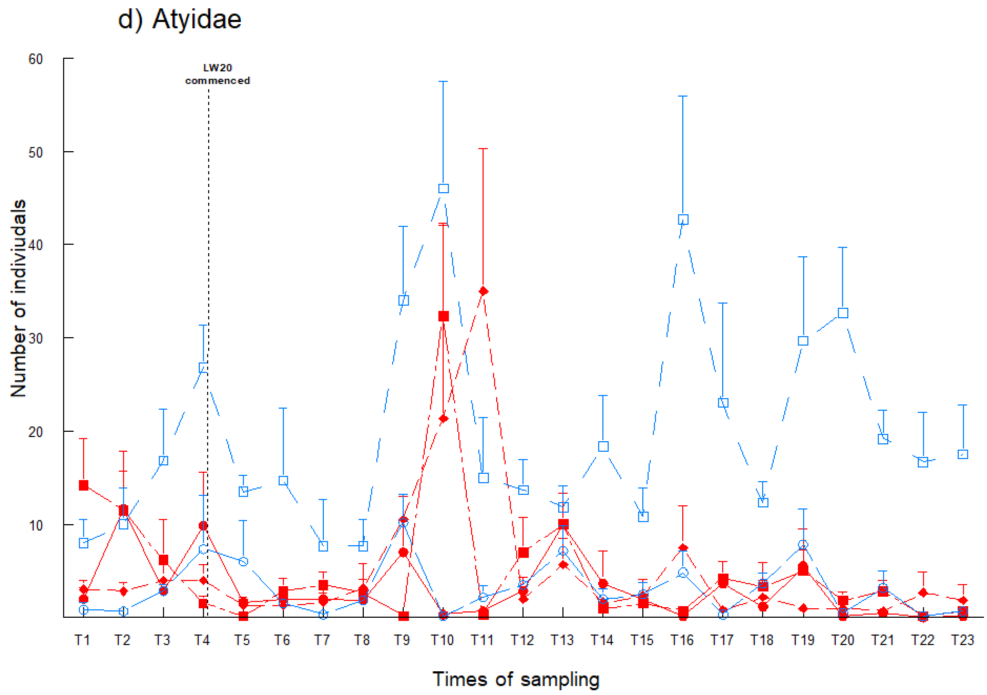
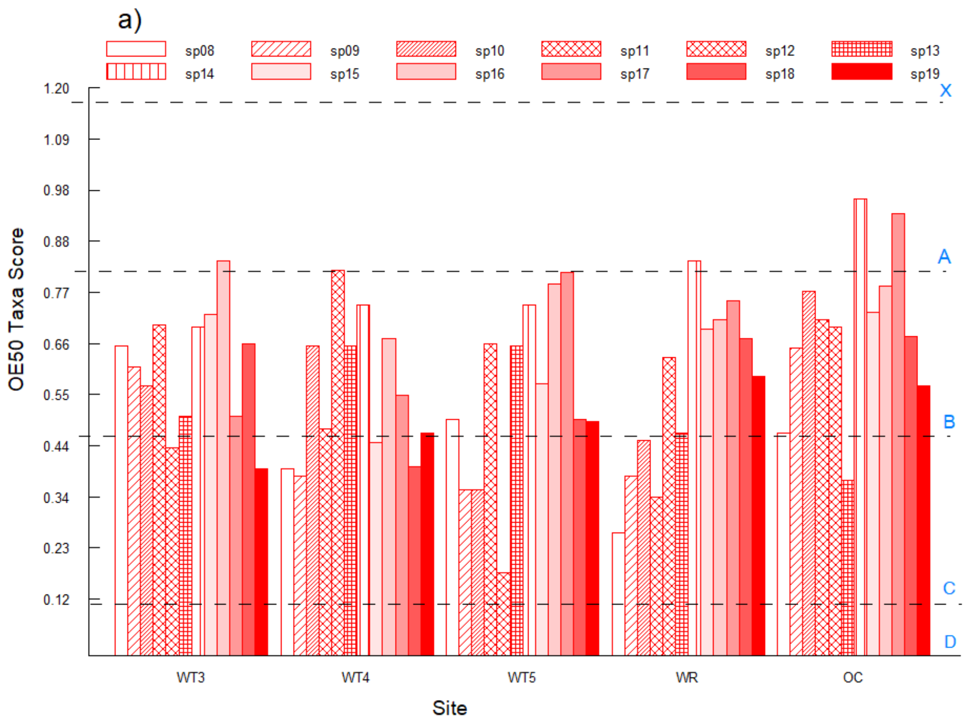


Figure 12 cont'd. Mean number (+SE) of c) Leptophlebiidae and d) Atyidae at locations on Waratah Rivulet (WT3: solid circle; WT4: diamond; WT5: solid square) and the reference locations (Woronora River: empty square; O'Hares Creek: empty circle) (*n* = 6). Time 1 = spring 2008, T2 = autumn 2009 etc.

