Benthic Communities Monitoring - Statistical Review 2022

Chain Valley Colliery

Prepared for Delta Coal

January 2023

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Delta Coal

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1	29 November 2022	Dr Paul.Goldsworthy	Nathan Garvey	Draft
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Executive Summary

Chain Valley Colliery (CVC) is an underground coal mine located beneath the southern end of Lake Macquarie, approximately 60 kilometres (km) south of Newcastle, NSW. CVC produces thermal coal for the domestic and export markets. As part of CVC's environmental performance, and to satisfy Condition 7(h), Schedule 4 of Development Consent for SSD-5465 (Modification 2), a Benthic Communities Management Plan (BCMP) was developed (CVC 2019).

Since 2012, CVC has monitored the soft sediment benthic community in shallow lake environments above their coal workings. The overall aim of the monitoring is to assess potential impacts of underground coal operations (primarily subsidence) on aquatic ecology using benthic communities as the specific indicator of impact. CVC undertakes six-monthly sampling of lake sediments for analysis of benthic community composition and environmental variables (water depth and sediment grain size). Samples are collected in Spring (March) and Autumn (September) at (potential) Impact, Reference, and Control sites.

At each site, Laxton Environmental Consultants P/L collected five replicate sediment samples by diver using 200 x 200 x 100 millimetre (mm) sieve boxes with 1 mm mesh. Samples were sieved to remove particles less than 1 mm and captured material preserved for laboratory sorting and enumeration of infauna. Sediment grain size analysis was undertaken on one 250 millilitre (mL) sample of sediment from each site. Infauna were categorised into operational taxonomic units (OTUs) comprising molluscs, polychaete worms and various higher taxa, such as terebellids, ophiuroids, echinoids, sponges, crabs, barnacles and fish.

EMM Consulting Pty Ltd (EMM) conducted statistical analysis on the full benthic dataset from September 2012–September 2022. Descriptive statistics (means, standard deviations, standard errors, minimums, maximums, and counts) were calculated within MS Excel. Summary statistics were subsequently used for visualisation of trends by site and by treatment (Impact versus Control versus Reference).

Raw abundance data were imported into PRIMER v6 for univariate and multivariate analysis. Raw benthic counts were used to calculate univariate diversity indices comprising total number of species (S), total number of individuals (N), species richness (Margalef, d), evenness (Pielou, J') and diversity (Shannon-Wiener, log e, H'). Multivariate analysis of the benthic community data included non-metric multidimensional scaling (nMDS), analysis of similarities (ANOSIM) and similarity percentage analysis (SIMPER), correlated with environmental variables (water depth and grain size) using principal components analysis (PCA).

From 19 sampling events between September 2012 and September 2022, a total of 24,265 benthic individuals from the 26 OTUs were counted in sediment samples from across the study area. The three most abundant taxa were the bivalve mollusc *Soletellina* (7,021 individuals), bivalve *Corbula* (6,167 individuals) and polychaetes-thin (6,126 individuals). Together these taxa accounted for 79% of the total number of benthic individuals collected. Each of these biodiversity indices were broadly similar across the sampling sites.

Ongoing development of CVC's underground coal extraction led to the redesignation of several Reference sites as Impact sites, with R3, R4, R5 and R6 becoming IM5, IM6, IM7 and IM8, respectively. To help discern greater relationship information, EMM focused univariate and multivariate statistical analysis of the different site treatments (Impact, Reference, and Control) for the monitoring period after redesignation of these sites (from September 2016 onwards).

From 13 sampling events between September 2016 and September 2022, a total of 16,347 benthic individuals from 26 OTUs were counted. Between 7 and 16 OTUs (mean 12.0) were reported per site, with the lowest number of OTUs at IM4 (7) and C2 (8) and the highest number of OTUs at IM2 and R7 (16). There was no clear spatial pattern associated with the number of OTUs per site treatments.

The total number of individuals per site varied between 494 (R8 and R11) and 1,152 (IM2). Abundances were lowest (<500 individuals) at R8 and R11 (494) and highest (>1,000 individuals) at C2 (1,017), IM2 (1,152) and IM5 (1,046). There was no clear spatial pattern associated with the abundance of benthic species per site treatments.

Multivariate pairwise test results (ANOSIM) indicated highly non-significant differences between all treatment pairs – Control versus Reference (99.2%), Control versus Impact (25.4%) and Reference versus Impact (30.1%) with a global R value of –0.019 at a significance level (p) of 0.6. Negative R-values are attributed to benthic habitats that are patchy and exhibit high variability between replicates (Chapman & Underwood 1999).

Cluster analysis of pair-wise Bray Curtis similarity between sites indicated that at 75% similarity level there were four site clusters: C5-C7-R11; IM2-IM5-R7; R10; and all remaining sites. Importantly, the Impact sites did not cluster together as a discrete group but were spread along the x-axis, interspersed amongst Reference and Control sites. Similarity patterns evident in the cluster analysis were further explored using an nMDS plot for abundance data at each site. The distribution (in nMDS space) of Impact, Reference and Control sites did not indicate site groupings attributed to impacts from CVC operations since benthic communities at most sites were tightly grouped (similar) and, except for IM2 and IM5, most sites with benthic communities that were significantly different to the main cluster of sites were Reference (R7, R10, R11) and Control (C5 and C7) sites.

SIMPER analysis of square-root transformed biological data indicated that more than 80% of the differences between the site clusters were mostly attributed to abundances of two polychaetes (mud and thin) and three bivalve molluscs, *Corbula*, *Soletellina* and *Trichomya*.

Mud polychaete abundances varied between site groups and over time, with notably lower abundances (and variability) apparent in the IM2-IM5-R7 cluster and at R10 compared to the other site clusters. Thin polychaete abundances were broadly similar for three of the four site clusters at each monitoring event, with higher abundances apparent within the 'all other' site cluster at most sampling times.

Abundances of bivalves *Corbula* and *Soletellina* were significantly higher within the 'all other' site cluster compared to the other three site clusters. *Trichomya* abundances were significantly higher within the IM2-IM5-R7 site cluster, driving the separation of this site's benthic community from the other sites. *Trichomya* abundances varied across time, being notably higher in samples collected in 2020 and 2021. This may be driven by changes in benthic habitats, such as higher levels of fine sediment, or a recruitment pulse due to favourable conditions in previous years. The exact cause is difficult to determine.

Importantly, the IM2-IM5-R7 cluster, that differed in benthic community structure from all other sites, did not change consistently over time (abundances neither increasing or decreasing) and therefore was not indicative of impacts from the CVC operations.

PCA, undertaken on the normalised environmental data, indicated that the three main site groups differentiated primarily due to silt, sand, and shell content, with minor differences in water depth being less important. The site groupings based on environmental variables (PCA) were different to the site groupings evident in benthic community structure (nMDS) which suggests that factors other than, or in addition to, sediment composition are driving the benthic structure.

From an ecological perspective, the benthic assemblages across the monitoring area fell into several groups that did not appear to be a response to CVC operations but were most likely due to subtle environmental variations driven by unknown environmental factors.

Monitoring sites C5-C7-R11 and R10 are furthest north and likely to be exposed to greater water circulation within greater Lake Macquarie that may provide increased food availability and/or better water quality that influences benthic community composition. Three of these sites – C5, C7 and R10 – had sediments with higher sand content which may support different benthic communities compared to the high silt areas further south and/or reflect greater water circulation likely at those sites.

Statistical analysis of CVC's benthic monitoring data did not indicate exceedance of the BCMP (CVC 2019) subsidence impact performance measure of "minor environmental consequences, including minor changes to species composition and/or distribution" has occurred.

EMM recommends that benthic monitoring could be scaled-back to annual data collection (in March) since the non-photosynthetic benthic communities are unlikely to exhibit strong seasonal variability.

TABLE OF CONTENTS

Appendices

Tables

Figures

1 Introduction

Chain Valley Colliery (CVC) is an underground coal mine located beneath the southern end of Lake Macquarie, approximately 60 kilometres (km) south of Newcastle, NSW. CVC produces thermal coal for the domestic and export markets.

As part of CVC's environmental performance, and to satisfy Condition 7(h), Schedule 4 of Development Consent for SSD-5465 (Modification 2), a Benthic Communities Management Plan (BCMP) has been developed (CVC 2019). The stated purpose of this BCMP is to:

- outline details of the benthic communities monitoring data collected;
- outline existing and predicted subsidence levels;
- outline the methodology to be used to identify depth changes at monitoring locations;
- identify benthic community monitoring locations;
- identify reporting requirements;
- detail benthic community management measures;
- identify the requirements for incident or exceedances reporting and reviews of the document; and
- identify persons responsible for implementation of requirements.

Since 2012, CVC has monitored the soft sediment benthic community in shallow lake environments above their coal workings. The overall aim of the monitoring is to assess potential impacts of underground coal operations (primarily subsidence) on aquatic ecology, with benthic community composition as the specific indicator of impact.

CVC undertakes six-monthly sampling of lake sediments for analysis of benthic community composition and environmental variables (water depth and sediment grain size). Samples are collected in Spring (March) and Autumn (September) at (potential) Impact, Reference, and Control sites (**Appendix A**).

