

**Benthic Macroinvertebrate Monitoring
for the Goolwa Channel Water Level Management
Project, year two, and Barrage Releases within the
Coorong, Lower Lakes and Murray Mouth region**



**Final Report
for the Department of Environment and Natural Resources**

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June 2011



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1. Executive Summary

This report presents data for the second year of the Goolwa Channel Water Level Management Project (GCWLMP) and the first year following the flow from barrage releases within the Coorong and Murray Mouth region. The objectives of this study were to monitor the macroinvertebrate communities, environmental conditions and habitat quality of the sediments in the Goolwa Channel and Murray Mouth regions, in order to ascertain whether benthic recovery takes place with the restoration of freshwater flow. The study also assessed the presence of *Ficopomatus enigmaticus* by its settlement.

This report covers the survey period from December 2010 to May 2011. For the GCWLMP, six sites were sampled and for the barrage release project, nine sites. One site (Monument Road) is co-shared for the GCWLMP and barrage release surveys. For the barrage release, samples were taken from both the subtidal and nearshore areas (intertidal), with the latter mostly submerged due to the high water level. At all sites samples were taken for macrobenthos, sediment and water characteristics as described in previous reports for GCWLMP and TLM monitoring.

Salinities were below 5 ppt at most sites, and further reduced to freshwater conditions over time, in the deeper water as well. The freshening also occurred at the northern end of the North Lagoon, where it brought down the hypersaline conditions. Dissolved oxygen saturation was frequently below the recommended 90% saturation of the ANZECC guidelines and causes as well as consequences of oxygen depletion should be further investigated.

The Goolwa Channel was primarily inhabited by freshwater macroinvertebrates, dominated by insect larvae and amphipods, with few estuarine polychaetes occurring near the barrage. Abundances were significantly different across sites and decreased at most sites since commencement of the GCWLMP. Diversity was low for most of the entire study period, but a little higher around the Goolwa barrage. No distinct assemblages became apparent in the current survey period, but over the entire GCWLMP timeframe a shift in assemblages over time occurred.

The habitat in the Murray Mouth was characterised by freshwater conditions, which also penetrated into the North Lagoon. Apart from salinity, dissolved oxygen as well pH and temperature contributed to the characterisation of environmental conditions. Sediments became coarser towards the North Lagoon, which contributed to differentiation of sites by their environmental characteristics.

Environmental conditions did not change with depth of the sampling locations.

Several phases of macroinvertebrate response were apparent. Indications for an initially positive response were given by the presence of juvenile polychaetes and amphipods in December, as well as some high amphipod abundances. However, the system changed further with continued freshwater flow by a decline in macroinvertebrates in subtidal sediments while the intertidal or nearshore sediments started to be recolonised, largely by amphipods and chironomid larvae. Polychaetes shifted their distribution range towards and into the North Lagoon.

The response of macroinvertebrates was not explainable by single environmental parameters, instead several water and sediment parameter had to be considered to explain the variability in benthic data.

This highlights the relevance of a comprehensive approach to the ecosystem. The further recovery of macroinvertebrate populations and communities in the system will rely on the environmental improvement as well as species specific life histories and tolerances. The absence of the pollution indicator *Capitella* sp. from the sampling sites in the Murray Mouth can be seen as a sign of improved environmental conditions in the estuary following the flushing that accompanied the water release, or a reduced tolerance of this species to freshwater.

To assess whether the tubeworm *Ficopomatus enigmaticus* was still present under the freshwater flow conditions, an experimental analysis of their settlement was carried out as the high water level prevented access to measure their reef structures. The settlement frames were deployed in January 2011 at two sites in the Goolwa Channel, where the tubeworms occurred in early 2010, and near Pelican Point where live *F. enigmaticus* were found in December 2010. The settlement frames were retrieved monthly until April 2011, but no indication of tubeworm settlement present.

Following the overall cleansing effect of the flood for the Coorong, Lower Lakes and Murray Mouth, a return to more estuarine mixing of freshwater and oceanic water is expected to facilitate the recovery of macroinvertebrates. The reduced salinities in the Coorong have allowed refuge for macroinvertebrates needing higher salinities, and recolonisation of the Murray Mouth may thus happen from the Coorong as well as through the mouth from adjacent habitats.

2. Introduction

Reduced freshwater flow in the Murray River in past years had led to unprecedented low water levels in Lake Alexandrina and Lake Albert (South Australia), exposing acid sulphate soils in the Lower Lakes, with a particular risk of acidification in the Goolwa Channel and lower reaches of the tributaries (Finniss River and Currency Creek). The entire wetlands of the Coorong, Lower Lakes and Murray Mouth were in crisis (Lester & Fairweather 2009, Kingsford et al. 2011), with different stressors (salinity, acidity, reduced water level) prevailing in different parts of the system. Recent increase in rainfall over winter/spring 2010 and inflow of freshwater from recent flooding events along the Murray River has resulted in rising water levels breaching the regulator at Clayton and allowing the barrages to open, thus restoring connectivity to the Lower Lakes and Murray Mouth. Reconnection at the Goolwa barrage linked a freshwater system created by the Goolwa Channel Water Level Management Project (GCWLMP) since mid 2009 with the Murray Mouth region. Reconnection through the remaining barrages linked sections of Lake Alexandrina which had dried out and had partly contained MBS (monosulfidic black ooze) (Fitzpatrick et al. 2008). The habitat changes resulting from restored flow included an increase in aquatic (submerged) habitat and a decline in salinity.

Benthic macroinvertebrates are known to respond quickly to changes in water quality, sediment structure and other environmental changes (Boesch & Rosenberg 1981, Diaz & Rosenberg 1995, Ysebaert et al. 2002, Mackay et al. 2010). (Geddes 1987) described the substantial changes in abundances and distribution of macrofauna and fish in the Murray Mouth and Coorong following extended river flow in the early 1980's. Smaller water releases over the barrages in 2003 and 2003 had moderate effects on the biota in the Murray Mouth and Coorong (Geddes 2005a, Dittmann et al. 2006). The objectives of this study were to

- (i) continue monitoring effects of the GCWLMP on macroinvertebrates in the Goolwa Channel, following on from previous assessment of changes induced by the water level management (Dittmann et al. 2010b),
- (ii) monitor the macroinvertebrate communities, environmental conditions and habitat quality of the sediments between the Murray Mouth and North Lagoon of the Coorong, to ascertain whether benthic recovery takes place in response to the restored flow creating estuarine or freshwater conditions in the Murray Mouth, and
- (iii) look at the response of *Ficopomatus enigmaticus* to the reduced salinity levels in the Goolwa Channel and Murray Mouth region.

In the Goolwa Channel, a subset of sites from previous monitoring was continued to be sampled for macroinvertebrates. For the barrage release, the composition and abundance of benthic macroinvertebrate assemblages was sampled over several months as the environmental conditions in the system changed. Following continued heavy rainfall in the catchment and water availability, the water release over the barrages turned into a regulated flood passing through the barrages. Not only were the overall volumes of freshwater released much higher than initially anticipated, the duration of the release period also exceeded the timeframe known at the start of the project.

The spread of *F. enigmaticus* into Lake Alexandrina and the Goolwa Channel resulted from a long drought and a decrease in water levels with subsequent increased salinity through the leakage of seawater at the Goolwa Barrage in 2007. This allowed *F. enigmaticus* larvae to enter parts of Lake Alexandrina which they quickly colonised (Dittmann et al. 2009). When surveys were conducted during 2010, live worms were found in the Goolwa Channel in high densities, just adjacent to the Clayton regulator (Benger et al. 2010, Dittmann et al. 2010b). With the restored connectivity between the Lower Lakes, Goolwa Channel and Murray Mouth region, the response of *F. enigmaticus* to lower salinities is of particular concern, with the possibility that the established adult aggregations could have adapted to lower salinity and their larvae could potentially spread.

While the barrages opened in late August, contractual delays did not allow sampling to commence until December. This report covers the second year of the Goolwa Channel Water Level Management Project (GCWLMP), and the first response to the barrage releases within the Coorong and Murray Mouth region between December 2010 and April 2011. The report presents data for the December 2010, February and April 2011 sampling in the GCWLMP, and the December 2010, and February, March and April 2011 sampling events for the barrage release project. Results are presented separately for the GCWLMP and barrage release project.

3. Materials and Methods

3.1 Sampling sites and dates

Benthic macroinvertebrate fauna were sampled during December 2010 and February and April 2011 for the GCWLMF at six sites within the Goolwa Channel region (Table 1, Appendix 1), continued from the previous monitoring (Dittmann et al. 2010b). Sites included Clayton West (CW: site 2), mouth of the Finniss (MF: site 4), Currency Creek (CC: site 7), Goolwa Channel (GC: site 9) and the two control sites which were located on the eastern side of the Clayton weir (CE: site 1) and on the Coorong side of the Goolwa barrage at Monument Road (MR: site 10) (Figure 1). Due to the high water level, the exact sampling locations at the sites moved further inshore.

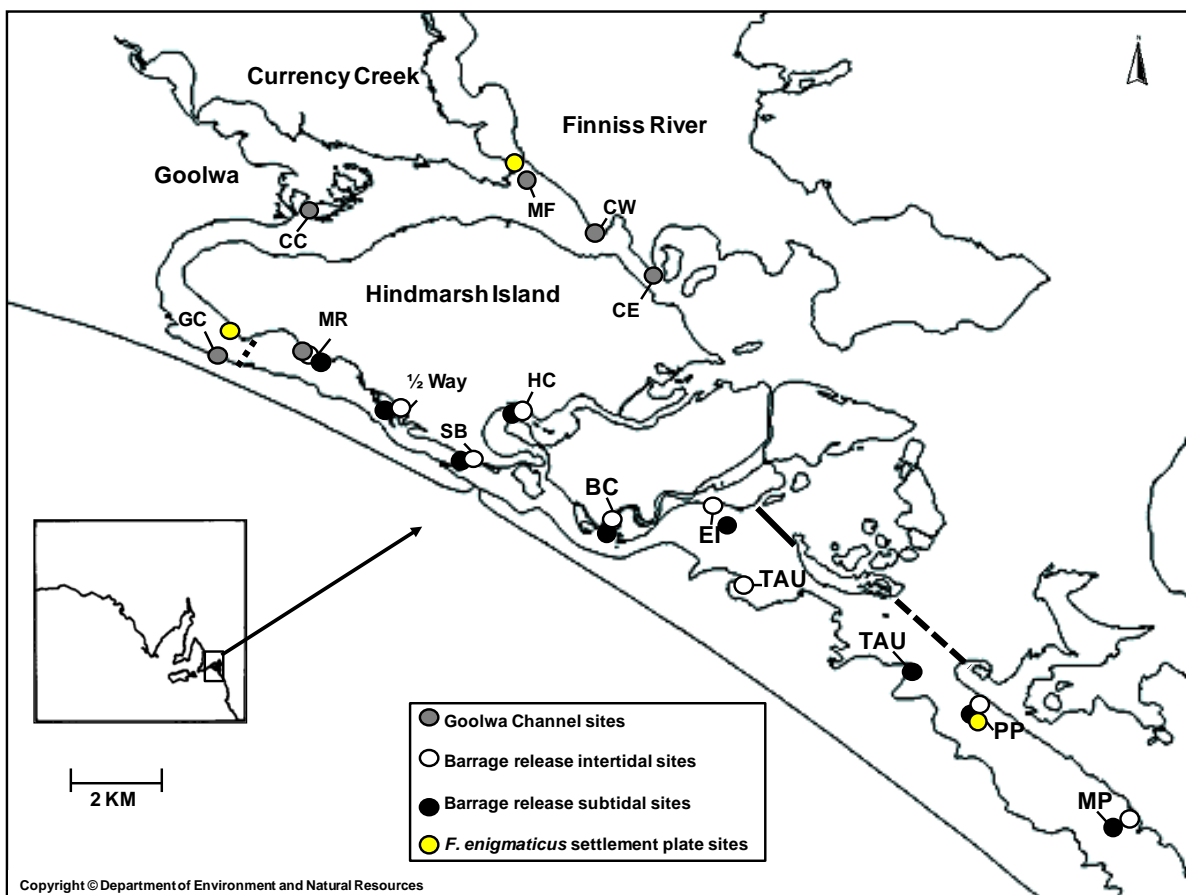


Figure 1: Study site locations in the Goolwa Channel, Lower Lakes and Murray Mouth region for the Goolwa Channel and barrage release projects. Sites include Clayton East (CE: site 1), Clayton West (CW: site 2), Mouth of the Finniss (MF: site 4), Currency Creek Mouth (CC: site 7), Goolwa Channel (GC: site 9), Monument Road (MR: site 10), Half Way (½ Way), Sugars Beach (SB), Hunters Creek (HC), Boundary Creek (BC), Ewe Island (EI), Tauwichee (TAU), Pelican Point (PP) and Mark Point (MP). Goolwa Barrage = Black dotted line, Ewe Island Barrage = Black line, Tauwichee Barrage = Black dashed line.

For the barrage release survey, benthic macroinvertebrate fauna were sampled during December 2010, and February, March and April 2011 at nine sites located throughout the Murray Mouth and into the North Lagoon of the Coorong (Table 1, Appendix 1). Sites included Monument Road (MR), Half Way (½ Way), Sugars Beach (SB), Hunters Creek (HC), Boundary Creek (BC), Ewe Island (EI), Tauwichee (TAU), Pelican Point (PP) and Mark Point (MP). At all of these sites, samples were taken at two water depths, from the nearshore intertidal and subtidal (Figure 1). As water levels were high

and tides not well pronounced, many of the intertidal sites were not exposed mudflats and had to be accessed by boat.

The settlement of *Ficopomatus enigmaticus* was investigated from February to April 2011 at three sites where *F. enigmaticus* were previously known to occur (Benger et al. 2010), or live ones seen in December 2010 (Pelican Point). Sites were mouth of the Finniss River (MF), west of the Goolwa Channel barrage (GC) and Pelican Point (PP) (Figure 1).

Table 1: Environmental, sediment and biological samples collected for the GCWLMF and barrage release survey during December (D) 2010, and February, (F), March (M), April (A) 2011. See Appendix 1 for detail on sampling dates and locations.

Goolwa Channel	Water Characteristics	Chlorophyll-a	Sediment Characteristics	Abundance	Biomass
East of Clayton Regulator (CE) - Site 1	D F A	D F A	D A	D F A	D A
West of Clayton Regulator (CW) - Site 2	D F A	D F A	D A	D F A	D A
Mouth of Finniss (MF) - Site 4	D F A	D F A	D A	D F A	D A
Mouth of Currency Creek (CC) - Site 7	D F A	D F A	D A	D F A	D A
Goolwa Barrage (GB) - Site 9	D F A	D F A	D A	D F A	D A
Monument Road (MR) - Site 10	D F A	D F A	D A	D F A	D A

Barrage Release	Water Characteristics	Chlorophyll-a	Sediment Characteristics	Abundance	Biomass
Monument Rd (MR) - Intertidal	D F M A	D A	D A	D F M A	D F A
Subtidal	D F M A	A	D A	D F M A	D F A
1/2 Way - Intertidal	D F M A	D A	D A	D F M A	D F A
Subtidal	D F M A	A	D A	D F M A	D F A
Sugars Beach (SB) - Intertidal	D F M A	D A	D A	D F M A	D F A
Subtidal	D F M A	A	D A	D F M A	D F A
Hunters Creek (HC) - Intertidal	D F M A	D A	D A	D F M A	D F A
Subtidal	D F M A	A	D A	D F M A	D F A
Boundary Creek (BC) - Intertidal	D F M A	D A	D A	D F M A	D F A
Subtidal	D F M A	A	D A	D F M A	D F A
Ewe Island (EI) - Intertidal	D F M A	D A	D A	D F M A	D F A
Subtidal	D F M A	A	D A	D F M A	D F A
Tauwitchere (TAU) - Intertidal	D F M A	D A	D A	D F M A	D F A
Subtidal	D F M A	A	D A	D F M A	D F A
Pelican Point (PP) - Intertidal	D F M A	D A	D A	D F M A	D F A
Subtidal	D F M A	A	D A	D F M A	D F A
Mark Point (MP) - Intertidal	D F M A	D A	D A	D F M A	D F A
Subtidal	D F M A	A	D A	D F M A	D F A

3.2 Environmental parameters

At each site, water quality parameters including temperature (°C), salinity (ppt), oxygen concentration (O₂ mg/l) and saturation (O₂ %) of the water overlying the mudflats were measured monthly using both a TPS WP -81 (for pH, temperature and salinity) and WP-82Y (for dissolved oxygen) electrode (Table 1). Due to concerns about the accuracy of the oxygen electrode, additional measurements were

carried out with an YSI 85 handheld electrode at most sites and sampling dates. pH of the overlying water was also recorded using the TPS WP-81 electrode.

To characterise the sedimentary environment for benthic organisms, grain size, organic matter and chlorophyll-*a* were sampled in December 2010 and April 2011 for both projects, taking three replicates of each parameter per site (Table 1). Sediment samples for grain size were taken with a 60 ml cut-off syringe and for organic matter using a 3 ml cut-off syringe. Samples of the sediment parameters were stored on ice in the field, and later frozen when the samples were returned to the lab until further analysis. For sediment chlorophyll-*a*, three replicate samples were taken per site by inserting a 5 ml vial about 1 mm into the sediment. 5 ml of methanol were added to extract the chlorophyll, and the vial was heavily shaken before being wrapped in aluminium foil and frozen for later analysis.

3.3 Macrofauna

To investigate macroinvertebrate species composition and abundance within sediments, handheld PVC corers (83.32 cm² surface area) were used for intertidal sediments at each site. For each of the land-based sites that were intertidal or under shallow water, ten haphazardly placed replicate samples were taken. Each replicate core was inserted into the sediment to approximately 15 cm depth, sealed with a stopper to avoid disturbance of the sample and dug out with a shovel or by hand. For the barrage release study, subtidal locations within each site were located approximately 50 m towards the deeper channel from the intertidal location. Due to the changing water levels over time, the amount of water overlying subtidal locations varied between 50 cm to over 2 m in depth. When the overlying water was deeper than 1 m, submerged sites were sampled with a benthic Ekman grab (225 cm² surface area), deployed from a small boat. The grab penetrated approximately 10 cm into the sediment. When water levels were below 1 m, subtidal locations were sampled using the PVC corers (83.32 cm² surface area) in the vicinity of the boat. In the Goolwa Channel, sediments at the Mouth of the Finniss and in Currency Creek were also sampled by Ekman grab. All samples were sieved through a 500 µm mesh size *in situ*. Sorting of live samples was carried out within 1-5 days and organisms were identified to the lowest taxonomic level possible and individual numbers for each species counted. Amphipods were not differentiated into family or species. For insects, larval and pupae life stages encountered during sorting were noted in macrobenthic analyses, but not counted separately for chironomids.

For each sample, specimens of all phyla were preserved together to obtain a biomass value for all macroinvertebrates per sample. They were dried in an oven at 80 °C until constant dry weights (d.w.) were achieved (at least 24 hours). Samples were then placed in a muffle furnace at 450 °C for 5 hours. Samples were removed from the furnace and left to cool in a dessicator before final weighing. The weight after burning is subtracted from the dry weight to obtain biomass as ash free dry weight (AFDW).

Juvenile macrobenthic samples were taken using a 60 mL syringe with cut off end (surface area 7.07 cm²), inserted approximately two centimetres into the sediment, following the procedure used by Rolston and Dittmann (2009) and Dittmann et al. (2010a). Ten replicate samples were taken at each

location around the water edge. For submerged sites a 60 mL syringe was used to extract samples from the top layer of the sediment collected by the Ekman grab. Samples were individually stored in jars and transported to the lab for processing. Due to the large number of samples, juvenile samples that could not be processed within two days were frozen and thawed for further analysis. In the laboratory, samples were sieved through 250 μm mesh and repeatedly washed in freshwater and decanted five times to separate organisms from sediments. The sieved sample was caught in a beaker and sieved through a 100 μm mesh. All juveniles remaining in both sieves were sorted under the microscope and specimens identified to species level where possible.

Three replicate *F. enigmaticus* settlement frames were deployed (top of the frame was approximately 30 cm under the water surface) at GC, MF and PP. The frame design followed the experiments by Rolston (see Dittmann et al. 2009) which consisted of a 81 cm x 58 cm PVC frame deployed 30 cm below the water surface (Figure 2). Tiles were randomly selected to be replaced after one month (Tile number 2, 4 and 8) and two month intervals (Tile number 3, 7, and 9). All remaining tiles were left on for the full three month period. At the end of each sampling period the individual tiles were photographed (Olympus MJU- 8000) and analysed for settlement of any *F. enigmaticus*.