AUSRIVAS Analyses

For AUSRIVAS surveys done in spring, OE50 scores ranged between 0.18 (Site WT5 in spring 2012) and 0.965 (Site OC in spring 2014) (Figure 13a). OE50 Taxa Scores for samples collected in autumn ranged from 0.30 (Site WR in autumn 2014) to 0.91 (Site WT5 in autumn 2015) (Figure 35b). All of the OE50 Taxa scores were below 1.00 (Figure 13a&b), indicating that the number of taxa observed was less than would be expected relative to the AUSRIVAS reference watercourses.

Five Band A scores (equivalent to AUSRIVAS reference condition) were obtained in spring (Site WT3 in 2016, WT4 in 2012, WR and OC in spring 2014 and Site OC in spring 2017) (Figure 13a). Four locations achieved a Band A score in autumn (WT3 in autumn 2013, WT4 in 2012, WT5 in 2015 and OC in autumn 2017) (Figure 13b). Notably, the O'Hares Creek location only scored lower than a Band B in spring 2013 and autumn 2019 (Figures 13a&b). On this sampling occasion, the OE50 score for Location WT3 was lower than the score obtained by any other spring survey (Figure 13a).



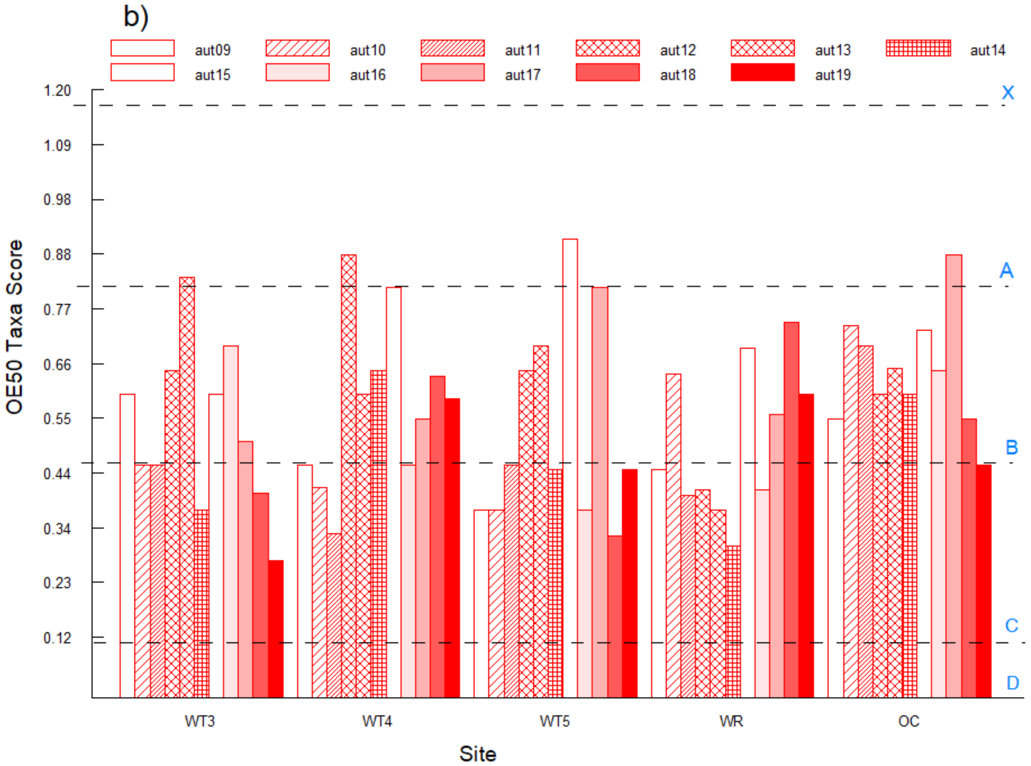


Figure 13. Mean OE50 Taxa Scores and their respective Band Scores (X-D) from AUSRIVAS samples collected from edge habitat at each site in a) spring and b) spring between 2008 and 2019 (*n* = 2).

4.1.2.2 Aquatic Macrophytes

Waratah Rivulet v Controls

Prior Surveys (Spring 2009 – Autumn 2019)

To date, there have been no detectable impacts to macrophytes at the Waratah Rivulet locations (i.e. Locations WT3, WT4 and WT5) in relation to the control locations that could be associated with mining of the Longwalls 20-22 mining area.