The BCMP defines the three site types:

- Impact sites – potentially currently or historically impacted upon by subsidence.
- Reference sites – not currently impacted by subsidence but fall within the proposed future mining footprint. Following undermining, Reference sites are redesignated as Impact sites.
- Control sites - will not be impacted upon by subsidence, comprising those areas lying outside the footprint of current and future coal workings.

Full details of the benthic sampling and analysis regime are provided in Section 4 of the BCMP (CVC 2019). At each site, five replicate sediment samples are collected by diver using 200 x 200 x 100 mm sieve boxes with 1 mm mesh. Samples are sieved to remove particles less than 1 mm and captured material is preserved in formaldehyde for laboratory sorting and enumeration of infauna. Sediment grain size analysis is undertaken on one 250 millilitres (mL) sample of sediment from each site.

The BCMP prescribes statistical analysis methods for univariate and multivariate analysis of the benthic monitoring data – biological and environmental (Table 1.1).

Table 1.1 Benthic monitoring data statistical methods (BCMP, CVC 2019)

Variable type	Analysis	Description
Environmental: Water Quality	ANZECC/ARMCANZ Guidelines	Trigger values for slightly – moderately disturbed
Biotic and Environmental	Univariate	Descriptive graphical statistics Analysis of Variance (2-way nested) Analysis of Similarity (2-way nested)
Biotic and Environmental	Multivariate	Square-root transformed, Bray-Curtis similarity matrices, Cluster analysis and dendrograms
	Multidimensional Scaling Ordination	Sites represented as points in space, relative distances indicate similarity
	BIOENV	Correlation between biotic and environmental data using PRIMER

EMM has undertaken statistical analysis of the supplied benthic monitoring data in accordance with the BCMP.

2 Methods

Statistical analysis was undertaken on the full benthic dataset (September 2012–September 2022) from Laxton Environmental, who undertake the field sampling programs on behalf of Delta Coal. The supplied benthic data were checked and reordered within multiple MS Excel worksheets to facilitate statistical analysis in accordance with the BCMP (CVC 2019).

Descriptive statistics (ie means, standard deviations, standard errors, minimums, maximums and counts) were calculated within MS Excel. Summary statistics were subsequently used for visualisation of trends by site and by treatment (impact versus control versus reference sites). For the purposes of analysis, the control and reference groups have been kept separate.

Raw data were imported into PRIMER v6 for univariate and multivariate analysis. PRIMER (Plymouth Routines in Multivariate Ecological Research) consists of a “wide range of univariate, graphical and multivariate routines for analysing arrays of species-by-samples data from community ecology” (Clarke & Gorley 2006) and is the software of choice for benthic ecology.

Univariate diversity indices were calculated from the raw benthic count data. Total number of species (S), total number of individuals (N), species richness (Margalef, d), evenness (Pielou, J’) and diversity (Shannon-Wiener, log e, H’) were calculated within PRIMER v6 (DIVERSE) and graphically presented to identify any site-by-site trends in benthic community structure.

Due to the high frequency of zeros in the benthic count data, the data were square root transformed to downplay taxa with comparatively high counts and to increase the statistical visibility of the rarer taxonomic groups. Bray-Curtis similarity (resemblance) matrices were subsequently developed from the transformed data and statistical analysis of these multivariate data was undertaken using PRIMER v6 routines.

Simple agglomerative hierarchical clustering was undertaken, producing dendrograms to visually identify distinct data groups based on different levels of similarity in benthic community structure. Dendrograms (also known as tree diagrams) display groups of samples in successively smaller numbers of clusters as the threshold of similarity at which two groups merge decreases. Groups (clusters) of sites (or other factors) can be identified for further data exploration with respect to the potential drivers of the groupings.

Non-metric multidimensional scaling (nMDS) was undertaken using PRIMER v6. nMDS is a powerful multivariate tool used to analyse benthic community data whereby points (eg sites) are plotted in 2-dimensional space such that the relative distance between points is relative to the same rank order as relative dissimilarities of each sample; ie points close together represent samples that are very similar in community composition and points further apart are more different. Distance between points cannot be used an absolute measure of similarity or dissimilarity, rather relative distance between points indicates relative similarity/dissimilarity.

The PRIMER v6 routine for analysis of similarities (ANOSIM) provides an approximate analogue of standard univariate analysis of variance (ANOVA). Using the resemblance matrix calculated from benthic count data, ANOSIM was used to test the null hypothesis that there are no differences between treatments (ie CVC’s Impact, Reference and Control sites) allowing for potential differences between individual sites. A two-way crossed design – sites within treatments – was used. ANOSIM produces p and R values, where p indicates the level of significance for differences between benthic communities, in this case grouped into the three different site types, and R values indicate the strength of any differences. As R values approach 1 the strength of the difference between groups increases. R values close to zero indicate no difference between the groups. This is an important consideration given the inherently variable nature of benthic community data where small-scale variability (between replicates) can often be as great (or greater) than the larger scale differences between sites.

Subsequent interpretation of which individual benthic taxa are driving any of the observed differences between treatments and/or sites was undertaken using similarity percentage analysis (SIMPER) within PRIMER v6. SIMPER outputs indicate the percentage that each taxa contributes to the observed pairwise differences and informs the investigation of why the abundance (or absence) of certain species occur at individual sites.

Environmental data – water depth and sediment grain size – collected at each of CVC's benthic monitoring sites were investigated as potential influencing factors in benthic community composition. The environmental data were normalised (subtract mean and divide by standard deviation) to allow comparison between factors with different units of measure, such as metres water depth, percent silt and percent sand. Principle components analysis (PCA) was used to visualise site-by-site groupings based on water depth and sediment grain size (PRIMER v6).

The variation in environmental data was subsequently used to help identify potential factors, for example water depth, that are driving the development of the benthic assemblages. This approach is critical in defining the potential reasons for variation in benthic community structure within the context of natural variability, driven by environmental factors, and potential impacts from project-related activities.

Ongoing development of CVC's underground coal extraction has led to the redesignation of several of the early Reference sites as Impact sites (Table 2.1). Sites R3, R4, R5 and R6 have become IM5, IM6, IM7 and IM8, respectively and to compensate for the loss of reference sites, additional reference and control sites were added.

Changing the 'treatment' designation of sites is necessary to account for the ongoing expansion of the coal workings but it does complicate the statistical analysis process since the potential for impacts from subsidence at these sites changes over time. To help discern greater relationship information, EMM focused statistical analysis of the different site types (impact, reference, and control) for the monitoring period after redesignation of the earlier reference sites to impact sites and after the addition of most of the new reference and control sites (from September 2016 onwards).

Table 2.1 List of benthic monitoring sites indicating sites redesignated due to expansion of CVC mining operations

Reference sites	Control sites	Potential impact sites
R1	**C1**	**IM1**
R2	**C2**	**IM2**
R3 *(becomes IM5 in March 2014)*	**C3**	**IM3**
R4 *(becomes IM6 in March 2014)*	**C4**	**IM4**
R5 *(becomes IM7 in September 2015)*	**C5** *(added in March 2016)*	**IM5** *(=R3 prior to September 2015)*
R6 *(becomes IM8 in September 2015)*	**C6** *(added in September 2016)*	**IM6** *(=R4 prior to September 2015)*
R7 *(added in March 2016)*	**C7** *(added in March 2018)*	**IM7** *(=R5 prior to September 2015)*
R8 *(added in September 2016)*		**IM8** *(=R6 prior to September 2015)*
R9 *(added in September 2016)*		
R10 *(added in March 2018)*		
R11 *(added in March 2018)*		

3 Analysis results

In the laboratory, biological samples were sorted into different taxonomic groups - operational taxonomic units (OTUs) – comprising, molluscs and four polychaete worms sorted to genus level and all other fauna split into broader groups. These broader groups were higher taxa (such as terebellids, ophiuroids), general organism groups (sponges, crabs, barnacles, fish) or specific descriptive types (such as mud polychaetes, thin polychaetes and thick polychaetes).

Infauna were categorised into 26 OTUs (Table 3.1) and these have been used to differentiate sites during statistical analysis.

