Environment Protection Authority

The influence of acidified sediments and other environmental factors on aquatic invertebrates in the Lower Lakes

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EPA South Australia

The influence of acidified sediments and other environmental factors on aquatic invertebrates in the Lower Lakes

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1 Executive Summary

The severe drought that occurred in the Murray-Darling Basin between 2007 and 2010, led to reduced water levels in Lake Alexandrina and Lake Albert (collectively known as the Lower Lakes) and acidification of sediment on the exposed lake margins of some areas in the region. During the drought, both water and sediment pH declined to less than 4 at some sites and elevated concentrations of metals were also detected. Following the break in the drought, ongoing monitoring conducted by CSIRO and EPA has shown continued low pH and high dissolved metal levels at some sites, particularly at depth in the sediment profile. The effect of these acid sulfate soils on the aquatic invertebrate community following inundation was largely unknown for the Lower Lakes.

An assessment of the condition of the sediment across the Lakes, three years after the drought broke, was undertaken by the EPA and CSIRO in 2013 using the sediment quality triad approach, including biological, chemical and ecotoxicological measurements. This report presents the biological work conducted as part of this larger triad study, and investigates the relationships between the biological and environmental (including chemical) data.

Sediment core samples and sweep net samples of aquatic invertebrates were collected from 17 sites in the Lower Lakes on two occasions (March and November) in 2013. The chemical composition of the sediment was investigated by CSIRO and Southern Cross University by testing concentrations of various ions, metals and acidity metrics. Field observations including substrate composition and some basic water chemistry measurements were also recorded when the biotic samples were collected.

The results from this study suggest:

- Of the 17 sites monitored in the region, seven were acidic (sediment pH < 6.5) in March and five in November. Only three sites (Milang, Poltalloch and Dog Lake in Lake Alexandrina) had acidic surface sediment during both seasons.

- The highest species richness and abundance of taxa, was present in the top 2 cm of sediment of sites across the Lower Lakes, irrespective of sediment pH or substrate type (grain size). Some taxa were found deeper in the sediment, however always in low abundances, and there were no overall patterns to suggest that certain taxa were more likely to be found living deeper in the sediment profile.

- Species richness and abundance varied between sites. The high abundances observed at some sites, particularly at coarse (sandy) sediment sites, were usually due to the large numbers of nematodes collected. At some sites the nematode population accounted for more than 99% of the individuals found. In March, 93% of the benthic community across the Lower Lakes comprised of worms and nematodes. This reduced to 76% in November due to the increase in abundance of microcrustaceans.

- Significant differences in the community composition due to acidity was found for the March core samples but not the November core samples.

- Significant differences in community composition occurred between substrate types (coarse, medium and fine grain sizes) and region (Finniss River, Lake Alexandrina, Lake Albert, but rarely Currency Creek) for both March and November core data. A significant difference also occurred between seasons, with greater taxon richness occurring in November.

- Reduced Inorganic Sulfur (RIS) was the chemical variable that best explained the March core data, explaining 80% of the variation in the invertebrate community, and sediment redox potential (Eh) was the variable that best explained the November core data, explaining 45% of the variability. The sites with high

RIS were also characterised by fine substrate, high TOC and high taxa richness, while the sites with high Eh (positive) were characterised by coarse substrate, low TOC and low taxon richness. These findings were found to be related to the type of environment sampled; lacustrine or riverine/wetland habitat, and the general patterns in the monitoring data can be summarised as:

Lacustrine environment	**Adjacent wetlands and riverine environment**
Coarse substrate (coarse sand)	Fine substrate (silts and clays)
Low total organic carbon	High total organic carbon
Low Reduced Inorganic Sulfur	High Reduced Inorganic Sulfur
Oxidising environment	Reducing environment
Low invertebrate richness	High invertebrate richness

- The invertebrate community collected with sweep nets from submerged/marsh macrophyte types was significantly different from emergent macrophyte types and bank habitat. The submerged and marsh macrophytes provide a unique habitat for some species, such as some hemipterans (true bugs) and beetles that will not be found in other habitat types. When assessing micro-habitats individually, samples collected from *Typha* micro-habitat were often significantly different from samples collected from other emergent macrophytes sampled at the same site.

This study found that the invertebrate community present in the Lower Lakes is primarily being determined by the substrate type at each site. Whether the site is a lacustrine (lake), or riverine or wetland environment is likely to be the main driver in determining the main substrate types at each site. The main area of Lake Alexandrina and parts of Lake Albert is dominated by coarse to medium sands and exposed to seiching. The more sheltered areas such as the tributaries entering the Lakes and the wetlands adjacent to the Lakes are dominated by fine substrate and organic material. These more sheltered areas provide more suitable habitat to a range of invertebrate taxa and are the areas of increased species richness but also the areas of poorer sediment quality.

There was evidence of sediment acidity impacting on the invertebrate community of the Lower Lakes, particularly in March, however, the impacts appear to be minor. Should sediment pH or water pH levels decline further in the future, or more prolonged acidification events occur it is likely that on-going alterations to the community structure will occur, changing it from one comprising of mostly acid–sensitive taxa to one dominated by taxa tolerant of low pH levels.

It is recommended that periodic monitoring of surface sediment pH occur at some sites across the Lower Lakes to monitor for changing pH conditions, particularly during low flow events, and document seasonal changes as well as noting if pH levels decline further to values below 6.0. Sampling of the invertebrate community at some of these sites in 3-5 years would provide additional information about the recovery of the Lower Lakes from drought and acidification issues and provide more baseline information about the natural state of the system. Future monitoring should focus on the areas of most concern, primarily the more sheltered areas across the Lakes.

2 Introduction

A severe drought occurred in the Murray-Darling Basin between 2007 and 2010, restricting flow to the Lower Lakes and reducing the water level in the lake to as low as -1.0 m AHD. Water quality deteriorated across the region, significantly increasing nutrient concentrations and salinity levels, with salinity reaching more than 8000 µS/cm in Lake Alexandrina and 20,000 µS/cm in Lake Albert during the drought (Mosley *et al.* 2013). During late 2010 and early 2011, the drought was broken in the Murray-Darling Basin and water levels were reinstated to pre-drought levels. Salinity levels have since reduced but are still above pre-drought concentrations and nutrient concentrations have also declined (EPA unpublished data).