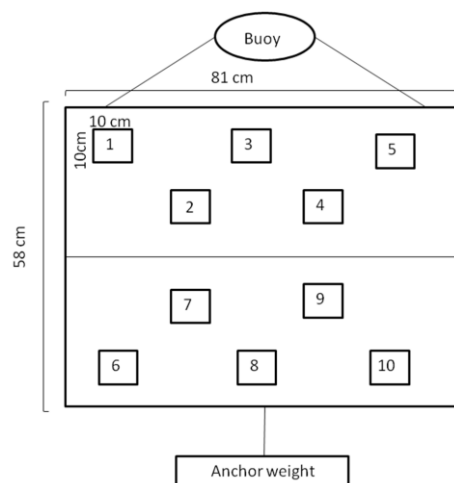


Figure 2: Diagram of *F. enigmaticus* settlement plates deployed for three months at sites Mouth of the Finniss (MF), Goolwa Channel (GC) and Pelican Point (PP).

3.4 Data analysis

The following diversity indices for macroinvertebrates were calculated; total species number (S), Shannon-Wiener diversity (H') and Pielou's index for equitability (J'), using PRIMER v6 software.

Differences in macroinvertebrate abundances of the major taxa (Annelida, Crustacea and Insecta) and total biomass between Goolwa Channel monitoring sites and sampling months were tested by permutational analysis of variance using a two-way PERMANOVA on fourth-root transformed data with a Bray-Curtis similarity matrix, dummy value (+ 1) due to many zero values, and with 9999 permutations. The factors tested in PERMANOVA were 'site' (a fixed factor with six levels) and 'month' (a fixed factor with either two or three levels, biomass was not sampled in February). Differences in the benthic assemblage data between sites and months was then tested using a two-way

PERMANOVA on fourth-root transformed assemblage data. Non-parametric multi-dimensional scaling (MDS) ordination plots were used to display differences in macroinvertebrate assemblages between the six sites (CE, CW, MF, CC, GC, and MR) and across the three months (December 2010, February and April 2011). Similarities of macroinvertebrate assemblages between the six sites in the present survey (December 2010 – April 2011), as well as between months from the previous year's survey between August 2009 and August 2010, were explored with the traditional cluster analysis, whereby a similarity profile test (SIMPROF) was applied to differentiate statistically significant and genuine clusters in *a priori* unstructured data sets. Macroinvertebrate data were again fourth-root transformed prior to analysis and a dummy value of 1 added for Bray-Curtis similarity because of too many zero values in the data sets. Principal coordinate analysis (PCO) was used instead of nMDS to better illustrate species contributing to the principal coordinates differentiating sites, illustrated by overlying vectors (Spearman correlation) in the PCO plots. ANOSIM tests were carried out to further explore differentiation of assemblages.

Differences in the abundances of the total macroinvertebrates, major taxa (Annelida, Crustacea, Mollusca and Insecta) and individual taxa between barrage release monitoring sites and months were tested using a three-way PERMANOVA. The three factors tested in PERMANOVA were 'sites' (a fixed factor with nine levels), 'depth' (a fixed factor with two levels: intertidal and subtidal) and 'month' (a fixed factor with four levels: December 2010, and February, March and April 2011). Differences in total macroinvertebrate biomass between barrage release monitoring sites and sampling months, was also tested using a three-way PERMANOVA, with 'site', 'month' and 'depth' as factors, however only across December 2010, and February and April 2011.

Principle Coordinate plots (PCO) were used to display differences in macroinvertebrate assemblages across the barrage release monitoring sites in December 2010, and February, March and April 2011. Vector overlays depict species that are important for the assemblage patterns. Differences in the macroinvertebrate assemblage data between sites, months and depths were then tested by using a three-way PERMANOVA. The two factors tested were 'site', 'month' and 'depth'.

Differences in environmental conditions, including sediment organic matter content, chlorophyll-*a*, salinity (ppt), temperature (°C), oxygen saturation (O₂ %) and content (O₂ mg/L) and pH were tested separately using PERMANOVA with two factors 'site' and 'month' for samples taken in the Goolwa Channel and with three factors 'site', 'month' and 'depth' for samples taken in the barrage release project. Similarity of sampling sites based on several of the measured environmental factors was explored with Principle component analysis (PCA). Environmental data had been transformed as needed (log transformation for salinity and grain size fractions) and square root for pH, temperature and chlorophyll-*a*) and were further normalised for PCA. Euclidean distance was used prior to further PERMANOVA analysis on differences between the factors as above. Chlorophyll-*a* was omitted from the analysis as not measured for subtidal sites in December. To explore links between macroinvertebrate assemblages and environmental data, distance-based linear models (DISTLM) were calculated and visualised using distance-based redundancy analysis (dbRDA).

4. Results

4.1 Goolwa Channel

General observations

The high water levels in the Goolwa Channel persisted since mid 2010 and the continued freshwater conditions led to a regrowth of reed along the shores, which made access to the sedimentary sampling sites difficult. During the sampling events, little bird life was noticed at the study sites.

4.1.1 Environmental parameters

The flow of the Murray River into the Lower Lakes led to freshwater conditions prevailing across all sites over time, yet a particular increase in salinity was noticed at Goolwa Channel (GC) during December 2010 (19.50 ppt \pm 0.10 S.E., Figure 3). Water temperatures showed little variability across sites and reflected seasonal temperature variations (Figure 3). Dissolved oxygen concentrations fluctuated over time at each site and were lowest (\sim 4 mg/L) at Clayton West (CW) and Monument Road (MR) during December 2010. Fluctuations in oxygen saturation were also observed at several

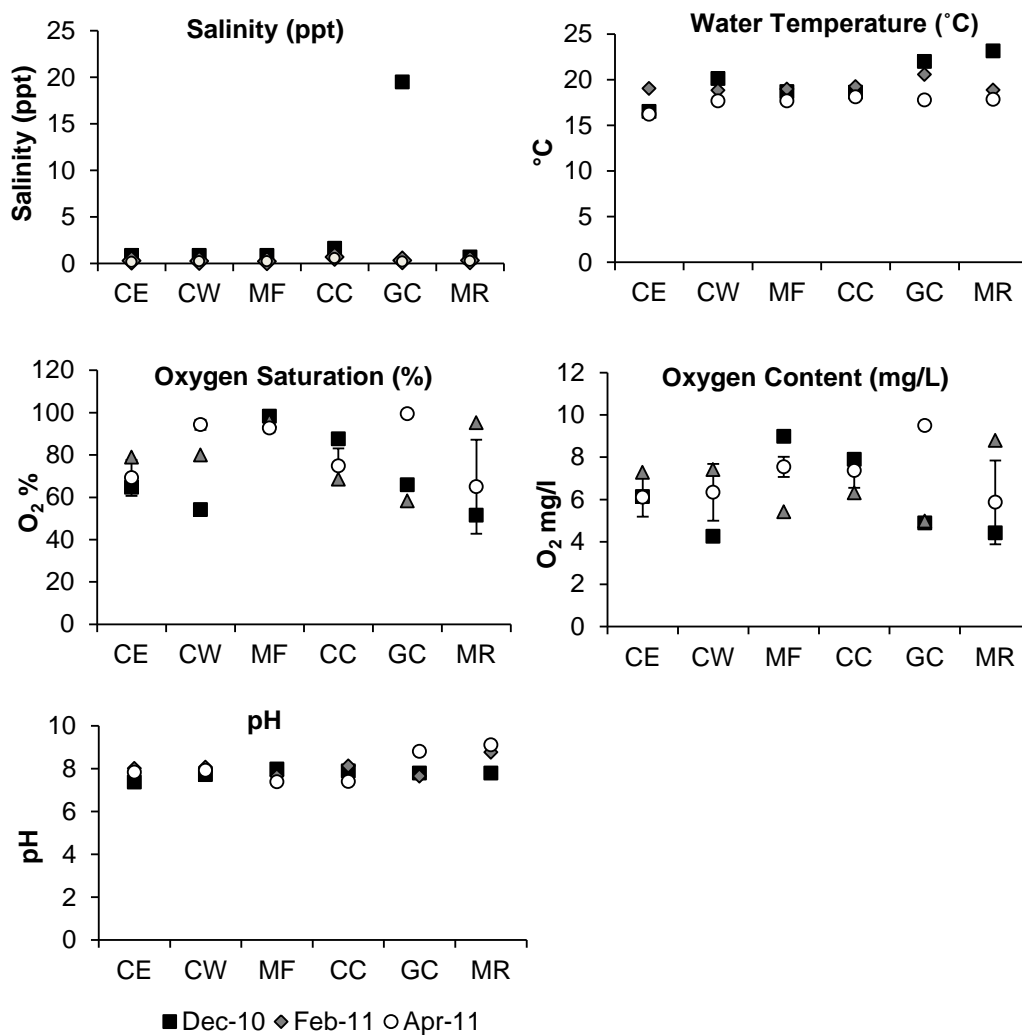


Figure 3: Average water parameters (\pm S.E.); salinity (ppt), temperature ($^{\circ}$ C), oxygen saturation (O_2 %) and content (O_2 mg/L) and pH across the six monitoring sites (CE, CW, MF, CC, GC, MR, see Figure 1) and three sampling events (December 2010, and February and April 2011).

sites including MR, GC and CW (Figure 2), but were below the 90 % threshold of the ANZECC guidelines at almost all sites and dates. For pH, levels were neutral across all sites and did not vary much. There were significant differences across sites and sampling months for each of the environmental variables (Figure 3, Table 2).

The significant interaction terms for site and month for all water parameters measured (Table 2) indicates very site specific changes in environmental conditions in the Goolwa Channel and adjacent control sites.

Table 2: Results from PERMANOVA analyses on environmental parameters including salinity (ppt), water temperature (°C), oxygen saturation (O₂ %), oxygen content (O₂ mg/l) and pH recorded in samples of the six sites of the Goolwa Channel across months (December 2010, and February and April 2011). Significant P-values are highlighted in bold.

	Source	df	MS	Pseudo-F	P (perm)
ppt	Site	5	5.9	201.09	0.0001
	Month	2	16.1	552.27	0.0001
	Site x Month	10	4.9	168.32	0.0001
	Residual	75	0.0		
°C	Site	5	8.3	203.73	0.0001
	Month	2	1.4	34.94	0.0001
	Site x Month	10	4.7	114.58	0.0001
	Residual	75			
O₂ %	Site	5	2.3	3.46	0.007
	Month	2	1.8	2.75	0.0764
	Site x Month	10	2.5	3.81	0.0003
	Residual	75	0.7		
O₂ mg/l	Site	5	0.7	0.94	0.4593
	Month	2	1.3	1.78	0.1782
	Site x Month	10	3.3	4.59	0.0002
	Residual	75	0.7		
pH	Site	5	7.0	270.24	0.0001
	Month	2	0.0	0.43	0.6505
	Site x Month	10	1.7	64.80	0.0001
	Residual	36	0.0		

Chlorophyll-a

The average chlorophyll-a content across all sites in December 2010 was 1.03 mg m⁻², and 0.50 mg m⁻² in April 2011. Chlorophyll-a content varied significantly between sites and sampling months ($F_{5,24} = 4.284$, $P < 0.05$), with high variation at Clayton East (CE) between months (Figure 4).

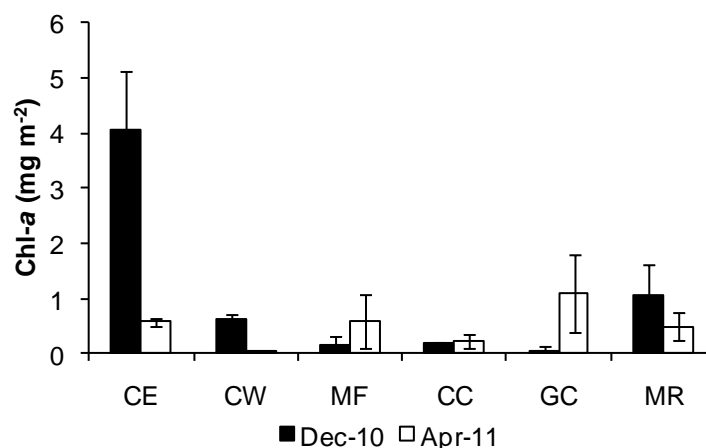


Figure 4: Average sediment chlorophyll-a content (mg.m⁻², ± S.E.) at the study sites surveyed in the Goolwa Channel during December 2010 and April 2011.

Sediment Grain Size

Sediments sampled in the Goolwa Channel were moderately well to very well sorted with a fine sand consistency towards the Murray Mouth and medium to coarser sand towards Clayton and the Finniss River (Table 3) in both sampling months. Sites within the Murray Mouth area including site Currency Creek (CC), Goolwa Channel (GC) and Monument Road (MR) contained very fine sand particles (125-250 µm, Figure 5), while sediments at Clayton West (CW) and mouth of the Finniss (MF) had a more prominent medium sand fraction (250-500 µm). Clayton East (CE) was the only site that mostly contained very coarse sand particles (>1000 µm, Figure 5).

Table 3: Sediment characteristics of the Goolwa Channel during the sampling months of December 2010 and April 2011. The median grain size of sediment in µm along with the sorting coefficient, are provided as characteristics of mudflat sediment. The verbal description of sediment grain size and sorting follows (Blott & Pye 2001).

Site	December 2010					April 2011			
	Median			Sorting		Median		Sorting	
1 CE	1636.93	Very Coarse Sand	0.17	Very Well Sorted	1651.64	Very Coarse Sand	0.03	Very Well Sorted	
2 CW	429.87	Medium Sand	0.61	Moderately Well Sorted	333.98	Medium Sand	0.64	Moderately Well Sorted	
4 MF	266.19	Medium Sand	0.38	Well Sorted	186.18	Fine Sand	0.1	Very Well Sorted	
7 CC	211.44	Fine Sand	0.4	Well Sorted	190.86	Fine Sand	0.59	Moderately Well Sorted	
9 GC	189.56	Fine Sand	0.6	Moderately Well Sorted	218.8	Fine Sand	0.34	Very Well Sorted	
10 MR	168.15	Fine Sand	0.22	Very Well Sorted	208.63	Fine Sand	0.51	Moderately Well Sorted	

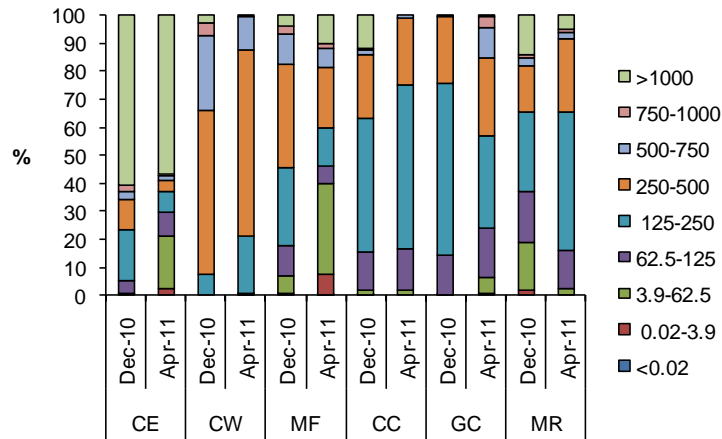


Figure 5: Sediment grain size composition (% of major fractions, size in µm) at the sites in the Goolwa Channel during the December 2010 and April 2011 surveys.

Sediment Organic Matter

Average sediment organic matter was 3.22 % (dw) in December 2010 and 0.54 % (dw) in April 2011 (Figure 6). Significantly higher values were recorded at Clayton East (CE) in December (13.91 ± 3.51 % dw), supported by a significant site and month interaction ($F_{5,24} = 20.994$, $P < 0.001$). Yet in April, CE recorded the lowest sediment organic matter on average (0.02 % dw).

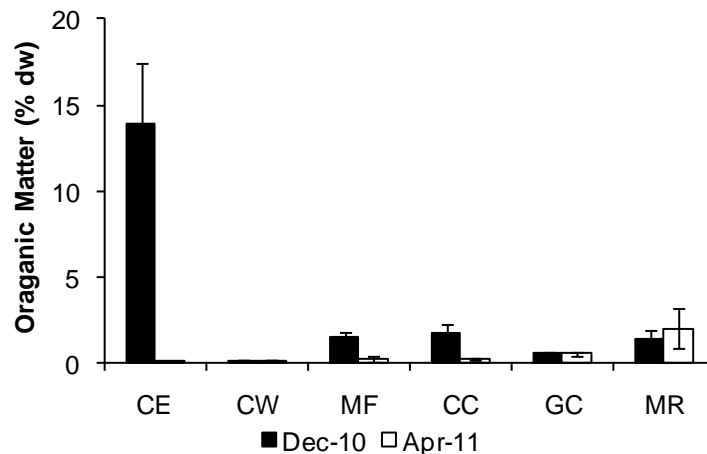


Figure 6: Sediment organic matter (% dry weight) at study sites of the Goolwa Channel in December 2010 and April 2011.

4.1.2 Macroinvertebrate diversity

A total of 22 macroinvertebrate taxa were recorded during the Goolwa Channel project, with insect larvae and pupae accounting for a total of 13 taxa. Annelids were represented by four species and were present at all sites and months, with the exception of Clayton East (CE) (Table 4). Crustaceans and arachnids were each represented by three and two species, respectively (Table 4), with the Amphipoda crustaceans found at all sampling sites and months, with the exception of sites CE and Goolwa Channel (GC) in February 2011. Chironomid larvae and pupae were found throughout the Goolwa Channel at all sites and sampling periods except at site Currency Creek (CC) during February 2011 (Figure 7, Table 4).

Table 4: Occurrence of macrobenthic taxa and species numbers during the December 2010, and February and April 2011 survey months across the six monitoring sites including Clayton East (CE: site 1), Clayton West (CW: site 2), Mouth of the Finnis (MF: site 4), Currency Creek Mouth (CC: site 7), Goolwa Channel (GC; site 9) and Monument Road (MR: site 10).

PHYLA	SPECIES	CE (1)			CW (2)			MF (4)			CC (7)			GC (9)			MR (10)		
		D	F	A	D	F	A	D	F	A	D	F	A	D	F	A	D	F	A
Annelida	<i>Simplisetia aequisetis</i>												X				X	X	X
	<i>Australoneis ehlersi</i>																	X	
	<i>Boccardiella limnicola</i>						X	X	X		X		X						
	Oligochaeta				X			X	X	X	X		X					X	
Crustacea	Amphipoda	X		X	X	X	X	X	X	X	X	X	X		X		X	X	X
	Ostracoda	X																	
	Unidentified crustacean														X				
Insecta	Zygoptera sp.	X									X								
	Water boatman Larvae	X																	
	Hydrophilidae	X			X	X													
	Ceratopogonidae Larvae												X	X	X				
	Chironomid (Larvae + pupae)	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X
	Culicidae													X					
	Staphylinidae				X														
	Leptophlebiidae													X					
	Nymphulinae							X											
	Microcaddis Larvae										X								
	Tipulid larvae																	X	
	Winged insect indet.				X														
Unidentified insect pupa											X								
Arachnida	Spider pisauridae		X																
	Pseudoscorpion									X									
Species number per sampling event		6	2	2	6	3	3	5	4	4	6	2	2	8	2	4	5	4	3
Species number per site		7			7			6			7			9			6		

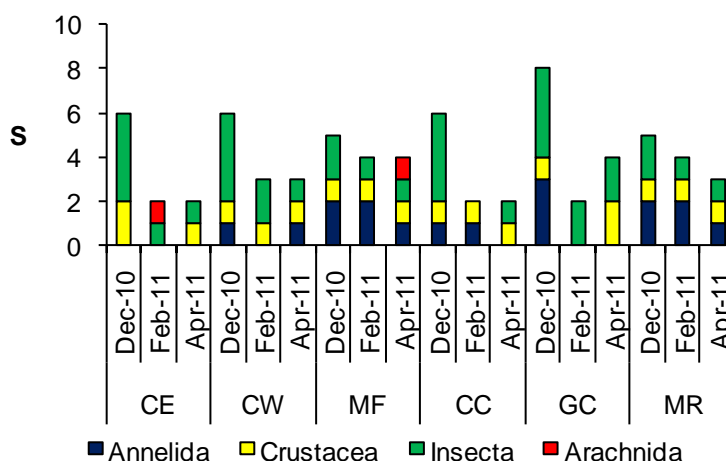


Figure 7: Total species number of major phyla of benthic macroinvertebrates identified at each sampling site in December 2010, and February and April 2011.

Diversity, as measured by the Shannon-Wiener index H' , was consistently low at all sites, due to the low species numbers and dominance of one or two species, in particular amphipods and chironomid larvae (Figure 7, Table 5). While the dominance of these taxa were still pronounced, the occurrence of other species lead to slightly higher species numbers in December 2010 (Figure 7).