Table 3.1 Operational taxonomic units (OTUs) derived for CVC benthos, 2016-2022

Polychaetes – thin (P)	*Nassarius jonasii* (G)	prawns (C)
Polychaetes – thick (P)	*Anadara trapezia* (B)	crabs (C)
Polychaetes – mud (P)	*Corbula truncata* (B)	barnacles (C)
terebellids (P)	*Cyamiomactra mactroides* (B)	ophiuroids (E)
Chaetopterus sp. (P)	*Dosinia sculpta* (B)	echinoids (E)
Cirratulidae (P)	*Paphia undulata* (B)	planaria (F)
Pectinaria sp. (P)	*Saccostrea glomerata* (B)	sponges
Sthenelais pettiboneae (P)	*Soletellina alba* (B)	fish
Lepsiella (Bedeva) hanleyi (G)	*Trichomya hirsuta* (B)	

Key: (P) = polychaete worm
(G) = gastropod mollusc
(B) = bivalve mollusc
(C) = crustacean
(E) = Echinoderm
(F) = flatworm (Platyhelminth)

3.1 Benthic data 2012-2022

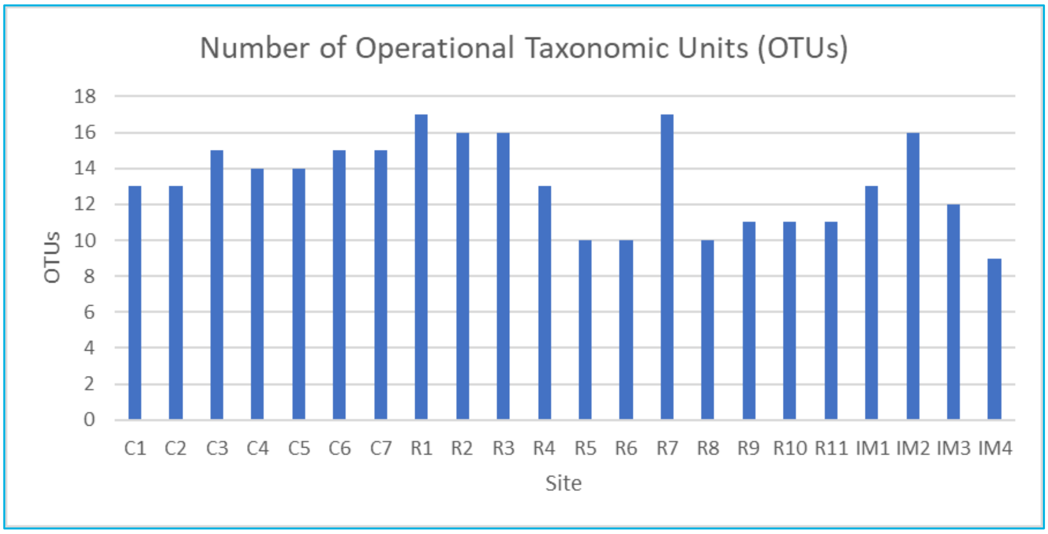
From 19 sampling events between September 2012 and September 2022, a total of 24,265 benthic individuals from the 26 different taxonomic groups were counted in sediment samples from across the study area. The three most abundant taxa were the bivalve mollusc *Soletellina* (7,021 individuals), bivalve *Corbula* (6,167 individuals), and polychaetes-thin (6,126 individuals). Together these taxa account for 79% of the total number of benthic individuals collected in sediment samples throughout the monitoring period. The most speciose faunal group is bivalve molluscs, with seven species collected.

The number of OTUs and individuals identified for each site over time are shown in Figure 3.1. In these figures the site numbering reflects the original reference site designations rather than changes that were made due to subsequent undermining.

Between 9 and 17 OTUs (mean 13.2) were reported per site. The lowest number of OTUs was at IM4 (9) and R5, R6 and R8 (10), and the highest number of OTUs was at R1 and R7 (17) and R2, R3 and IM2 (16). There is no clear spatial pattern associated with the number of OTUs per site type.

The total number of individuals per site varied between 494 (R8 and R11) and 1,976 (C2). Abundances were lowest (<750 individuals) at C6 (679), C7 (648), R7 (666), R8 (494) and R11 (494).

Abundances were highest (>1,500) at C2 (1,976), C4 (1,559), R2 (1,557) and IM2 (1,583). There is no clear spatial pattern associated with the abundance of benthic species per site type.



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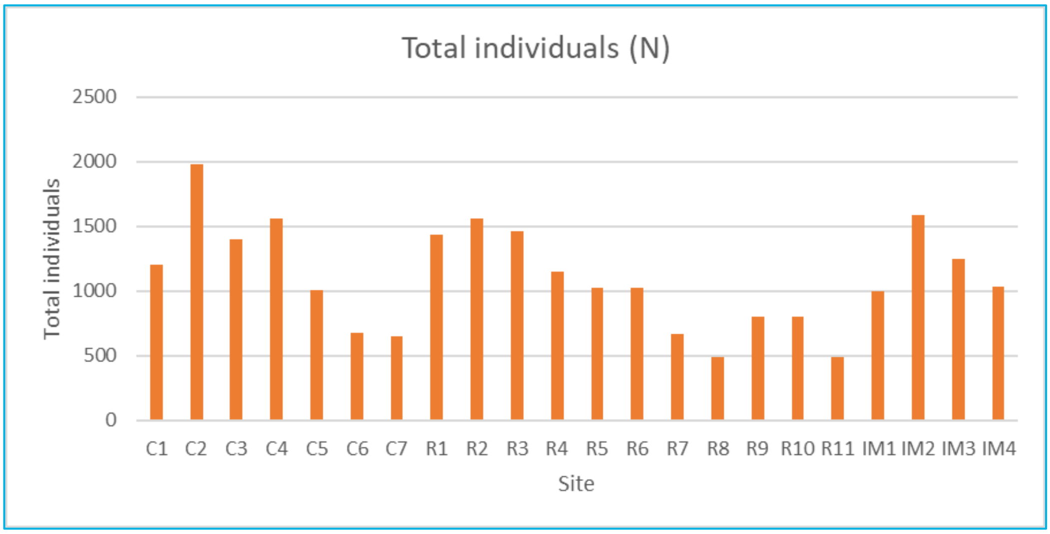


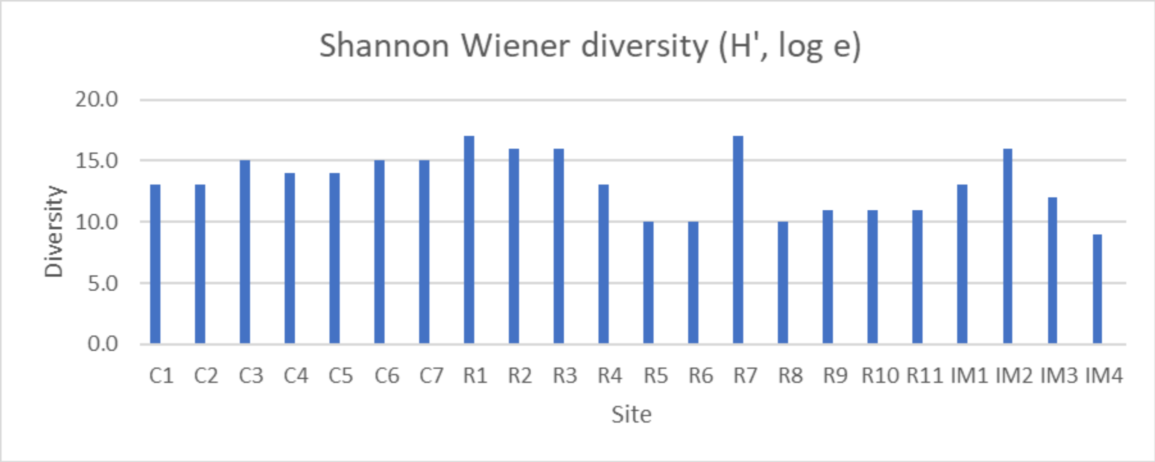
(b)

C = control sites R = reference sites IM = impact sites

Figure 3.1 Total number of (a) operational taxonomic units (OTUs) and (b) individuals identified in benthic samples from each CVC monitoring site for the period 2012–2022

Shannon Wiener diversity (H’, log e), Margalef richness (d) and Pielou’s evenness (J’) values for each site over time are shown in Figure 3.2.

Each of these biodiversity indices are broadly similar across all sampling sites. Margalef richness most closely follows the distribution of OTUs per site, with marginally lower richness (<1.3) apparent at R5, R6 and IM4. The diversity and evenness vary across the sites within a narrow range of 1.24−1.81 and 0.49−0.69, respectively.



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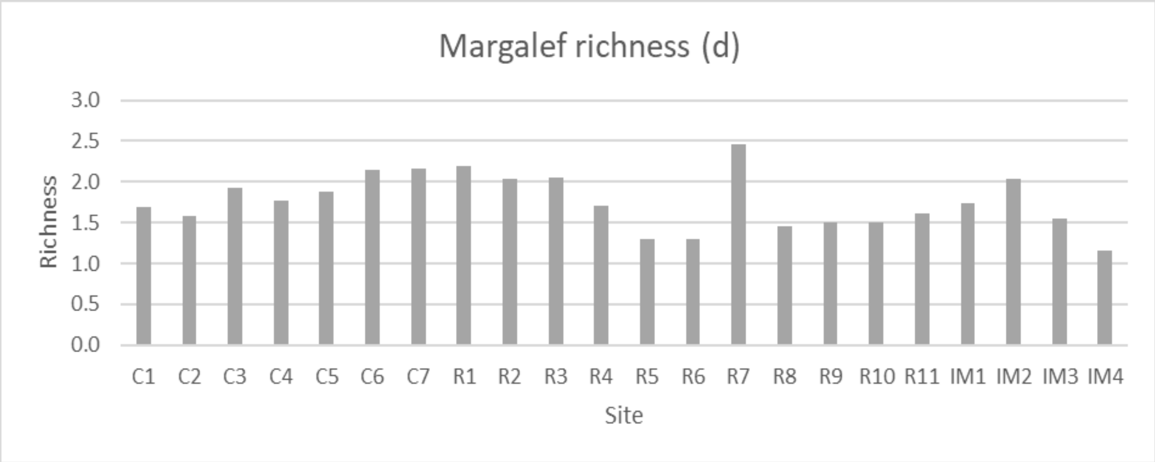

