Benthic Communities Monitoring - Statistical Review 2024

Chain Valley Colliery

Prepared for Delta Coal

January 2025

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Delta Coal

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1	12 December 2024	Paul Goldsworthy	Andrea McPherson	Draft – CVC comments
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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 4), a Benthic Communities Management Plan (BCMP) was developed (CVC 2023).

Since 2012, CVC has monitored the soft sediment benthic community in shallow lake environments above their underground mine 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 historically has undertaken 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. Since 2023, the monitoring frequency has reduced to annual sampling in autumn.

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–March 2024. 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).

Ongoing development of CVC's underground coal extraction led to the redesignation of some Reference sites as Impact sites, with R3, R4, R5 and R6 becoming IM5, IM6, IM7 and IM8, respectively, in 2015, and R2, R7, R8 and R11 becoming IM10, IM11, IM9 and IM12, respectively, in March 2024. Also, new Reference sites, R12 and R13, were added in March 2024. 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 21 sampling events between September 2012 and March 2024, a total of 26,816 benthic individuals from the 30 different taxonomic groups were counted in sediment samples from across the study area. The three most abundant taxa were the bivalve mollusc *Soletellina* (7,829 individuals), bivalve *Corbula* (6,481 individuals), and polychaetes-thin (6,175 individuals). Together these taxa account for 76% of the total number of benthic individuals collected in sediment samples throughout the monitoring period. The most speciose faunal groups are polychaetes and bivalve molluscs, with ten species collected in both groups.

Between 5 and 21 OTUs (mean 15.0) were reported per site. The lowest number of OTUs were at the new sites established in March 2024 (R12 and R13) with 5 and 9 OTUs, respectively. The next lowest number of OTUs (11) was at IM4 and R5 (now IM7), and the highest number of OTUs, 20 and 21, was at R3 (now IM5) and IM2, respectively. With the lowest and the highest OTU counts occurring at Reference and Impact sites, there is no clear spatial pattern associated with the number of OTUs per site type.

The total number of individuals per site varied from 29 and 86 (R12 and R13) and between 575 (R8/IM9) and 2,210 (C2). Abundances were lowest (<1,000 individuals) at C6 (746), C7 (793), R7/IM11 (751), R8/IM9 (575), R9 (955), R11/IM12 (622), R12 (29) and R13 (86).

Abundances were highest (>1,500) at C2 (2,210), C3 (1,555), C4 (1,604), R1 (1,562), R2/IM10 (1,726), R3/IM5 (1,514) and IM2 (1,650). There is no clear spatial pattern associated with the abundance of benthic species per site type.

Multivariate pairwise test results (ANOSIM) highly non-significant differences between all treatment pairs – Control versus Reference (0.561), Control versus Impact (0.603) and Reference versus Impact (0.433). 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 five clusters: C7; C5-R11; IM2-R3-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.

The nMDS plots for benthic abundance data grouped by sampling event for each site indicated high variability (differences in nMDS space) between benthic community data over time at each site. Groupings were indicated for 70% similarity and indicated that benthic community structure differ between sampling events at each site and did not a follow an abrupt or sequential change over time that could be attributed to impacts from subsurface activities

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*.

Principle components analysis (PCA) undertaken on the normalised environmental data, indicated that the three main site groups differentiated primarily due to silt, sand, and shell content. 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.

The BEST analysis indicated that none of the environmental variables were significant drivers of the benthic community structure. The highest correlation (R 0.427, non-significant) between the biological and environmental data was associated with sediment type, primarily the percentage of sand, followed by percentage of silt and shell. This is not unexpected since most benthic fauna have preferences for particular types of sediment.

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.

Statistical analysis of CVC's benthic monitoring data did not indicate exceedance of the BCMP (CVC 2023) subsidence impact performance measure of "minor environmental consequences, including minor changes to species composition and/or distribution" has occurred.

EMM recommends continuation of the existing benthic monitoring program – annual monitoring in autumn each year.

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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. The mine produces thermal coal which is transported underground to the Manning Colliery where it is crushed and screened before being sent to Vales Point Power Station.

As part of CVC's environmental performance, and to satisfy Condition 7(h), Schedule 4 of Development Consent for SSD-5465 (Modification 4), a Benthic Communities Management Plan (BCMP) has been developed (CVC 2023). The stated purpose of the BCMP is to minimise the impact on Benthic Communities through:

- outlining the required data to be collected on monitored benthic communities
- identifying benthic community monitoring locations
- identifying reporting requirements
- detailing preventative management measures
- identifying the requirements for incident or exceedances reporting
- identifying the responsible persons for all required actions
- Identifying the review requirements for the BCMP.

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 initially undertook six-monthly sampling of lake sediments for analysis of benthic community composition and environmental variables (water depth and sediment grain size), with samples collected in Autumn (March) and Spring (September). The monitoring frequency was reduced to once per year (March) in 2023 due to recommendations from the 2022 statistical review of benthic community monitoring data (EMM 2022).

Benthic monitoring occurs at potentially Impacted, Reference, and Control sites (Appendix A).

The BCMP defines the three site types as follows:

- Impacted (prefix IM) – sites currently or historically potentially impacted upon by subsidence.
- Reference (prefix R) – sites not currently impacted by subsidence but fall within the proposed future mining footprint. Following undermining, Reference sites are redesignated as Impacted sites.
- Control (prefix C) – sites which 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 2023). 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 2023)

Variable type	Analysis	Description
Environmental: Water Quality	ANZECC/ARMCANZ Guidelines	Trigger values for slightly – moderately disturbed ecosystems: Estuaries.
Biotic and Environmental	Univariate	Descriptive graphical statistics. Analysis of Variance and 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 [1]	Correlation between biotic and environmental data using PRIMER.

[1] Primer v6 replaced the BIOENV routine with BEST (BIOENV + BVStep)

This report provides the results of 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 – March 2024) 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 2023).

Descriptive statistics (i.e. 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 monitoring.

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 (e.g. 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, or in other words, 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, but instead the relative distance between points indicates relative similarity and 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 (i.e. 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 many of the early Reference sites as Impact sites (Table 2.1). Sites R2, R3, R4, R5, R6, R7, R8 and R11 have become IM10, IM5, IM6, IM7, IM8, IM11, IM9 and IM12, 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	Impact sites
R1	**C1**	**IM1**
R9 *(added in September 2016)*	**C2**	**IM2**
R10 *(added in March 2018)*	**C3**	**IM3**
R12 *(added in March 2024)*	**C4**	**IM4**
R13 *(added in March 2024)*	**C5** *(added in March 2016)*	**IM5** *(=R3 prior to September 2015)*
	C6 *(added in September 2016)*	**IM6** *(=R4 prior to September 2015)*
	C7 *(added in March 2018)*	**IM7** *(=R5 prior to September 2015)*
		IM8 *(=R6 prior to September 2015)*
		IM9 *(=R8 prior to March 2024)*
		IM10 *(=R2 prior to March 2024)*
		IM11 *(=R7 prior to March 2024)*
		IM12 *(=R11 prior to March 2024)*

3 Analysis results

In the laboratory, biological samples were sorted into different taxonomic groups – operational taxonomic units (OTUs) – comprising some polychaete worms and molluscs sorted to genus or species 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 31 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, 2016–2024

Polychaetes – thin (P)	*Lepsiella (Bedeva) hanleyi* (G)	prawns (C)
Polychaetes – thick (P)	*Nassarius jonasii* (G)	crabs (C)
Polychaetes – mud (P)	*Philine angasi* (G)	barnacles (C)
terebellids (P)	*Anadara trapezia* (B)	ophiuroids (E)
Chaetopterus sp. (P)	*Corbula truncata* (B)	echinoids (E)*
Cirratulidae (P)	*Cyamiomactra mactroides* (B)	planaria (F)
Diopatra sp. (P)	*Dosinia sculpta* (B)	sponges (S)**
Gorgonorhynchus repens (P)	*Mactra* sp. (B)	fish
Pectinaria sp. (P)	*Paphia undulata* (B)	
Sthenelais pettiboneae (P)	*Saccostrea glomerata* (B)	* *Echinocardium cordatum* (E)
	Soletellina alba (B)	** *Dysidea* sp. (S)
	Theora lubrica (B)	** *Tetilla* sp. (S)
	Trichomya hirsuta (B)	

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

3.1 Benthic data 2012–2024

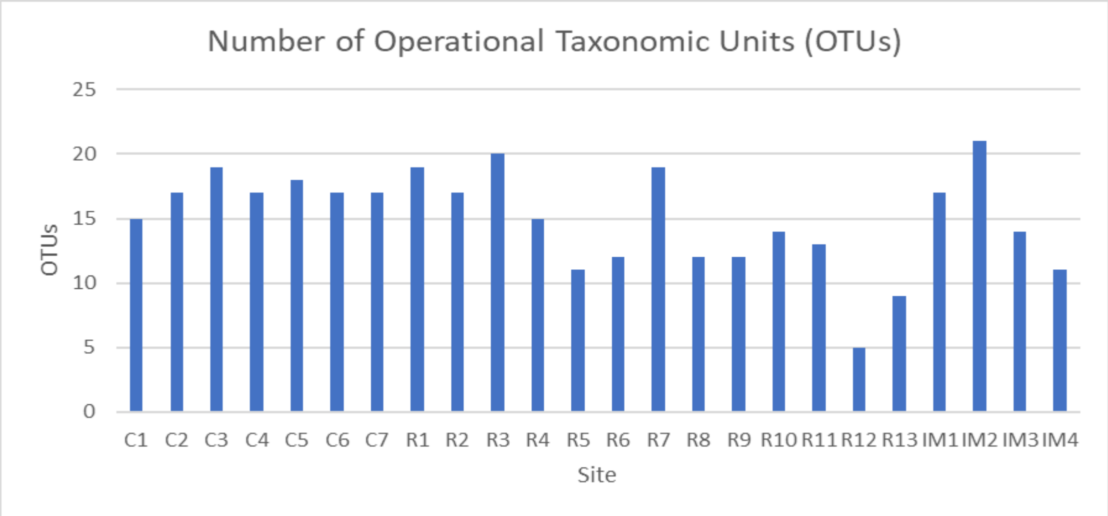
From 21 sampling events between September 2012 and March 2024, a total of 26,816 benthic individuals from the 30 different taxonomic groups were counted in sediment samples from across the study area. The three most abundant taxa were the bivalve mollusc *Soletellina* (7,829 individuals), bivalve *Corbula* (6,481 individuals), and polychaetes-thin (6,175 individuals). Together these taxa account for 76% of the total number of benthic individuals collected in sediment samples throughout the monitoring period. The most speciose faunal groups are polychaetes and bivalve molluscs, with ten species collected in both groups.