Current Survey (Spring 2019)

Consistent with the findings of the previous report, analyses comparing patterns of change in assemblages of aquatic macrophytes at each of the potential impact locations with the control locations found significant interactions between the factors Location nested within Impact and Time nested within Period (i.e. Ti(Pe) x Lo(Im)), indicating that there was significant variation among Locations, which depended on the time of sampling and *vice versa* (Table 9, Figures 14a-b).

The Principal Coordinate Analyses (PCoA) indicates that assemblages of aquatic macrophytes sampled at the Waratah Rivulet locations differed considerably from assemblages at the Woronora River location (Figures 14a-c). SIMPER has consistently indicated that differences were largely due to the presence of *Myriophyllum pedunculatum*, which has only occurred at the Woronora River location, and the floating-attached species, *Triglochin procerum,* which has commonly been more widespread at the Woronora River location than at the other locations sampled.

There were no conspicuous differences in mean total diversity or percentage cover of macrophytes at locations WT3, WT4 or WT5 in relation to the control locations between the 'Before' and 'After' mining periods (Table 10, Figures 15a&b). Fewer species of macrophytes have commonly been observed at Location WT4 than at the other locations sampled, most likely due to the paucity of species at the upstream site (i.e. Site WT4-1) (Figure 15a). Cover increased at all locations except WT4 between the autumn 2019 (T22) and spring 2019 (T23) surveys (Figure 15b).

Table 9. PERMANOVA on macrophyte assemblage data to compare locations on Waratah Rivulet (WT3, WT4 and WT5) with control locations (Woronora River and O'Hares Creek). Percentages of Components of Variation (% CV) are shown.

WT3					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	37263	1.86	0.05	7.36
Impact = Im	1	40864	1.33	0.21	5.63
Time (Pe) = Ti(Pe)	21	**6309**	**1.51**	**0.01**	**6.33**
Location (Im) = Lo(Im)	1	**30262**	**2.03**	**0.00**	**7.99**
Pe x Im	1	13664	0.91	0.62	0.00
Site (Lo(Im)) = Si(Lo(Im))	3	**12166**	**4.14**	**0.00**	**8.35**
Pe x Lo(Im)	1	**16010**	**1.95**	**0.00**	**8.36**
Ti(Pe) x Im	21	3590	0.86	0.84	0.00
Pe x Si(Lo(Im))	3	**5549**	**1.89**	**0.00**	**6.28**
Ti(Pe) x Lo(Im)	21	**4170**	**1.42**	**0.00**	**7.84**
Ti(Pe) x Si(Lo(Im))	63	2940	0.84	1.00	0.00
Residual	552	3508			41.87
WT4					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	27847	1.60	0.10	6.29
Impact = Im	1	30321	1.05	0.44	2.36
Time (Pe) = Ti(Pe)	21	4412	1.05	0.36	2.09
Location (Im) = Lo(Im)	1	**30050**	**1.54**	**0.04**	**7.11**
Pe x Im	1	10359	0.79	0.77	0.00
Site (Lo(Im)) = Si(Lo(Im))	3	**17241**	**5.88**	**0.00**	**11.21**
Pe x Lo(Im)	1	**15610**	**1.85**	**0.01**	**8.66**
Ti(Pe) x Im	21	2794	0.66	1.00	0.00
Pe x Si(Lo(Im))	3	**5790**	**1.97**	**0.00**	**7.08**
Ti(Pe) x Lo(Im)	21	**4211**	**1.44**	**0.00**	**8.61**
Ti(Pe) x Si(Lo(Im))	63	2932	0.78	1.00	0.00
Residual	552	3740			46.58
WT5					
Source	**df**	**MS**	***Pseudo-F***	***P***	**% CV**
Period = Pe	1	46880	1.94	0.05	8.60
Impact = Im	1	17389	0.65	0.91	0.00
Time (Pe) = Ti(Pe)	21	5317	1.29	0.08	4.85
Location (Im) = Lo(Im)	1	**29724**	**1.54**	**0.04**	**6.75**
Pe x Im	1	7748	0.49	0.99	0.00
Site (Lo(Im)) = Si(Lo(Im))	3	**17147**	**5.73**	**0.00**	**10.62**
Pe x Lo(Im)	1	**20962**	**2.19**	**0.00**	**10.19**
Ti(Pe) x Im	21	3416	0.83	0.88	0.00
Pe x Si(Lo(Im))	3	**6805**	**2.27**	**0.00**	**7.80**
Ti(Pe) x Lo(Im)	21	**4128**	**1.38**	**0.00**	**7.74**
Ti(Pe) x Si(Lo(Im))	63	2992	0.83	1.00	0.00
Residual	552	3586			43.46

Table 10. PERMANOVA on diversity and cover of aquatic macrophytes. Bold numbers indicate significant results at *P* < 0.05.