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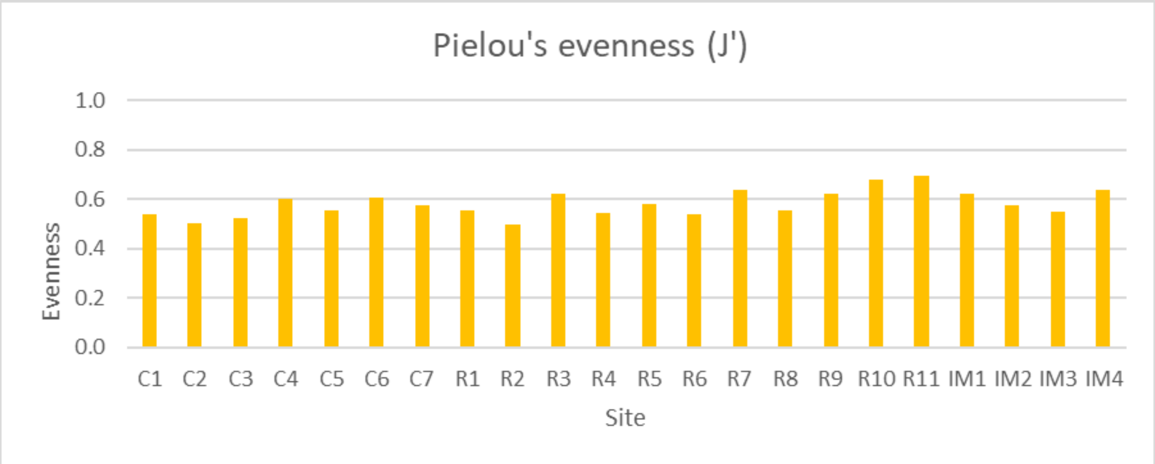


(c)

C = Control sites R = Reference sites IM = Impact sites

Figure 3.2 (a) Shannon Weiner diversity, (b) Margalef's richness and (c) Pielou's evenness for benthic samples from each CVC monitoring site for the period 2012-2022

As indicated in Methods (Section 2), to help discern greater relationship information EMM has focused statistical analysis of the different site types (Impact, Reference, and Control) on the monitoring period after redesignation of sites R3, R4, R5 and R6 as IM5, IM6, IM7 and IM8, respectively. The analysis focus was shifted to the monitoring period from September 2016 onwards.

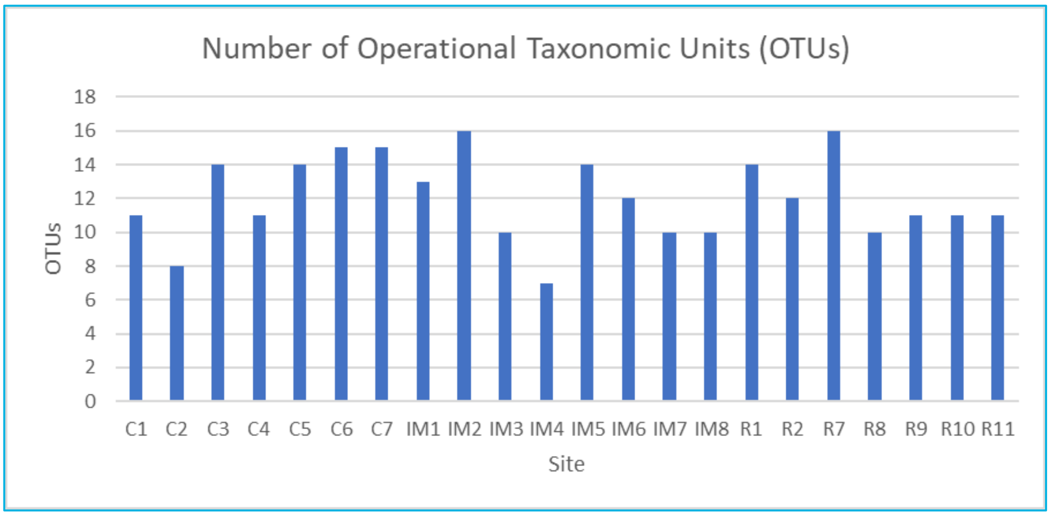
3.2 Benthic data 2016–2022

From 13 sampling events between September 2016 and September 2022, a total of 16,347 benthic individuals from 26 different taxonomic groups were counted in sediment samples from across the study area.

The number of OTUs and individuals identified for each site over time is shown in Figure 3.3. In these figures the site numbering reflects the redesignation of reference sites (R3-R6) as impact sites (IM5-IM8) due to ongoing expansion of the underground coal workings.

Between 7 and 16 OTUs (mean 12.0) were reported per site. The lowest number of OTUs were at IM4 (7) and C2 (8) and the highest number of OTUs were at IM2 and R7 (16). There is no clear spatial pattern associated with the number of OTUs per site.

The total number of individuals per site varied between 494 (R8 and R11) and 1,152 (IM2). Abundances were lowest (<500 individuals) at R8 and R11 (494). Abundances were highest (>1,000 individuals) at C2 (1,017), IM2 (1,152) and IM5 (1,046). There is no clear spatial pattern associated with the abundance of benthic species per site.



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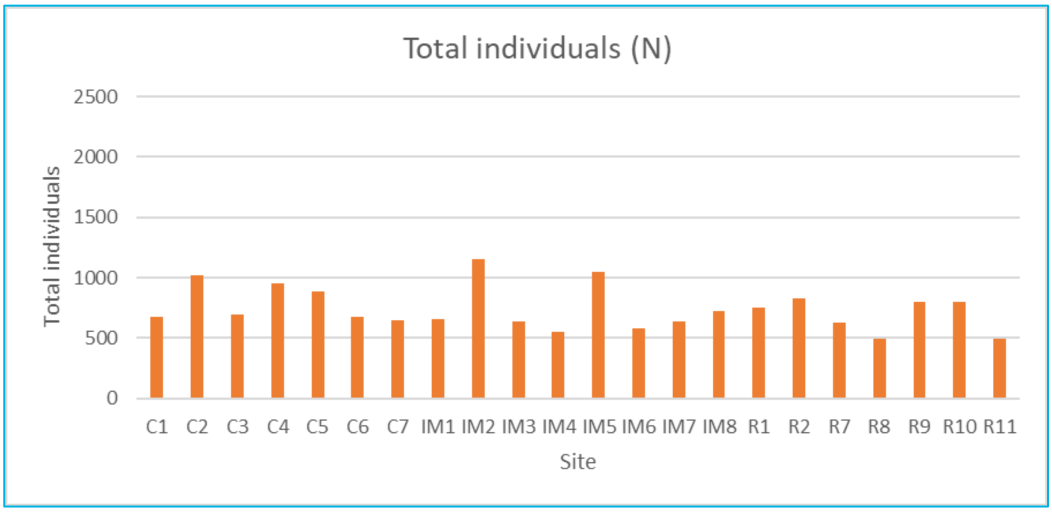


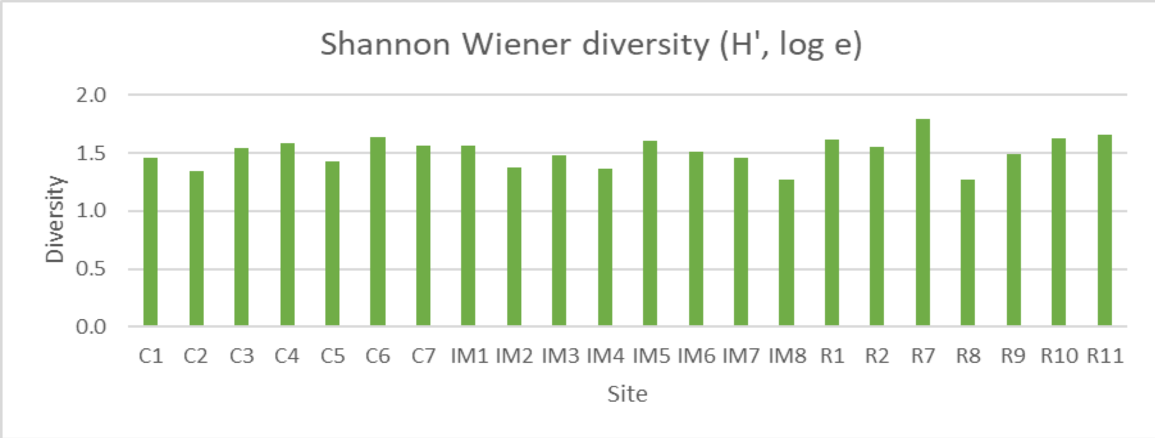
(b)

C = Control sites IM = Impact sites R = Reference sites

Figure 3.3 Total number of (a) operational taxonomic units (OTUs) and (b) individuals identified in benthic samples from each CVC monitoring site for the period 2016-2022

Shannon Wiener diversity, Margalef richness and Pielou's evenness values for each site over time (2016−2022) are shown in Figure 3.4.