Acid Sulfate Soils in the Murray Darling Basin

The reduced water levels in the lake led to exposure of sediments on the lake margins. This sediment contained pyritic minerals, which when oxidised produced acidic sediments. Soil acidification was extensive within the region, with soil pH reducing to below four at many sites (Fitzpatrick *et al*. 2010). Research by CSIRO post-drought found that acidic conditions still persisted at some sites, with the pore water of 75% of test sites in the region having a pH < 7 and 21% having a pH < 4. (Fitzpatrick *et al.* 2011). High concentrations of metal ions in the pore water were also recorded, with many concentrations exceeding the national water quality trigger values (Fitzpatrick *et al.* 2011; Mosley *et al.* 2014).

The presence of reduced pH and increased availability of metal ions in the sediment poses a potential toxicity hazard for the aquatic ecosystem of the Lower Lakes. Previous monitoring in the region (e.g. Mosley *et al.* 2014) has raised two main issues of concern in regard to acid sulfate soils in the Lower Lakes in regard to the potential impact to the ecosystem. These issues are: 1) those sites which are acidic on the surface of the sediment (i.e. at the sediment/water interface) and 2) those sites which are acidic deeper in the sediment profile but which may still be impacting on the biota living in the sediment or at the surface.

Benthic Community Structure

Aquatic invertebrates play an important role in the functioning of aquatic food-webs, being the major link between detrital organic materials and higher trophic levels, such as fish, birds, turtles, water rats and frogs. Invertebrates are often used to assess the ecological health of inland waters because they are common, easily sampled, can be readily identified and are known to have a wide range of environmental tolerances, with some able to survive in low oxygenated or polluted waters, while others are sensitive and require well-oxygenated, unpolluted waters. Aquatic invertebrates have been well studied in South Australia in recent decades and their presence, diversity and abundance can be readily used as indicators of aquatic ecosystem condition.

The effect of acid sulfate soils on aquatic invertebrates is largely unknown for the Lower Lakes but is crucial in understanding the ecological risks posed by exposure to acid sulfate soils in the region. Areas where sediments were previously exposed still contain acidic material and pore water in some locations, and may be hindering the recovery of aquatic life post-drought. Monitoring the benthic community structure between 2008 and 2010 had found that while improvements in benthic community diversity had occurred as water levels returned to normal, some molluscs and crustaceans had not yet returned to the system (Giglio 2011). As these groups are acid-sensitive due to the susceptibility for their shells and carapaces to be damaged by acidic water, their widespread absence may indicate a broad response to acidifications events in the region.

To investigate the potential impact that acid sulfate soils is having on the ecosystem of the Lower Lakes, three years after the drought broke, the sediment quality triad approach (a multiple line of evidence approach using biological, chemical and ecotoxicological endpoints) was used. This project was conducted in collaboration with CSIRO and the overall objectives of the study were to:

- Assess spatial and temporal variation of ecotoxicological effects due to acidification, and
- identify hot-spots based on the sediment quality triad approach.

This current report focuses on the biological component of the triad study and aimed to assess the following:

- To determine if acid sulfate soils are impacting on the aquatic invertebrate community of the Lower Lakes
- to determine which species were living in the sediment of the Lower Lakes and where in the sediment profile they occurred, and
- to determine the variation in invertebrate community structure between different micro-habitats, in particular, how vegetation type influenced the community structure.

3 Methods

Study Site

Seventeen sites were monitored across the Lower Lakes in March and November 2013, with sites located in Lake Alexandrina, Lake Albert, Currency Creek and Finniss River (Table 1, Figure 1). Sites were categorised according to their sediment characteristics (coarse, medium or fine) by Southern Cross University/CSIRO; coarse substrate was considered to be sands to loamy sands, medium substrate being sandy loams to light clays and fine substrate being medium to heavy clays and silty clays. Each site was also categorised as being either acidic (pH < 6.5, ANZECC & ARMCANZ 2000) or neutral (6.5 < pH < 9, ANZECC & ARMCANZ 2000), using pore water pH measurements from the top layers (< 10 cm) of sediment by CSIRO (see Table 1).

The substrate type varies considerably throughout the Lower Lakes region, with sites in Lake Alexandrina and Currency Creek being predominantly coarse substrate (Table 1). Lake Albert sites are mostly medium substrates with fine sludgy material on the surface at some locations, and Finniss River, Boggy Creek on Hindmarsh Island and both Dog Lake and Boggy Lake (small inlets at the top of Lake Alexandrina) consisting of predominantly fine silts and clays.

Table 1 Site characteristics of sampling locations in the Lower Lakes.

Code	Waterbody	Site name	Easting [a]	Northing [a]	Sediment acidity [b]	Dominant substrate[b]
LF01	Finniss River	Wally's Landing	303198	6079714	Neutral, Neutral	Fine, Fine
LF02	Lake Alexandrina	Point Sturt North	321247	6070294	Acidic, Neutral	Coarse, Coarse
LF03	Lake Alexandrina	Milang	316106 (316024)	6079440 (6079440)	Acidic, Acidic	Coarse, Coarse
LF04	Lake Alexandrina	Tolderol	331889	6083697	Neutral, Neutral	Coarse, Coarse
LF06	Lake Alexandrina	Poltalloch	339011 (338928)	6070334 (6070125)	Acidic, Acidic	Coarse, Coarse
LF07	Lake Albert	Waltowa	352376	6059074	Neutral, Neutral	Medium, Fine
LF08	Lake Albert	Meningie	349125	6049311	Neutral, Neutral	Medium, Medium
LF10	Lake Albert	Campbell Park	341261	6056503	Neutral, Acidic	Medium, Medium
LF12	Lake Alexandrina	Loveday Bay	326379	6061724	Acidic, Neutral	Coarse, Coarse
LF13	Lake Alexandrina	Tauwitcherie	319050 (319092)	6060550 (6060494)	Neutral, Neutral	Medium, Medium
LF15	Lake Alexandrina	Boggy Creek	311139	6065855	Acidic, Neutral	Fine, Medium
LF17	Lake Alexandrina	Point Sturt South	314849	6069780	Neutral, Neutral	Coarse, Coarse
LF19	Lake Alexandrina	Dog Lake	331551	6086656	Acidic, Acidic	Fine, Medium
LF20	Lake Alexandrina	Boggy Lake	334997	6089162	Neutral, Acidic	Fine, Fine
LF21	Lake Albert	Windmill Site	345597	6064184	Neutral, Neutral	Coarse, Coarse
LF23	Currency Creek	Currency Creek	301055	6072892	Acidic, Neutral	Coarse, Coarse
LF24	Finniss River	Finniss River South	305763	6073896	Neutral, Neutral	Fine, Fine

[a] = co-ordinates listed are the locations of the sediment core samples collected in March 2013, co-ordinates in brackets are the locations sampled in November, if they differed substantially from the March samples.