Over the entire study period of the GCWLMP since August 2009, species numbers were increased around August 2010 due to various different insect species, but decreased again since (Figure 8).

Average diversity over the study period was about $H'=1$ between Clayton east and Currency Creek, and about $H'=1.4$ on either side of the Goolwa barrage, yet variations in diversity were high at several sites (Figure 9).

Table 5: Diversity (Shannon-Wiener index H') and Pielou's evenness index (J) of macroinvertebrates in December 2010, February and April 2011 across the six monitoring sites.

Site	H' (Shannon-Wiener)			J (Evenness)		
	Dec-10	Feb-11	Apr-11	Dec-10	Feb-11	Apr-11
CE	0.64	0.22	0.45	0.36	0.32	0.65
CW	1.14	0.47	0.97	0.64	0.43	0.88
MF	0.70	0.66	1.10	0.44	0.47	0.79
CC	0.79	0.63	0.04	0.44	0.91	0.05
GC	0.89	0.13	0.63	0.43	0.19	0.45
MR	1.10	0.76	0.93	0.68	0.54	0.85

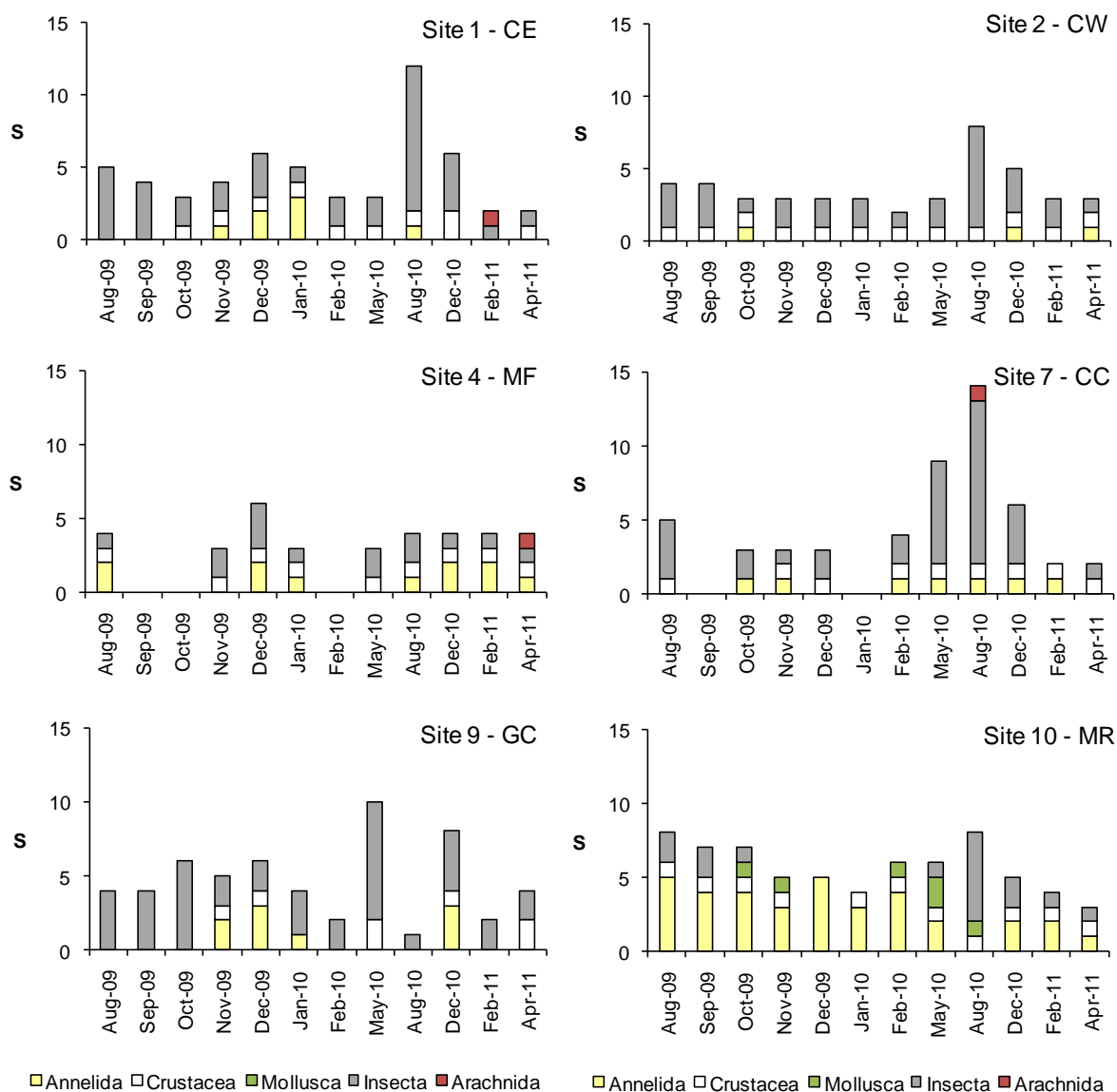


Figure 8: Species numbers per taxa found at each of the six Goolwa Channel monitoring sites (CE, CW, MF, CC, GC, MR) between August 2009 and April 2011.

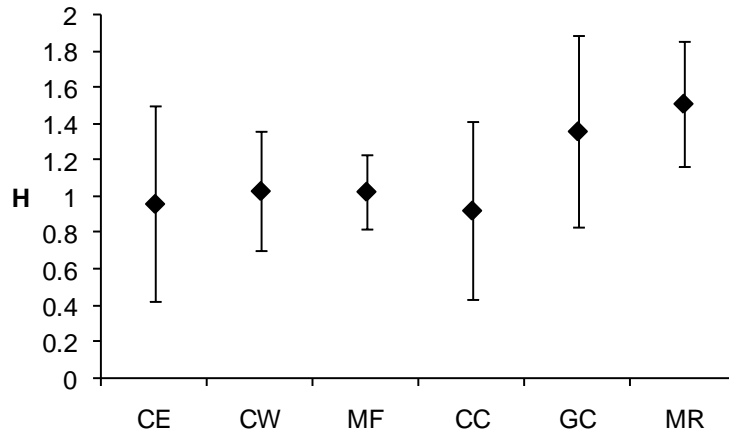


Figure 9: Average diversity (Shannon-Wiener diversity (H')) (± S.D.) from August 2009 – April 2011 at each of the six Goolwa Channel monitoring sites (CE – site 1, CW – site 2, MF – site 4, CC – site 7, GC – site 9, MR – site 10).

4.1.3 Macroinvertebrate abundances and distribution

Macroinvertebrate abundances were low and variable, with the minimum average $13.33 \pm 21.47 \text{ ind.m}^{-2}$ recorded at site CC (Currency Creek) during February and the maximum average $4908.79 \pm 5796.50 \text{ ind.m}^{-2}$ at site GC (Goolwa Channel) during December (Figure 10). Total macroinvertebrate abundances were higher during the December sampling month and decreased across the study period (Figure 10, Table 6). Abundances of annelids (polychaetes and oligochaetes) were higher closer to the Goolwa barrage and at Monument Road (control site in the Murray Mouth region, MR), yet decreased over the three sampling periods (Figure 11). Amphipods dominated at the mouth of the Finniss (MF) and on the inside of the Goolwa barrage during December 2010, decreasing throughout the sampling period at most sites except CC (Figure 11). Insect larvae (Chironomids) were abundant throughout the Goolwa Channel monitoring sites, less so at MR, the control site, but were also seen to decrease in abundance over the sampling period (Figure 11). A significant site and month interaction was obtained (Table 6) for total benthos, annelids, crustaceans and insects, which indicates that abundances varied at each site over the three sampling times.

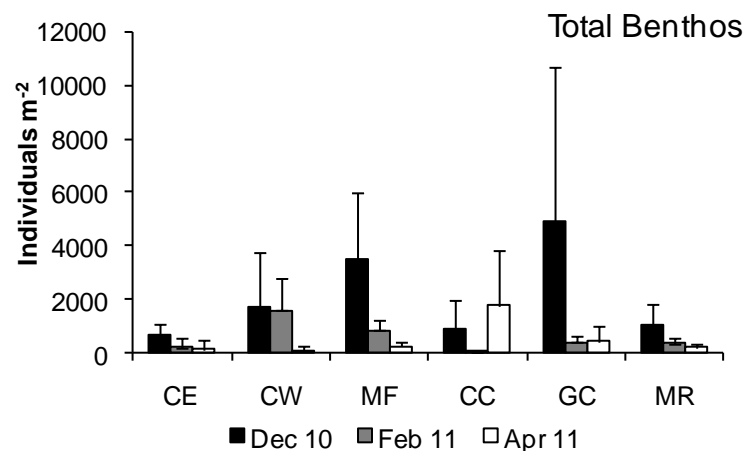


Figure 10: Average abundances (individuals m⁻²; ± S.D., n=10) of all macroinvertebrates at each of the six monitoring sites (CE, CW, MF, CC, GC, MR) in the Goolwa Channel in December 2010, and February and April 2011.

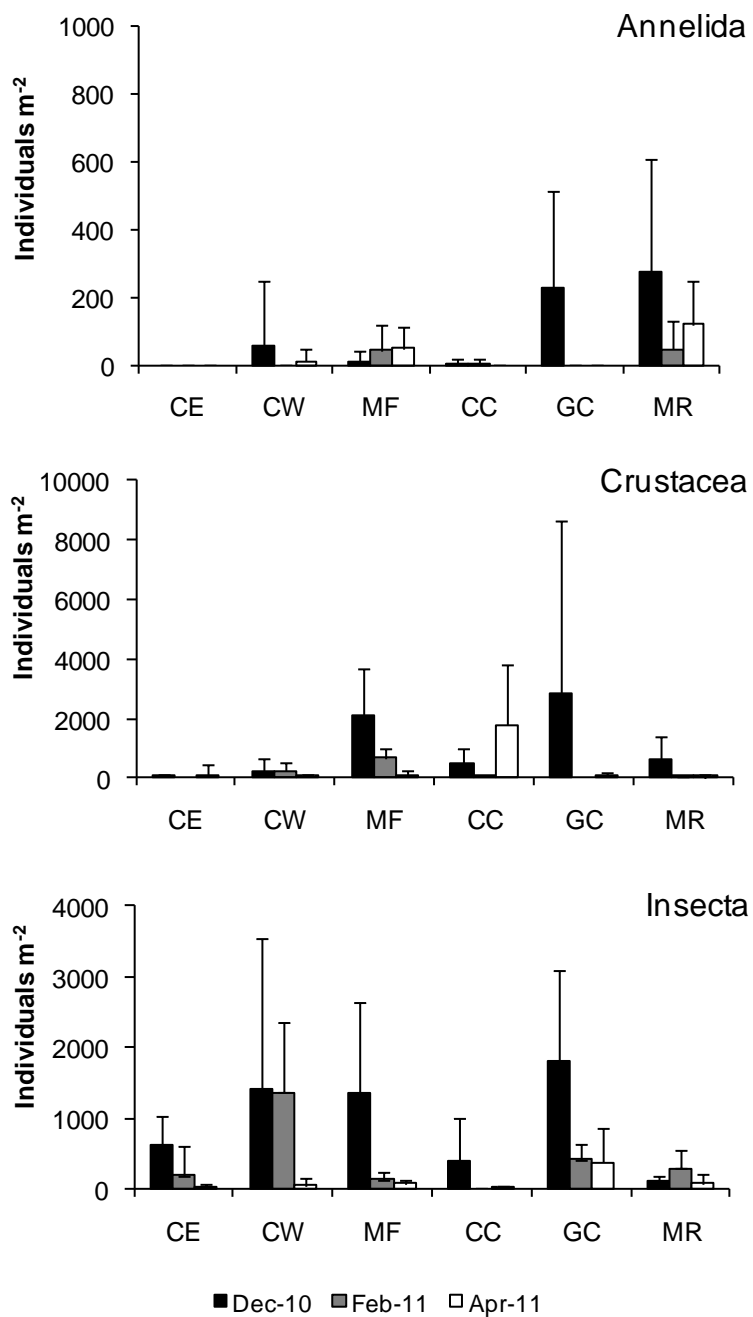


Figure 11: Average abundances (individuals m^{-2} ; \pm S.D., $n=10$) of the major taxa (Annelids, Crustaceans and Insects) at each of the six monitoring sites (CE, CW, MF, CC, GC, MR) in the Goolwa Channel in December 2010 and February and April 2011. Note the different scales of the y-axis.

Table 6: Results from PERMANOVA analyses for the total macroinvertebrate abundances as well as abundances of major phyla, with 'site' and 'month' as factors. Significant P-values are highlighted in bold.

	Source	df	MS	Pseudo-F	P (perm)
Total Benthos	Site	5	4169.2	8.33	0.0001
	Month	2	11351.0	22.69	0.0001
	Site x Month	10	4248.9	8.49	0.0001
	Residual	162	500.3		
Annelida	Site	5	4840.0	9.98	0.0001
	Month	2	1348.9	2.78	0.642
	Site x Month	10	1332.1	2.75	0.0036
	Residual	162	485.1		
Crustacea	Site	5	11277.0	17.18	0.0001
	Month	2	10867.0	16.55	0.0001
	Site x Month	10	3949.8	6.01	0.0001
	Residual	162	656.3		
Insecta	Site	5	7224.6	12.28	0.0001
	Month	2	23803.0	40.46	0.0001
	Site x Month	10	2437.9	4.14	0.0001
	Residual	162	588.3		

The macroinvertebrates in the Goolwa Channel had been monitored over the GCWLMP project since August 2009 (Dittmann et al. 2010b) and abundances can be compared over the entire timeframe since water level management began, for those sites continued to be sampled in the second year. Abundances of total macroinvertebrates were significantly different over all months sampled since August 2009 and between sites (Table 7). Apart from occasional high abundances of amphipods or chironomid larvae (being the main insect found) at various sites, a general decrease in abundances appears at all sites, including the two 'control' sites at Clayton east (CE) and Monument Road (MR) which are now reconnected with the Goolwa Channel (Figure 12).

Table 7: Results from PERMANOVA analysis for the total macroinvertebrate abundances at the six sites (CE, CE, MF, CC, GC, MR) between August 2009 and April 2011. Significant P-values are highlighted in bold.

Source	df	MS	Pseudo-F	P (perm)
Site	5	6432.0	20.75	0.0001
Month	11	15677.0	50.59	0.0001
Site x Month	52	2167.7	6.99	0.0001
Residual	771	309.8		

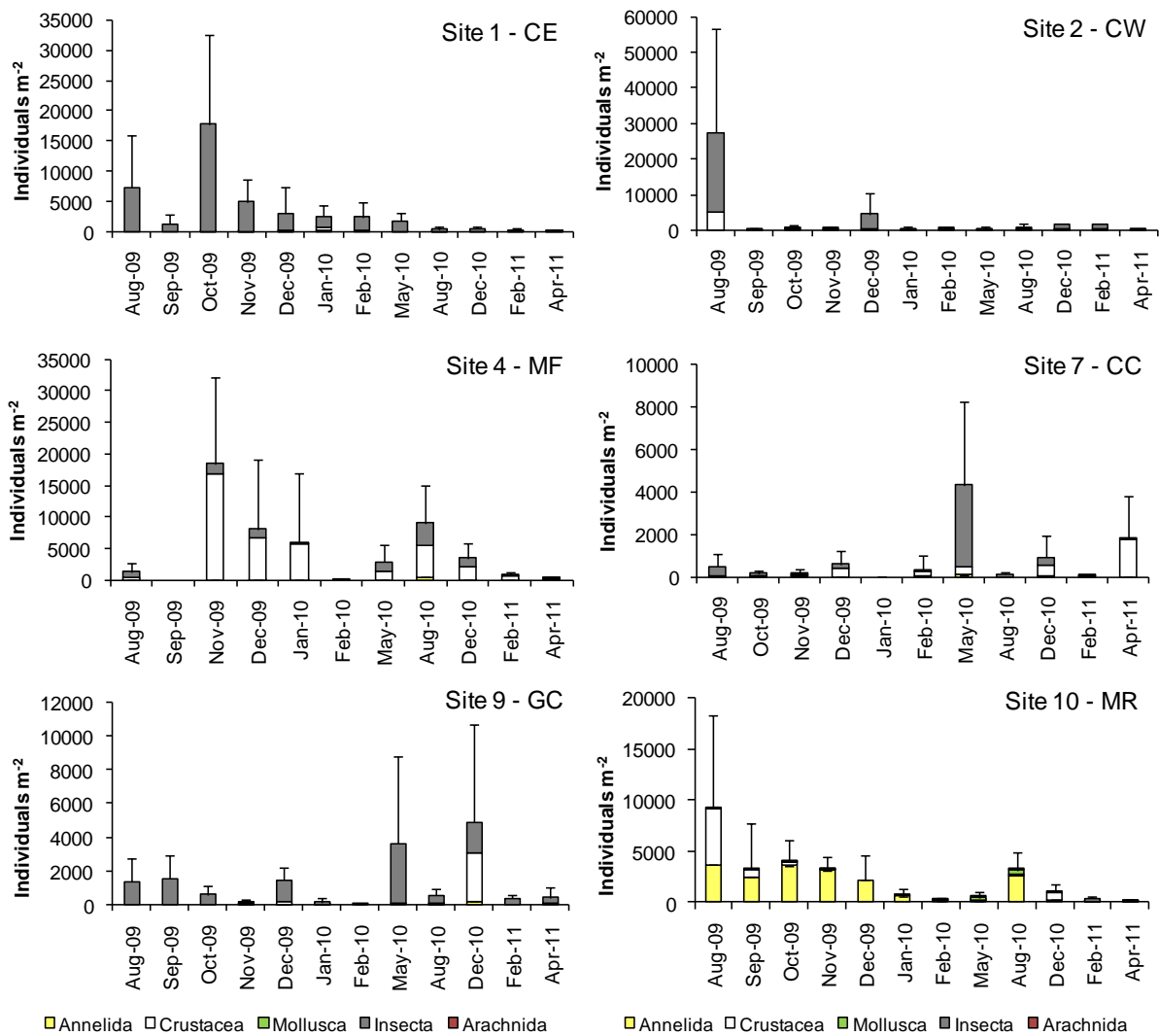


Figure 12: Abundances (mean individuals m^{-2} , \pm S.D., $n=10$) of macroinvertebrates recorded throughout the monitoring period from August 2009 to April 2011. The bars differentiate the major taxa constituting the individual density at the sites. Note that the scale of the y-axis varies substantially between sites. Site MF was not sampled in October 2009 and site CC was not sampled in September 2009. Only sites continued into the second year of sampling are shown.

4.1.4 Macroinvertebrate biomass

Total biomass of macroinvertebrates in the Goolwa Channel region was very low. Biomass was higher in December 2010 (0.31 ± 0.47 g AFDW m^{-2}) than April 2011, but varied less across sites (Table 8). Only the control site at Monument Road (MR) had a similar biomass in April 2011 than in December (Figure 13). This pattern follows the decrease in abundances over the sampling period described above. The higher biomass at Monument Road is mainly constituted of polychaetes.

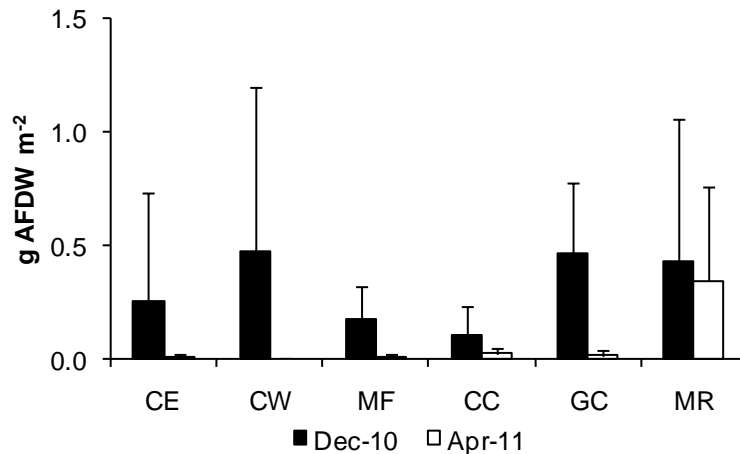


Figure 13: Average biomass (ash-free dry weight (AFDW) m⁻²; ± S.D., n=10) of macroinvertebrates for each of the six monitoring sites in the Goolwa Channel in December 2010 and April 2011.

Table 8: Results from PERMANOVA analysis on the macroinvertebrate biomass for the factors 'site' and 'month' in the GCWMLP. Significant interactions are highlighted in bold.