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 5 and 21 OTUs (mean 15.0) were reported per site. The lowest number of OTUs were at the new sites established in March 2024 (R12 and R13) with 5 and 9 OTUs, respectively. The next lowest number of OTUs (11) was at IM4 and R5 (now IM7), and the highest number of OTUs, 20 and 21, was at R3 (now IM5) and IM2, respectively. With the lowest and the highest OTU counts occurring at Reference and Impact sites, there is no clear spatial pattern associated with the number of OTUs per site type.

The total number of individuals per site varied from 29 and 86 (R12 and R13) and between 575 (R8/IM9) and 2,210 (C2). Abundances were lowest (<1,000 individuals) at C6 (746), C7 (793), R7/IM11 (751), R8/IM9 (575), R9 (955), R11/IM12 (622), R12 (29) and R13 (86).

Abundances were highest (>1,500) at C2 (2,210), C3 (1,555), C4 (1,604), R1 (1,562), R2/IM10 (1,726), R3/IM5 (1,514) and IM2 (1,650). There is no clear spatial pattern associated with the abundance of benthic species per site type.



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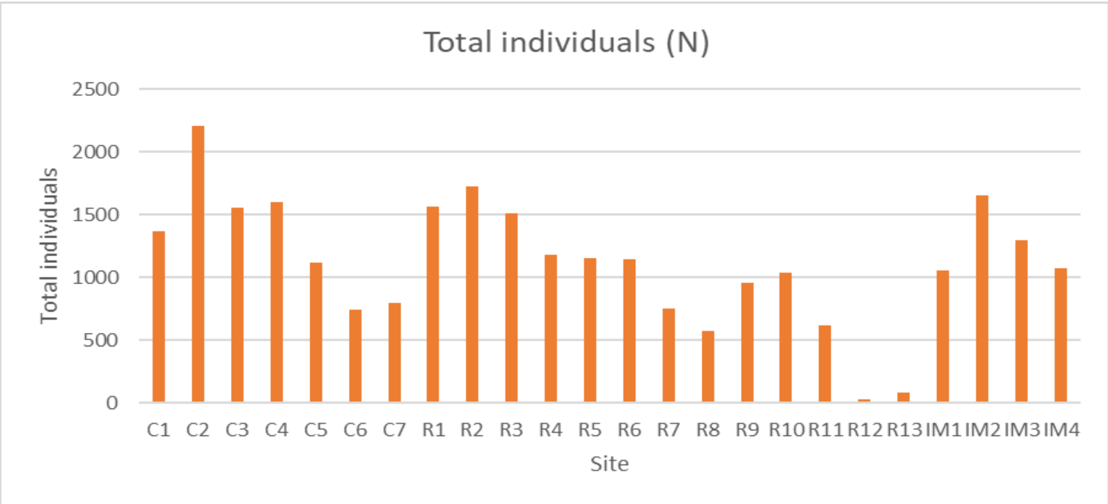


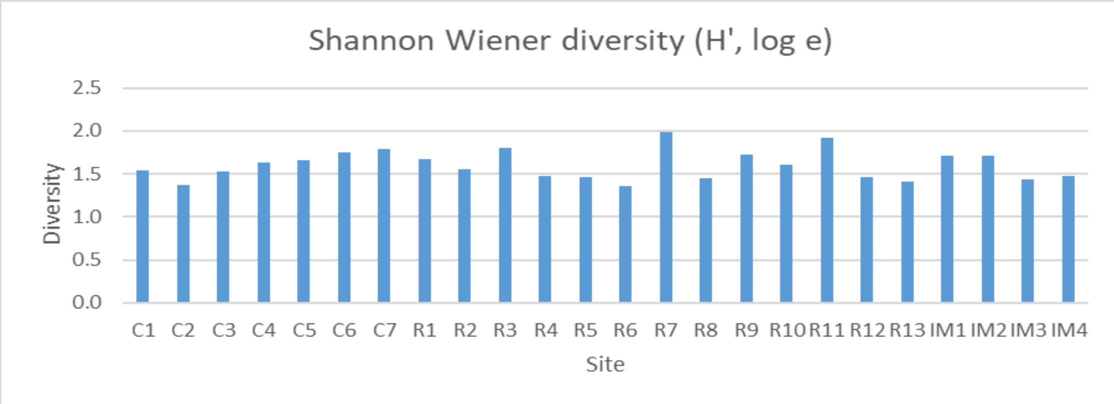
(b)

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 2012–2024

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 (<1.5) apparent at R5, R12 and IM4. The diversity and evenness vary across the sites within a narrow range of 1.36−1.98 and 0.48−0.90, respectively.



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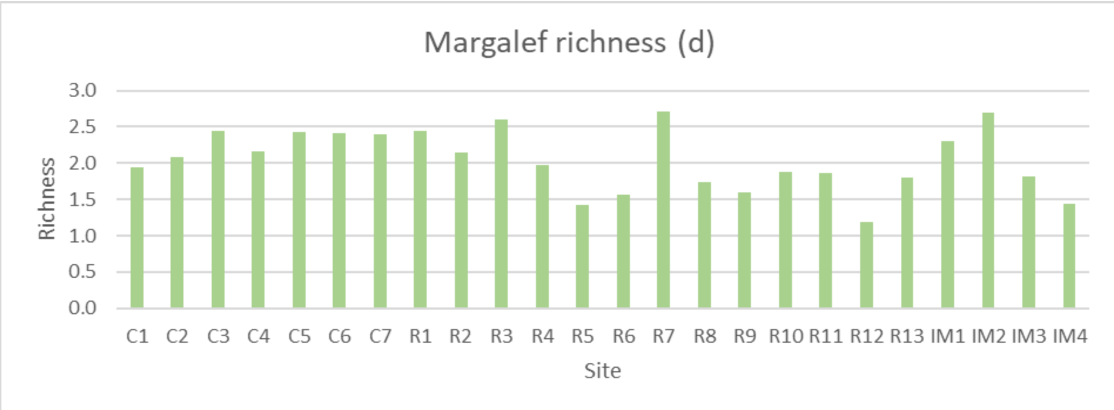

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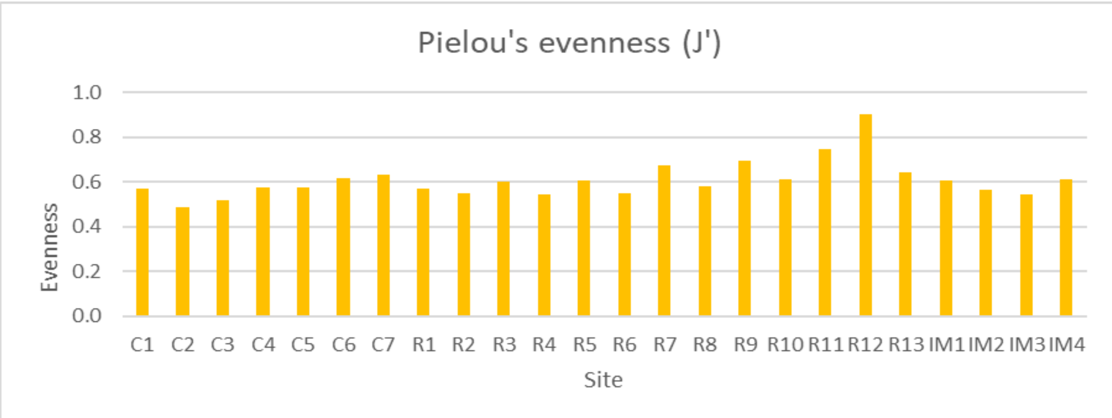


(c)

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

Figure 3.2 (a) Shannon Weiner diversity, (b) Margalef richness and (c) Pielou's evenness for benthic samples from each CVC monitoring site for the period 2012–2024

As indicated in Methods (Chapter 2), to help discern greater relationship information, EMM has focused statistical analysis of the different site types (Impact, Reference, and Control) for 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 2016–2024

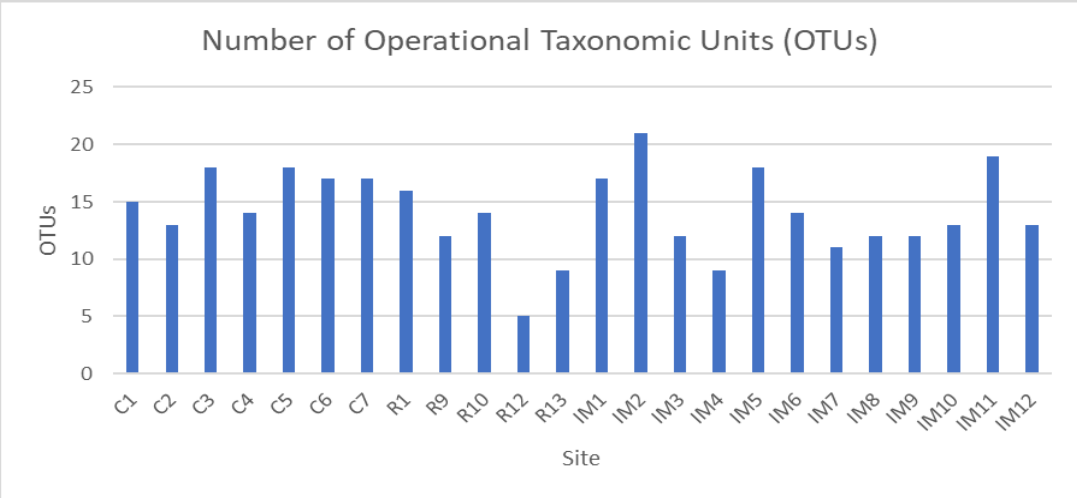
From 15 sampling events between September 2016 and March 2024, a total of 18,893 benthic individuals from 30 different taxonomic groups were counted in sediment samples from across the study area.