[b] = listed as 'results for March 2013, results for November 2013'. Measurements were recorded from the top 10 cm of sediment in March and the top 5 cm in November.

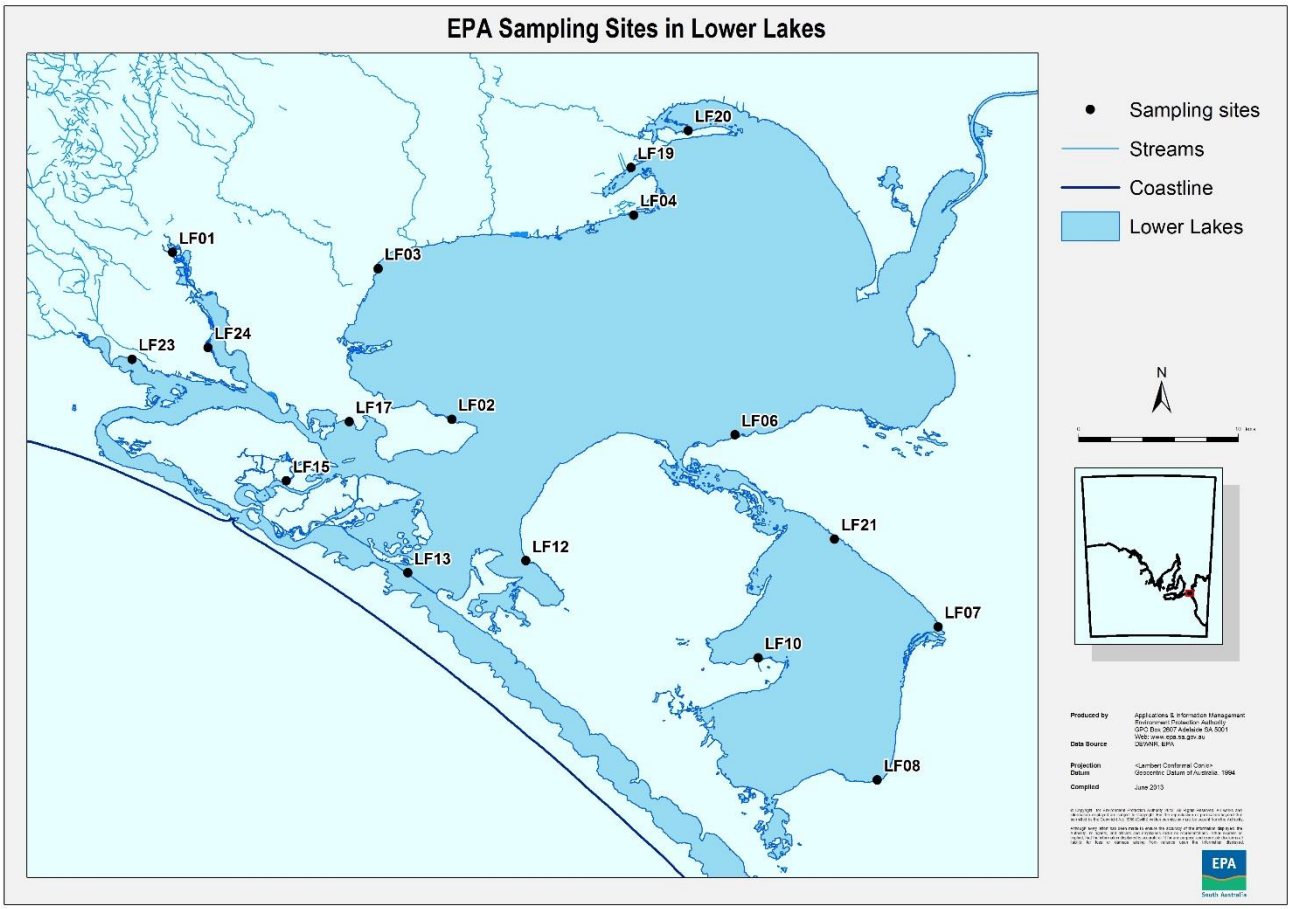

Figure 1 Map of the Lower Lakes region showing sampling sites.

Field Procedures

The aquatic invertebrate community at each site was sampled using two methods; sediment cores and sweep net samples. Sediment core samples were collected to target species that either live in the sediment or are closely associated with the surface layers of the sediment. Sweep net samples were included to collect a broader range of aquatic invertebrates, such as those that are associated with aquatic vegetation or more likely to be found in the water column rather than near the sediment. By collecting samples from these different locations we sought to determine if the potential impacts from acid sulfate soils were restricted to organisms associated with the sediment or if impacts could also be seen within the water column.

Sediment Cores

Sediment core samples were collected from each site with a shovel (Figure 2) in water between 50 and 120 cm deep. Samples were collected in triplicate spaced approximately 1 - 2 m apart and stored in individually marked containers containing methylated spirits for subsequent processing. In March, core samples were collected to a depth of 10 cm and divided into three horizons; 0 – 2 cm, 2 – 5 cm, and 5 – 10 cm. In November, 250 mL of sediment from the 0 - 2 cm layer only was collected from each site.

The site at Tauwitcherie (LF13) was difficult to sample in March due to problems with collecting an intact core from among the dense reed beds. Only one sample extending to a depth of 5 cm could be collected. In November, this site was then shifted to the outskirts of the reed bed where the sediment was more consolidated. The location of

the Milang and Poltalloch sites were also shifted slightly in November to be closer to the shoreline than where they were sampled in March. This was due to an increase in water level in Lake Alexandrina making it impossible to access and sample the original location.

At most sites the samples were individually sieved through a 250 µm mesh in the field to remove fine sediments. This was not necessary at the coarse substrate sites as most of the sand particles were greater than 250 µm and were retained in the net. At many sites a distinct colour change was noticed in the sediment sample with an obvious pale coloured oxic layer on the surface and a darker grey, sometimes black colouration occurring in the deeper layers (Figure 2).