Source	df	MS	Pseudo-F	<i>P</i> (perm)
Site	5	181.9	2.35	0.0406
Month	1	6677.1	86.18	0.0001
Site x Month	5	499.3	6.45	0.0001
Residual	108	77.5	6.44	

4.1.5 Macroinvertebrate community

While there was significant differentiation in the macroinvertebrate assemblages across the six sampling sites and three monitoring months in the Goolwa Channel and at the two control sites (Clayton East (CE) and Monument Road (MR)) on either side of it (Table 9), there was no obvious assemblage pattern (Figure 14). The macroinvertebrates did not occur in well defined assemblages at either site (ANOSIM Global R=0.383, P=0.001) or month (ANOSIM Global R=0.375, P=0.001).

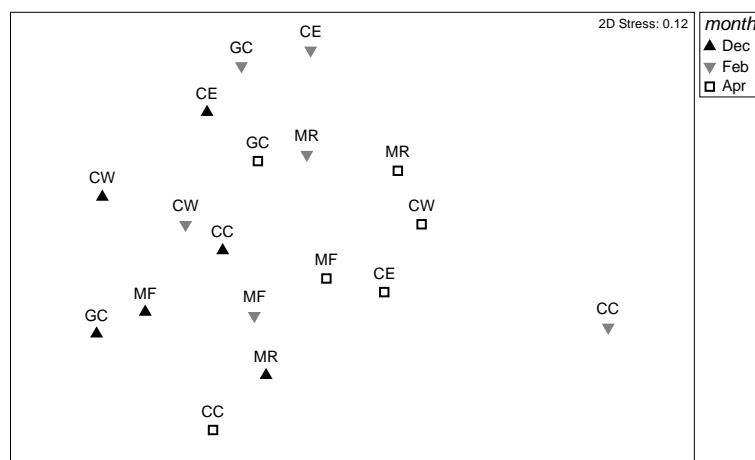


Figure 14: nMDS ordination plot showing macroinvertebrate assemblages at each of the Goolwa Channel monitoring sites (CE, CW, MF, CC, GC, MR) in December 2010, and February and April 2011.

Table 9: Results from PERMANOVA analysis on the macroinvertebrate assemblages for the factors site (CE, CW, MF, CC, GC, MR) and month (three sampling events December 2010, and February and April 2011). Significant P-values are highlighted in bold.

Source	df	MS	Pseudo-F	P (perm)
Site	5	14560.0	13.89	0.0001
Month	2	18129.0	17.29	0.0001
Site x Month	10	6863.8	6.55	0.0001
Residual	162	1048.4		

While the assemblages of macroinvertebrates were similar within the Goolwa Channel and Lower Lake control site across months between August 2009 and April 2011, they were distinct at the control site in the Murray Mouth (site 10: MR), largely due to the polychaetes *Capitella sp.*, *Simplisetia aequisetis* and *Nephtys australiensis* (Figures 15 and 16). Assemblages at all other sites were characterised more by amphipods and chironomid larvae (Figure 15). This temporal and spatial distinction was apparent in the PCO plot and cluster analysis, and a PERMANOVA analysis indicated significantly different macroinvertebrate assemblages between sites and months (Table 10). The change in the macroinvertebrate assemblage in the Goolwa Channel appears to have occurred between 2009 (samples clustered in the top left of the PCO plot) and 2011 (samples clustered towards the right in the PCO plot), and assemblages at the control site in the Murray Mouth (site 10) became more similar to the freshwater assemblage characterising the rest of the Goolwa Channel as freshwater flows continued through summer 2010/11 (Figure 15).

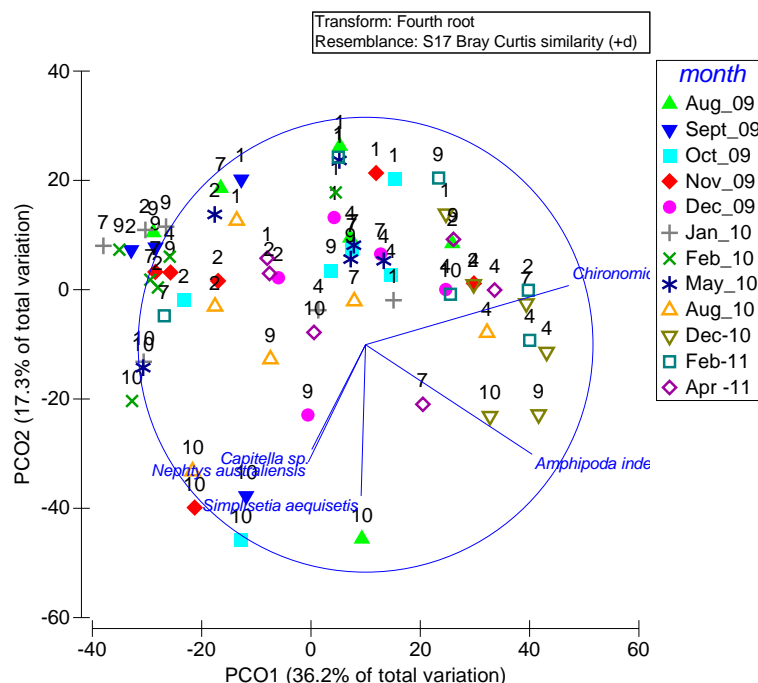


Figure 15: PCO plot of macroinvertebrate assemblages throughout the Goolwa Channel and control sites in the monitoring period between August 2009 and April 2011. The circle presents a vector overlay (Spearman correlation) illustrating the contribution of the respective species to the PCO axes.

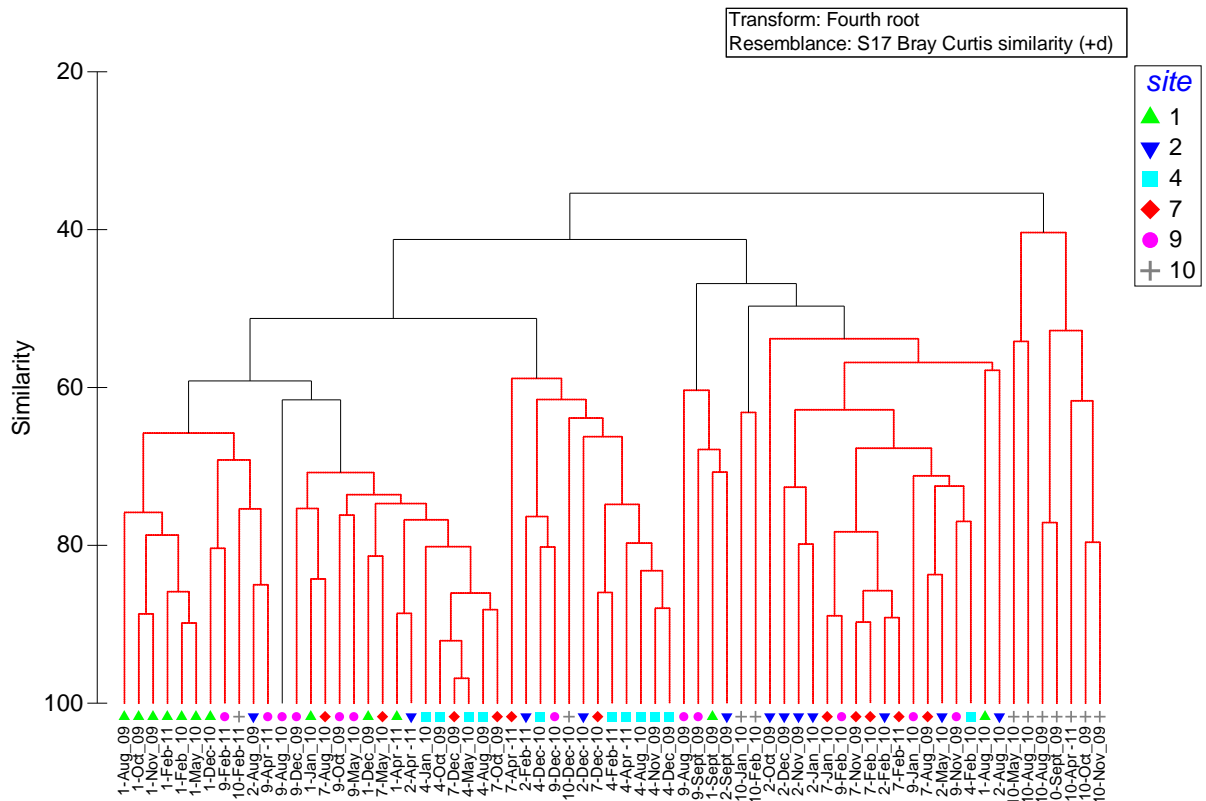


Figure 16: Dendrogram of mudflat macroinvertebrates over all surveys from August 2009 to April 2011. The sites are site 1 (CE), site 2 (CW), site 4 (MF), site 7 (CC), site 9 (GC) and site 10. The black lines indicate genuine clusters, while the red lines indicate samples that cannot be significantly differentiated (SIMPROF permutation test).

Table 10: Results from PERMANOVA analysis on the macroinvertebrate assemblages for the factors site (CE, CW, MF, CC, GC, MR) and month (August 2009 – April 2011). Significant P-values are highlighted in bold.

Source	<i>df</i>	MS	<i>Pseudo-F</i>	<i>P (perm)</i>
Site	5	41846.0	44.55	0.0001
Month	11	23752.0	25.28	0.0001
Site x Month	52	6491.7	6.91	0.0001
Residual	771	939.4		

4.2 *Ficopomatus enigmaticus* settlement

The high water level made it impossible to measure and count tubes from reefs in the field. The response of the tubeworms to the freshwater conditions was thus tested by attracting their larvae to settlement tiles, which has successfully recorded their presence in the Lower Lakes in the past (Dittmann et al. 2009). No colonisation of tubeworms was found after the three month deployment of settlement collectors in the Goolwa Channel (Figure 17). At Pelican Point, two arrays were lost after the first month; these were replaced by one extra array with settlement plates. One of the tiles retrieved from the remaining array appeared to have a few small tubes attached to the side, yet they were broken and empty. Thus no *Ficopomatus enigmaticus* were present at sites between the Goolwa Channel (GC) and Pelican Point (PP) after the three month survey.

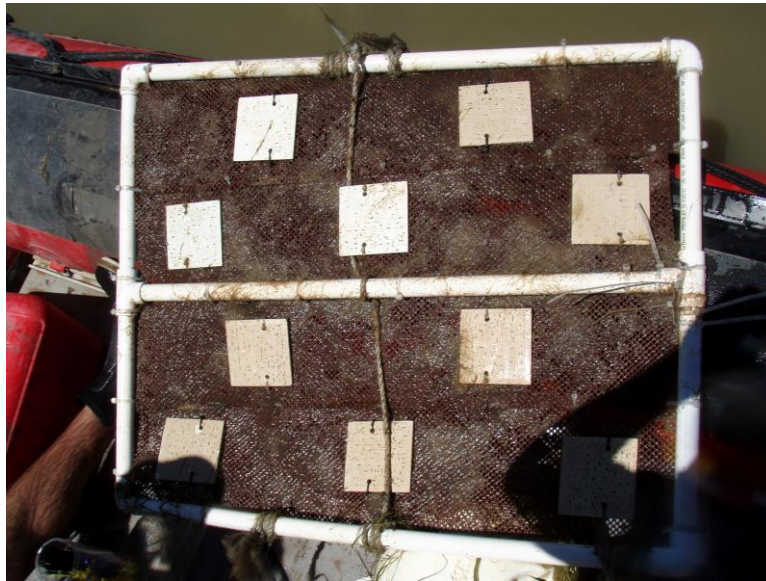


Figure 17: A *F. enigmaticus* settlement plate collected from Pelican Point (PP) after one month of deployment (23 February 2011).

4.3 Barrage release

General Observations

In December 2010, mudflats were not exposed at any site due to very high water levels and hence no foraging habitat available for shorebirds. By early February 2011, water levels were shallower and nearshore mudflat sites could be accessed by foot (from the channel side). Water birds (pelicans, gulls, shags, and swans) were present in high numbers at all sites during the December 2010 sampling period. Fur seals were often seen near Beacon 19, Ewe Island and Boundary Creek, and reef structures of *Ficopomatus enigmaticus* were seen but broken, with pieces found among the sediment at Monument Road, Pelican Point and Mark Point.

4.3.1 Environmental parameters

Throughout the Murray Mouth region, freshwater conditions prevailed, and no stratification was found as salinities were not significantly different with the depth of the water column (Table 11). At all sites and depths, salinities were higher in the December 2010 sampling (Figure 18, Table 11). Yet, as barrages had been open for several months prior to the commencement of this study, salinities in the Murray Mouth were already brackish to fresh (≤ 5 ppt) by December. The exception was Mark Point (MP), where salinities still dropped further from about 16 ppt in December to <1 ppt in April, as continued freshwater flow reached into the Coorong (Figure 18). Water temperatures varied little along the sampling sites over time, but increased significantly over summer to highest temperatures in February 2011. Seasonal temperature variations were particularly noticeable in the subtidal sampling stations between Monument Road (MR) and Hunters Creek (HC) (Figure 18, Table 11).

In December, dissolved oxygen concentrations (O_2 mg/L) and saturation (O_2 %) were low in the water overlying the sediment between MR and HC and also near the top end of the North Lagoon at Pelican Point (PP) and MP (Figure 18). Dissolved oxygen concentration and saturation increased at these sites over the following months, but appeared to drop again in April 2011. The range of variation in dissolved oxygen values over the sampling months was larger and more inconsistent between subtidal sites. An effect of the Murray Mouth and possibly flow over the Ewe Island and Tauwitchere barrages was apparent from higher dissolved oxygen values at the intertidal sampling stations between Boundary Creek (BC) and Tauwitchere (TAU) (Figure 18, Table 11). However, across all sites, months and depths there was a significant amount of variation in values of oxygen saturation and concentration (Figure 18, Table 11). The pH was around 8 at all sites, depths and across all sampling events, and only slightly below pH 7 at Hunters Creek (intertidal) in December (Figure 18).

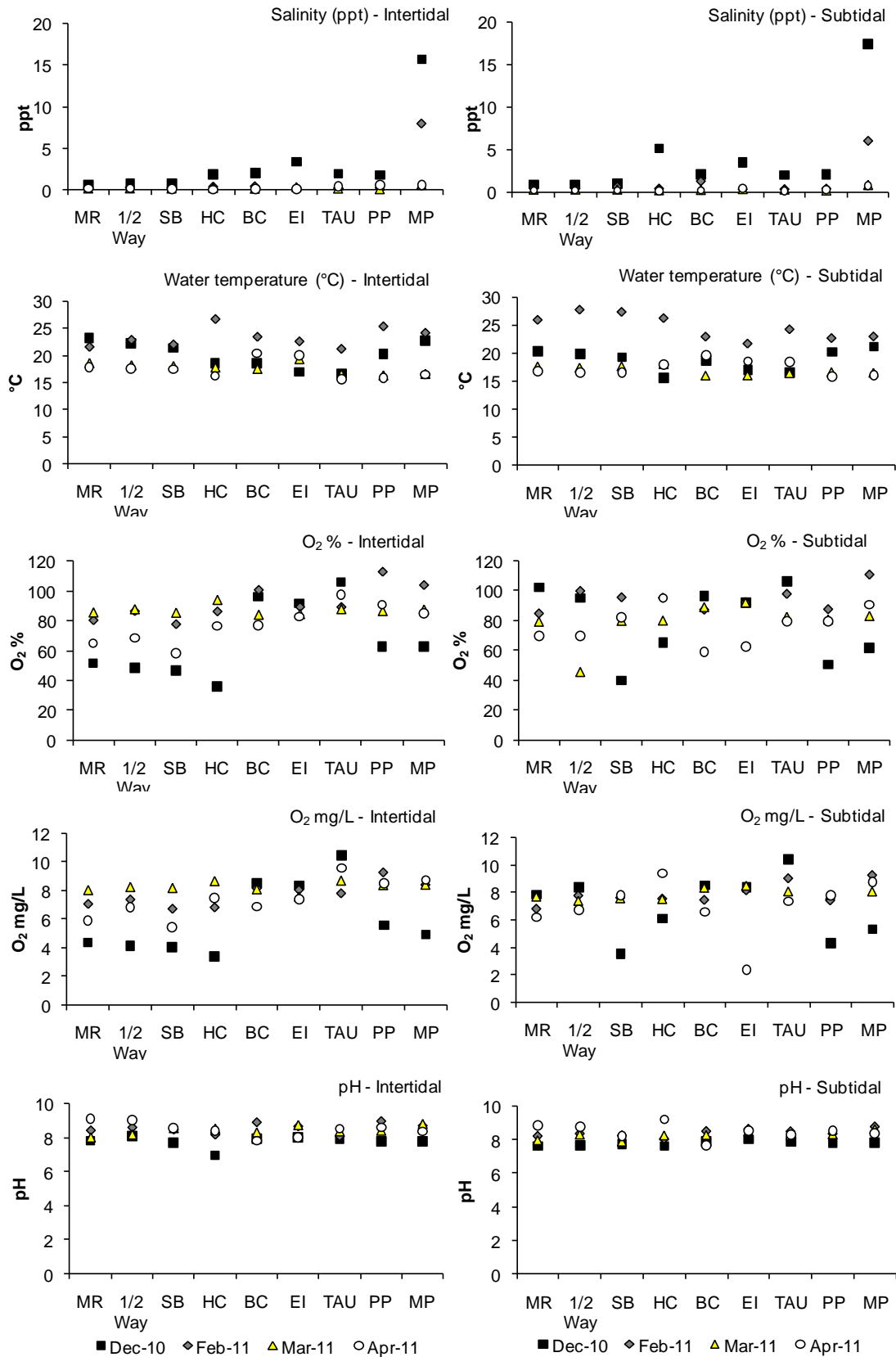


Figure 18: Average water parameters (\pm S.E.) including salinity (ppt), temperature ($^{\circ}$ C), oxygen saturation (O₂ %) and content (O₂ mg/L) and pH in the intertidal and subtidal zones at the nine monitoring sites in December 2010 and February, March and April 2011.

Table 11: Results from PERMANOVA analyses on environmental parameters including salinity (ppt), water temperature (°C), oxygen saturation (O₂ %), oxygen content (O₂ mg/l) and pH recorded in samples of the nine sites of the water release study, at two water depths (nearshore intertidal and subtidal) and across months (December 2010, and February, March and April 2011). Significant P-values are highlighted in bold.

	Source	df	MS	Pseudo-F	P (perm)
ppt	Site (Si)	8	14.6	176.58	0.0001
	Month (Mo)	3	46.7	563.97	0.0001
	Depth (De)	1	0.1	1.21	0.2846
	Si x Mo	24	4.2	51.12	0.0001
	Si x De	8	0.2	2.05	0.0414
	Mo x De	3	0.3	3.81	0.01
	Si x Mo x De	24	0.2	2.29	0.0015
	Residual	303	0.1		
°C	Site (Si)	8	1.5	43.40	0.0001
	Month (Mo)	3	79.6	2312.20	0.0001
	Depth (De)	1	0.4	12.81	0.0007
	Si x Mo	24	2.2	65.27	0.0001
	Si x De	8	0.6	17.45	0.0001
	Mo x De	3	2.5	71.99	0.0001
	Si x Mo x De	24	0.9	25.46	0.0001
	Residual	311	0.1		
O ₂ %	Site (Si)	8	3.8	5.96	0.0001
	Month (Mo)	3	9.1	14.09	0.0001
	Depth (De)	1	0.9	1.49	0.2273
	Si x Mo	24	2.1	3.27	0.0001
	Si x De	8	1.2	1.81	0.0741
	Mo x De	3	2.7	4.23	0.0051
	Si x Mo x De	24	1.5	2.26	0.0013
	Residual	303	0.6		
O ₂ mg/l	Site (Si)	8	3.9	6.38	0.0001
	Month (Mo)	3	8.7	13.97	0.0001
	Depth (De)	1	0.1	0.21	0.6469
	Si x Mo	24	2.8	4.54	0.0001
	Si x De	8	1.3	2.14	0.0342
	Mo x De	3	1.8	2.96	0.0307
	Si x Mo x De	24	1.2	1.96	0.0056
	Residual	300	0.6		
pH	Site (Si)	8	1.4	183.93	0.0001
	Month (Mo)	3	35.8	4531.40	0.0001
	Depth (De)	1	1.4	181.90	0.0001
	Si x Mo	24	2.7	344.69	0.0001
	Si x De	8	1.1	138.12	0.0001
	Mo x De	3	0.6	82.74	0.0001
	Si x Mo x De	24	0.7	90.82	0.0001
	Residual	144	0.1		

Chlorophyll-a

Average chlorophyll-a values of intertidal sediments differed significantly across sites ($F_{8,36} = 6.473$, $P < 0.001$) and months ($F_{1,36} = 5.664$, $P < 0.05$), however there was no significant interaction between the two factors. Chlorophyll-a was significantly higher at Pelican Point (PP) and Mark Point (MP) in the North Lagoon (Figure 19). No significant difference in Chl-a was found between sites for subtidal sediments during April 2011.