Each of these biodiversity indices are broadly similar across all sampling sites. Margalef richness most closely follows the distribution of OTUs per site, with marginally lower richness (<1.0) apparent at C2 and IM4. The diversity and evenness vary across the sites within a narrow range of 1.27−1.79 and 0.50−0.70, respectively.



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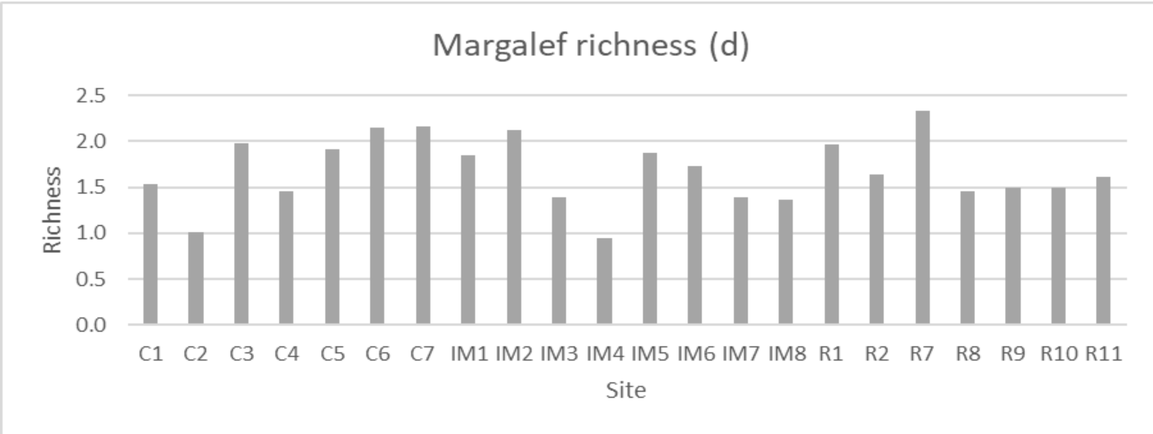

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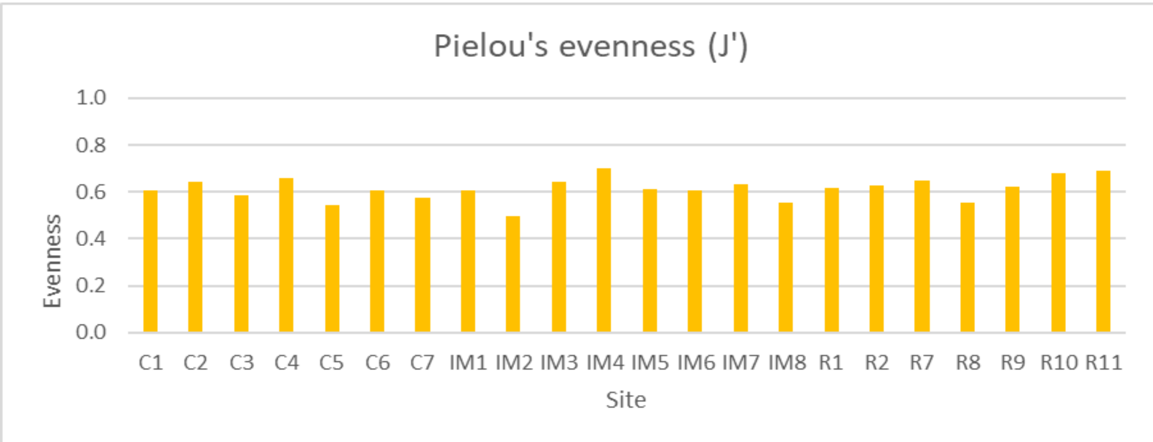


(c)

C = Control sites R = Reference sites IM = Impact sites

Figure 3.4 **(a) Shannon Weiner diversity, (b) Margalef's richness and (c) Pielou's evenness for benthic samples from each CVC monitoring site for the period 2016-2022**

3.3 Analysis of similarity

In accordance with the statistical analysis suite identified in Table 1.1 of the BCMP, benthic data were explored using ANOSIM, cluster analysis and nMDS.

For ANOSIM, a two-way nested design was used to test for similarities between the three different site types (Impact, Reference and Control). Testing for differences in benthic communities between the three site types during the period September 2016 to September 2022 derived a global R value of -0.019 at a significance level (p) of 0.6 (well above the statistical significance level of 0.05). Pairwise test results indicate highly non-significant differences between all treatment pairs – Control versus Reference (0.992), Control versus Impact (0.254) and Reference versus Impact (0.301). Negative R-values are attributed to benthic habitats that are patchy and exhibit high variability between replicates (Chapman & Underwood 1999).

The ANOSIM plot indicates that there are no significant differences between the three site types (Figure 3.5) since the global R value (black vertical line) falls within the wider distribution of R values (blue bars).

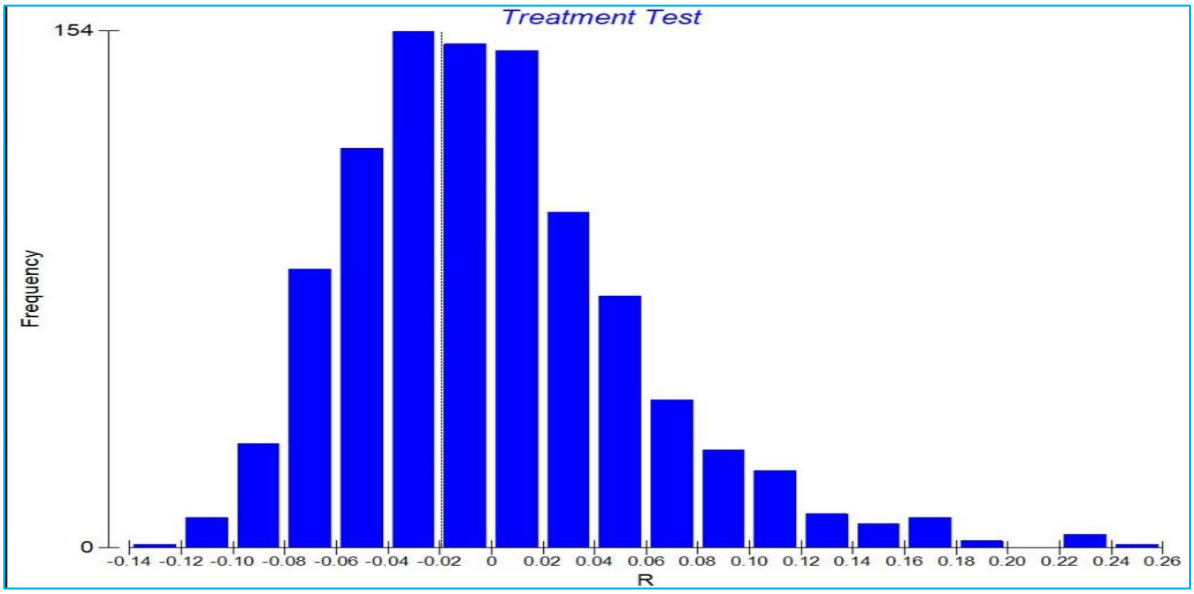


Figure 3.5 ANOSIM test results for benthic data across site types, September 2016 to September 2022. Vertical line indicates the global R value of -0.019

3.4 Cluster analysis

Cluster analysis was used to visualise pair-wise similarity between sites based on levels of Bray Curtis similarity for the monitoring period September 2016 to September 2022 (Figure 3.6). The dendrogram indicates that at 75% similarity level there are four clusters: C5-C7-R11; IM2-IM5-R7; R10; and all remaining sites.

Importantly, the Impact sites do not cluster together as a discrete cluster but rather are spread along the x-axis, interspersed amongst Reference and Control sites.