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), and subsequent redesignation of R2 (IM10), R7 (IM11), R8 (IM9) and R11 (IM12), due to ongoing expansion of the underground coal workings. Sites R12 and R13 were added in March 2024 to compensate for the ‘loss’ of reference sites during redesignation as impact sites.

Between 5 and 21 OTUs (mean 14.1) were reported per site. The lowest number of OTUs were at R12 (5), IM4 and R13 (9) and the highest number of OTUs were at IM2 (21) and IM11 (19). There is no clear spatial pattern associated with the number of OTUs per site.

The total number of individuals per site varied between 29 (R12) and 1,251 (C2). Abundances were lowest (<600 individuals) at R12 (29), R13 (86), IM9 (575) and IM4 (583). Abundances were highest (>1,000 individuals) at C2 (1,251), IM2 (1,219), IM5 (1,095) and R10 (1,035). The new reference sites (R12 and R13) had the lowest number of OTUs and lowest abundances of all sites based on one sampling event at each site.

There is no clear spatial pattern associated with the abundance of benthic species per site.



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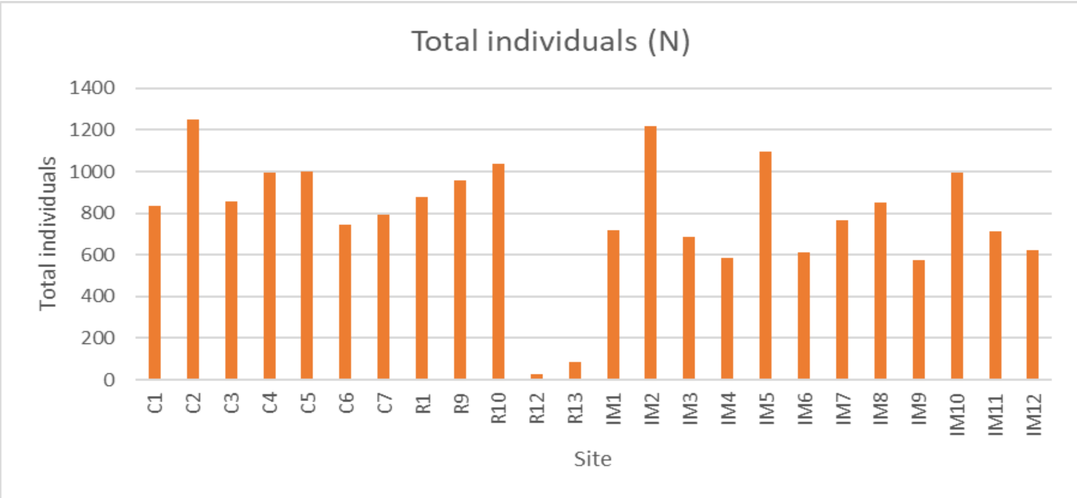


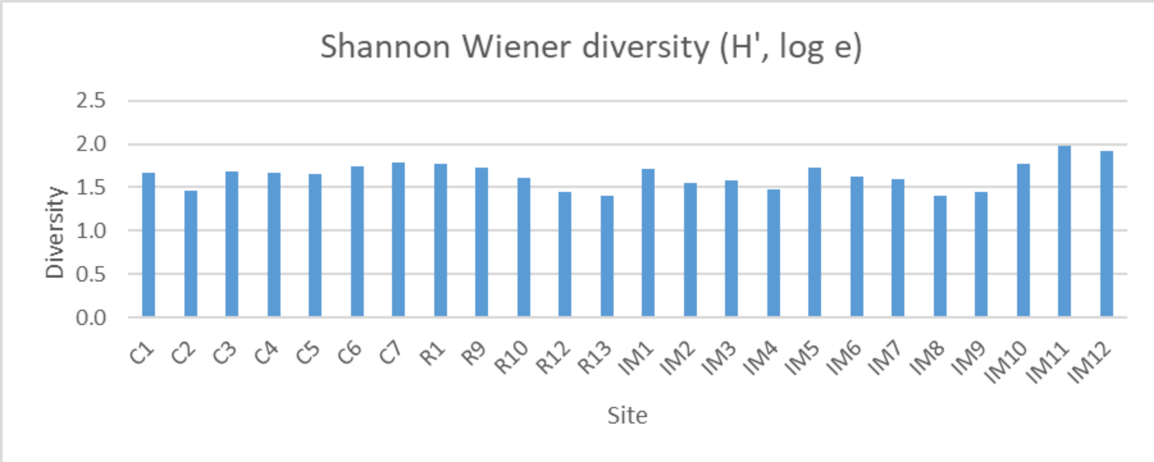
(b)

C = control sites R = reference sites IM = impact 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 2016-2024

Shannon Wiener diversity, Margalef richness and Pielou's evenness values for each site over time (2016−2024) 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 (<1.5) apparent at R12 and IM4. The diversity and evenness vary across the sites within a narrow range of 1.41−1.91 and 0.50−0.90, respectively.



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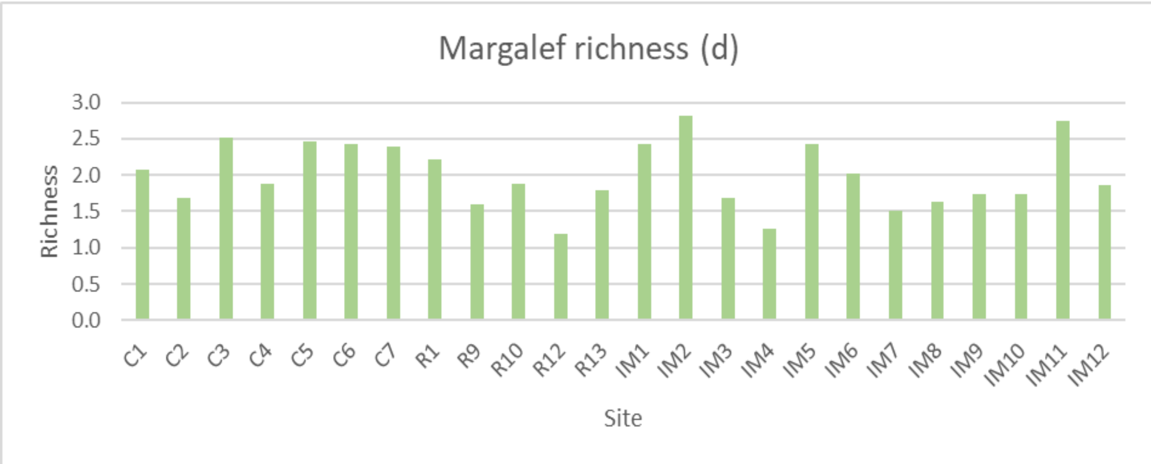

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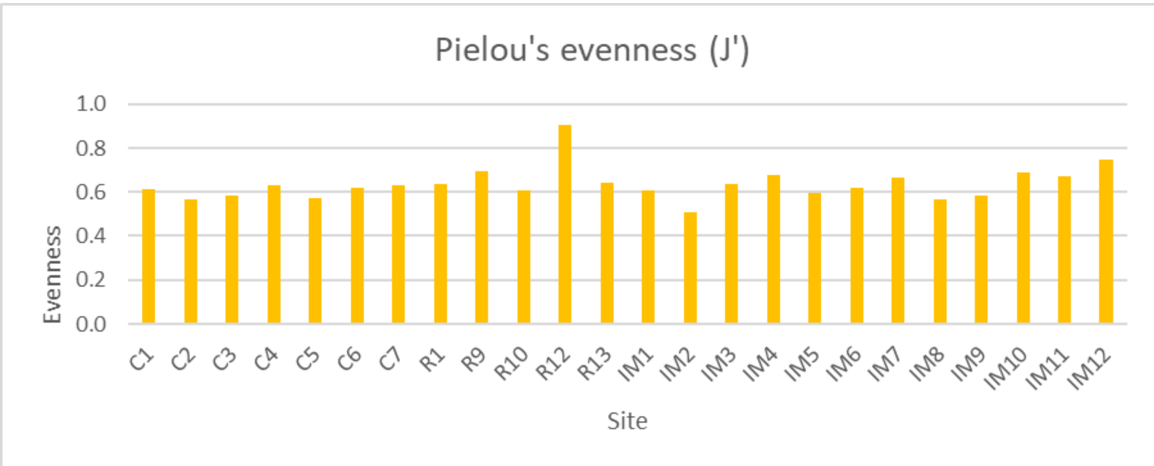


(c)

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

Figure 3.4 (a) Shannon Weiner diversity, (b) Margalef richness and (c) Pielou's evenness for benthic samples from each CVC monitoring site for the period 2016–2024

The limited data (one sampling event) currently available for the new reference sites (R12 and R13), skew the statistical analysis and prevent a meaningful assessment of differences between the other sites and treatments. Consequently, the statistical analysis shown below was undertaken without data from these two sites.

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 March 2024 derived a global R value of -0.023 at a significance level (p) of 0.537 (well above the statistical significance level of 0.05). Pairwise test results indicate highly non-significant differences between all treatment pairs – Control versus Reference (0.561), Control versus Impact (0.603) and Reference versus Impact (0.433). 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) because the global R value (black vertical line shown at x-axis position -0.023) falls within the wider distribution of R values (blue bars).