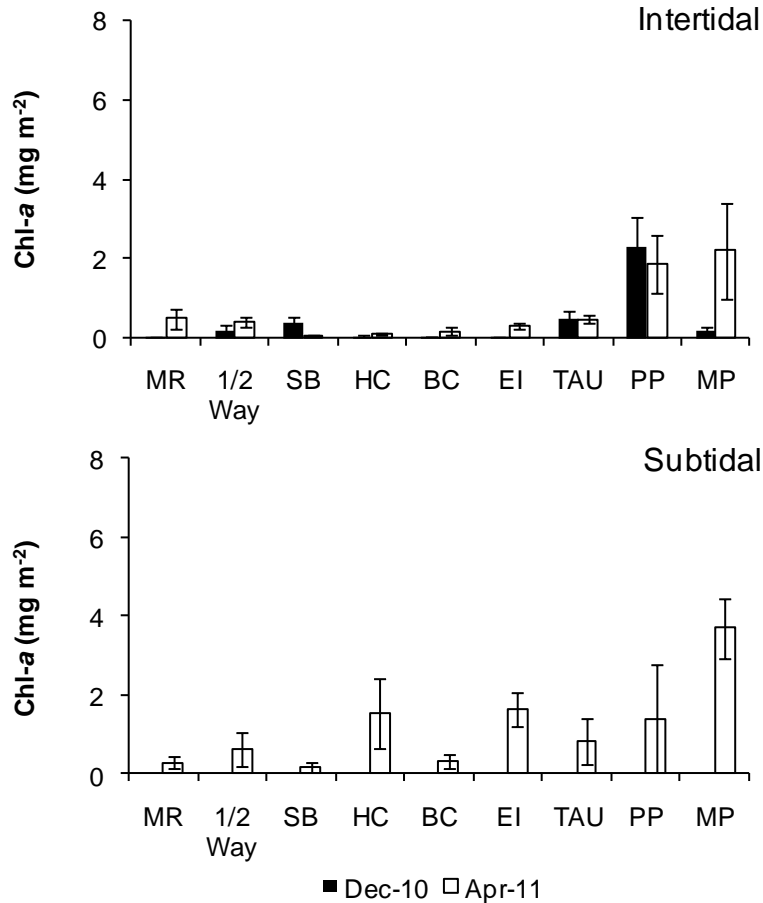


Figure 19: Average sediment chlorophyll-a content ($\text{mg}\cdot\text{m}^{-2}$, \pm S.E.) at the intertidal study sites surveyed in December 2010 and April 2011. No samples were taken in the subtidal sediments during December 2010 sampling.

Sediment Grain Size

Sediments sampled throughout the barrage release surveys were moderately well to very well sorted with a primarily fine sand consistency in the Murray Mouth region and medium to coarse sand towards the North Lagoon (Figure 20, Table 12). There was little variation in grain size classifications between December 2010 and April 2011.

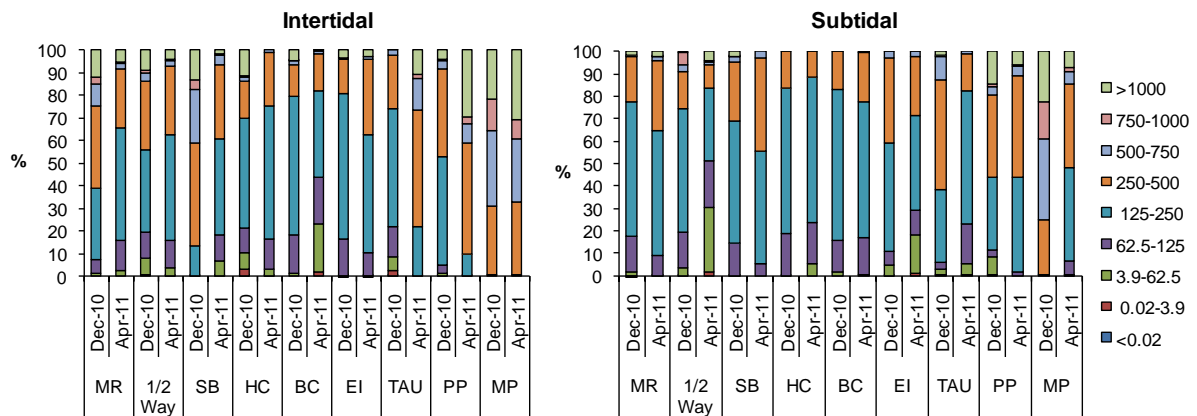


Figure 20: Sediment grain size composition (% of major fractions, size in μm) at the nine monitoring sites in the barrage release project during the December 2010 and April 2011 surveys.

Table 12: Sediment characteristics of the barrage release project during the sampling months of December 2010 and April 2011. The median grain size of sediment in μm along with the sorting coefficient, are provided as characteristics of mudflat sediment. The verbal description of sediment grain size and sorting follows (Blott & Pye 2001).

Site	December 2010				April 2011			
	Median		Sorting		Median		Sorting	
Intertidal								
Monument Rd	309.35	Medium Sand	0.47	Well Sorted	208.63	Fine Sand	0.51	Moderately Well Sorted
1/2 Way	230.08	Fine Sand	0.41	Well Sorted	214.67	Fine Sand	0.50	Moderately Well Sorted
Sugars Beach	456.16	Medium Sand	0.46	Well Sorted	217.26	Fine Sand	0.47	Well Sorted
Hunters Creek	194.25	Fine Sand	0.49	Well Sorted	190.71	Fine Sand	0.59	Moderately Well Sorted
Boundary Creek	179.72	Fine Sand	0.59	Moderately Well Sorted	141.75	Fine Sand	0.31	Very Well Sorted
Ewe Island	180.17	Fine Sand	0.62	Moderately Well Sorted	218.79	Fine Sand	0.56	Moderately Well Sorted
Tauwitechere	189.87	Fine Sand	0.53	Moderately Well Sorted	355.06	Medium Sand	0.51	Moderately Well Sorted
Pelican Point	243.56	Fine Sand	0.56	Moderately Well Sorted	431.14	Medium Sand	0.22	Very Well Sorted
Mark Point	638.47	Coarse Sand	0.49	Well Sorted	645.27	Coarse Sand	0.36	Well Sorted
Subtidal								
Monument Rd	181.83	Fine Sand	0.59	Moderately Well Sorted	215.00	Fine Sand	0.58	Moderately Well Sorted
1/2 Way	184.00	Fine Sand	0.54	Moderately Well Sorted	121.15	Very Fine Sand	0.22	Very Well Sorted
Sugars Beach	199.73	Fine Sand	0.55	Moderately Well Sorted	235.96	Fine Sand	0.57	Moderately Well Sorted
Hunters Creek	175.65	Fine Sand	0.62	Moderately Well Sorted	168.40	Fine Sand	0.61	Moderately Well Sorted
Boundary Creek	178.37	Fine Sand	0.64	Moderately Well Sorted	182.26	Fine Sand	0.59	Moderately Well Sorted
Ewe Island	226.34	Fine Sand	0.55	Moderately Well Sorted	171.43	Fine Sand	0.40	Well Sorted
Tauwitechere	289.46	Medium Sand	0.52	Moderately Well Sorted	173.36	Fine Sand	0.57	Moderately Well Sorted
Pelican Point	281.05	Medium Sand	0.38	Well Sorted	267.04	Medium Sand	0.56	Moderately Well Sorted
Mark Point	673.16	Coarse Sand	0.52	Moderately Well Sorted	256.35	Medium Sand	0.49	Well Sorted

Sediment Organic Matter

Sediment organic matter showed site specific variation over the sampling months (start and end of this benthic sampling survey), but there was no consistent pattern across the study sites for higher or lower sediment organic matter in either December or April (Figure 21, Table 13). Only intertidal sediments at Boundary Creek (BC) and Ewe Island (EI) had higher content in April, whereas sediments at Tauwitechere and Pelican Point had lower organic matter contents in April (Figure 21). Depth (inter- or subtidal) had no effect on the organic matter content of the sediment (Table 13).

Table 13: Results from PERMANOVA analysis on the organic matter content for the factors site (MR, 1/2Way, SB, HC, BC, EI, TAU, PP, MP), month (December 2010 and April 2011) and depth (intertidal and subtidal). Significant P-values are highlighted in bold.

Source	df	MS	Pseudo-F	P (perm)
Site (Si)	8	2.5	4.18	0.0006
Month (Mo)	1	0.9	1.43	0.2332
Depth (De)	1	0.5	0.88	0.3577
Si x Mo	8	2.4	4.05	0.0006
Si x De	8	0.8	1.31	0.2398
Mo x De	1	0.4	0.75	0.3928
Si x Mo x De	8	1.9	3.22	0.0025
Residual	72	0.6		

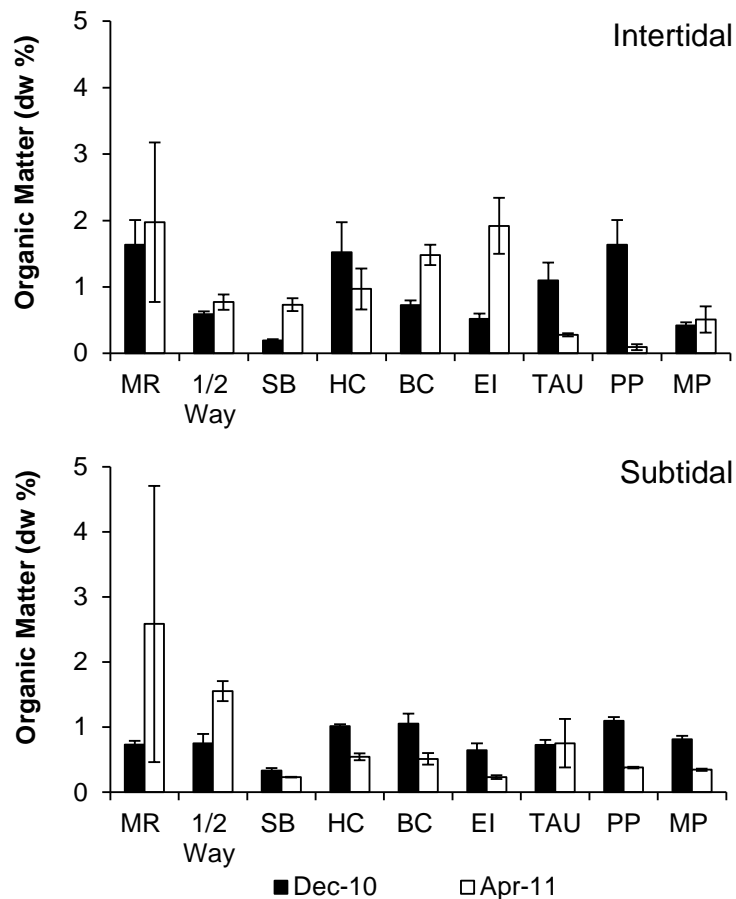


Figure 21: Sediment organic matter (as % dry weight, \pm S.E.) at intertidal and subtidal study sites during December 2010 and April 2011.

Habitat conditions

Based on environmental parameters in the water (measured every survey month) and sediment (measured in December and April), the combined conditions of the habitat can be assessed. For all of the months, the sites and months were significantly different with regards to their water quality (Figure 22, Table 14), whereby dissolved oxygen concentration and saturation as well as pH contributed to PC1, which explained 44 % of the data, and salinity and temperature on PC2 explaining a further 24% of the data. In December, sites with lower dissolved oxygen are grouped to the right of the PCO plots (compare Figure 18) and Mark Point was distinct at the first two sampling events when salinities were higher, but grouped with the other sites at the next sampling events when salinity was similar at all study sites.

Considering all water quality parameters and sediment grain size and organic matter for December and April, the sampling sites and months are again significantly different (Figure 23, Table 14), indicating changes in the habitat for macroinvertebrates. The first axis (PC1) explained 35 % of the data with the percentages of coarse and very coarse sand (which were more prevalent towards the North Lagoon, Table 12), temperature and salinity accounting for this distinction. Further differentiation

of sites over the months was due to dissolved oxygen concentration and saturation, accounting for an additional 23 % of the variability in the data explained.

As was apparent by analysis of single environmental parameters above, conditions did not change with depths, which respectively did not account for separating sampling sites or months according to their environmental conditions (Table 14).

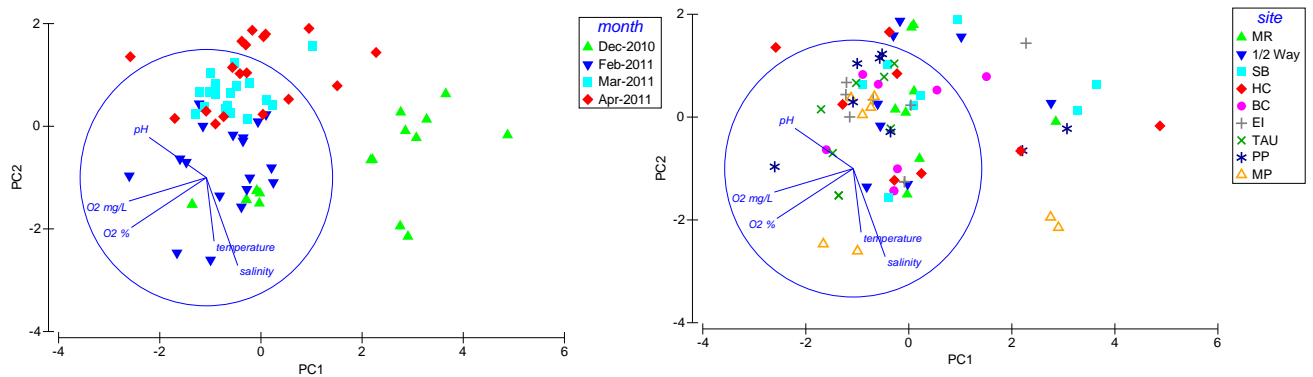


Figure 22: Principal component analysis (PCA) of water quality parameters at the study sites for the barrage release project, displayed for clarity by survey month (left) and site (right).

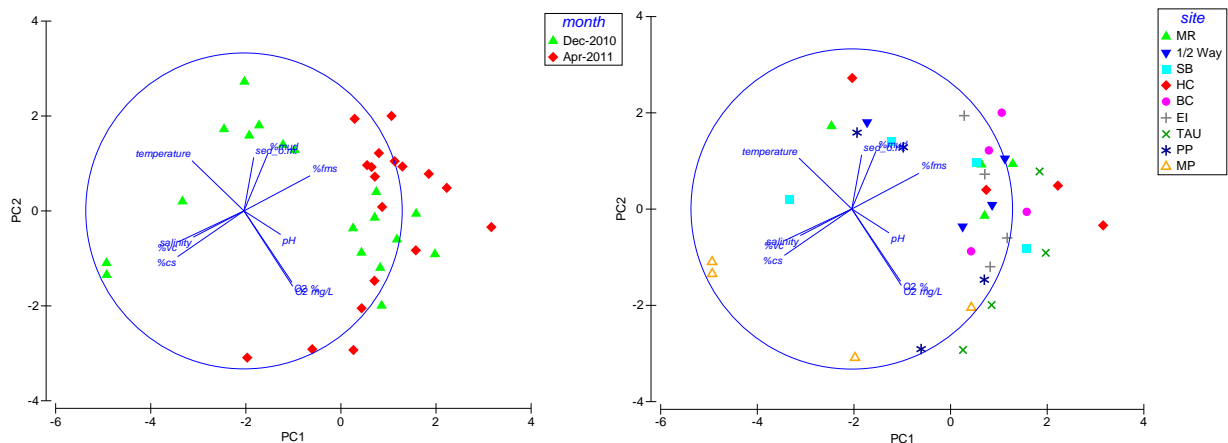


Figure 23: Principal component analysis (PCA) of water and sediment parameters (grain sizes and organic matter) at the study sites for the barrage release project, sampled at start and end of the project, displayed for clarity by survey month (left) and site (right).

Table 14: Results from PERMANOVA analysis on several environmental factors, water quality from all survey months, and water and sediment parameters from December 2010 and April 2011. Chlorophyll-a was not included in the analysis as not assessed for subtidal sediments in December. Significant P-values are highlighted in bold.

	Source	df	MS	Pseudo-F	P(perm)
water parameters all months	Site (Si)	8	6.2	4.16	0.0001
	Month (Mo)	3	49.9	33.62	0.0001
	Depth(De)	1	0.7	0.49	0.7277
	Si x Mo	24	4.1	2.77	0.0001
	Si x De	8	1.6	1.09	0.3709
	Mo x De	3	2.8	1.87	0.0605
	Residual	24	1.5		
water and sediment parameters December and April	Site (Si)	8	15.7	3.07	0.0003
	Month (Mo)	1	53.0	10.33	0.0001
	Depth(De)	1	12.0	2.35	0.0461
	Si x Mo	8	10.0	1.94	0.0133
	Si x De	8	4.4	0.85	0.7079
	Mo x De	1	3.6	0.70	0.6402
	Residual	8	5.1		

4.3.2 Macroinvertebrate diversity

A total of 22 taxa were recorded across the nine monitoring sites between December 2010 and April 2011 (Table 15). Insects were most diverse and numerous, represented by seven taxa. Yet, only chironomid larvae occurred regularly during the study sites and times, whereas larvae of other insects appeared sporadically in benthic samples (Table 15). Six taxa each of annelids and crustaceans were recorded, while only three molluscs were found within the samples. The polychaetes *Simplisetia aequisetis* and *Nephtys australiensis*, amphipods and chironomid larvae had the highest frequency of occurrence by being present at almost every site across months and depth (Table 15). The number of annelid species increased towards North Lagoon, where four species of annelids were present in the subtidal sediments at Mark Point (MP) (Figure 24, Table 15). *Capitella* sp., a prominent polychaete species and pollution indicator that had dominated the sediments in the past, was missing at the sites sampled for the barrage release.

At the single sites, the number of macroinvertebrate species found ranged from 0 to 10, with higher species numbers recorded in sub- than intertidal sediments in December 2010 (Figure 24). Yet in February, March and April 2011, no differences occurred in the number of benthic species between the two sampling depths, due to a drop in species numbers in subtidal sediments (Figure 24).

Diversity indices revealed a low diversity with dominance of few species at most sites, originating from the numerical abundance of *Simplisetia aequisetis*, amphipods, and chironomid larvae (Tables 15 and 16, Figure 27). There was no consistent pattern in diversity along the nine sampling sites, nor between the inter- and subtidal locations or the four sampling events (Table 16).

The shift towards a freshwater system was also noted by the presence of ehippia of Cladocera (freshwater zooplankton) in the benthic samples.

Table 15: Occurrence of macrobenthic taxa and species during the barrage release project for the December 2010 (D), and February (F), March (M) and April 2011 (A) sampling events. The presence of taxa is indicated for the nearshore (I) and subtidal (S) sampling locations.