Figure 2 **Sediment core collected from LF02 (Point Sturt, North) in November 2013, showing distinct layers of sediment.**

Sweep Net Samples

Sweep net samples were collected according to the Australian River Assessment System (AUSRIVAS) sampling protocol for South Australia (www.ausrivas.ewater.com.au/index.php/manuals-a-datasheets?id=58). This involved sampling a 10 m section of edge habitat using a triangular dip net with 30 cm sides and a 250 µm mesh (Anon 1997) (Figure 3). All available micro-habitats (e.g. sandy bank, individual vegetation types) were sampled in combination in March. However, to better understand which habitat types might contain the greatest diversity of taxa or if particular taxa are more likely to be present in a certain macrophyte type, the different micro-habitats present at a site were sampled separately in November. A 10 m section of each micro-habitat was sampled at most sites, however, at Finniss River, Wally's Landing only 5 m of each of two micro-habitats (*Myriophyllum* sp. and *Schoenoplectus* sp.) was available for sampling. All samples were placed in separate plastic screw-topped jars and preserved with methylated spirits in the field.

Figure 3 Collecting a sweep net sample along the edge of the bank at LF21 (Windmill Site) in March 2013.

Laboratory procedures

Sediment Cores

Rose Bengal solution (approximately 5 – 10 mL) was added to each core sample jar to stain the fauna present and assist in picking out specimens. To process the core samples, each sample was washed through a 250 µm sieve to ensure fine sediments were removed and then placed in a sorting tray. Under a dissecting microscope, specimens were picked out of the sample, enumerated and identified to the lowest practical level using available identification guides. Macroinvertebrates and zooplankton (microcrustacea) were both identified. As a 250 µm sieve was used all small microcrustaceans such as juveniles and rotifers were not retained in the sample and therefore not included in the data. A full list of all taxa identified is presented in Appendix 1.

Sweep Net Samples

All sweep net samples were processed in accordance with the AUSRIVAS sampling and processing manual. This included a sub-sampling technique where a minimum of 10% of the sample was processed or until at least 200 individuals were counted. The remainder of the sample was inspected with the naked eye with any additional taxa not seen in the subsample picked out and identified. The sample was processed using a dissecting microscope with specimens enumerated and identified to the lowest practical level using available identification guides. In March, microcrustacea were identified in each sample but not enumerated, however in November an estimated abundance of each of the microcrustacea was made.

For some of the sites, particularly the sandy sites, 100% of the sample needed to be processed to ensure at least 200 individuals were identified and counted. However, for other sites, only 10% needed to be processed. For this reason all data has been adjusted to 100% prior to analysis to enable comparisons between sites.

Field Observations

Basic water chemistry was recorded in the field using a YSI 556 multimeter probe. Water temperature (degrees Celsius), specific conductivity (µS/cm), dissolved oxygen (% saturation) and water pH were recorded. Other observations made included percent coverage of site by biofilm and detritus, macrophytes, or filamentous algae, and an estimated percentage of each substrate type: boulder, algae, gravel, sand, silt, clay, detritus. Macrophytes present at each site were also identified to at least genus. In November, sediment pH and redox potential (Eh) were also recorded in the field using a handheld probe and an estimation was made of the depth of the oxic layer at each site.

Chemistry

Sediment pH values were recorded by CSIRO in March and by EPA and CSIRO staff in November using a hand-held probe. A pH less than 6.5 was considered acidic (ANZECC & ARMCANZ 2000). Metal concentrations in the sediment were measured by CSIRO in both March and November using an acid extraction method (1M HCl). The acid extracted metal data represents the potentially biologically available fraction of metals, specifically this includes metals on soil exchange sites and a range of reactive minerals (AVS, carbonates, Fe and Mn oxides) and represents the pool of metals that may be mobilised following oxidation and acidification, or subsequent reduction during rewetting, of acid sulfate materials. In November, metal concentrations in pore water were also measured. Metals were analysed using ICP-OES and ICP-MS. Sub-samples of soil were dried (at 60°C for 48hrs) prior to crushing and analysis for acid-base characteristics using methods from Ahern *et al*. (2004), for titratable actual acidity (TAA, a measure of soluble and exchangeable acidity), reduced inorganic sulfur (RIS, assumed to be pyrite, FeS_2), acid neutralising capacity (ANC_{BT}) and acid volatile sulfur (AVS, November only). Net acidity was calculated as the potential sulfidic acidity (ie. Scrs or Sox) plus actual acidity plus retained acidity minus measured acid neutralising capacity/fineness factor (the fineness factor was defaulted to 1.5). TOC was measured using methods described in Rayment and Higginson (1992).

A full list of environmental variables measured in this study is provided in Appendix 2.

Data Analysis

Abundance and richness information was calculated using an Excel spreadsheet. The remaining data analyses were performed in PRIMER v6 (Clarke and Gorley 2006) with the PERMANOVA+ add on (Anderson *et al.* 2008). As the amount of sediment collected for each horizon in each core varied in March, the data were transformed prior to analysis. Data were standardised in PRIMER by the total abundance for each sample, which converted the abundances of each taxa to a percentage of the total abundance in each horizon. A Draftsmans plot was created to assess for homoscedasticity in the invertebrate data, and following evaluation the data was log(x+1) transformed, to reduce the effect of taxa with high abundances. Resemblance matrices were produced for the biological data using both the Bray-Curtis (a measure of community composition which includes the abundance of each species present in a sample) and the Jaccard (presence/absence data) similarity measures.

Non-metric Multi-Dimensional Scaling (MDS) plots were produced from each biological similarity measure to display the relationships in community composition in a 2-dimensional format. A 2D stress value lower than 0.20 is considered to be an acceptable representation of the data in two dimensions. Hierarchical cluster analyses were undertaken to determine which samples had high similarities and grouped together using the CLUSTER routine with the group average linkage option and a SIMPROF (similarity profile) test. This cluster analysis was then overlayed onto the MDS to illustrate groupings of samples at the, arbitrarily chosen, 50% similarity level.

The PERMANOVA routine was used to determine where statistically significant differences ($p < 0.05$) occurred. The differences between sites grouped together based on specific variables of interest were explored (e.g. pH (acidic, pH < 6.5 or neutral, 6.5 < pH < 9.0), substrate type (fine, medium and coarse; determined by CSIRO), region (Finniss River, Currency Creek, Lake Alexandrina and Lake Albert). When a significant PERMANOVA test was identified, pair-wise PERMANOVA tests and SIMPER analyses were also performed to further investigate the patterns in the data. Pair-wise tests identified which pairs of the relevant factors showed significant differences, at the $p < 0.05$ level, and SIMPER analyses determined which taxa contributed to the significant differences; those with a dissimilarity/SD coefficient of greater than one. SIMPER analysis requires the abundances of each species to determine which taxa have contributed to the differences seen in the PERMANOVA. Results from the SIMPER analyses are presented in Appendix 3.