WT3		**Richness**		**Abundance**	
Source	**df**	**MS**	***PseudoF***	**MS**	***PseudoF***
Period = Pe	1	18.93	4.40	3958	2.60
Impact = Im	1	**3.24**	**4.30**	7437	4.11
Time (Pe) = Ti(Pe)	21	3.43	1.65	440	0.31
Location (Im) = Lo(Im)	1	0.07	0.40	636	0.30
Pe x Im	1	1.28	1.34	1785	1.02
Site (Lo(Im)) = Si(Lo(Im))	3	1.31	1.03	**2035**	**5.08**
Pe x Lo(Im)	1	1.35	1.05	1637	0.61
Ti(Pe) x Im	21	1.17	0.56	1522	1.06
Pe x Si(Lo(Im))	3	0.42	0.33	**1906**	**4.76**
Ti(Pe) x Lo(Im)	21	2.08	1.62	**1439**	**3.59**
Ti(Pe) x Si(Lo(Im))	63	1.28	1.00	401	0.80
Residual	552	1.28		500	
WT4		**Richness**		**Abundance**	
Source	**df**	**MS**	***PseudoF***	**MS**	***PseudoF***
Period = Pe	1	0.99	1.23	443	0.70
Impact = Im	1	**27.72**	**24.61**	1	1.29
Time (Pe) = Ti(Pe)	21	1.74	0.83	1238	0.85
Location (Im) = Lo(Im)	1	0.29	0.49	551	0.27
Pe x Im	1	4.40	3.82	24	0.71
Site (Lo(Im)) = Si(Lo(Im))	3	0.78	0.69	**2434**	**4.90**
Pe x Lo(Im)	1	0.78	0.76	1498	0.77
Ti(Pe) x Im	21	0.92	0.44	582	0.40
Pe x Si(Lo(Im))	3	0.41	0.37	1148	2.31
Ti(Pe) x Lo(Im)	21	**2.09**	**1.85**	**1461**	**2.94**
Ti(Pe) x Si(Lo(Im))	63	1.13	0.93	497	0.92
Residual	552	1.21		538	
WT5		**Richness**		**Abundance**	
Source	**df**	**MS**	***PseudoF***	**MS**	***PseudoF***
Period = Pe	1	**22.41**	**5.65**	6873	1.21
Impact = Im	1	**8.62**	**4.19**	**5984**	**7.63**
Time (Pe) = Ti(Pe)	21	2.12	0.98	923	0.68
Location (Im) = Lo(Im)	1	1.32	0.88	214	0.18
Pe x Im	1	0.22	0.68	2437	0.57
Site (Lo(Im)) = Si(Lo(Im))	3	0.96	0.67	**2692**	**5.28**
Pe x Lo(Im)	1	2.23	0.47	5900	1.64
Ti(Pe) x Im	21	1.25	0.58	749	0.55
Pe x Si(Lo(Im))	3	**5.61**	**3.90**	**2541**	**4.99**
Ti(Pe) x Lo(Im)	21	2.17	1.50	**1366**	**2.68**
Ti(Pe) x Si(Lo(Im))	63	1.44	1.13	510	0.97
Residual	552	1.27		527	