PHYLA	SPECIES	Monument Road				1/2 Way				Sugars Beach				Hunters Creek				Boundary Creek				Ewe Island				Tauwitechere				Pelican Point				Mark Point						
		D	F	M	A	D	F	M	A	D	F	M	A	D	F	M	A	D	F	M	A	D	F	M	A	D	F	M	A	D	F	M	A	D	F	M	A			
Annelida	<i>Simplisetia aequisetis</i>	I	S	I	S	S	I			S	I			S	S	S		I	S			I			S	I	S	I	S	I	I	S	S	I	S	S	S	I	S	I
	<i>Australonereis ehlersi</i>																																							
	<i>Nephtys australiensis</i>		I	S		S	I		S	S	I	S	I	S	S		S		I	S	S	S				I	S	I			I		S	S	S	S				
	<i>Boccardiella limicola</i>					S										S																				S	S			
	<i>Ficopomatus enigmaticus</i>																														S	S				S		S	S	
	<i>Oligochaeta</i>					I			I				I	S							I	S				S	I						S	S	S	S				
Crustacea	Amphipoda	I	S	I	S	I	S		S	I	S	I	S	I	S	S	I	S	S	I	I	S	I	S	I	I	S	I	S	I	I	S	I	S	I	I	S	I	S	I
	Ostracoda									S	I											I																		
	Mysidacea indet.									S	I											I																		
	<i>Paragrapsus gaimardii</i>									I	S	I																												
	<i>Helograpsus haswellianus</i>	S																																						
	<i>Amarinus laevis</i>									S	I																													
Mollusca	<i>Arthritica helmsi</i>									S				S								I				I														
	<i>Salinator</i> sp.					I	S			S	I	S		S								I				S				S										
	Hydrobiidae									S	I	S		S								I				I				S					S					
Insecta	Culicidae				I		S											S	S												S			S	S					
	Ceratopognidae indet.																																							
	Chironomid (Larvae + pupae)	S	I	I	S	I	S	I	S	I	S	I	I	I	S	I	I	S	I	I	S	I	S	I	S	I	S	I	I	S	I	S	I	S	I	S	I	S		
	Tipulid Larvae					I																																		
	Ephydriidae pupae	I																																						
	Corixidae																I																							
Insect indet.				I																																				
Species number per sampling event		6	4	4	4	8	5	1	4	9	9	3	4	6	4	5	3	6	5	3	3	8	3	3	5	8	3	4	4	8	4	5	4	8	6	6	7			
Species number per site		9				9				10				9				7				9				9				8				8						

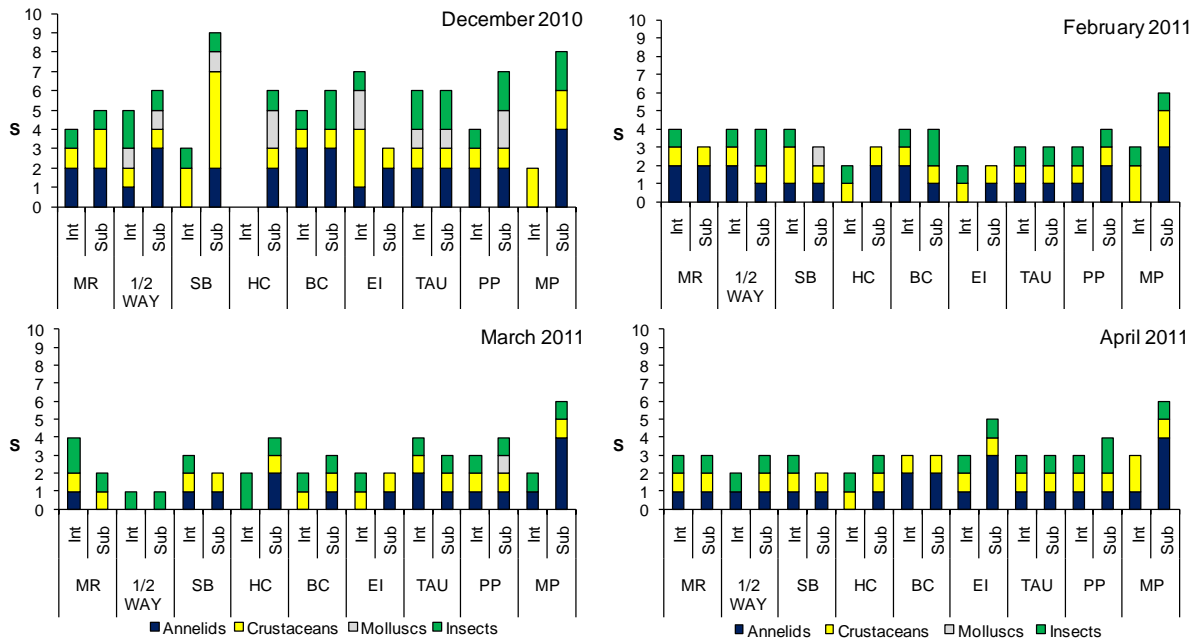


Figure 24: Numbers of macroinvertebrate species and their major taxonomic composition found in the intertidal and subtidal locations at each of the monitoring sites in December 2010, and February, March and April 2011. No benthic organisms were recorded at the Hunters Creek (HC) intertidal site during the December 2010 sampling.

Table 16: Diversity (Shannon-Wiener index H') and Pielou's evenness index (J) of macroinvertebrates in December 2010, and February, March and April 2011 in the intertidal and subtidal sampling stations at all nine monitoring sites. No macroinvertebrates were found in the intertidal sediments at Hunters Creek (HC) in December 2010, and only one species at both the intertidal and subtidal sites for 1/2 Way in March 2011.

<i>H'</i> (Shannon-Wiener)								
Site	Dec-10		Feb-11		Mar-11		Apr-11	
	Intertida	Subtidal	Intertida	Subtidal	Intertida	Subtidal	Intertida	Subtidal
MR	1.10	0.97	1.24	0.86	1.01	0.66	0.85	0.68
1/2 Way	1.13	0.75	1.24	1.35			0.10	1.04
SB	0.66	0.84	0.50	0.79	0.44	0.61	0.65	0.23
HC		0.18	0.38	0.72	0.69	1.21	0.69	0.63
BC	0.73	0.45	0.57	1.17	0.10	0.36	0.05	0.69
EI	0.46	0.12	0.28	0.39	0.15	0.26	0.37	1.20
TAU	0.34	0.51	0.95	0.52	1.08	0.78	0.83	0.78
PP	0.62	1.28	0.47	0.69	0.82	1.14	0.53	0.63
MP	0.56	1.58	1.08	1.32	0.69	0.83	1.04	0.38

<i>J</i> (evenness)								
Site	Dec-10		Feb-11		Mar-11		Apr-11	
	Intertida	Subtidal	Intertida	Subtidal	Intertida	Subtidal	Intertida	Subtidal
MR	0.79	0.60	0.89	0.78	0.73	0.95	0.77	0.62
1/2 Way	0.70	0.42	0.89	0.98			0.15	0.94
SB	0.60	0.38	0.36	0.72	0.41	0.88	0.59	0.33
HC		0.10	0.54	0.66	1.00	0.87	0.99	0.58
BC	0.45	0.25	0.41	0.84	0.14	0.33	0.05	0.63
EI	0.23	0.11	0.41	0.57	0.22	0.37	0.34	0.75
TAU	0.19	0.28	0.87	0.47	0.78	0.71	0.75	0.71
PP	0.45	0.66	0.43	0.50	0.74	0.82	0.47	0.45
MP	0.81	0.76	0.98	0.74	1.00	0.46	0.94	0.21

4.3.3 Macroinvertebrate abundances and distribution

The macroinvertebrate abundances were low (1219 ± 3915 individuals m^{-2} on average) throughout the barrage release monitoring project, apart from subtidal sediments in December 2010 (Figure 25), which was due to very high densities of amphipod crustaceans present in the deeper water sediments of Hunters Creek (HC) (Figure 26). The abundances of all macroinvertebrates, as well as major taxa (annelids, crustaceans, molluscs and insects) were significantly different across sites and months (Table 17) and, apart from insects, also for depths (Table 17). Interactions between all three factor levels were significantly different for all major macroinvertebrate taxa, yet for molluscs not between months and depth (Table 17). The analyses indicate a high level of variation in macroinvertebrate abundances and distribution over the project sites and sampling times.

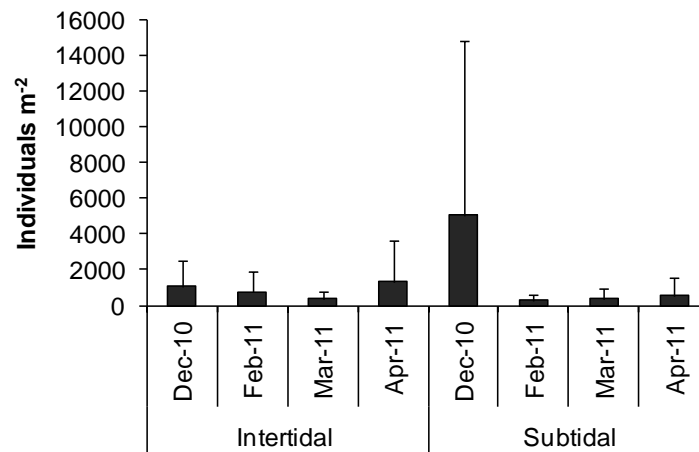


Figure 25: Average abundances (individuals m^{-2} , \pm S.D., $n=10$) of total macroinvertebrates recorded in the intertidal and subtidal sampling stations in December 2010, and February, March and April 2011.

Some patterns are still emerging. The highest abundances recorded occurred mostly in subtidal sediments, with the exception of insects and amphipods in the intertidal sediments in April 2011 (Figure 26). Particular taxa were more abundant in sediments at specific sites; crustacea and molluscs at Hunters Creek, annelids at Monument Road, Tauwitchere, Pelican and Mark Point, and insect larvae mainly between $\frac{1}{2}$ Way and Hunters Creek (Figure 26).

The pattern of distribution and abundance for higher taxa was driven by particular species (note that not all higher taxa were differentiated to species level, such as chironomids and amphipods) (Figure 26 and 27). Crustacea were mainly represented by amphipods and insect larvae by chironomids. For annelids, single species had different patterns, with *Simplisetia aequisetis* occurring at Monument Road and south of the Tauwitchere barrage, *Nephtys australiensis* being present at all sites in low numbers and oligochaetes being more prevalent in subtidal sediments at Mark Point (Figure 27).

The prolonged freshwater conditions over the study period, occurring also in deeper water, caused unfavourable environmental conditions for estuarine macroinvertebrates, leading to continued low abundances (Figures 25 to 27). Annelids appeared to persist in subtidal sediments between the Tauwitchere barrage and Mark Point. Species specific responses to the changing environmental conditions with the flow are best documented by the absence of *Capitella* sp., which had numerically dominated the benthos in the past. Amphipod numbers were increasing again by April in intertidal

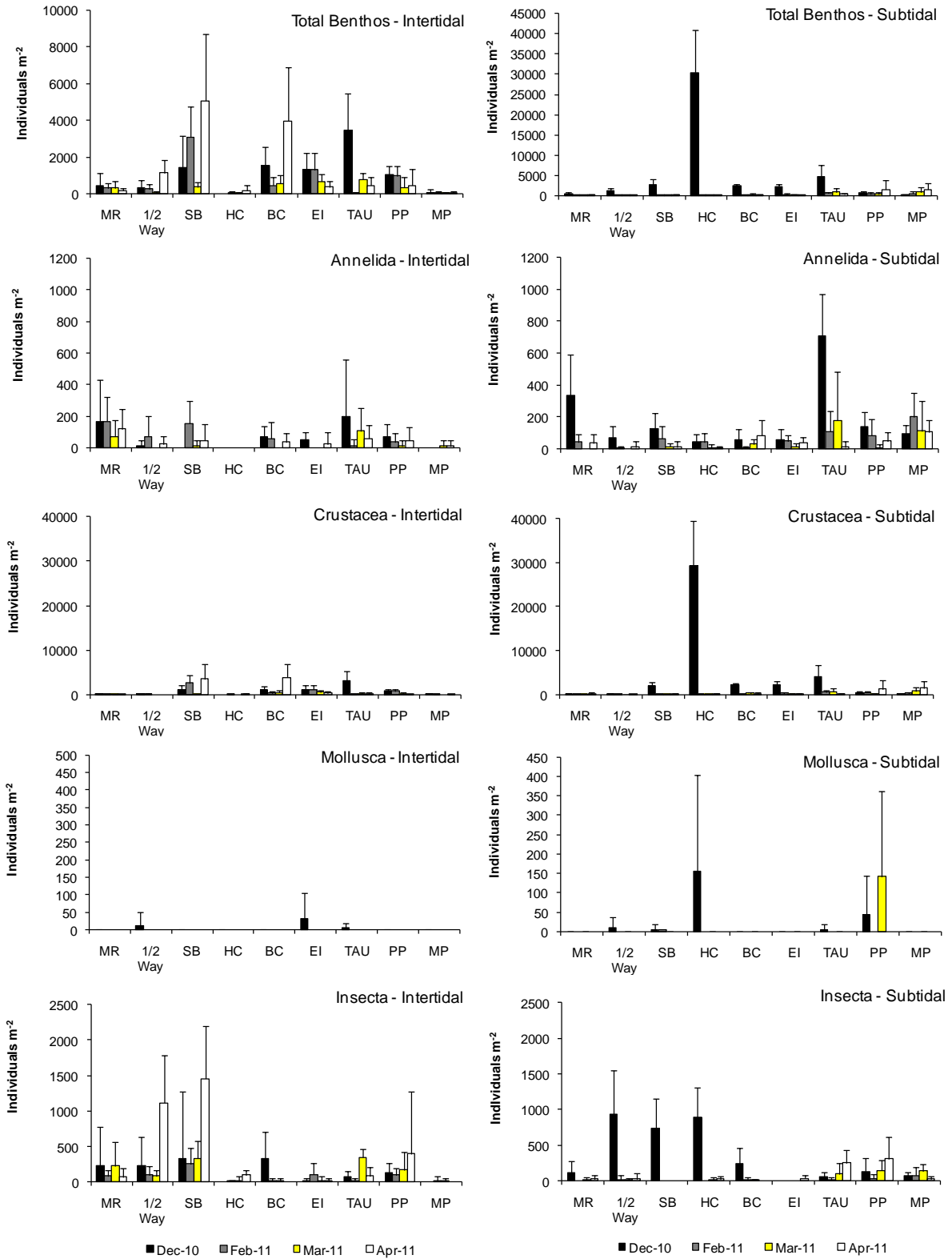


Figure 26: Average abundances (individuals m⁻², ± S.D., n=10) of all macroinvertebrates and major taxa (Annelida, Crustacea, Mollusca and Insecta) recorded in the intertidal and subtidal sampling stations in December 2010, and February, March and April 2011. Site codes are: Monument Road (MR), Half Way (1/2 Way), Sugars Beach (SB), Hunters Creek (HC), Boundary Creek (BC), Ewe Island (EI), Tauwichee (TAU), Pelican Point (PP) and Mark Point (MP). Note that the scale of the y-axis varies substantially between taxa and depths.

sediments at Sugars Beach and Boundary Creek (Figure 27, notice scale bar). The increase in insect numbers in April in intertidal sediments could be related to their seasonal life histories, as only the larval stages are aquatic. Yet, comparably high numbers had been found in subtidal sediments in December. Further personal observations from Mundoo Channel in early February indicate the replacement of an estuarine macroinvertebrate community with a freshwater one.

Table 17: Results from PERMANOVA analyses on abundances of all macroinvertebrates and major taxa recorded in samples of the nine sites of the water release, at two water depths (nearshore intertidal and subtidal) and months (December 2010, and February, March and April 2011). Significant P-values are highlighted in bold.

	Source	df	MS	Pseudo-F	P (perm)
Total Benthos	Site (Si)	8	13266.0	28.53	0.0001
	Month (Mo)	3	12017.0	25.85	0.0001
	Depth (De)	1	2071.2	4.45	0.0256
	Si x Mo	24	1839.6	3.96	0.0001
	Si x De	8	10821.0	23.27	0.0001
	Mo x De	3	7343.2	15.79	0.0001
	Si x Mo x De	24	2513.2	5.41	0.0001
	Residual	648	464.9		
Annelida	Site (Si)	8	4951.5	8.14	0.0001
	Month (Mo)	3	16904.0	27.78	0.0001
	Depth (De)	1	39797.0	65.39	0.0001
	Si x Mo	24	1661.6	2.73	0.0001
	Si x De	8	4917.9	8.08	0.0001
	Mo x De	3	3846.2	6.32	0.0002
	Si x Mo x De	24	1934.5	3.18	0.0002
	Residual	648	608.6		
Crustacea	Site (Si)	8	29179.0	53.59	0.0001
	Month (Mo)	3	16814.0	30.88	0.0001
	Depth (De)	1	17333.0	31.84	0.0001
	Si x Mo	24	3188.7	5.86	0.0001
	Si x De	8	7367.1	13.53	0.0001
	Mo x De	3	8621.3	15.84	0.0001
	Si x Mo x De	24	2857.3	5.25	0.0001
	Residual	648	544.4		
Mollusca	Site (Si)	8	332.8	3.95	0.0003
	Month (Mo)	3	874.8	10.38	0.0001
	Depth (De)	1	798.5	9.47	0.0017
	Si x Mo	24	233.2	1.76	0.0001
	Si x De	8	459.5	5.45	0.0001
	Mo x De	3	199.9	2.37	0.0684
	Si x Mo x De	24	274.1	3.25	0.0001
	Residual	648	84.3		
Insecta	Site (Si)	8	6633.5	11.52	0.0001
	Month (Mo)	3	11311.0	19.64	0.0001
	Depth (De)	1	1960.9	3.40	0.0623
	Si x Mo	24	8345.5	6.00	0.0001
	Si x De	8	13446.0	14.48	0.0001
	Mo x De	3	4463.6	23.34	0.0001
	Si x Mo x De	24	576.1	7.75	0.0001
	Residual	648			

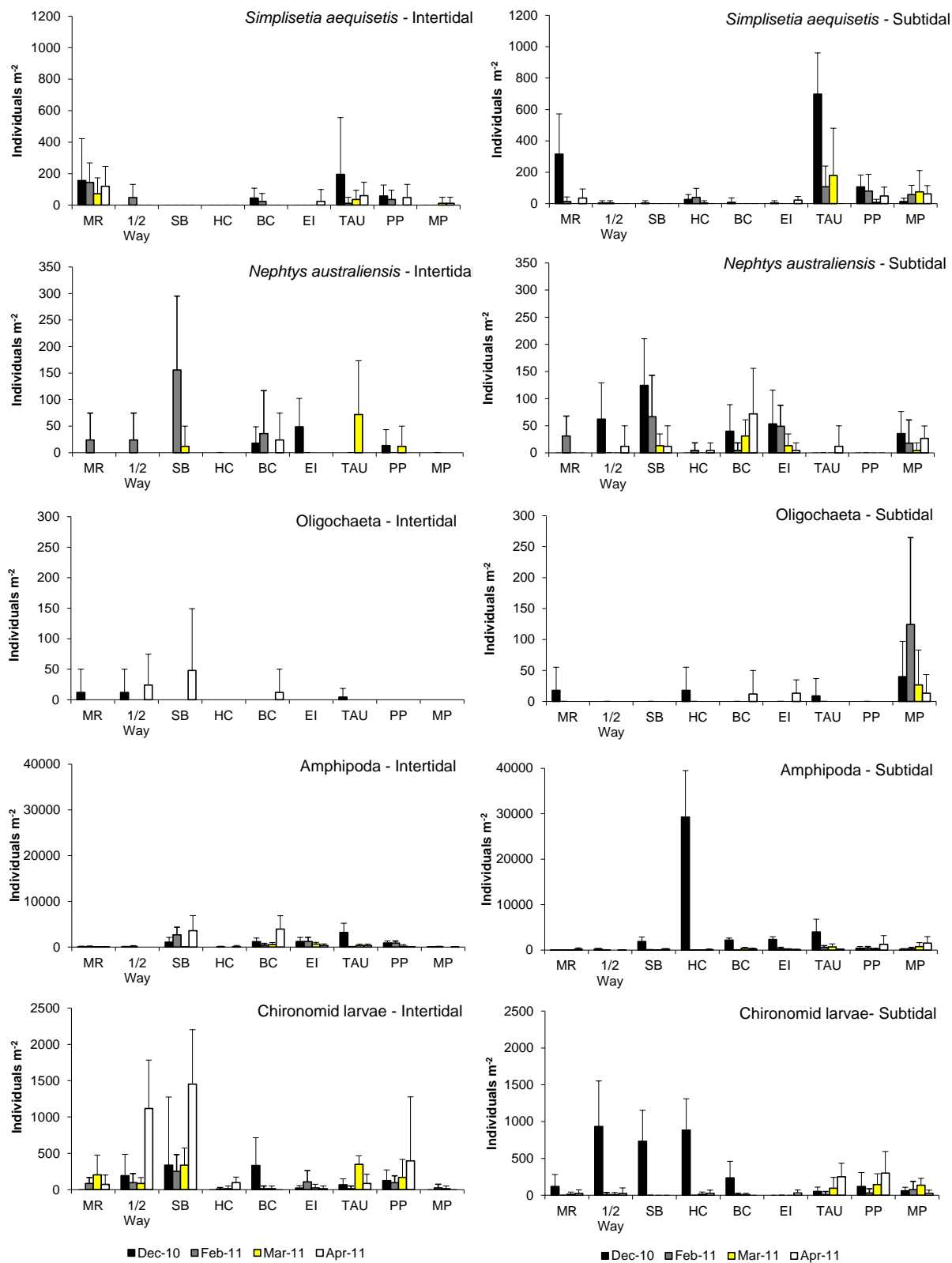


Figure 27: Average abundances (individuals m^{-2} , \pm S.D., $n=10$) of individual key taxa (*Simplisetia aequisetis*, *Nephtys australiensis*, Oligochaeta, Amphipoda and chironomid larvae) recorded in the intertidal and subtidal sampling stations in December 2010, and February, March and April 2011. See Figures 1 and 26 for site codes. Note that the scale of the y-axis varies substantially between taxa.

Table 18: Results from PERMANOVA analyses on abundances of individual taxa recorded in samples of the nine sites of the water release, at two water depths (nearshore intertidal and subtidal) and months (December 2010, and February, March and April 2011). Significant P-values are highlighted in bold.