BEST analysis was also used to determine which environmental variables best explained the patterns in the invertebrate data. All environmental data was normalised before analysis and less than detection limit results for any variable were substituted for zero. For environmental data variables were first checked to determine which were strongly correlated (> 0.95) and collinears were removed from the dataset before further analysis. Euclidean distance was used to create a resemblance matrix for the environmental dataset. A Spearman rank correlation was used to test one variable at a time and also to determine which combination of five variables best explained the community composition.

4 Results

pH results

The surface sediment pH varied considerably across the Lower Lakes in March, ranging from 6.07 at LF19 (Dog Lake) to 7.74 at LF10 (Campbell Park). Seven sites had a pH < 6.5 in March and were therefore considered acidic, being below the listed trigger values for south-central Australia in the national guidelines (ANZECC/ARMCANZ 2000). Similar patterns were evident in November with five sites having a pH < 6.5 (Figure 4). Only three sites were consistently below this pH trigger value, all being located on Lake Alexandrina (LF03 (Milang), LF06 (Poltalloch) and LF19 (Dog Lake)).

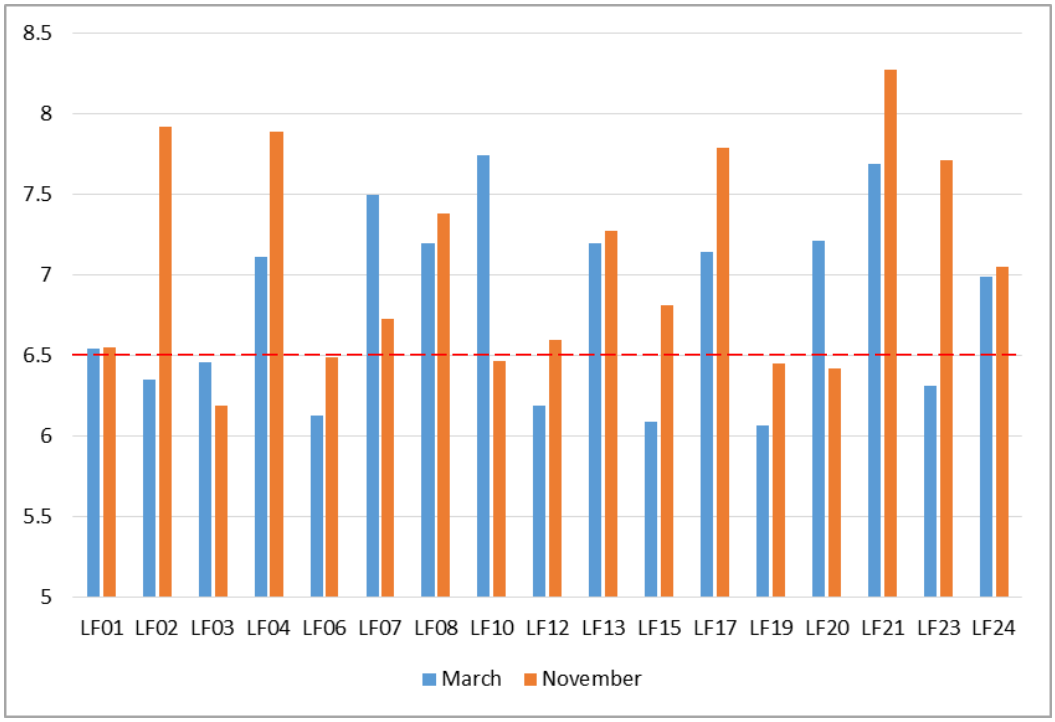

Figure 4 **Surface sediment pH values recorded at each of the 17 sampling sites in March and November, 2013. (ANZECC trigger value of pH 6.5 is represented by the dashed horizontal red line).**

Sediment core samples

The aquatic invertebrate fauna collected from sediment in the Lower Lakes in March consisted of 68 taxa, comprising 7 identified species from 36 genera, 28 families and 17 orders. The samples collected in November were similar with a total of 70 taxa identified (9 species from 36 genera, 29 families and 15 orders). Nematodes, nemerteans and tardigrads were not identified beyond the phylum level.

Vertical distribution of invertebrate taxa

The vertical distribution of benthic invertebrates was investigated in only one of the three replicates collected in March from each monitoring sites. At least 40% of the invertebrate richness occurred in the top 2 cm of sediment at most sites, with few taxa collected from the deeper horizons (Figure 5). This top horizon also consistently contained a higher abundance of taxa than the deeper horizons, with between 53 and 98% of the total abundance in the core sample occurring in the top 2 cm. A few sites (LF02, LF06 and LF08) had no taxa present in the deepest horizon (5 – 10 cm) and LF13, (Tauwitcherie) is presented in Figure 5 as one whole layer as only a 0 – 5 cm layer could be collected from this site.

A PERMANOVA test showed no significant interaction between the different horizons and either sediment pH or grain size, suggesting that the differences in the community structure between the horizons is not influenced by pH or substrate type. However, significant differences were seen due to the depth of the horizon (Bray-Curtis: pseudo-*F* = 3.44, df = 2,44, p = 0.0001), with pair-wise tests showing the community in the top horizon (0 – 2 cm) to be significantly different to both the 2 – 5 cm and 5 – 10 cm layers (p = 0.0074 and 0.0001 respectively). No significant difference was seen between the 2 – 5 cm layer and the 5 – 10 cm layer (p = 0.0881).

There were a few taxa that were found in deeper horizons that had not been found in the top horizon at that site. These included chironomids, worms, copepods, cladocerans, platyhelminths, a biting midge and a mite, however, those taxa collected in the deeper horizons were usually only collected in very low numbers (< 10 individuals). No taxa were found to be consistently living in the deeper horizons across the Lower Lakes region.