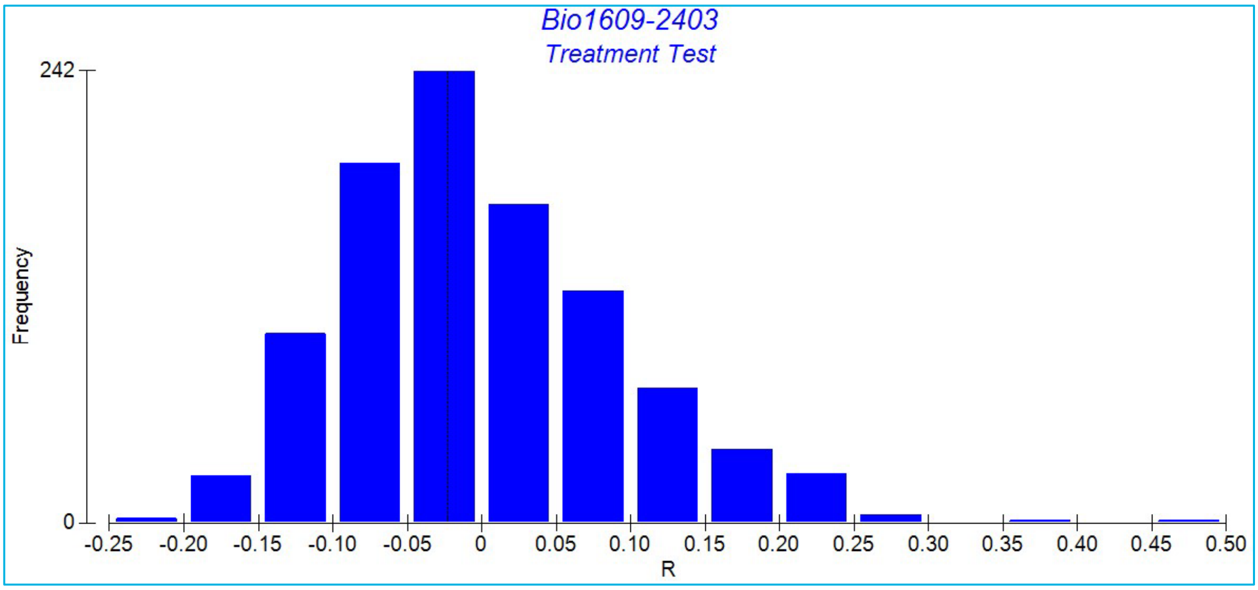


Figure 3.5 ANOSIM test results for benthic data across site types, September 2016 to March 2024. Vertical black line indicates the global R value of -0.023

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 March 2024 (Figure 3.6). The dendrogram indicates that at 75% similarity level, there are five clusters: C7; C5-R11; IM2-R3-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.

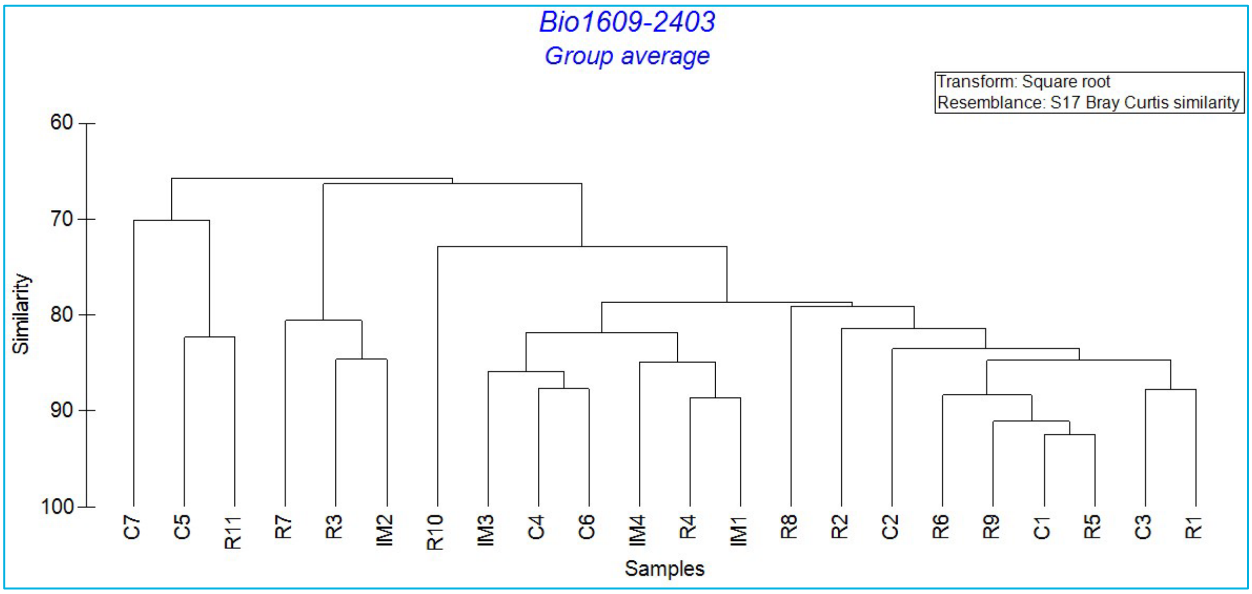


Figure 3.6 **Dendrogram derived from cluster analysis of CVC benthic community monitoring data across all sites between September 2016 and March 2024**

Averaging benthic species abundances across all sites for each sampling event (Figure 3.7) indicates the recent sampling events are most dissimilar, with four event clusters apparent at 75% similarity:

- March 2024 [2403]
- September 2022 [2209] + March 2023 [2303]
- March 2021 [2103] + March 2022 [2203]
- all other events.

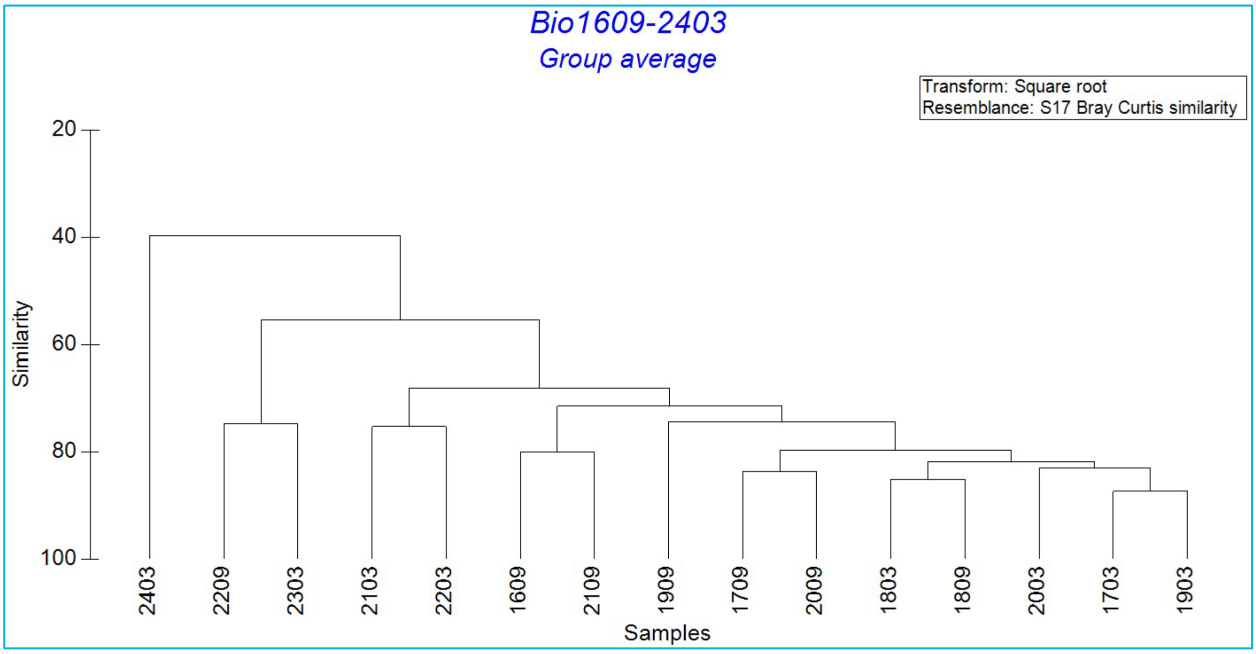


Figure 3.7 **Dendrogram derived from cluster analysis of CVC benthic community monitoring data across all events between September 2016 and March 2024**

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 similar in community composition (Clarke & Gorley 2006). The similarity patterns indicated in the cluster analysis of abundance data grouped by site were further explored using an nMDS plot for abundance data for all sampling events at each site (Figure 3.8). The green circles indicate site groupings that correspond to the 75% similarity level.

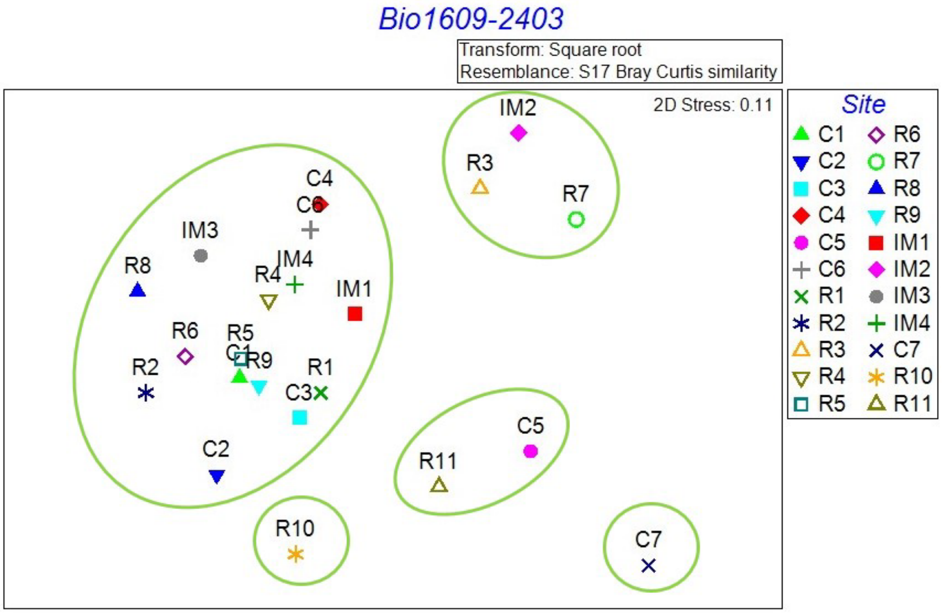


Figure 3.8 Patterns in community structure depicted as nMDS plot based on square-root transformed abundance data of all taxa (OTUs) for each site (all events), September 2016–March 2024

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) and several smaller groups of similar sites. Six out of the seven sites that exhibit significantly different benthic communities to the main group were originally designated as reference (R3, R7, R10 and R11) or control (C5 and C7) site types; although, R3, R7 and R11 were subsequently redesignated as impact sites (IM5, IM11 and IM12, respectively).