	Source	df	MS	Pseudo-F	P (perm)
<i>Simplisetia aequisetis</i>	Site (Si)	8	10072	23.966	0.0001
	Month (Mo)	3	6064	14.429	0.0001
	Depth (De)	1	6775.6	16.122	0.0004
	Si x Mo	24	1860.3	4.426	0.0001
	Si x De	8	3025.3	7.198	0.0001
	Mo x De	3	1239.5	2.949	0.0307
	Si x Mo x De	24	1442.5	3.432	0.0001
	Residual	648	420.28		
<i>Nephtys australiensis</i>	Site (Si)	8	4295	13.558	0.0001
	Month (Mo)	3	2912.8	9.194	0.0001
	Depth (De)	1	11459	36.173	0.0001
	Si x Mo	24	1641.9	5.182	0.0001
	Si x De	8	1700.5	5.367	0.0001
	Mo x De	3	874.84	2.761	0.0392
	Si x Mo x De	24	1348.9	4.257	0.0001
	Residual	648	316.8		
Oligochaeta	Site (Si)	8	1026.8	8.216	0.0001
	Month (Mo)	3	461	3.688	0.0093
	Depth (De)	1	1407	11.259	0.0008
	Si x Mo	24	304.12	2.433	0.0005
	Si x De	8	1456.2	11.652	0.0001
	Mo x De	3	237.91	1.903	0.128
	Si x Mo x De	24	239.31	1.914	0.0049
	Residual	648	124.97		
Amphipoda	Site (Si)	8	29413	54.974	0.0001
	Month (Mo)	3	15202	28.413	0.0001
	Depth (De)	1	21218	39.656	0.0001
	Si x Mo	24	3240.4	6.056	0.0001
	Si x De	8	8743.3	16.341	0.0001
	Mo x De	3	8458.7	15.809	0.0001
	Si x Mo x De	24	2848.2	5.323	0.0001
	Residual	648	535.04		
Chironomid larvae	Site (Si)	8	6953.3	12.568	0.0001
	Month (Mo)	3	10686	19.314	0.0001
	Depth (De)	1	1529.5	2.764	0.0987
	Si x Mo	24	3576.2	6.463	0.0001
	Si x De	8	8301.5	15.005	0.0001
	Mo x De	3	14805	26.76	0.0001
	Si x Mo x De	24	4553.7	8.23	0.0001
	Residual	648	553.27		

4.3.4 Macroinvertebrate biomass

Biomass of macroinvertebrates was low and varied significantly between sites, months and depth (Figure 28, Table 19). Biomass was higher in the subtidal sediments in December, especially between Monument Road (MR) and Hunters Creek (HC), largely due to amphipods, chironomids and larger polychaetes present (Figures 27 and 28). On average, intertidal biomass was 0.25 g AFDW m⁻² and subtidal biomass 0.41 g AFDW m⁻².

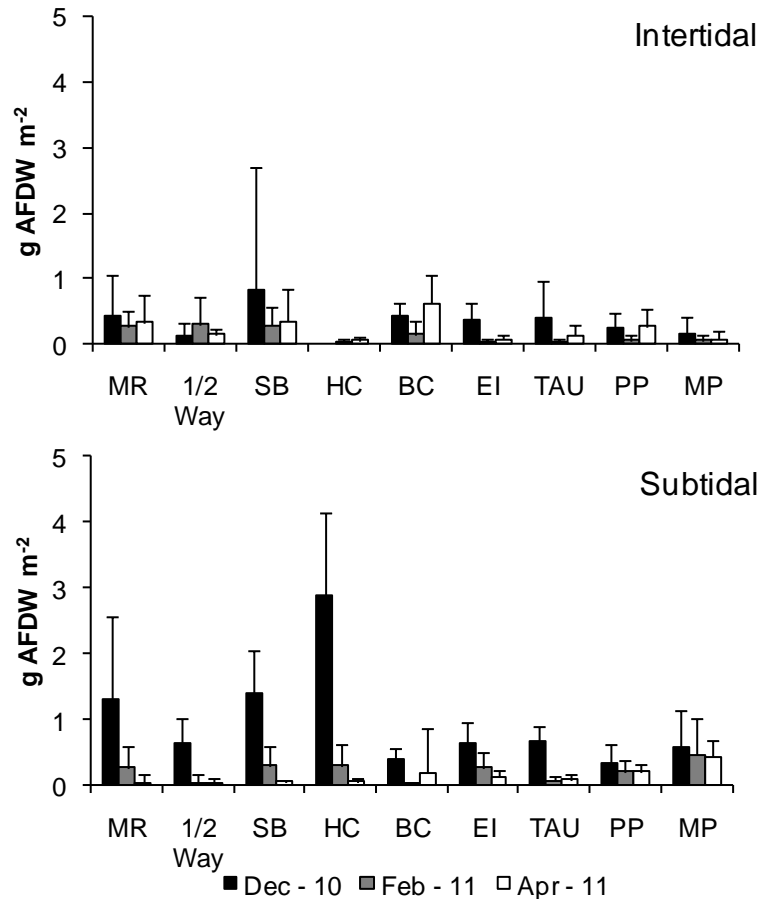


Figure 28: Average biomass (ash-free dry weight (AFDW) per m⁻²; (\pm S.D., $n=10$) of macroinvertebrates for each of the nine monitoring sites in the intertidal and subtidal zones in December 2010 and February and April 2011.

Table 19: Results from PERMANOVA analysis on the macroinvertebrate biomass for the factors site (MR, 1/2Way, SB, HC, BC, EI, TAU, PP, MP), month (December 2010, and February, March and April 2011) and depth (Intertidal and Subtidal). Significant P-values are highlighted in bold.

Source	df	MS	Pseudo-F	<i>P</i> (perm)
Site (Si)	8	590.5	7.64	0.0001
Month (Mo)	2	3700.6	47.87	0.0001
Depth (De)	1	1271.6	16.45	0.0001
Si x Mo	16	164.9	2.13	0.0054
Si x De	8	1080.7	13.98	0.0001
Mo x De	2	2493.4	32.26	0.0001
Si x Mo x De	16	436.1	5.64	0.0001
Residual	486	77.3		

4.3.5 Macroinvertebrate community

Over the entire barrage release monitoring time frame from December 2010 to April 2011, the macroinvertebrate assemblages were significantly different between sites, water depth of sampling location and the four sampling months (Table 20). Yet, the PCO plot shows little clear structure of assemblages by month or depth (Figure 29). Analysing benthic communities separately for the four sampling events (figures not shown) did not show separations of assemblages by depth at either of

the four months. The species responsible for differentiating some of the sites over time and depths were amphipods, the polychaetes *Simplisetia aequisetis* and *Nephtys australiensis*, and chironomid larvae (Figure 29).

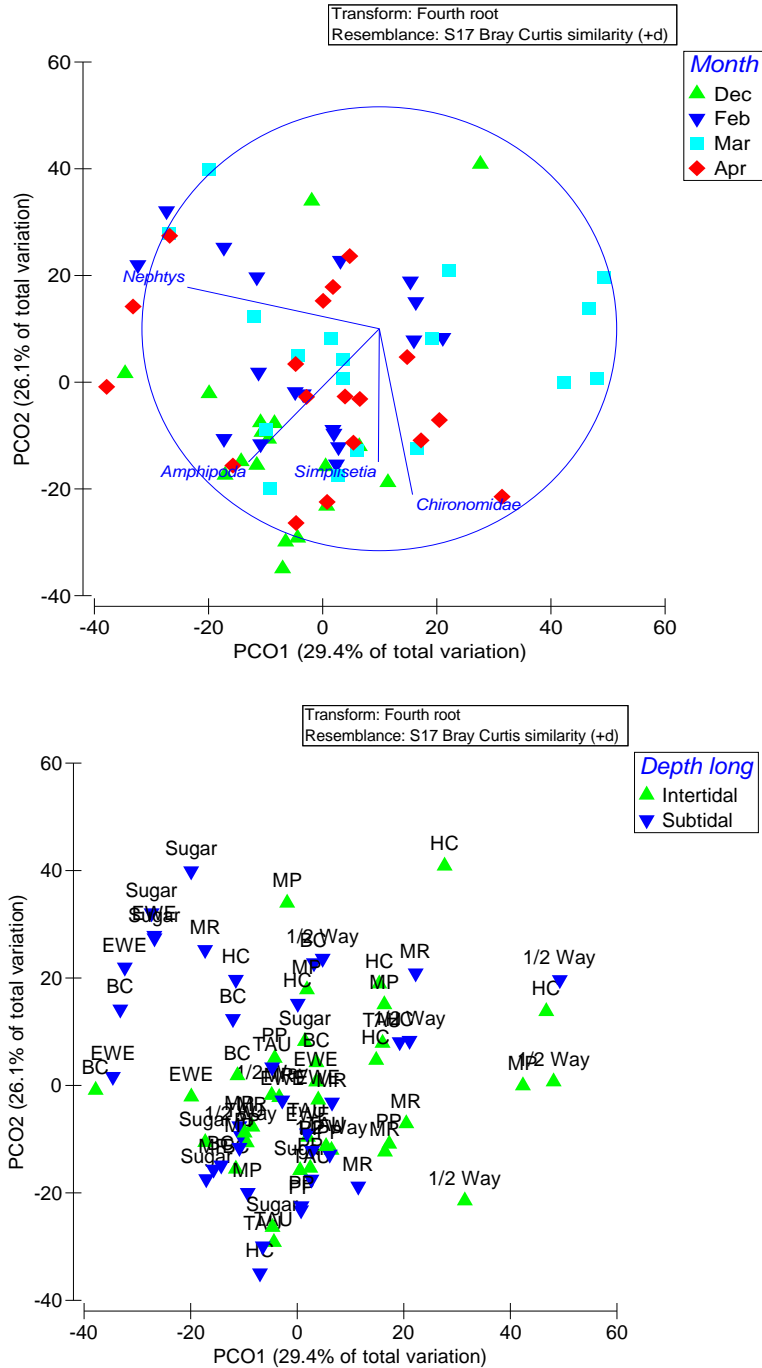


Figure 29: Principle coordinate analysis (PCO) of macroinvertebrate data showing macroinvertebrate assemblages during the barrage release monitoring displayed for clarity for each of the sites in December 2010, and February, March and April 2011 (top figure) and the intertidal and subtidal depth (bottom figure). The contribution of respective species to the PCO axes is indicated by the vector overlay.

Table 20: Result from PERMANOVA analysis on the macroinvertebrate assemblages for the factors site (MR, 1/2Way, SB, HC, BC, EI, TAU, PP, MP), depth (inter- and subtidal) and month (four sampling events December 2010, and February, March and April 2011). Significant P-values are highlighted in bold.

Source	df	MS	Pseudo-F	P (perm)
Site (Si)	8	32613.0	33.61	0.0001
Month (Mo)	3	20008.0	20.62	0.0001
Depth (De)	1	23099.0	23.80	0.0001
Si x Mo	24	5288.0	5.45	0.0001
Si x De	8	15026.0	15.48	0.0001
Mo x De	3	15219.0	15.68	0.0001
Si x Mo x De	24	5082.5	5.24	0.0001
Residual	648	970.4		

4.3.6 Link between macroinvertebrate assemblage and environmental conditions

While no clear patterns in macroinvertebrate assemblages were detected by the multivariate analysis, significant differences were present on all levels (Table 20) and it was explored whether and which environmental factors have contributed to it. Based on water quality parameters, which were determined at every sampling occasion, a temporal change in assemblages is apparent, splitting December from the following month, largely driven by salinity (Figure 30, left). Mark Point (MP), and to a lesser extent Hunters Creek (HC), had slightly higher salinities than the other sites in December (Figure 18), and these sites were the most distinct (Figure 30, right). Salinity did contribute significantly to the differentiation of macroinvertebrate assemblages (DISTLM: Pseudo- $F=3.499$, $P=0.005$), yet only about 5 % of the variation in benthic data was explained this way. Even with all water quality parameters taken together, only 13 % of the variation in macroinvertebrate patterns was explained. This can be an indication that from the start of the sampling for this project, more homogenous water conditions already existed in the entire study area.

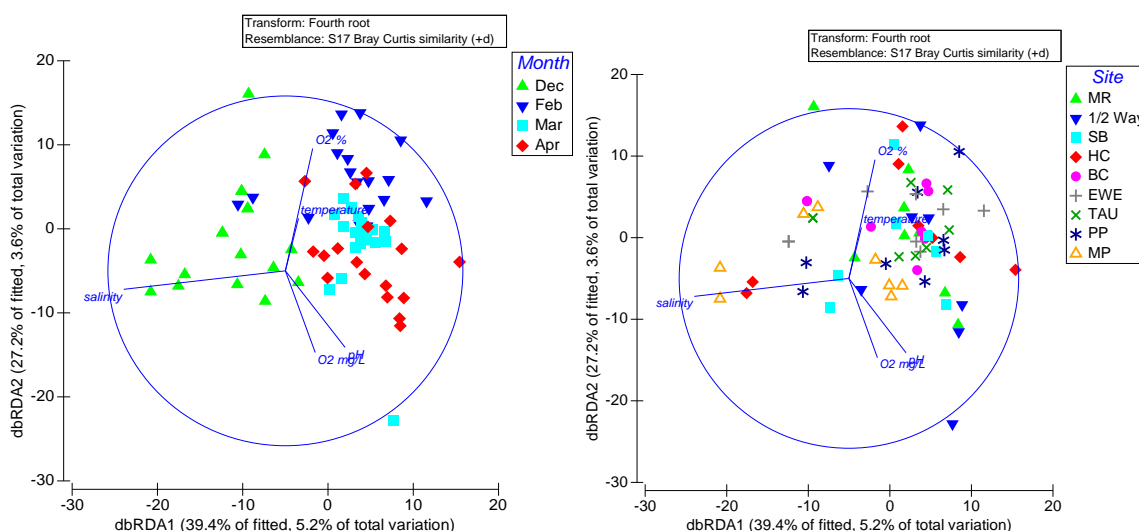


Figure 30: dbRDA plot illustrating relationships between environmental parameters and macroinvertebrates during the water release study, based on water quality parameters determined for every sampling month. For clarity, the figure is shown by month (left) and site (right) separately. Several parameters were transformed prior to analysis. The vector overlay uses base variables of environmental data, Spearman rank correlation.

As benthic macroinvertebrates are also affected by sediment conditions, the analyses were carried out for December and April when grain size and organic matter characteristics were determined. Chlorophyll-a was omitted from the analysis as it was not sampled in December from subtidal sites. The DISTLM analysis indicated that no single parameter contributed significantly to the pattern of macroinvertebrate assemblages, but all parameters together gave a much higher 44% of the variation explained. The dbRDA plot is, however, giving no clear picture of the link between the macroinvertebrate assemblage and the considered environmental factors that matches either the location of study sites in the Murray Mouth or the sampling month, with the exception of Mark Point (MP) from the North Lagoon (Figure 31).

For none of the analyses (water parameters all months or with sediment parameters for December and April) did the dbRDA plots indicate any pattern for the depth factor (inter- and subtidal locations).

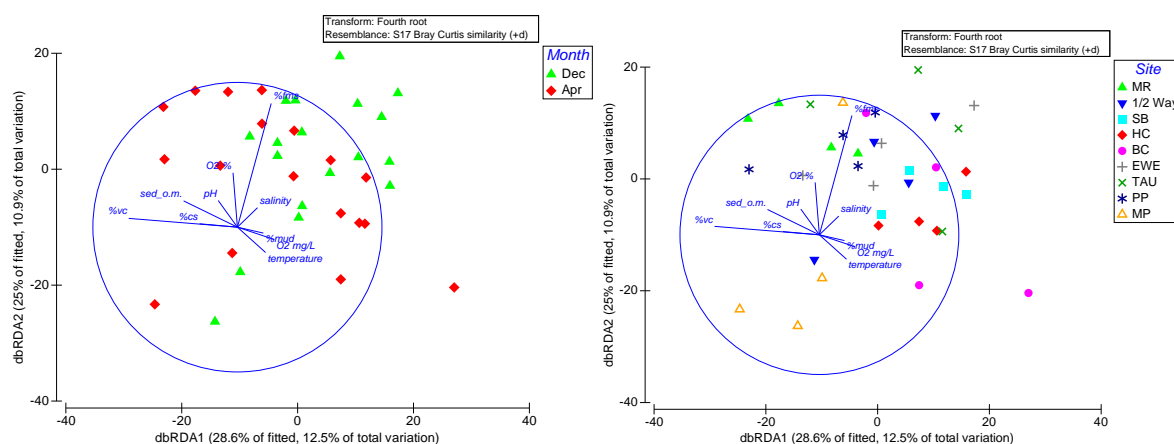


Figure 31: dbRDA plot illustrating relationships between environmental parameters and macroinvertebrates during the water release study, based on water quality and sediment parameters determined for December and April. Sediment grain size components are % mud, % fms (very fine to medium sand), % cs (coarse sand) and % vc (very coarse sand); sediment organic matter shown as sed_o.m. For clarity, the figure is shown by month (left) and site (right) separately. Several parameters were transformed prior to analysis. The vector overlay uses base variables of environmental data, Spearman rank correlation.

4.3.7 Juvenile macroinvertebrate abundances

An indication for an initially positive response to the water release was the presence of several size classes of polychaetes and amphipods in December samples, showing that recruitment had occurred prior to the commencement of the project. Throughout the barrage release project smaller sized polychaetes were almost always found in subtidal sediments, especially between Tauwitschere (TAU) and Mark Point (MP) in the North Lagoon.

Specific sampling with smaller cores and mesh sizes for the presence of juveniles as an indication for recovery taking place, gave a distribution and abundance pattern similar to the one obtained from larger macrobenthic samples (Figures 26 and 32). The higher number of individuals in the juvenile samples at subtidal sediments of Hunters Creek (HC) in December was also due to amphipods (Figures 27 and 32).

The species found in the juvenile samples (Table 21) were a subset of those recorded in the macrobenthic samples (Table 15). Yet, while no *Capitella* sp. was found in the macrobenthic samples, some individuals were present in the juvenile intertidal sediment samples in early February.

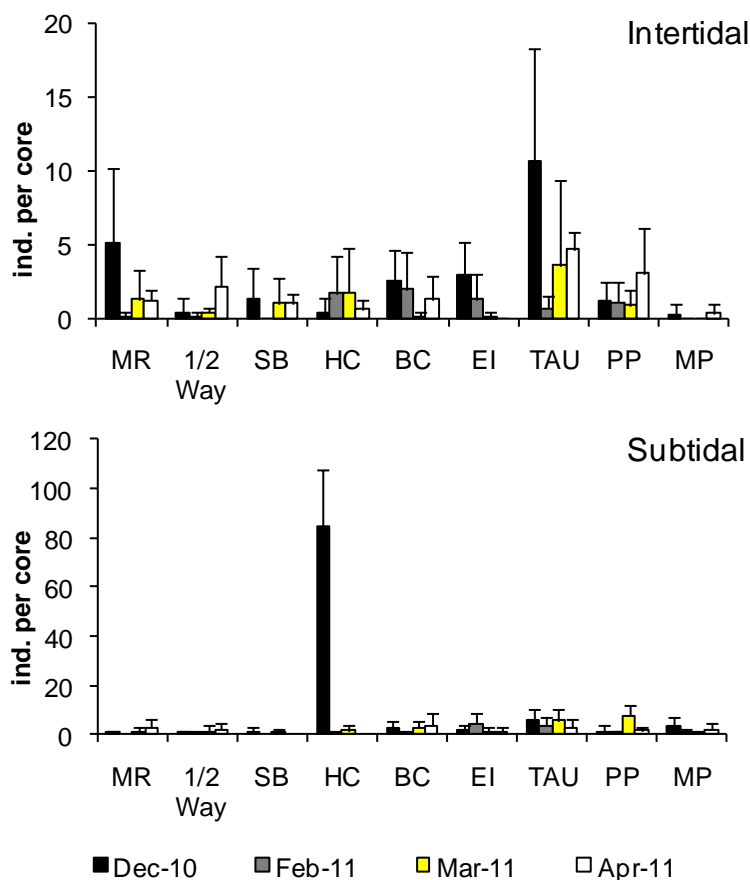


Figure 32: Average abundances (individuals per core (surface area 7.07 cm²), \pm S.D., $n=10$) of macroinvertebrates recorded in 'juvenile' samples at the intertidal and subtidal sampling stations in December 2010, and February, March and April 2011. Note the different scales of the y-axis.

Table 21: List of species recorded in samples for 'juveniles' in samples in December 2010, and February, March and April 2011 at intertidal (Int) and Subtidal (Sub) sampling sites.