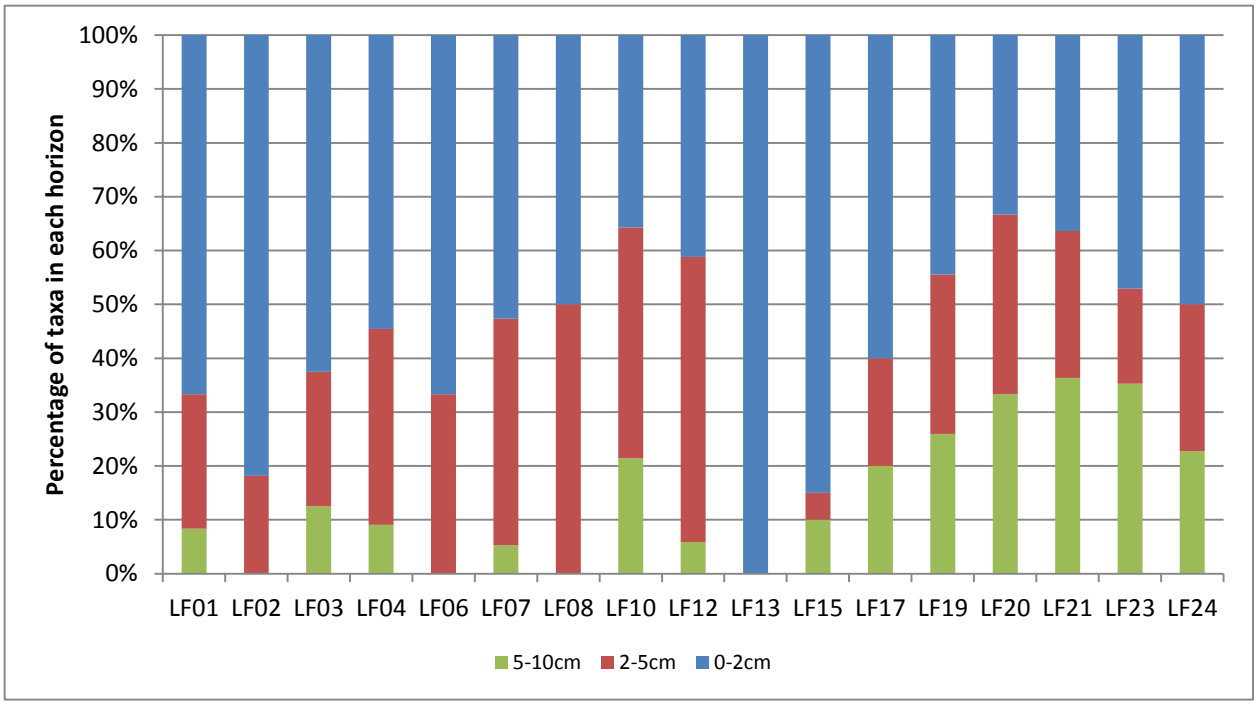

Figure 5 Percentage of taxa identified in each horizon of one core sample (replicate 1) collected from each site.

As the majority of taxa collected in March (in terms of both abundance and richness) were found in the top 2 cm of sediment, samples collected in November were from only the 0 – 2 cm horizon. Furthermore, data relating only to this top horizon has been analysed and presented in this report.

Aquatic invertebrate community composition

Taxon richness ranged from two taxa, collected from LF17 (Point Sturt, South) in November, to 26 taxa from LF07 (Waltowa) in November, with a general increase in richness occurring from autumn to spring (Figure 6). For both seasons, richness was higher at sites with fine substrate than at sites with coarse substrate and sites with the lowest richness were dominated by nematodes.

The degree of seiching that occurs in Lake Alexandrina leads to reduced deposition of fine sediments and organic matter and also hinders biofilm growth on the sediment surface, which is evident by the low total organic carbon concentrations measured at the coarse substrate sites in this study (Figure 7). This reduction in available food for invertebrates results in reduced diversity at these sites, allowing nematodes to flourish in an environment with little competition.

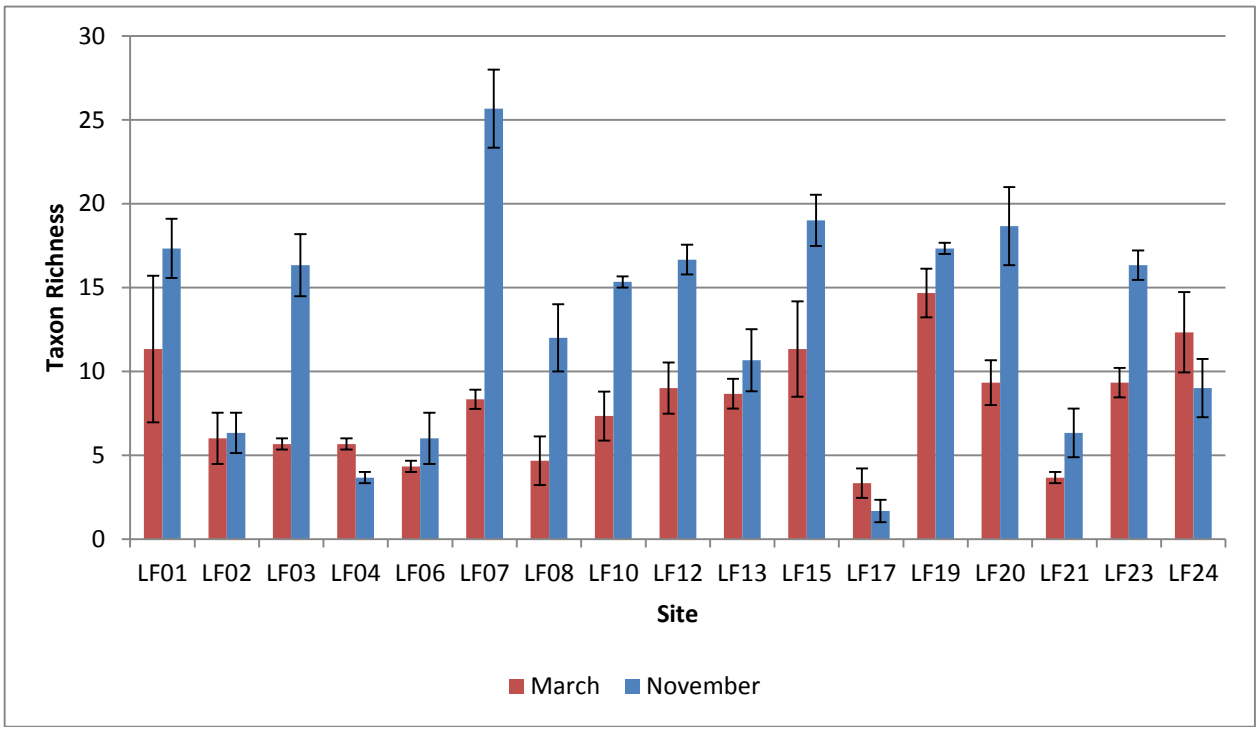

Figure 6 **Mean (± standard error) taxon richness for the 0 – 2 cm layer of the Lower Lakes benthic samples collected in March and November, 2013.**