The nMDS plots for benthic abundance data grouped by sampling event for each site (Figure 3.9, Figure 3.10 and Figure 3.11) indicate high variability (differences in nMDS space) between benthic community data over time at each site. Groupings are indicated for 70% similarity and indicate that benthic community structure differ between sampling events at each site and do not a follow an abrupt or sequential change over time that could be attributed to impacts from subsurface activities.

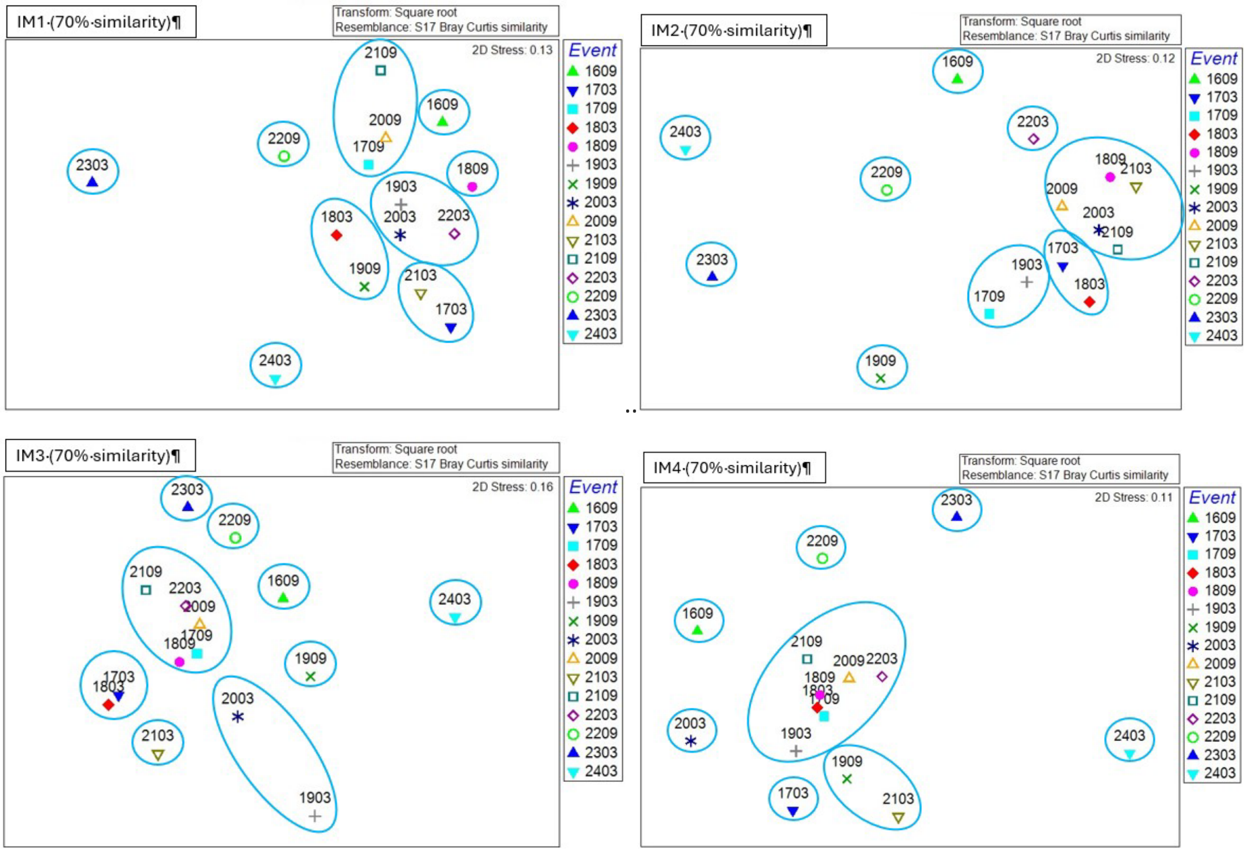


Figure 3.9 nMDS plots of square root transformed benthic abundance data grouped by sampling event (YYMM) at each Impact site

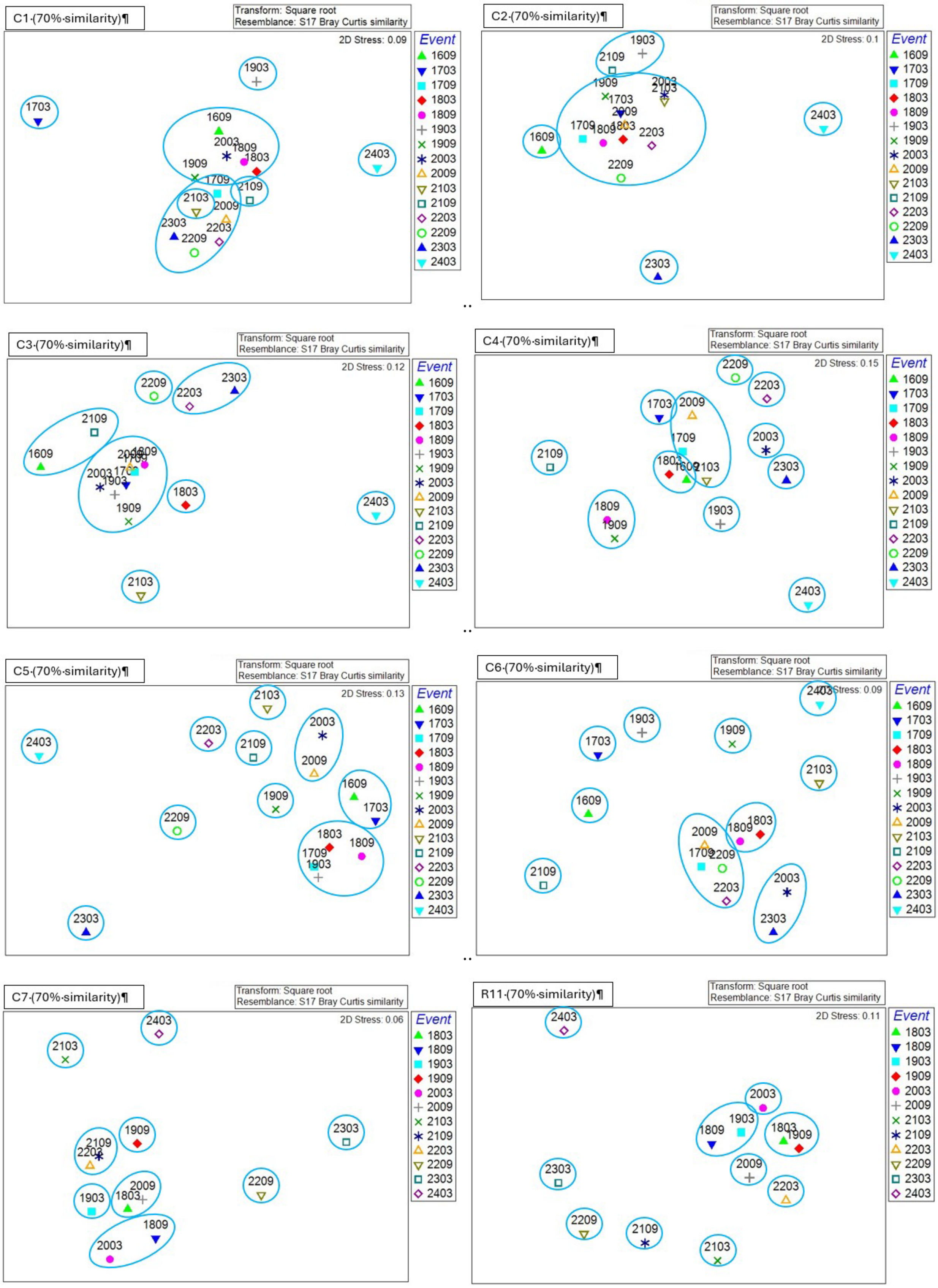


Figure 3.10 nMDS plots of square root transformed benthic abundance data grouped by sampling event (YYMM) at each Control site and Reference site R11