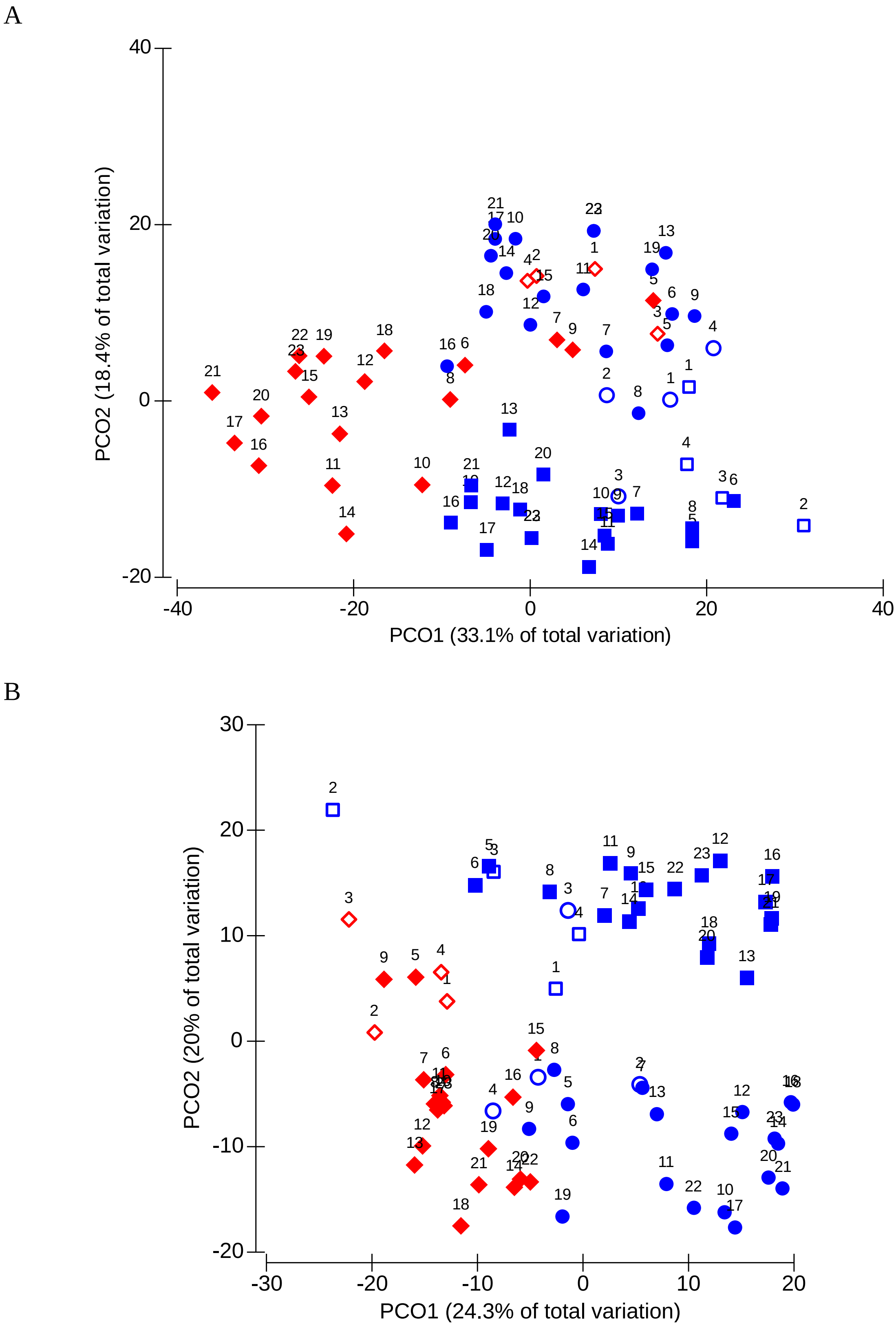


Figure 14. PCoA of centroids for macrophyte data for a) Location WT3 and b) Location WT4 and two control locations for each time of sampling. Red symbols: Potential 'impact' locations; Woronora River: Blue squares; O'Hares Creek: Blue circles. Empty symbols: 'Before' mining; Filled symbols: 'After' mining. Numbers indicate sampling time. Time 1 = spring 2008, T2 = autumn 2009, etc.

C

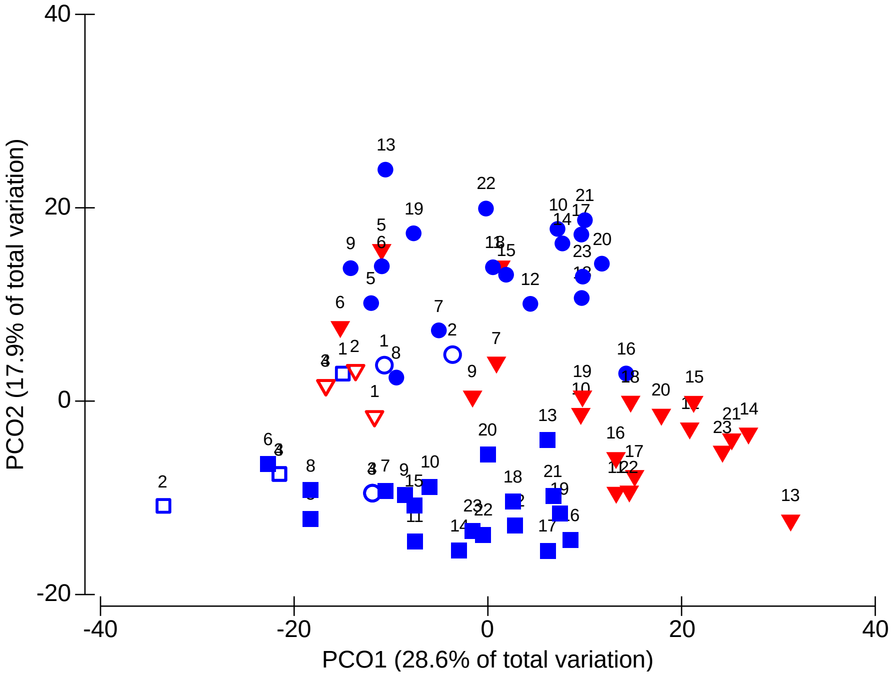


Figure 14 (Cont'd). PCoA of centroids for macrophyte data for c) Location WT5. Red triangles: Potential 'impact' location; Blue squares: Woronora River; Blue circles: O'Hares Creek. Empty symbols: 'Before' mining; Filled symbols: 'After' mining. Numbers indicate sampling time. Time 1 = spring 2008, T2 = autumn 2009, etc.