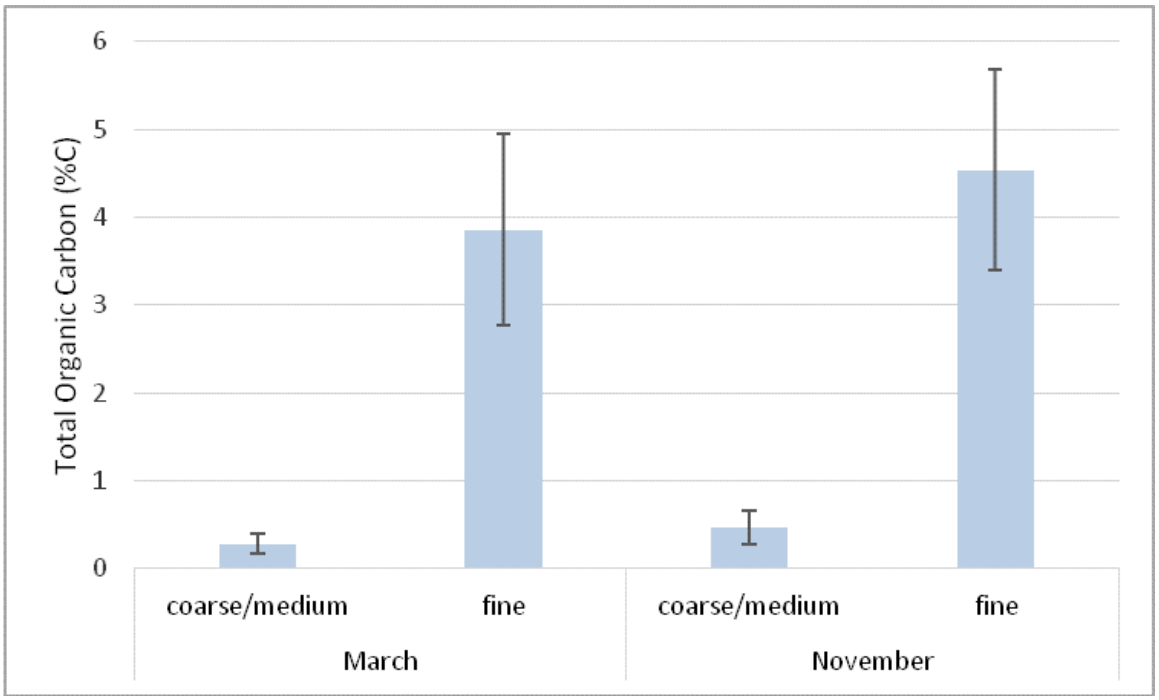

Figure 7 **Mean (± standard error) total organic carbon concentrations for March and November at sites dominated by coarse/medium substrate versus sites dominated by fine substrate.**

Nematodes and worms dominated the invertebrate community in both seasons (93% in March and 76% in November). The highest percent composition (> 95%) occurred in coarse substrate sites (LF02, LF03, LF04, LF06, LF12, LF17, LF21 and LF23) and one medium substrate site (LF08, Meningie) in March (Figure 8), and at LF04 (Tolderol), LF06 (Poltalloch) and LF17 (Dog Lake) in November (Figure 9). While the percent abundance of nematodes and worms decreased from autumn to spring, the true abundance remained approximately the same (ca 31,000 in March and ca 32,000 in November). This reduced percentage was due to the increase in abundance of zooplankton in November, due to natural seasonal variation (Figure 9 cf. Figure 8).

Chironomids (non-biting midges) made up just 2% of the benthic community in March and 1.2% in November. The highest abundances of chironomids occurred at LF13 (Tauwitcherie) and LF15 (Boggy Creek), where they comprised 27% and 25% of invertebrate community respectively. Other taxa collected in the sediment cores included polychaetes, mites, springtails, biting midges, molluscs, mayflies, caddisflies, odonates and crustaceans.