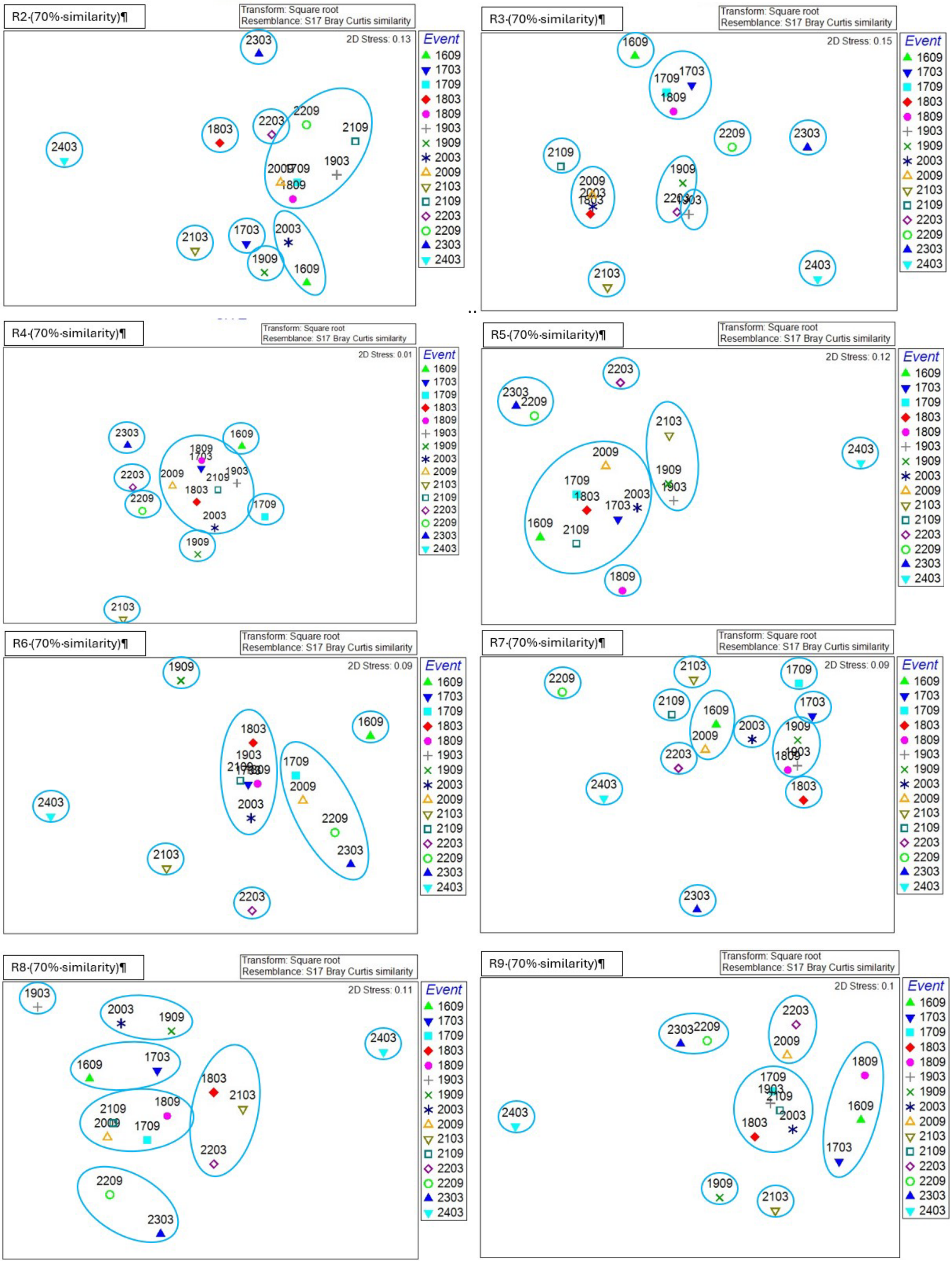


Figure 3.11 nMDS plots of square root transformed benthic abundance data grouped by sampling event (YYMM) at each Reference site (R11 is shown in previous figure)

The nMDS plots indicate that there are differences in benthic abundance data through time at all sites (Control, Impact and Reference). The groupings (at 70% similarity) are unrelated to specific periods of time and therefore indicate natural changes in benthic community structure evident at all sites across the multiple years. Such widespread variability over time is highly unlikely to be associated with subsurface activity by CVC.

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(IM12), IM2-IM5(R3)-IM11(R7) and R10 site groups identified during cluster analysis and confirmed by nMDS (Figure 3.8).

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

Specific differences between the site groups are as follows:

- <u>C5-C7-IM12 (Group A)</u>:
  - Much higher abundances of 'polychaete-mud' compared to other site groups.
  - Lower abundances of *Corbula and Soletellina* compared to Group D.
- <u>IM2-IM5-IM11 (Group B)</u>:
  - Higher abundances of *Trichomya* compared to other site groups.
  - Lower abundances of *Soletellina* compared to Group D.
  - Lower abundances of *Corbula* compared to Group D.
- <u>R10 (Group C)</u>:
  - Higher abundances of *Soletellina* compared to other site groups.
  - Higher abundances of *Corbula* compared to Group A and Group B.
- <u>All other sites (Group D)</u>:
  - Higher abundances of *Corbula* and *Soletellina* compared to other groups at most sampling times.

3.7 Temporal comparison of site groups

Comparison of temporal variation in abundances (mean + standard deviation) for each site group (C5-C7-IM12, IM2-IM5-IM11, R10, and 'all other') are provided for the five most abundant OTUs reported across the benthic monitoring area (Figure 3.12 and Figure 3.13).

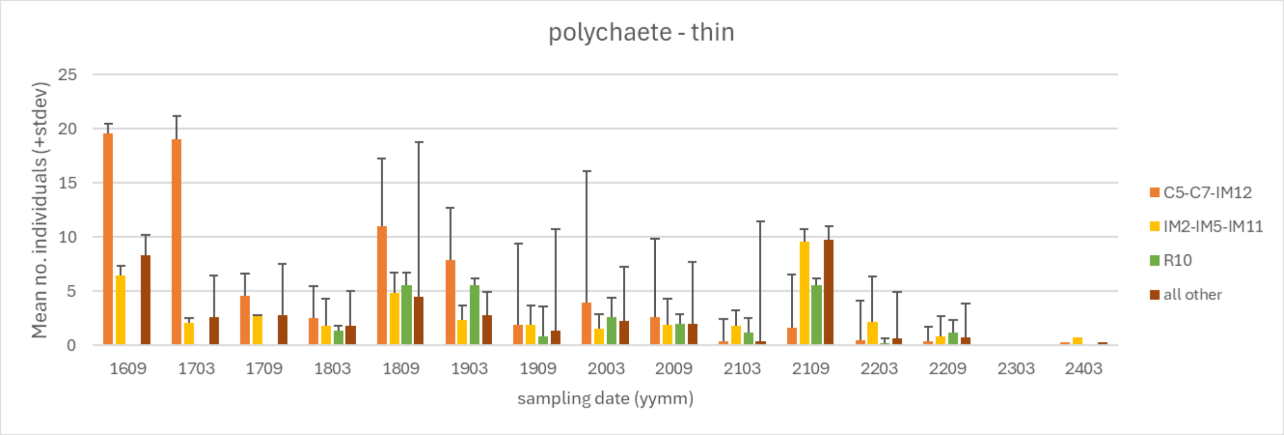
Thin polychaete abundances are broadly similar for three of the four site groups at each monitoring event, with more variability apparent over time in the C5-C7-IM12 group, higher in the earliest monitoring periods and lower abundances more recently, but with fluctuating abundances across the entire monitoring period.

Mud polychaete abundances are variable between site groups and over time. The notable differences are higher abundances (and variability) apparent in the C5-C7-IM12 group and at R10 compared to the other site groups. Large variability between sites is apparent, as shown by the often-large standard deviation lines above the bars.

Abundances of bivalves *Corbula* and *Soletellina* are similar across all site groups for most monitoring events; although mostly higher in the 'all other' site group, except in 2018 when R10 had higher mean abundances of *Corbula* and in September 2022 (2209) and March 2023 (2303) when R10 had higher abundances of *Soletellina*.

Trichomya abundances are significantly higher within the IM2-IM5-IM11 site group, 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. *Trichomya* (commonly called the hairy mussel) attaches to hard substrate such as rocks and shell, and the higher abundance of this species at IM2-IM5-IM11 is likely to be associated with the availability of suitable hard substrate.

The important aspect to note from these plots is that the IM2-IM5-IM11 group, 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.