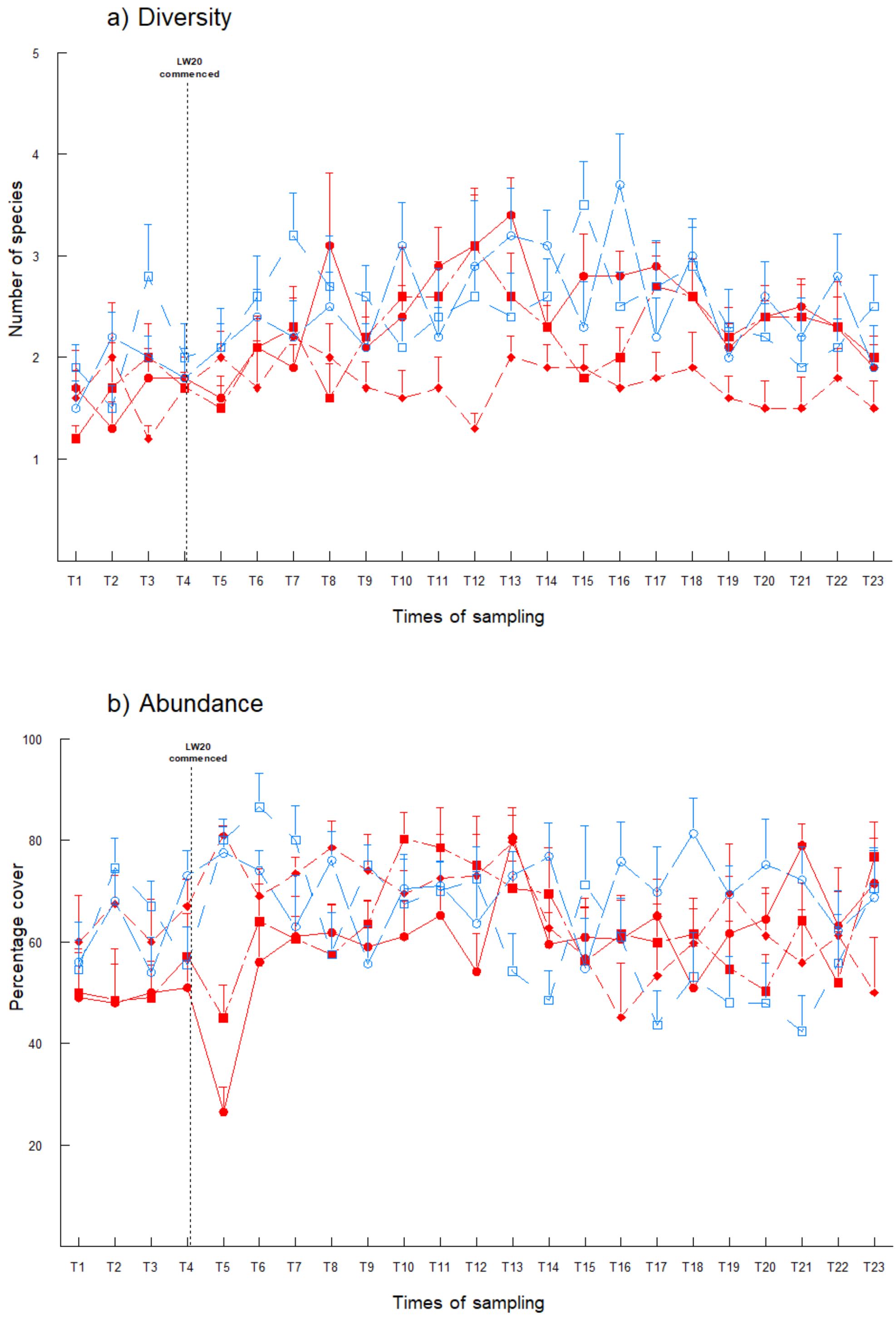


Figure 15. Mean (+SE) a) diversity and b) cover of aquatic macrophytes at locations on Waratah Rivulet (WT3: circle; WT4: diamond; WT5: solid square) and the reference locations (Woronora River: empty square; O'Hares Creek: empty circle) (*n* = 10). Time 1 = spring 2008, T2 = autumn 2009, etc.