Phyla	Species	Dec-10		Feb-11		Mar-11		Apr-11	
		Int	Sub	Int	Sub	Int	Sub	Int	Sub
Annelida	<i>Capitella</i> sp.			X					
	<i>Simplisetia aequisetis</i>	X	X		X				
	<i>Nephtys australiensis</i>		X	X					
	Oligochaeta	X	X						X
Crustacea	Amphipoda	X	X	X	X		X		X
	Ostracoda	X	X	X	X	X	X	X	X
	Mysidacea indet.			X					X
Mollusca	<i>Arthritica helmsi</i>	X	X		X				
Insecta	Ephydriidae	X						X	X
	Chironomid larvae	X	X	X	X				X
Arachnida	Acarina	X							

No post larvae were found in the juvenile samples, but macrobenthic specimens of varying size, indicating that a recruitment event occurred already several weeks or months before the first sampling

event. Meiofauna was also observed to occur, especially nematodes but also copepods and foraminifera, yet they were not counted.

These samples gave an indication for continued presence of some macroinvertebrates in the Murray Mouth region. The combination of this finding and macroinvertebrate samples indicate the area from Tauwichee (TAU) to Mark Point (MP) could possibly be a refuge and recruitment area from where recolonisation could commence.

5. Discussion

This project focussed on the investigation of macroinvertebrates which are mainly estuarine or marine and occur in sediments of estuaries and nearshore tidal wetlands, including larval stages of insects which can be found in sediments. The response described in the report is thus a response of organisms occurring mainly in brackish to marine salinities, which has to be considered for the interpretation of the decline in species occurrence and their abundances under prolonged freshwater conditions.

This report is documenting the changes in the macroinvertebrates following the release of water from the Murray River through the Lower Lakes into the Southern Ocean. The release volume and duration are more akin to a flood event than a temporary water release, and the observed effects in the system are thus more comparable to a flood disturbances as they were observed in tropical systems after hurricanes or intensive wet seasons, which have recorded major declines in macrobenthic abundances (Mallin et al. 2002, Currie & Small 2005, Grilo et al. 2011).

From the response shown by the macroinvertebrates so far, several phases can be differentiated:

- (1) At the first sampling in December, several months after the flow commenced, subtidal benthos revealed a positive response to the flow, with higher abundances of certain taxa and the occurrence of juveniles of several polychaetes and amphipods.
- (2) Over time, mudflats are starting to be recolonised, as indicated by amphipod and chironomid numbers at some sites in April, while species numbers and abundances declined in submerged sediments in most of the Murray Mouth region with prolonged freshwater conditions.
- (3) Estuarine and marine polychaetes in particular are shifting their distribution into the North Lagoon of the Coorong.

These responses are more comparable to findings from (Geddes 1987) to an outflow of the Murray River in the early 1980s, rather than to smaller releases in 2003 and 2005 which functioned like a pulse event (Gillanders & Kingsford 2002) with limited effects (Geddes 2005a, b, Dittmann et al. 2006), yet still being crucial for macroinvertebrate abundances in the Murray Mouth detectable for over a year after the event (Dittmann et al. 2011). Chironomids have responded to past freshwater releases in the Murray Mouth (Dittmann et al. 2006), and were the winners of the current change in the system, with increased abundances over a wider distribution range.

In the Goolwa Channel, the transition to a freshwater ecosystem continued, which had emerged during the first year of monitoring after the water level management and includes the reversal of the brackish fauna initially found inside of the Goolwa barrage (Baring et al. 2009, Dittmann et al. 2010b). The patterns of diversity and abundances of macrobenthos seen in the Goolwa Channel and Murray Mouth corresponds with established salinity patterns observed in many other estuaries (Ysebaert et al. 2003) and combined in a well known diagram (Figure 33).

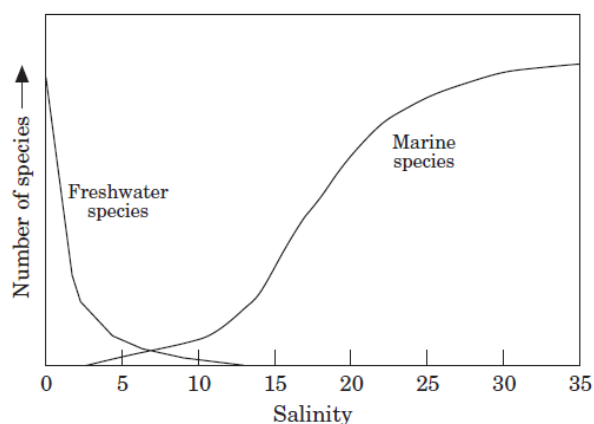


Figure 33: Relationship between macrofaunal species numbers and salinity, modified further from the classic Remane diagram by (Attrill & Rundle 2002).

Based on six years of macroinvertebrate monitoring in the Murray Mouth and Coorong (Dittmann et al. 2011), an understanding of their distribution in relation to salinity and dissolved oxygen had been derived. The key species identified as characteristic for the system in annual monitoring and the present study include the polychaetes *Simplisetia aequisetis*, *Nephtys australiensis*, amphipods and chironomid larvae. The polychaetes never occurred under freshwater conditions as they now prevailed in the system (Figure 34), while low numbers of amphipods have been found in low salinities before and chironomids were more tolerant towards a wider range of salinities (Figure 34). The figure helps to illustrate the scarcity of some benthic macroinvertebrates currently observed in the system, while it also indicates that these species are able to occur under salinities below marine conditions, once more mixing with seawater resumes and restores the estuarine character.

A further environmental factor emerging as influential for characterising the conditions in the course of the flood was dissolved oxygen, with values close to hypoxic conditions ($<2 \text{ mg L}^{-1}$, lowest value recorded in the Murray Mouth was 2.35 mg L^{-1}), similar to observations following hurricane induced floods (Mallin et al. 1999). Low oxygen conditions can have detrimental effects on benthic macrofauna (Nilsson & Rosenberg 1994, Diaz & Rosenberg 1995). Many study sites were characterised by dissolved oxygen concentrations of around 4 mg L^{-1} , and past records again indicate that, apart from chironomids, few macroinvertebrates in the Murray Mouth and Coorong occurred in such low oxygen conditions (Figure 34).

Further environmental factors were affected by the floods, and microphytobenthic biomass was low in the sediments of the Murray Mouth and Coorong, also compared to previous years (Dittmann et al. 2011). Benthic diatoms and microbes respond to river floods and ensuing changes in turbidity and nutrients (Sagan & Thouzeau 1998, Hillebrand & Sommer 2000, Cebrian et al. 2009). Sediment organic matter in the study area varied mainly between sites and values were similar to previous years (Dittmann et al. 2011), thus indicating that restoration of a benthic food web in the system is possible (Hermand et al. 2008).

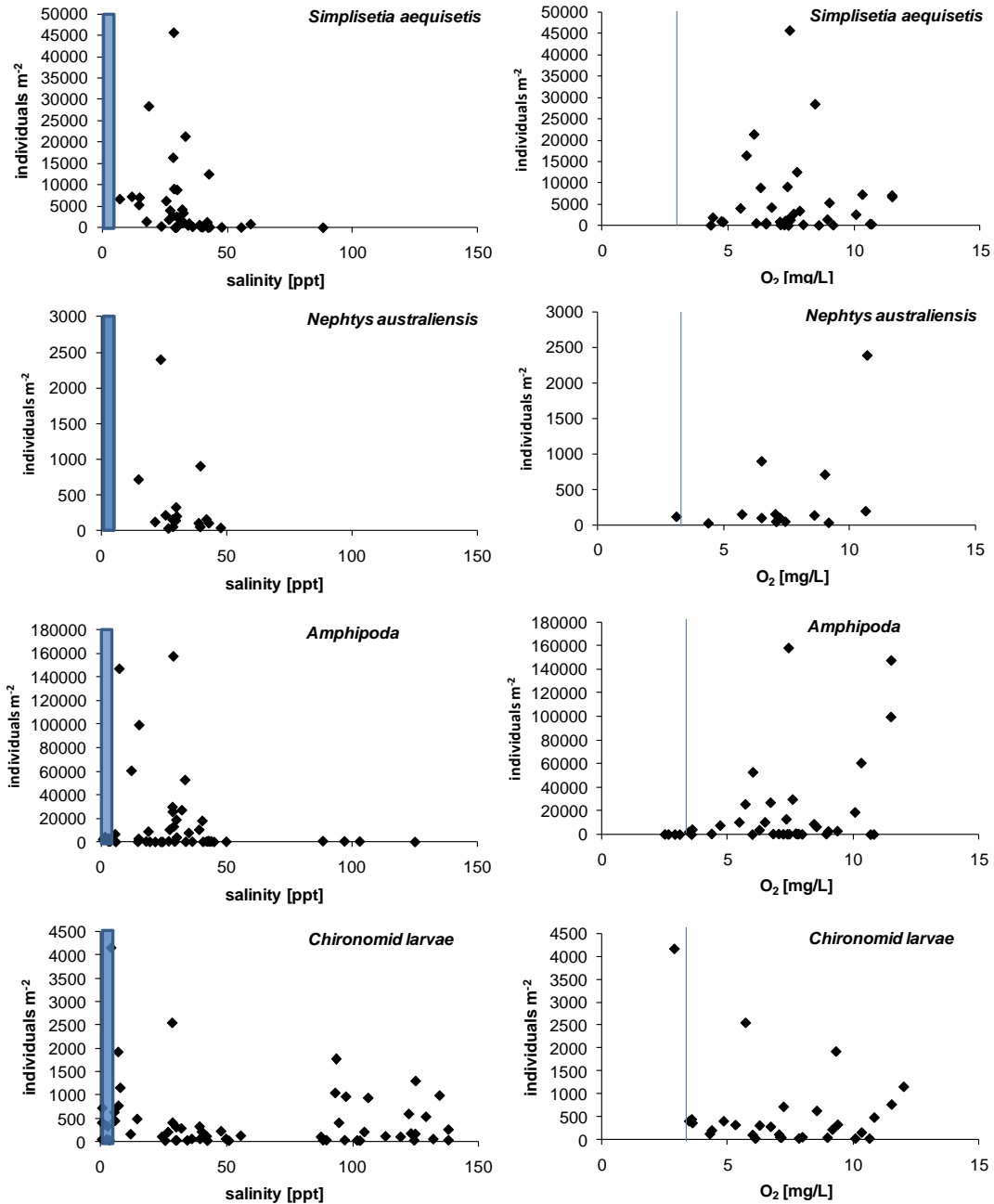


Figure 34: Average abundances of key species for the Murray Mouth and Coorong macroinvertebrate assemblages, in relation to salinities and dissolved oxygen in the water overlying the sediments, based on six years of monitoring from 2004 to 2009 (Dittmann et al., various reports). The area shaded in blue indicates the salinity in the study area of this barrage release project, and the blue line indicates the average lower dissolved oxygen concentrations recorded at several sites in the Murray Mouth and North Lagoon between December 2010 and April 2011.

Apart from the above, the link between environmental data and macroinvertebrate assemblages revealed that no single factor is responsible for the observed pattern. Salinity played a significant role, but could explain only a small part of the variation in benthic data, and while the inclusion of sediment parameter resulted in a higher explanation of the variation, none of the single factors did so with statistical significance. These findings are an important indication that the Murray Mouth and Coorong requires a system approach whereby the entire abiotic and biotic environment has to improve and be in the focus of management activities.

Estuaries are transition areas (Attrill & Rundle 2002) and estuarine organisms adapted to a continuously changing environment, with seasonal or annual variation in hydrological conditions (Kanandjembo et al. 2001), and can shift their spatial distribution pattern to favourable reaches of the estuary (Pech et al. 2007, Cyrus et al. 2010). The capability of the Murray Mouth region to recover from the flood respond are subject to restored estuarine conditions and recolonisation of macroinvertebrates by dispersal of larvae from unaffected areas. The flood now happened after the system had been under severe stress for several years (Kingsford et al. 2011) which had caused a major decline in benthic abundances (Dittmann et al. 2010a). The accumulation and frequency of drought and flood events could reduce the capacity to recover and restore ecosystem structure and function, as observed in estuaries in Portugal (Dolbeth et al. 2011, Grilo et al. 2011). Recovery can take several months and vary in river and estuarine section of different halinity (Mallin et al. 1999). The previously hypersaline North Lagoon, which is currently functioning like a refuge for marine macroinvertebrates, may thus show a different longer term trajectory of response to the Murray Mouth regions. In St. Lucia, South Africa, life histories and dispersal strategies were important for recovery from hypersaline conditions (Bolt 1975), and similar patterns may be observed in the Murray Mouth and Coorong. To evaluate the effects of the current flood on the system and advise future water management of the lower Murray, ongoing studies are required to observe the patterns and processes of macrobenthic recovery and infer effects on higher trophic levels of the ecosystem.

6. Acknowledgements

Accomplishment of the enormous sampling and sorting effort intrinsic in this monitoring is not possible without helping hands. Shaun Schroeder, Nathan Gloede, Jason Earl and Luke Silvester gave some of their time, energy and enthusiasm to help out. Their spontaneous ability to help is greatly acknowledged. The monitoring was funded through the Department of Environment and Natural Resources, and the entire CLLMM team, in particular Alec Rolston are acknowledged for ongoing discussion and support of the project.

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Appendix 1: Sampling dates, times and site coordinates for the GCWLMP and barrage release project for macroinvertebrates.

GCWLMP 2010-2011						
	Site #	Latitude	Longitude	Sampling Date	Sampling Time	
Dec-10	CE 1	-35°30'17.30"	138°55'8.95"	20/12/2010	1200	
	CW 2	-35°29'6.08"	138°54'6.62"	20/12/2010	1330	
	MF 4	-35°29'11.10"	138°53'3.24"	22/12/2010	1300	
	CC 7	-35°29'5.83"	138°49'4.25"	22/12/2010		
	GC 9	-35°31'6.30"	138°48'12.40"	20/12/2010		
	MR 10	-35°29'5.83"	138°49'4.25"	15/12/2010	1330	
Feb-11	CE 1	-35°31'16.70"	138°55'8.97"	21/02/2011	930	
	CW 2	-35°29'5.96"	138°54'6.57"	21/02/2011	1030	
	MF 4	-35°29'11.20"	138°52'9.21"	23/02/2011	1100	
	CC 7	-35°29'6.59"	138°49'3.15"	21/02/2011	1100	
	GC 9	-35°31'6.33"	138°48'12.7"	21/02/2011	1230	
	MR 10	-35°31'5.21"	138°49'7.51"	21/02/2011	1330	
April/ May- 11	CE 1	-35°32'18.30"	138°53'4.50"	4/05/2011	1030	
	CW 2	-35°29'5.96"	138°54'6.57"	29/04/2011	1130	
	MF 4	-35°29'11.20"	138°52'9.21"	4/05/2011	1145	
	CC 7	-35°31'6.30"	138°52'2.45"	4/05/2011	1300	
	GC 9	-35°31'6.25"	138°48'12.40"	29/04/2011	1200	
	MR 10	-35°31'5.84"	138°49'7.49"	29/04/2011		

Barrage Release 2010/ 2011

Site #	Dec-10				Jan/Feb- 11				Mar-11				Apl/May- 11			
	Latitude	Longitude	Sampling	Sampling	Latitude	Longitude	Sampling	Sampling	Latitude	Longitude	Sampling	Sampling	Latitude	Longitude	Sampling	Sampling
			Date	Time			Date	Time			Date	Time			Date	Time
Monument Rd MR-i	-35°29'5.83"	138°49'4.25"	15/12/2010	1330	-35°29'5.18"	138°49'7.47"	27/01/2011	830	-35°31'5.16"	138°49'7.46"	21/03/2011	1215	-35°31'5.18"	138°49'7.49"	28/04/2011	1200
Monument Rd sub MR-s	-35°31'5.86"	138°49'7.64"	16/12/2010	1200	-35°31'5.83"	138°49'7.52"	1/02/2011		-35°31'5.01"	138°49'7.51"	22/03/2011	710	-35°31'5.84"	138°49'7.49"	29/04/2011	1030
1/2 Way 1/2 Way-i	-35°32'1.39"	138°51'2.66"	15/12/2010	1200	-35°32'1.39"	138°51'2.66"	15/12/2010	1200	-35°32'1.40"	138°51'2.68"	21/03/2011	1130	-35°32'1.40"	138°51'2.68"	28/04/2011	1030
1/2 Way sub 1/2 Way-s	-35°32'3.60"	138°51'1.86"	16/12/2010	1030	-35°32'3.62"	138°51'3.75"	31/01/2011	1230	-35°32'5.03"	138°51'3.76"	22/03/2011	900	-35°32'5.03"	138°51'3.76"	22/04/2011	900
Sugars Beach SB-i	-35°32'9.40"	138°52'6.88"	15/12/2010	1100	-35°32'9.44"	138°52'6.77"	27/01/2011	910	-35°32'9.49"	138°52'6.92"	21/03/2011	1100	-35°32'3.92"	138°52'3.56"	29/04/2011	1000
Sugars Beach Sub SB-s	-35°32'9.66"	138°52'6.56"	16/12/2010	945	-35°33'0.05"	138°52'6.80"	31/01/2011		-35°32'9.95"	138°52'6.62"	22/03/2011	1000	-35°33'0.07"	138°52'6.82"	29/04/2011	930
Hunters Ck HC-i	-35°32'12.2"	138°53'4.40"	15/12/2010	900	-35°32'15.5"	138°53'4.56"	31/01/2011		-35°32'10.3"	138°53'4.66"	21/03/2011	1000	-35°32'10.9"	138°53'4.48"	28/04/2011	
Hunters Ck Sub HC-s	-35°32'20.0"	138°53'4.13"	21/12/2010		-35°32'1.34"	138°53'4.98"	31/11/2011	900	-35°32'2.02"	138°53'4.19"	22/03/2011	1100	-35°32'1.84"	138°53'4.49"	29/04/2011	1330
Boundary Ck BC-i	-35°33'7.80"	138°55'1.35"	22/12/2010		-35°33'7.98"	138°55'1.79"	2/02/2011		-35°33'7.99"	138°55'1.80"	28/03/2011	1100	-35°33'8.08"	138°55'0.47"	27/04/2011	1330
Boundary Ck Sub BC-s	-35°33'8.40"	138°55'2.41"	21/12/2010		-35°33'9.61"	138°55'1.51"	2/02/2011		-35°33'9.62"	138°55'1.52"	28/03/2011	1000	-35°33'9.05"	138°55'2.01"	27/04/2011	1400
Ewe Is. EI-i	-35°33'4.48"	138°56'5.29"	22/12/2010		-35°33'4.48"	138°56'5.12"	2/02/2011		-35°33'3.59"	138°56'3.15"	28/03/2011	1200	-35°33'4.37"	138°56'5.11"	27/04/2011	1300
Ewe Is. Sub EI-s	-35°33'7.85"	138°57'5.99"	21/12/2010		-35°33'7.61"	138°57'3.75"	2/02/2011		-35°33'3.34"	138°57'3.41"	28/03/2011	1100	-35°33'4.90"	138°57'6.50"	27/04/2011	1130
Tauwitschere TAU-i	-35°34'5.45"	138°57'6.27"	22/12/2010		-35°34'0.96"	138°57'3.90"	10/02/2011		-35°35'1.18"	138°59'3.89"	28/03/2011	930	-35°35'6.51"	138°59'7.21"	5/05/2011	930
Tauwitschere Sub TAU-s	-35°35'6.33"	139°00'7.46"	21/12/2010	1300	-35°35'6.34"	139°00'7.19"	10/10/2011	1100	-35°35'6.46"	139°00'7.23"	25/03/2011	1000	-35°35'3.14"	139°00'6.25"	27/04/2011	1200
Pelican Point PP-i	-35°35'9.78"	139°01'6.89"	5/01/2011		-35°35'8.87"	139°01'6.26"	10/02/2011	1200	-35°35'8.89"	139°01'6.40"	25/03/2011	1100	-35°35'8.87"	139°01'6.39"	5/05/2011	
Pelican Point Sub PP-s	-35°35'9.35"	139°01'6.13"	5/01/2011		-35°35'2.19"	139°01'6.18"	10/02/2011	1200	-35°35'9.44"	139°01'6.48"	25/03/2011	1030	-35°35'9.35"	139°01'6.23"	5/05/2011	
Mark Point MP-i	-35°37'9.01"	139°04'3.19"	5/01/2011		-35°37'8.03"	139°04'8.17"	10/02/2011		-35°37'7.86"	139°04'8.21"	25/03/2011	1230	-35°37'8.12"	139°04'8.22"	5/05/2011	
Mark Point Sub MP-s	-35°37'8.06"	139°04'7.64"	5/01/2011		-35°37'8.02"	139°04'7.61"	10/02/2011	1230	-35°37'8.07"	139°04'7.65"	25/03/2011	1200	-35°37'8.06"	139°04'7.59"	5/05/2011	