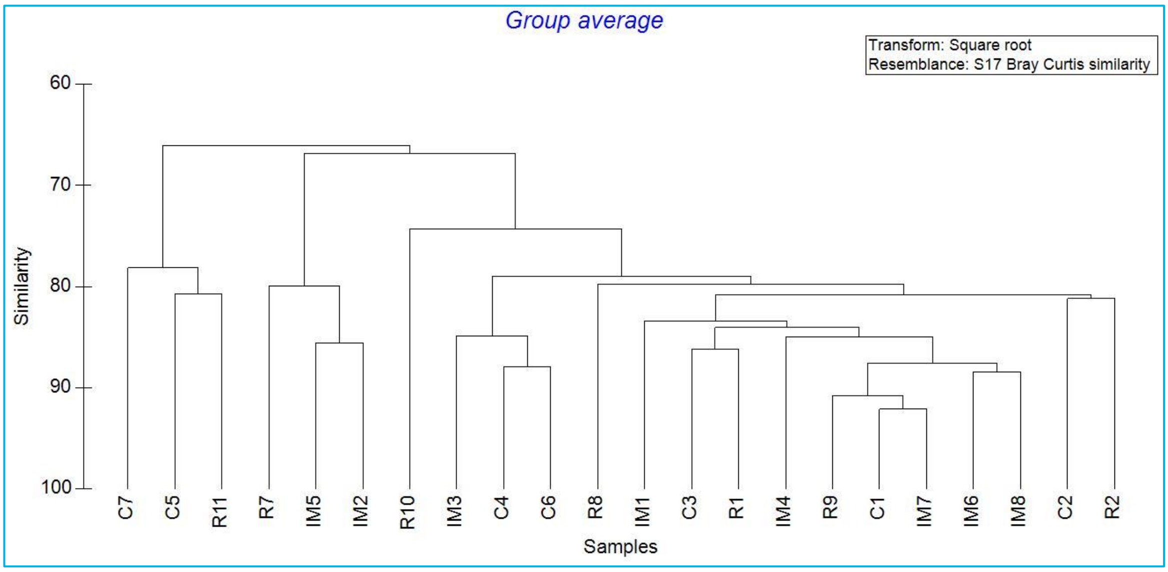


Figure 3.6 **Dendrogram derived from cluster analysis of CVC benthic community monitoring data across all sites between September 2016 and September 2022**

3.5 Multi-dimensional scaling

Non-metric multi-dimensional scaling (nMDS) is used to represent samples as points in 2D space such that points that are close together represent samples that are very similar in community composition (Clarke & Gorley 2006). The similarity patterns indicated in the cluster analysis are further explored using an nMDS plot for abundance data at each site (Figure 3.7). The green circles indicate site groupings that correspond to the 75% similarity level.

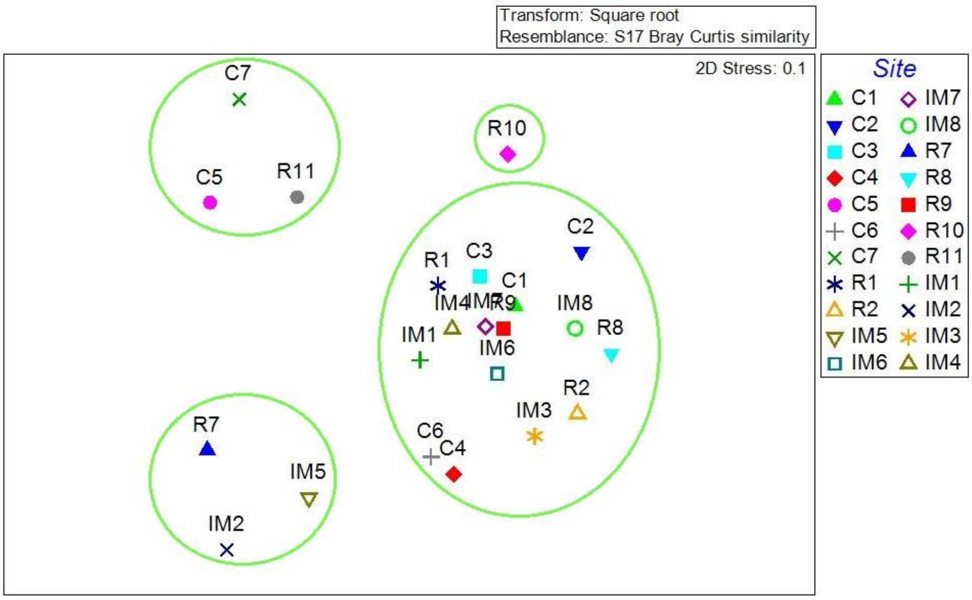


Figure 3.7 **Patterns in community structure depicted as nMDS plot based on square-root transformed abundance data of all taxa (OTUs) for each site, September 2016–September 2022**

The distribution (in nMDS space) of Impact, Reference and Control sites does not indicate site groupings that could be attributed to impacts from CVC operations since most sites have similar benthic communities (tightly grouped). Except for IM2 and IM5, most of the sites that exhibit significantly different benthic communities to the main group are designated as reference (R7, R10, R11) and control (C5 and C7) site types.

3.6 SIMPER

SIMPER analysis was undertaken on the square-root transformed biological data to identify which taxa are contributing to the separation between benthic communities evident at the C5-C7-R11, IM2-IM5-R7 and R10 site clusters identified during cluster analysis and confirmed by nMDS.

SIMPER results indicate that more than 80% of the differences between the site clusters are mostly attributed to abundances of two polychaetes (mud and thin) and three bivalve molluscs, *Corbula*, *Soletellina* and *Trichomya*.

Specific differences between the clusters are:

- C5-C7-R11 (Cluster A):
  - much higher abundances of polychaete-mud compared to other site clusters; and
  - lower abundances of *Corbula and Soletellina* compared to Cluster D.
- IM2-IM5-R7 (Cluster B):
  - higher abundances of *Trichomya* compared to other site clusters;
  - lower abundances of *Soletellina* compared to Cluster C; and
  - lower abundances of *Corbula* compared to Cluster D.
- R10 (Cluster C):
  - higher abundances of *Soletellina* compared to other site clusters; and
  - higher abundances of *Corbula* compared to Cluster A and Cluster B.
- All other sites (Cluster D):
  - higher abundances of polychaete thin, *Corbula* and *Soletellina* compared to other clusters at most sampling times.

3.7 Temporal comparison of site groups

Comparison of temporal variation in abundances (mean + standard deviation) for each site cluster (C5-C7-R11, IM2-IM5-R7, R10, and 'all other') are provided for the five most abundant OTUs reported across the benthic monitoring area (Figure 3.8 and Figure 3.9).

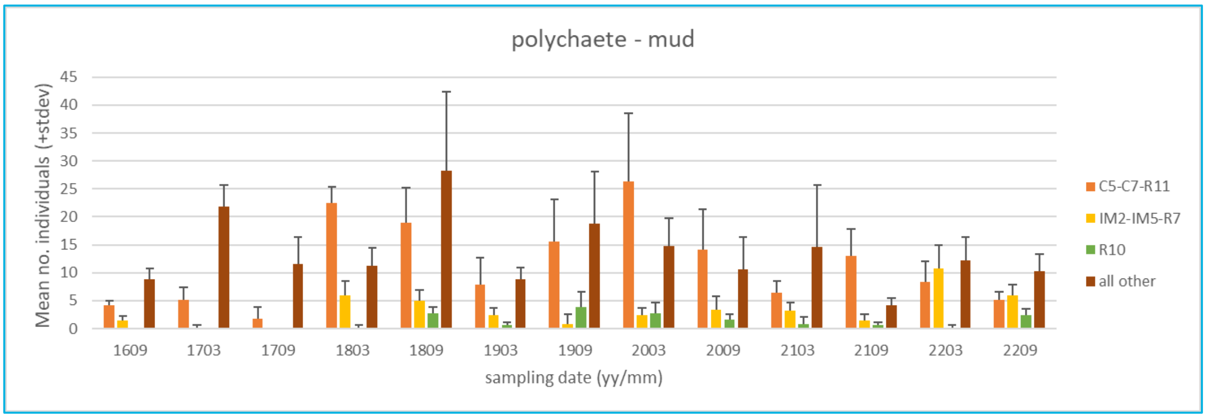
Mud polychaete abundances are variable between site clusters and over time. The notable differences are lower abundances (and variability) apparent in the IM2-IM5-R7 cluster and at R10 compared to the other site clusters.

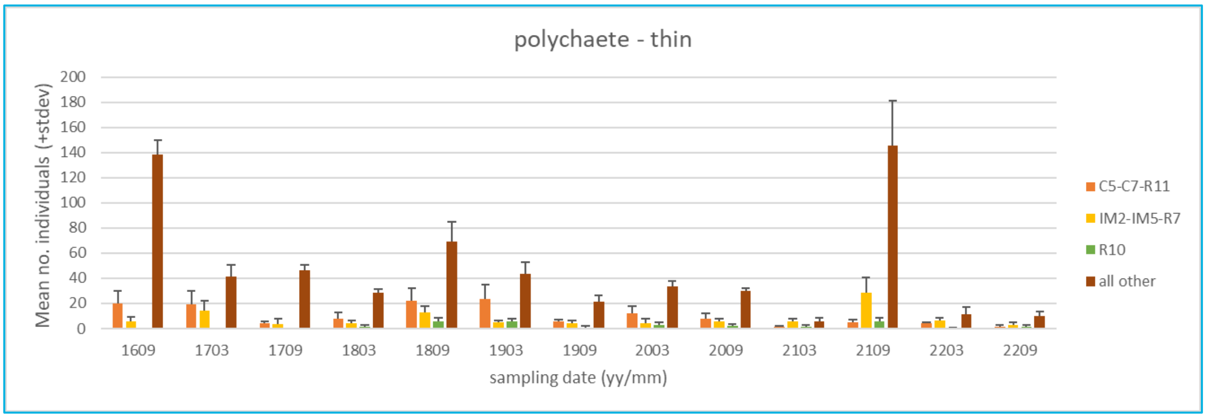
Thin polychaete abundances are broadly similar for three of the four site clusters at each monitoring event, with higher abundances apparent within the 'all other' site cluster at most sampling times.

Abundances of bivalves *Corbula* and *Soletellina* are significantly higher within the 'all other' site cluster compared to the other three site clusters.