5.0 SUMMARY & CONCLUSIONS

The primary objective of monitoring was to determine whether the extent and nature of any mining-related impacts, primarily subsidence-induced fracturing of bedrock, diversion and loss of aquatic habitat are consistent tent with the predictions made in the Extraction Plan and the *Metropolitan Coal Longwalls 20-22 Biodiversity Management Plan (2014)*[9] (Metropolitan Coal, 2015). 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.

Mining of the Longwalls 20-22 area commenced in May 2010 and was completed in April 2014. Subsequently, mining of the Longwalls 23-27 area commenced in May 2014 and was completed in March 2017. Longwalls 301-303 area commenced in April 2017 and was completed in June 2019. Longwall 304 commenced in July 2019.

To date, up to four monitoring surveys have been carried out prior to commencement of mining of Longwalls 20-22 and up to nineteen surveys within the 'After-mining' monitoring phase. The sampling outlined in this assessment was undertaken at control and potential impact places.

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

5.1 Tributary C/Eastern Tributary Locations (C1, C2 & C3)

Multivariate and univariate analyses of the monitoring data were used to test whether or not there was evidence of significant change in aquatic macroinvertebrate and macrophyte indicators at Tributary C/Eastern Tributary locations before- versus after-commencement of mining of Longwalls 20-22, in relation to Control locations.

[9] The *Metropolitan Coal Longwalls 20-22 Biodiversity Management Plan* is superseded by the *Metropolitan Coal Longwall 304 Biodiversity Management Plan.* Mining of Longwall 304 commenced in July 2019.

Evidence of mining related impacts were first noted at Location C1 in autumn 2014. Impacts included iron staining, fracturing of the streambed (predominantly bedrock), occasional falls in pool water levels below pre-mining levels and decreased cover of aquatic plants within the riparian zone. The condition of the aquatic macroinvertebrate fauna at Location C1 ranged from Band B (below AUSRIVAS reference condition) in spring 2014 to Band D (extremely impaired) in spring 2018.

Analyses have consistently found no changes to aquatic macroinvertebrate or macrophyte indicators at Location C1 that would indicate a significant impact from mining of the Longwalls 20-22 area. A significant Before-to-After mining change in the structure of the assemblage of aquatic macrophytes was detected at Location C1 in spring 2014 (T13) in relation to the control locations, but not subsequently. This result did not appear to be related to mining activities.

At Location C2, iron staining and falls in pool water levels below pre-mining levels were first noted by the spring 2016 and spring 2017 surveys, respectively. 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.

To date, analyses have detected a significant decrease in mean numbers of the freshwater shrimp family, Atyidae, at Location C2 within the after-mining period in spring 2015 and autumn 2017, but not subsequently. There have been no measurable changes to macrophyte indicators that would indicate an impact from mining.

At Location C3 in autumn 2013, dieback of riparian vegetation was noted at one of the sites sampled (i.e. Site C3-2), thought to be associated with tilting of the stream bank by mine subsidence. Occasional falls in pool water levels below pre-mining levels have been recorded at Site C3-2 since spring 2015, but not in spring 2019. To date, analyses have not detected significant changes in aquatic macroinvertebrate or aquatic macrophyte indicators sampled at Location C3 that would indicate an impact from mining. The condition of the aquatic macroinvertebrate fauna at Location C3 ranged between 0.29 (Band C) in spring 2010 to 1.02 (Band A) in spring 2014.

It is noted that Locations C1 and C2 are also sampled to assess potential impacts associated with mining within the Longwalls 23-27 area. To date, the main findings from analyses include:

- 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. Mean numbers of the mayfly family, Leptophlebiidae, were significantly more variable within the after-period at Location C1 between autumn 2015 and autumn 2019. Assemblages of macrophytes at Location C1 appear to have experienced a degree of environmental stress (desiccation) since spring 2017 as a result of mining activities.
- A significant change in assemblages of macroinvertebrates at Location C2 in autumn and spring 2019, which indicates an impact from mining. Analyses for Location C2 detected a significant decrease in mean numbers of the freshwater shrimp family, Atyidae, within the after-period between autumn 2016 and 2018 but not in spring 2019. Assemblages of macrophytes at Location C2 appeared to have changed significantly in spring 2014, autumn 2016 to spring 2019. Changes prior to the spring 2017 survey did not appear to be related to mining activities.

5.2 Waratah Rivulet Locations (WT3, WT4 & WT5)

An iron precipitate/micro-organism complex has commonly been observed at Locations WT3, WT4 and WT5 since sampling commenced in spring 2008. Cracking of bedrock in the stream channel due to subsidence was first noted at Location WT3 in spring 2013.