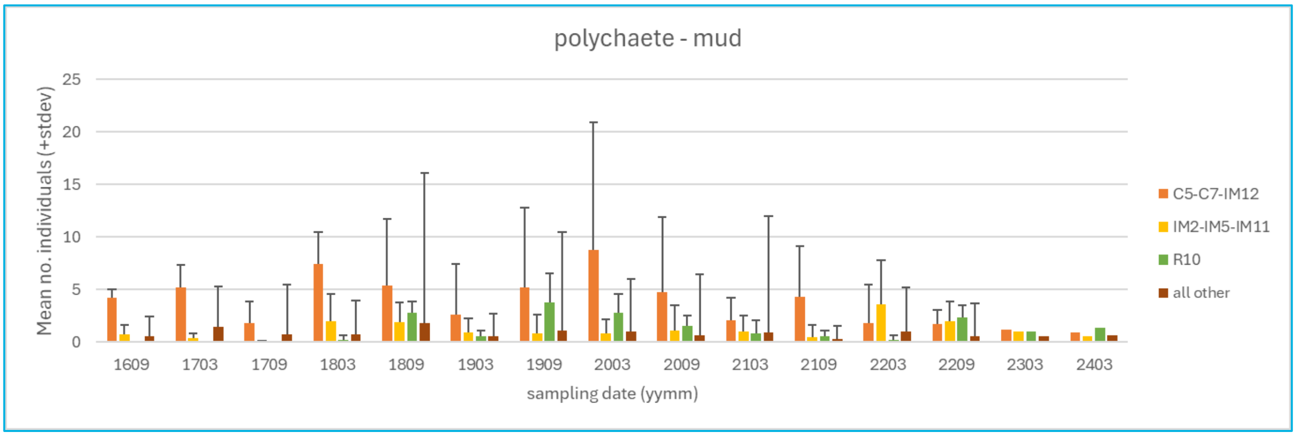
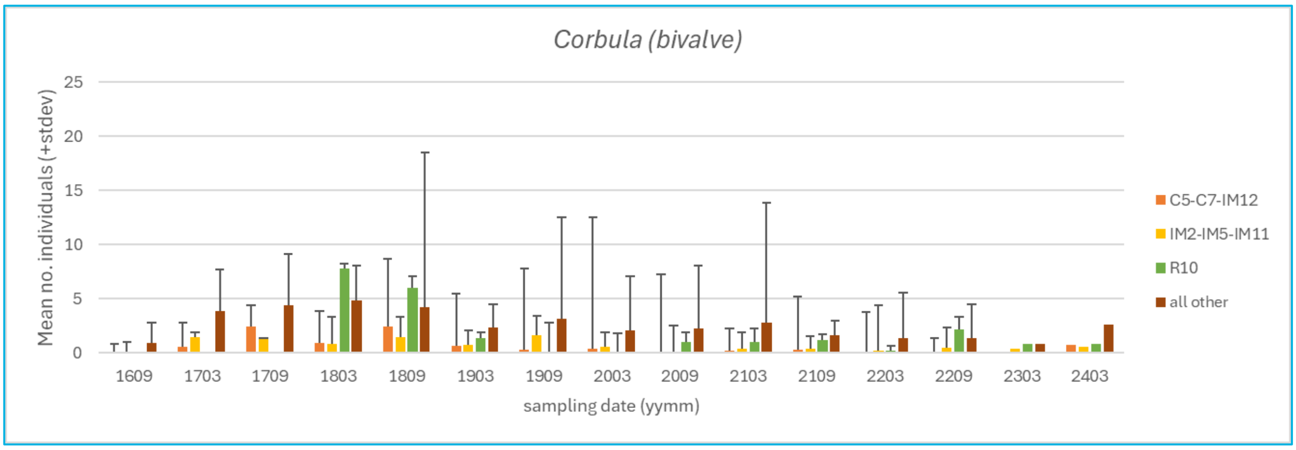
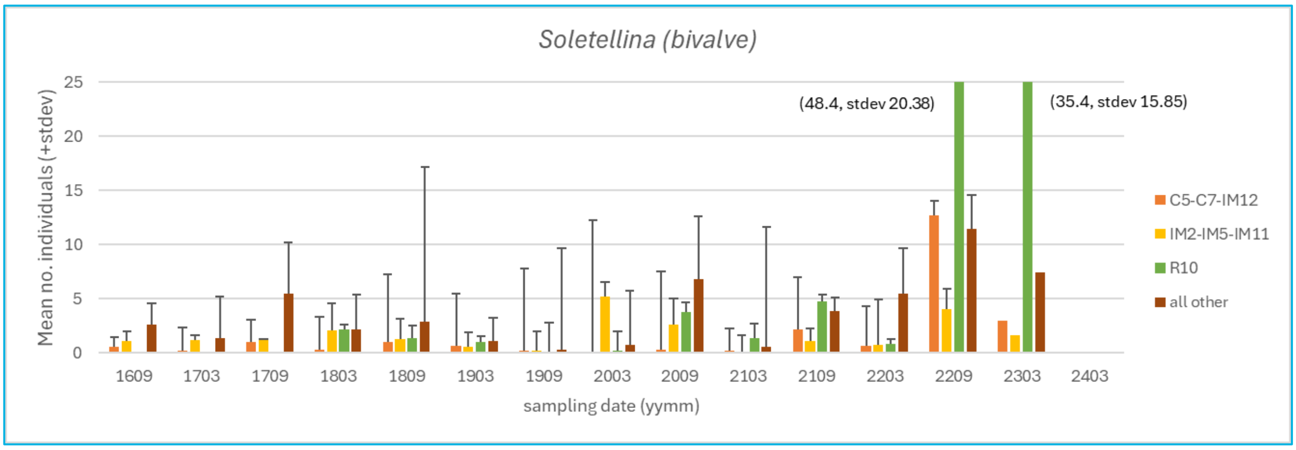


Figure 3.12 Temporal comparison of benthic abundances by site group for thin and mud polychaetes





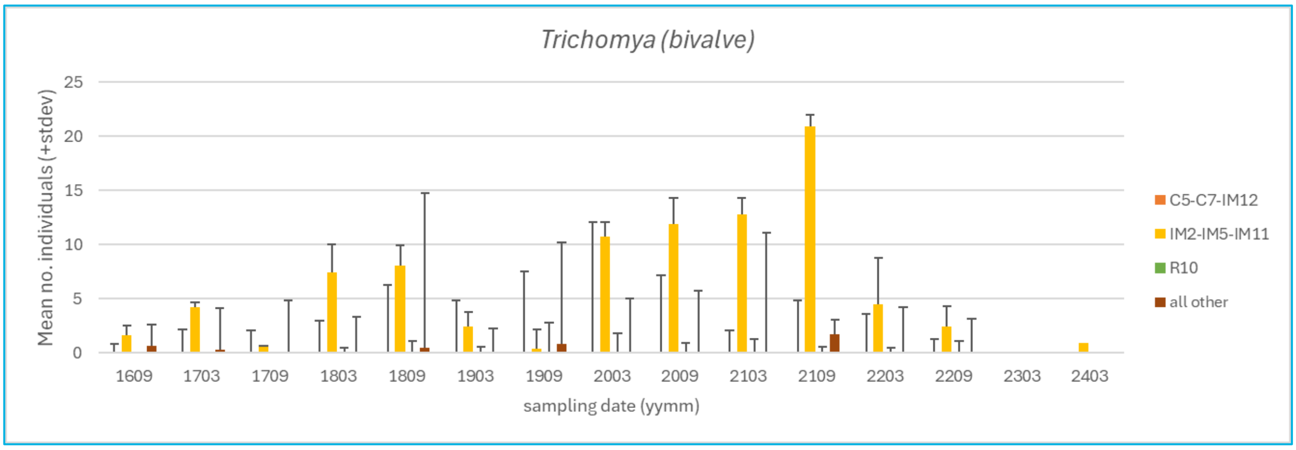


Figure 3.13 Temporal comparison of benthic abundances by site group for the bivalve molluscs *Corbula, Soletellina* and *Trichomya*

3.8 Environmental data

Environmental data were reported for each site and were analysed to discern potential environmental drivers of the observed site groupings evident in the benthic community data.

3.8.1 Sediment composition

The sediments at all sites sampled in March 2024 were described as 'largely composed of fine grey silt' with 'small to large shell fragments…at most stations' (Laxton & Laxton 2024).

Principal component analysis (PCA) was undertaken for sediment composition and four main site groups were differentiated due to silt, sand, and shell content:

- Group 1: IM8 and IM11 – high shell (95–98%), no sand (0%) and low silt (2–5%).
- Group 2: C5 and C7 – low shell (0–3%), medium to high sand (44–64%) and medium silt (36–53%).
- Group 3: C4 and IM5 – medium shell (35–50%), no sand (0%) and medium silt (50–65%).
- Group 4: all other sites – low shell (0–20%), low sand (<4%) and high silt (>80%).

The grouping of most sites is associated with the relative contribution of silt and shell: however, two sites (C5 and C7) were distinctly separated from the other sites due to higher sand component (Figure 3.14). Group 3 (C4 and IM5) falls midway along the silt–shell continuum.

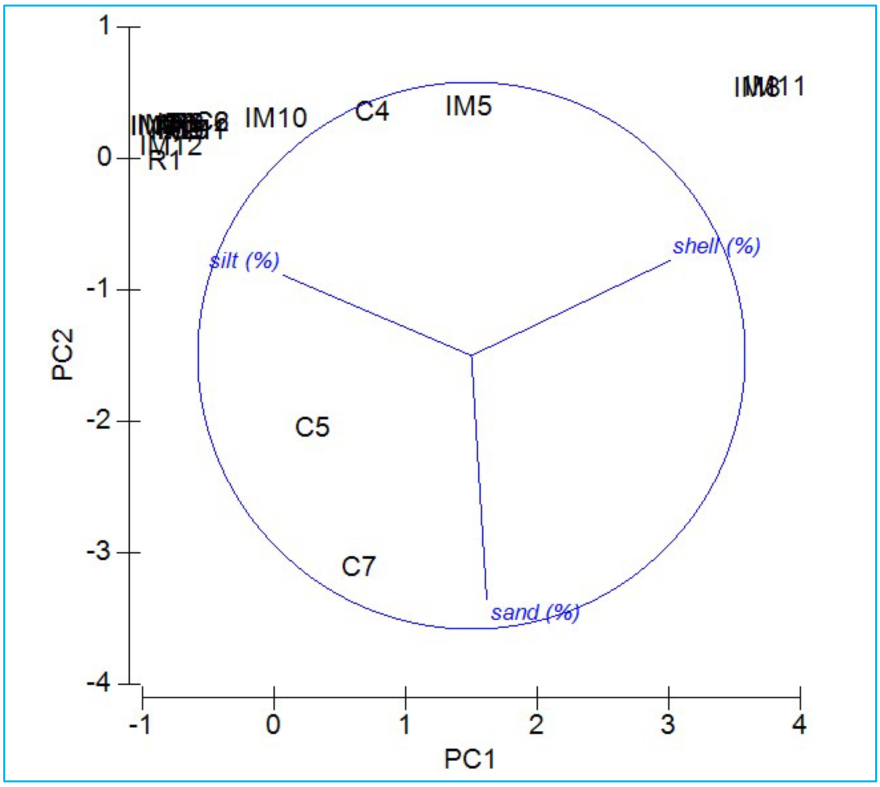


Figure 3.14 Principal components analysis (PCA) plot for sediment composition in March 2024.

3.8.2 Water quality

The measured physico-chemical quality of bottom waters was largely consistent across all sites, with only minor variability evident:

- temperature (°C): 25.18 to 26.48 (mean 25.80)
- conductivity (µS/cm): 55.17 to 55.90 (mean 55.53)
- salinity (parts per thousand, ppt): 36.55 to 37.10 (mean 36.82)

- pH: 7.55 to 8.72 (mean 7.90)
- dissolved oxygen (DO, mg/L): 4.67 to 5.9 (mean 5.4)
- turbidity (NTU): 0.20 to 17.70 (mean 8.41)
- oxidation reduction potential (ORP, mV): 257 to 496 (mean 304.46).

For clarity, PCA was undertaken for water quality, on two sets of indicators: 'conductivity, temperature and pH' and 'ORP, turbidity and DO'.

PCA results for conductivity, temperature and pH indicated most sites are similar for these parameters and fall within the centre of the PCA plot (Figure 3.15). There are some minor variations apparent with water at IM7 and IM8 being slightly warmer and with higher conductivity, water at IM1, IM10 and C1 being slightly cooler and water at C5, C6 and R13 having slightly higher pH than most sites. Variations in the water quality are only minor in magnitude and unlikely to drive significant differences in benthic community structure.