Trichomya abundances are significantly higher within the IM2-IM5-R7 site cluster, which drives the separation of this site's benthic community from the other sites. Abundances vary across time, notably higher in samples collected in 2020 and 2021.

The important aspect to note from these plots is that the IM2-IM5-R7 cluster, that differs in benthic community structure from all other sites, is not changing consistently over time (abundances neither increasing or decreasing) and therefore is not indicative of an impact from the CVC operations.





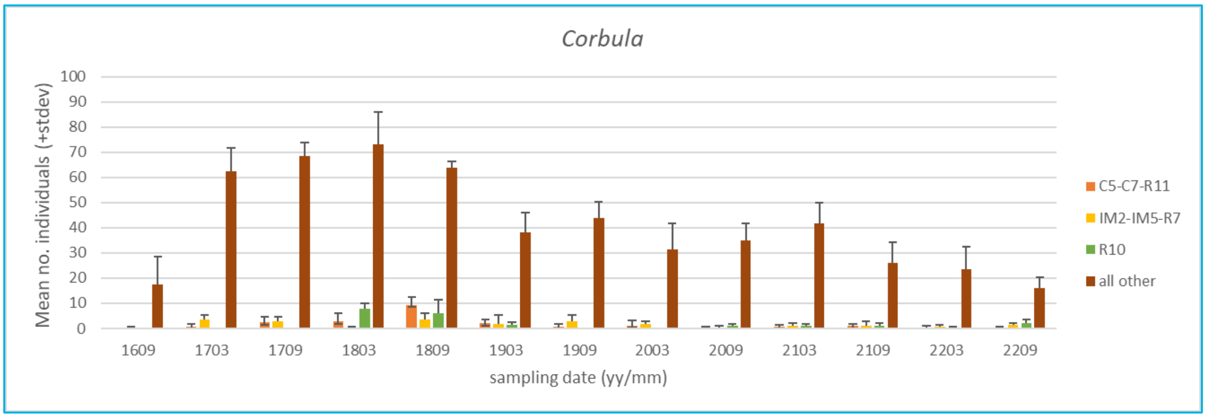
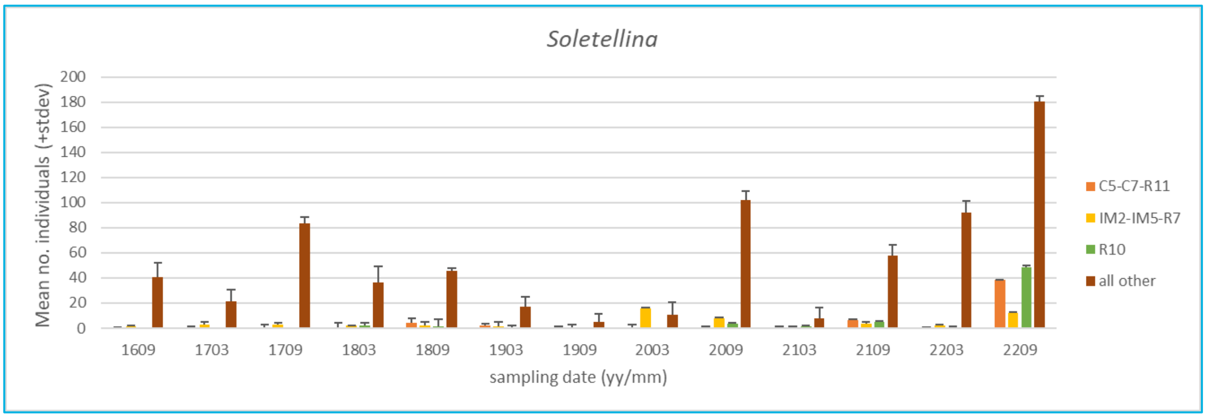


Figure 3.8 Temporal comparison of benthic abundances by site group for mud and thin polychaetes and the bivalve mollusc *Corbula*



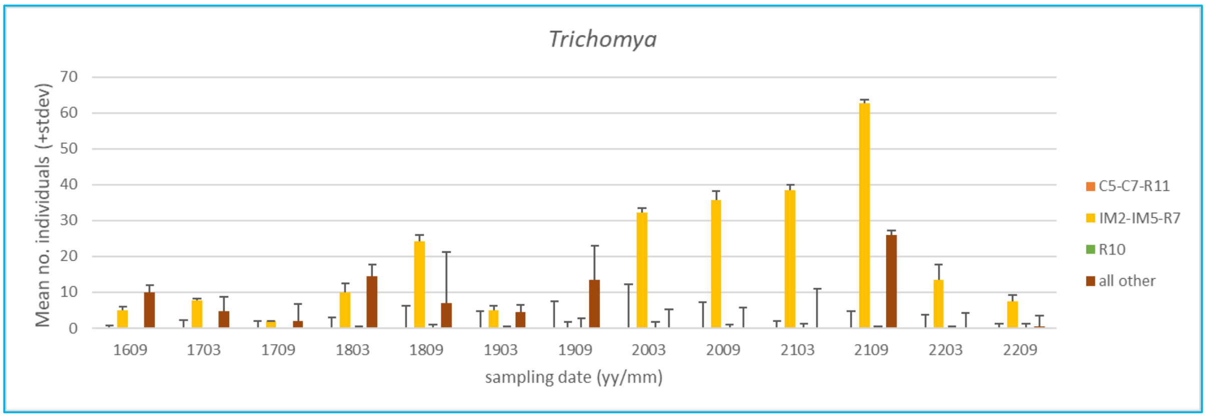


Figure 3.9 **Temporal comparison of benthic abundances by site group for the bivalve molluscs *Soletellina* and *Trichomya***

3.8 Environmental data

Water depths and sediment grain size were reported for each site and these environmental variables were analysed alongside the biological data to discern potential environmental drivers of the observed variation in benthic community structure (Figure 3.10).

The sediments at all sites sampled in September 2022 were described as *'largely composed of fine grey silt'* with *'small to large shell fragments…at most stations'* (Laxton & Laxton 2022).

PCA was undertaken on the normalised environmental data (Figure 3.10). The results indicate three main site groups that are differentiated primarily due to silt, sand, and shell content, with minor differences in water depth being less important:

- Group 1: high shell (98%), no sand (0%) and low silt (2%) – R7.
- Group 2: low shell (<1%), moderate sand (39–43%) and medium silt (56–61%) – C7 and R10.
- Group 3: variable shell (<20%), low sand (<4%) and high silt (>80%) – all other sites.

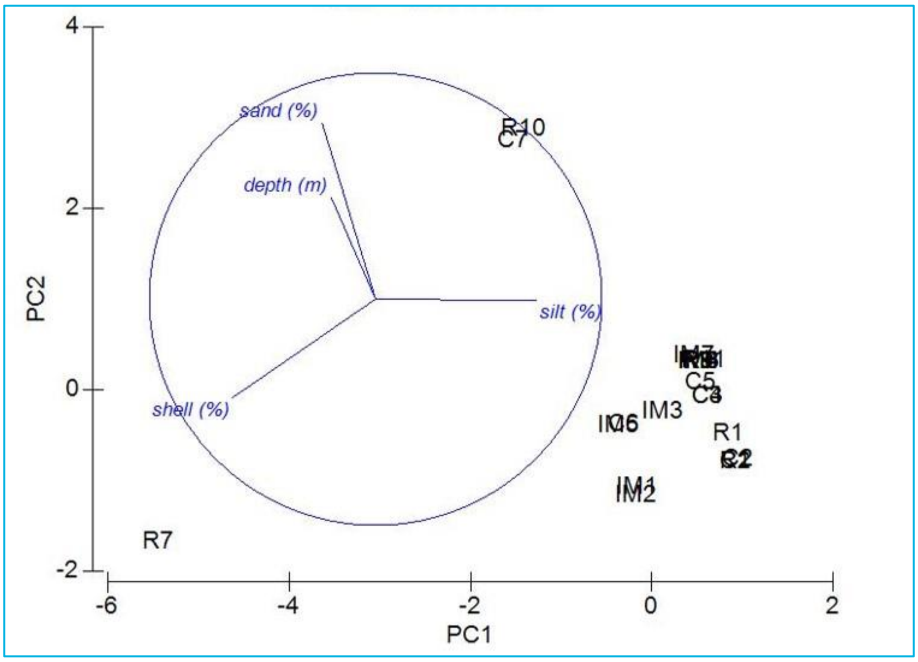


Figure 3.10 Principle components analysis (PCA) plot for normalised September 2022 environmental data – water depth (m), percentage shell, sand and silt for all sites

The site groupings based on environmental variables (PCA) are different to the site groupings evident in benthic community structure (nMDS). This suggests that factors other than, or in addition to, sediment composition are driving the benthic structure.

4 Discussion and recommendations

4.1 Discussion

Benthic communities are inherently variable across different spatial and temporal scales, typically in response to differences in local environmental conditions such as water depths, water circulation, tides and sediment characteristics. When assessing potential impacts from project activities it is important not to attribute site-by-site differences to project impacts without due consideration of the environmental and biological context.