The OE50 Taxa Scores obtained at the rivulet locations from spring 2008 to spring 2019 ranged between:

- Location WT3: 0.27 (Band C) in autumn 2019 to 0.84 (Band A) in spring 2016;
- Location WT4: 0.33 (Band C) in autumn 2011 to 0.88 (Band A) in autumn 2012;
- Location WT5: 0.18 (Band C) in spring 2012 to 0.91 (Band A) in autumn 2015.

To date, analyses comparing temporal changes in components of assemblages of macroinvertebrates and macrophytes at Locations WT3, WT4 and WT5 on the Waratah Rivulet with control locations have not detected significant changes from before- to after-mining of the Longwalls 20-22 underground mining area.

Univariate analyses however, have detected a significant change in mean diversity of macroinvertebrates at Location WT3 in spring 2016, autumn 2018, spring 2018 and autumn 2019. Differences appear to be related to diversity decreasing at Location WT3 within the after-mining period whilst there was an increase at the control locations.

There were no conspicuous differences in mean diversity at Locations WT4 or WT5 in relation to the control locations.

Mean abundance of macroinvertebrates and mean numbers of Leptophlebiidae and Atyidae did not differ at locations WT3, WT4 or WT5 in relation to the control locations between the before- and after-mining periods.

There were no detectable changes to aquatic macrophytes at the Waratah Rivulet locations in relation to the control locations that could be associated with mining.

5.3 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 in Table 11.

Table 11. Assessment of Environmental Performance – Underground Mining Area and Surrounds against the Longwall 304 Trigger Action Response Plan – Monitoring of Aquatic Biota, Stream Monitoring

Performance Measure	Performance Indicator	Monitoring Site(s) being Assessed	Parameters	Highest Significance Level/Trigger Recorded		Comments	Subsidence Impact Performance Indicator Exceeded?	Action/Response
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: • Location WT3 on Waratah Rivulet and Locations ET1 and ET3 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	Level 1	Data analysis indicates no significant changes in relation to control places pre-mining[1] compared to post-extraction[2] occur in the aquatic macroinvertebrate and/or macrophyte assemblages at Locations WT3, WT4 or WT5 on the Waratah Rivulet or Locations ET1, ET2 or ET3 on the Eastern Tributary during the mining of LW304.	Locations WT4, WT5, ET1 and ET3. Locations WT3 and ET2 (all parameters excluding those noted in Level 2 below).	No	Continue monitoring. Six monthly reporting.
				Level 2	Data analysis indicates significant (not long-term[3]), changes in relation to control places pre-mining[1] compared to post-extraction[2] occur in the aquatic macroinvertebrate and/or macrophyte assemblages at Locations WT3, WT4 or WT5 on the Waratah Rivulet or Locations ET1, ET2 or ET3 on the Eastern Tributary during the mining of LW304.	Location WT3 (change in diversity in spring 2016, autumn 2018, spring 2018, autumn 2019 and spring 2019). Location ET2 (change in Atyidae in spring 2015 and autumn 2017 but not subsequently; altered macrophyte assemblage in spring 2014 but not subsequently).	No	Continue monitoring. Six monthly reporting.
				Level 3	Data analysis indicates significant long-term[3] changes in relation to control places pre-mining[1] compared to post-extraction[2] occur in the aquatic macroinvertebrate and/or macrophyte assemblages at Locations WT3, WT4 or WT5 on the Waratah Rivulet or Locations ET1, ET2 or ET3 on the Eastern Tributary during the mining of LW304.	Nil	No	None required

[1] Pre-mining data is as follows: sites WT3 and ET1 (spring 2008 to autumn 2010); site ET3 (spring 2009 to autumn 2010); 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]).

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

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

6.0 ACKNOWLEDGEMENTS

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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 surveys[3]. • LW23-27 stream sites, as detailed in the LW23-27 aquatic ecology monitoring reports for the spring 2009 to spring 2013 surveys[4].	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.

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

System	Location	Site	Easting	Northing
Tributary C	1	1	03116117	6214117
	1	2	0311642	6214197
	2	1	0312163	6215413
	2	2	0312167	6215577
	3	1	0311434	6213617
	3	2	0311464	6213712
Waratah Rivulet	3	1	0309863	6214299
	3	2	0309842	6214447
	4	1	0310426	6215080
	4	2	0310322	6215160
	5	1	0310525	6215340
	5	2	0310687	6215363
Tributary B	1	1	0309554	6215153
	1	2	0309645	6215045
Bee Creek	1	1	0308947	6220285
	1	2	0308978	6220292
Woronora Tributary	1	1	0307680	6219108
	1	2	0307689	6219107
Woronora River	1	1	0307747	6219154
	1	2	0307943	6219054
O'Hares Creek	1	1	0303812	6213711
	1	2	0303636	6213577