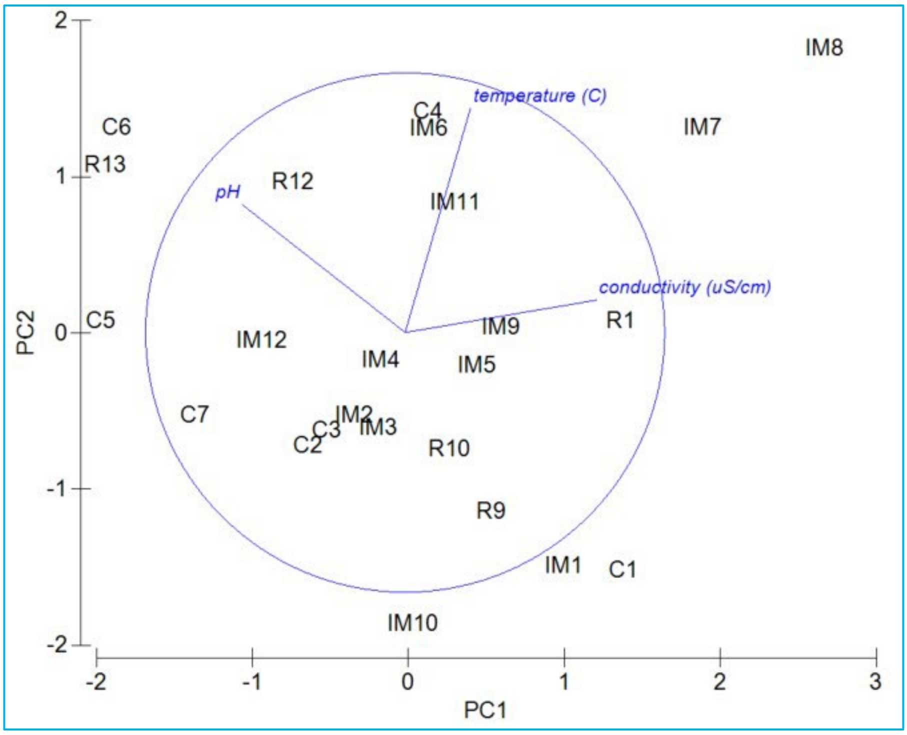


Figure 3.15 Principal component analysis plot for pH, temperature and conductivity at each site in March 2024.

PCA results for ORP, turbidity and DO indicated most sites are similar for these parameters and fall within the centre of the PCA plot (Figure 3.16). There are some minor variations apparent with water at C6 having higher ORP, water at IM6 having slightly higher DO and marginally higher turbidity apparent at IM1, C1 and C3 compared to most sites. These minor differences in water quality are unlikely to cause significant differences in benthic community structure.

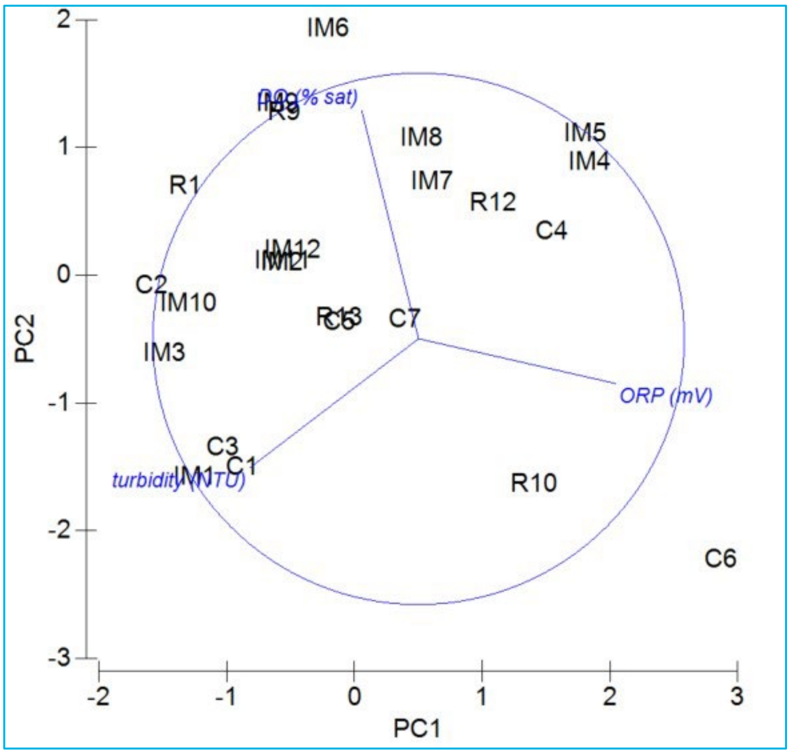


Figure 3.16 Principal component analysis plot for ORP, turbidity and DO at each site in March 2024.

3.8.3 BEST

The Primer v6 routine 'BEST' (which replaces the BIOENV routine in earlier Primer versions) finds a best match between the multivariate among-sample patterns of an assemblage and that from environmental variables associated with those samples (Clarke & Gorely 2006).

BEST was used to assess the biological data against the following environmental variables:

- silt (%)
- sand (%)
- shell (%)
- water depth (m)
- pH
- ORP (mV)
- turbidity (NTU)
- dissolved oxygen (mg/L).

Water temperature and conductivity varied so slightly between sites (1.3°C and 0.73 µS/cm, respectively) that they were deemed unlikely to drive differences in the benthic community data and were not included in the BEST analysis.

The BEST results (Figure 3.17) indicate that none of the environmental variables were significant drivers of the benthic community structure. The highest correlation between the biological and environmental data had an R value of 0.427 at a significance level of 0.07 (non-significant) and was associated with sediment type, primarily the percentage of sand, followed by percentage of silt and shell. This is not unexpected since most benthic fauna have preferences for particular types of sediment. Importantly, sediment type would not be affected by CVC operations beneath the lake.

Parameters
Rank correlation method: Spearman
Method: BIOENV
Maximum number of variables: 5
Resemblance:
Analyse between: Samples
Resemblance measure: D1 Euclidean distance

Variables
1 silt (%)
2 sand (%)
3 shell (%)
4 depth (m)
5 pH
6 ORP (mV)
7 turbidity (NTU)
8 DO (mg/L)

Global Test
Sample statistic (Rho): 0.427
Significance level of sample statistic: 7%
Number of permutations: 99 (Random sample)
Number of permuted statistics greater than or equal to Rho: 6

Best results

No.Vars	Corr.	Selections
1	0.427	2
2	0.414	1,2
2	0.375	2,3
3	0.354	1-3
3	0.347	1,2,5
3	0.347	1,2,7
3	0.344	1,2,4
3	0.340	1,2,6
4	0.323	1-4
4	0.320	1,2,5,7

Figure 3.17 Primer v6 BEST routine results

The only monitored environmental variable that could be affected by CVC operations is water depth (if subsidence was to occur). The BEST routine results indicate that water depth is not contributing to the observed differences in benthic community structure between sites – water depth, when combined with percentage of silt and sand, has a non-significant correlation of less than 35% of the observed differences in benthic community structure (R value 0.344).

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. 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 slightly 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.

Temporal variability is high across the monitoring period (2016–2024) at each site, regardless of the treatment type. Benthic abundances vary between sampling events but do not follow specific trajectories that might indicate a changing benthic habitat. If CVC activities were causing changes in the shallow environment in Lake Macquarie that affect the benthic communities, it might reasonably be expected that the community structure would switch relatively quickly (over a few sampling events) and develop into a different community structure that reflects the new environment. This is clearly not the case since the community structure at all sites varies through time and similarities are apparent between sampling events at different times throughout the eight-year period of monitoring. The data do not indicate a gradually (or rapidly) changing benthic environment that could be attributed to CVC operations beneath the lake.

Indeed, the best correlation (albeit non-significant) between the benthic community data and the site-specific environmental conditions is attributed to sediment type, specifically percentage of sand, silt and shell. The sediment composition of the lake bed is not associated with CVC operations and would be largely driven by the sources of sediment from the local catchment and the circulation patterns in the lake, whereby more sheltered sites accumulate finer grained sediments compared to the more exposed sites.

The high natural variability between sites and between sampling events indicates fluctuating species abundances that are likely to be driven by wider spatial and temporal drivers such as circulation patterns in Lake Macquarie, climate (wetter and/or drier periods) and associated variability in inter-year breeding success of benthic species that drive the cohort size in subsequent years.

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 furthest north are 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. Sites that have sediments with higher sand content may support different benthic communities compared to the high silt areas further south. Higher sand content is often correlated with higher water movement.

These large-scale drivers are too large to be adequately assessed by the monitoring program and are not the aim of CVC's environmental monitoring program, which necessarily targets detectable impacts of their operations at impact sites relative to control and reference sites. Awareness of natural environmental drivers for benthic community structure is important when assessing the potential impacts from activities.

Importantly, there is no indication from the benthic community data collected since 2016 that coal extraction by CVC beneath Lake Macquarie is impacting benthic communities within areas of Lake Macquarie directly above the workings.

4.2 Recommendations

Based on the statistical analysis of benthic community monitoring data, it is recommended that monitoring continues in its current form (annual sampling in autumn) to capture any annual variation in benthic assemblages that might occur following any summer temperature and/or rainfall extremes.

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.

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 (2023) *Environmental Management System, Chain Valley Colliery – Benthic Communities Management Plan*. Document ID ENV00021, 28 July 2023.

Laxton JH & Laxton ES (2024) *Lake Macquarie Benthos Survey Results No. 24.* Report prepared for Delta Coal, Mannering & CVC Collieries by JH & ES Laxton Environmental Consultants P/L, March 2024. 56pp.

Appendix A

Map of benthic sampling locations (source: Delta Coal)

Delta coal

Delta Coal
Benthic Communities
Monitoring

Mine Name: Chain Valley Colliery

Plan Name: Benthic Communities Monitoring

Drawn by: LM

Date: 28/07/2023

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Newcastle NSW 2300
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15 London Circuit
Canberra City ACT 2601

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Adelaide SA 5000
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Melbourne VIC 3000
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