The soft sediment benthic communities within the CVC monitoring area are dominated by polychaete worms and bivalve molluscs. Fauna abundances and diversity indices (richness, evenness, and diversity) differ between each site (as expected) although were found to be within a relatively narrow range across the monitoring area. Statistical analysis of the benthic data indicates a level of variability within the treatment groups (Impact, Reference and Control) that is similar to or greater than the variability between treatment groups. There are no significant differences between the treatment groups – Impact versus Reference versus Control.

From an ecological perspective, the benthic assemblages across the monitoring area fall into several groups that do not appear to be a response to CVC operations but are most likely grouping because of currently unconfirmed environmental factors.

For example, monitoring sites C5-C7-R11 and R10 are furthest north and likely to be exposed to greater water circulation within greater Lake Macquarie that may provide increased food availability and/or better water quality that is reflected in benthic community composition. In addition, three of these sites – C5, C7 and R10 – have sediments with higher sand content which may support different benthic communities compared to the high silt areas further south. Higher sand content is often correlated with higher water movement.

Monitoring sites IM2-IM5-R7 have benthic communities that are statistically distinct from the other monitoring sites although the contributing factors driving the differences are unclear, as evidenced by the results of the PCA. Importantly, these monitoring sites are not distinctly associated with CVC operations and the differences in benthic community are unlikely to be attributed to mining activities. Differences are more likely associated with local environmental conditions such as a combination of water depth, sediment characteristics, water circulation and/or water quality.

Statistical analysis of CVC's benthic monitoring data, primarily undertaken for the period September 2016 to September 2022, did not identify statistical differences between the benthic assemblages evident at sites designated as Impact, Reference and Control.

In conclusion, the results of statistical analysis of CVC's benthic monitoring data indicate that no exceedance of the BCMP (CVC 2019) subsidence impact performance measure of "minor environmental consequences, including minor changes to species composition and/or distribution" has occurred. Consequently, CVC is not required to implement any additional investigations of benthic communities within the project study area at this time and should continue the routine monitoring of benthic assemblages.

4.2 Recommendations

Currently, CVC conducts twice annual (seasonal) monitoring of benthic communities in southern Lake Macquarie. The overarching aim of the project is to monitor for detectable changes in benthic assemblages associated with potential subsidence of the lakebed due to undermining.

Subtidal benthic habitats, like those monitored by CVC, that are not dominated by benthic primary producers (such as seagrass and/or macroalgae), typically do not exhibit strong seasonal variation since the benthic species do not photosynthesise (and are therefore largely unaffected by changing light levels). Additionally, benthic environments are often quite stable with respect to sediment conditions that do not change on a regular cyclical nature with the seasons.

For these reasons and given the current absence of statistically relevant differences between benthic assemblages at CVC's impact monitoring sites when compared to the reference and control sites, EMM recommends that the frequency of CVC's benthic monitoring could be reduced to once per year. The recommended timing of annual monitoring is March (Autumn) to capture any variation in benthic assemblages that might occur following summer temperature extremes, while allowing ongoing statistical analysis of the historical and future March monitoring data.

Importantly, the frequency of monitoring should be reviewed if future monitoring results indicate impacts to benthic assemblages that are potentially associated with CVC operations, or if the local benthic environmental conditions change substantially.

5 References

Chapman MG & Underwood MJ (1999) Ecological patterns in multivariate assemblages: information and interpretation of negative values in ANOSIM tests. *Mar Ecol Prog Ser* 180:257-265

Clarke KR & Gorley RN (2006) *PRIMER v6: User Manual/Tutorial*. PRIMER-E, Plymouth 190pp.

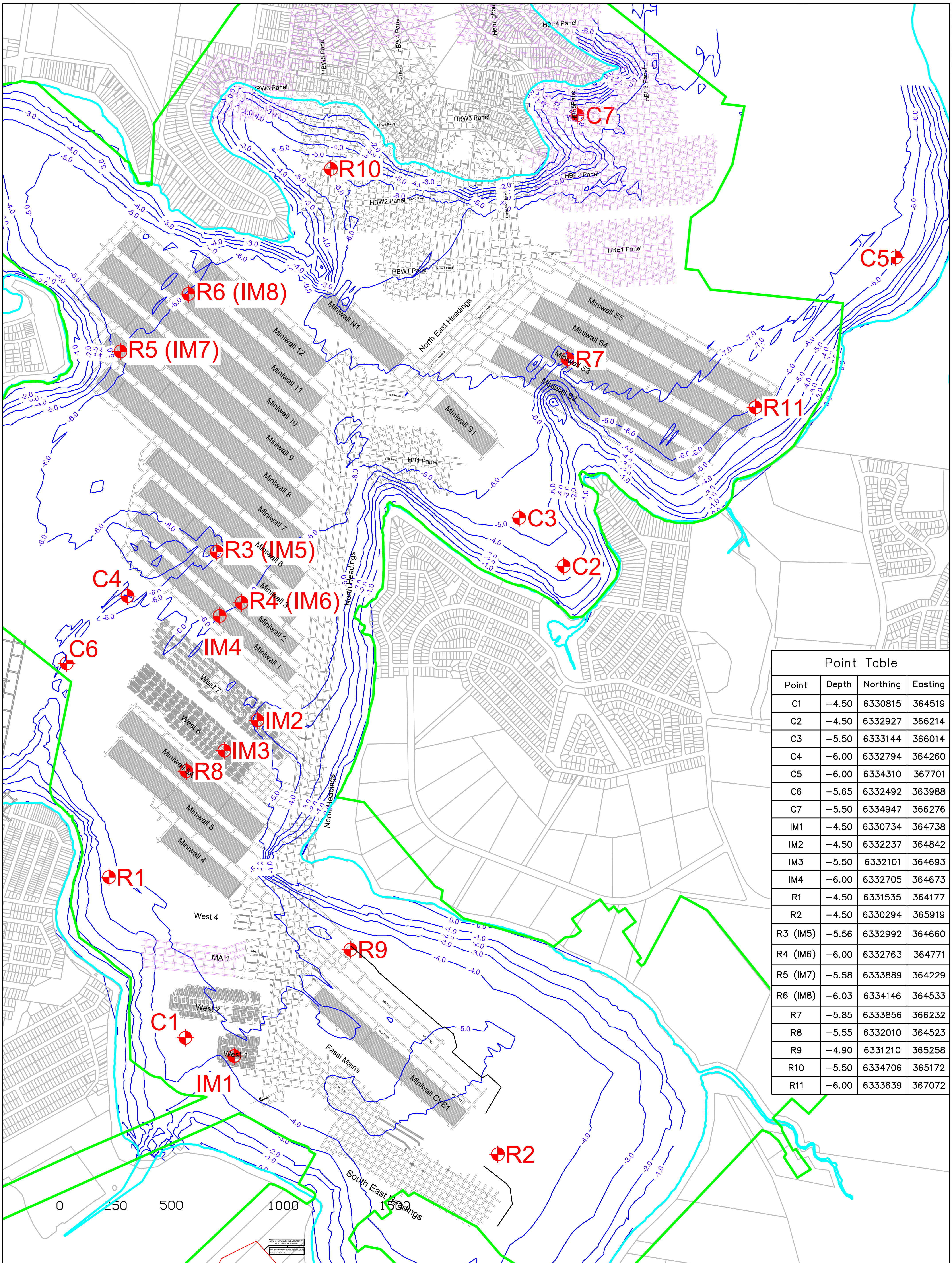
CVC (2019) *Benthic Communities Management Plan – Environmental Management Plan*, Chain Valley Colliery (14 November 2019)

Laxton JH & Laxton ES (2022) *Lake Macquarie Benthos Survey Results No. 22.* Report prepared for Delta Coal, Mannering & CVC Collieries by JH & ES Laxton Environmental Consultants P/L, September 2022. 54pp.

Appendix A

CVC benthic monitoring sites map

EMM
creating opportunities



Point	Depth	Northing	Easting
C1	−4.50	6330815	364519
C2	−4.50	6332927	366214
C3	−5.50	6333144	366014
C4	−6.00	6332794	364260
C5	−6.00	6334310	367701
C6	−5.65	6332492	363988
C7	−5.50	6334947	366276
IM1	−4.50	6330734	364738
IM2	−4.50	6332237	364842
IM3	−5.50	6332101	364693
IM4	−6.00	6332705	364673
R1	−4.50	6331535	364177
R2	−4.50	6330294	365919
R3 (IM5)	−5.56	6332992	364660
R4 (IM6)	−6.00	6332763	364771
R5 (IM7)	−5.58	6333889	364229
R6 (IM8)	−6.03	6334146	364533
R7	−5.85	6333856	366232
R8	−5.55	6332010	364523
R9	−4.90	6331210	365258
R10	−5.50	6334706	365172
R11	−6.00	6333639	367072

Legend
CVC Fassifern Seam Workings
Proposed Fassifern Seam Workings
Project Boundary
Lake Edge

DELTA COAL
CHAIN VALLEY COLLIERY
BENTHIC MONITORING POINTS
LAKE MACQUARIE

SCALE:	1:15000	DATE:	11 January 2023
DRAWN:	R Tubridy	DRG NO:	C1S0120_17
CHECKED:	L McWha	REV NO:	17
		SIZE:	A3

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