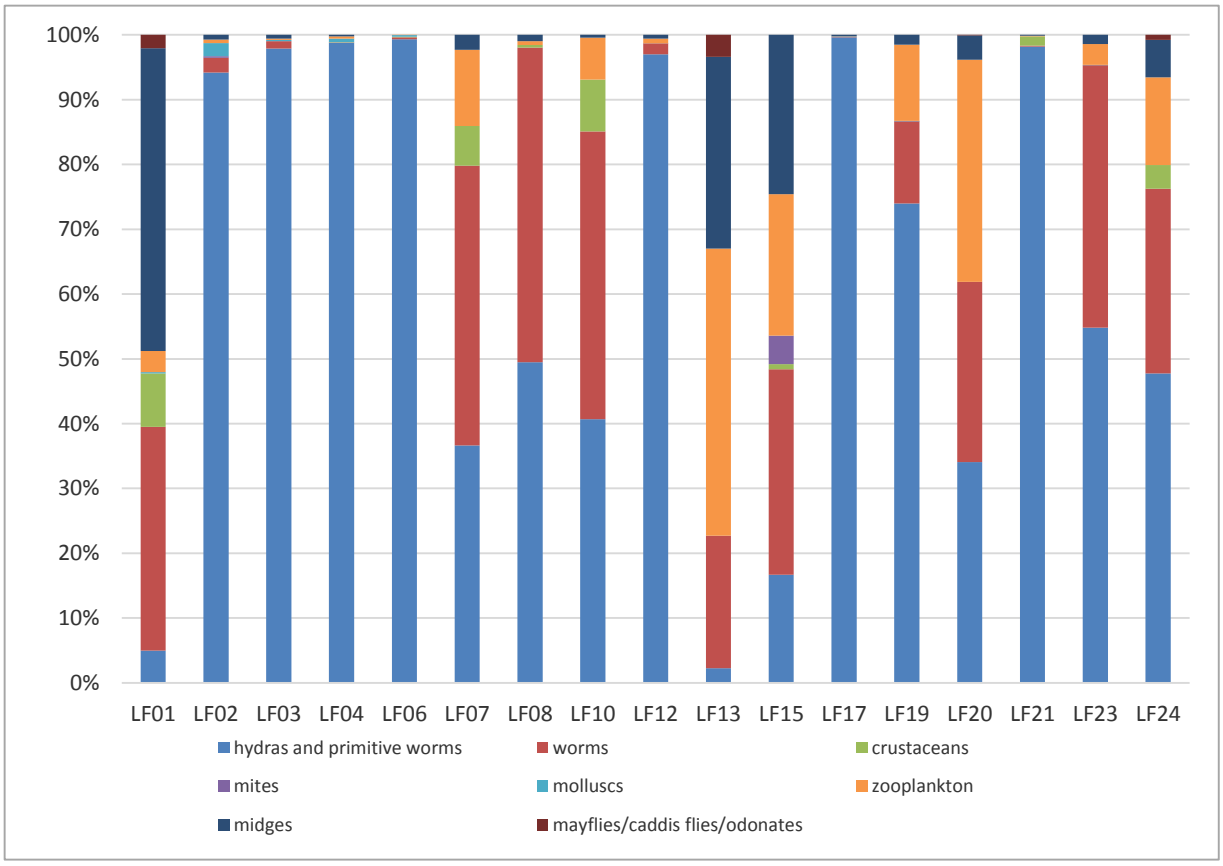

Figure 8 **Percent composition of invertebrate abundance by each taxon group for the 0 – 2 cm layer sediment core collected in March, 2013.**

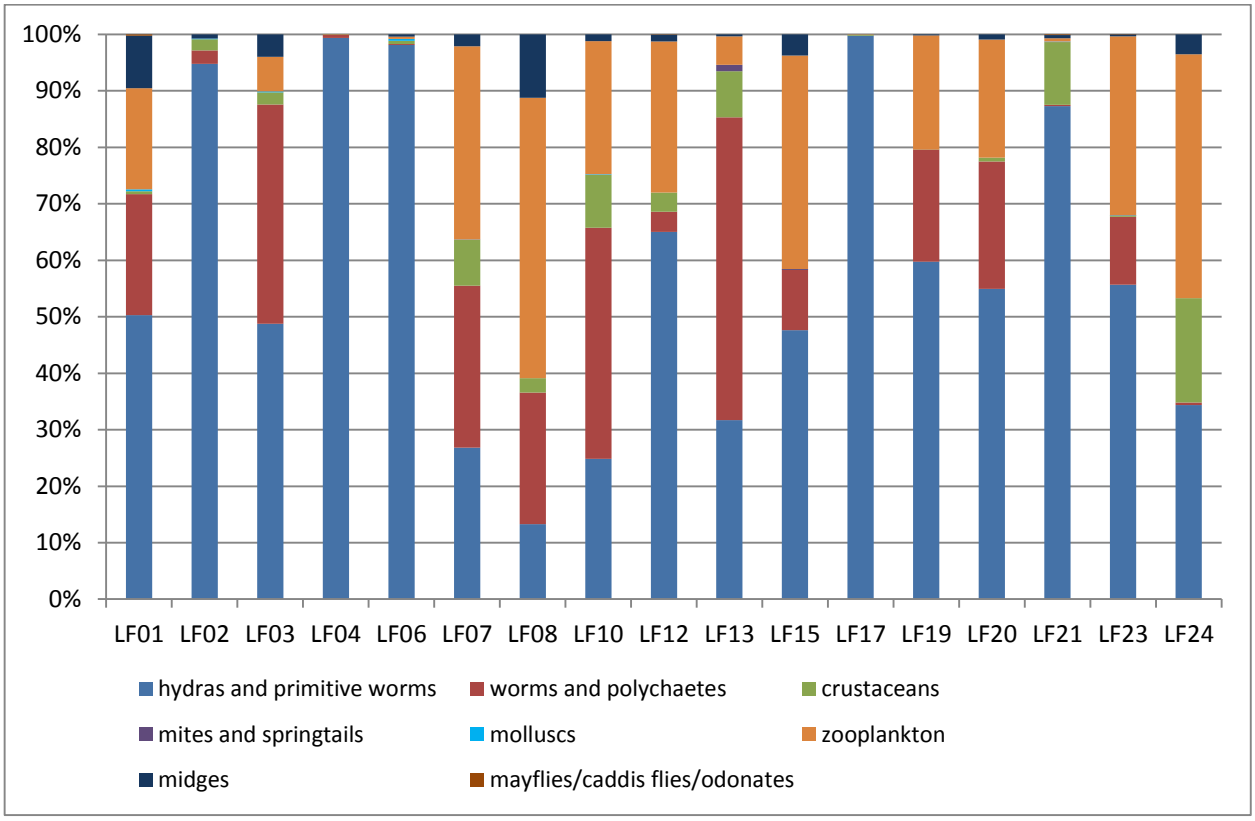

Figure 9 **Percent composition of invertebrate abundance by each taxon group for the 0 – 2 cm layer sediment core collected in November.**

To investigate the patterns in the distribution of aquatic invertebrate communities across the Lower Lakes hierarchical clustering was performed using the CLUSTER routine. The March core data (using the Bray-Curtis similarity measure) clustered into nine significantly different groups (Appendix 4), with all samples considered to have 21.5% similarity, while the November core samples clustered into 16 statistically significant groups, with 38% similarity overall. Generally, the three replicate samples collected from each site fused together before then being grouped with samples from other sites, indicating the variability within site was less than the variation between sites on most occasions. However, at some sites, such as Boggy Creek (LF15) on Hindmarsh Island in March, considerable differences were seen between replicate samples.

Relationship between the aquatic invertebrate core data and pH

The invertebrate data from the March core samples has been presented graphically in Figure 10 using both the Bray-Curtis and Jaccard resemblance measures. Most of the acidic samples are well concentrated at the lower-right side of the plot (Figure 10a), suggesting strong similarity in community composition, however, samples from Boggy Creek (LF15) were plotted further to the left. Of the acidic sites, all core replicates collected from Dog Lake (LF19) and Currency Creek (LF23) grouped together with more than 60% similarity and Loveday Bay (LF12), Point Sturt North (LF02), Milang (LF03) and Poltalloch (LF06) grouped together with 79% similarity. An increase in the dispersion can also be observed amongst the samples towards the left of the plot, which include samples from both of the Finniss River sites (LF01, LF24), Boggy Creek (LF15) and Tauwitcherie (LF13), demonstrating the variability in the composition seen in the replicate samples collected from these sites.

Using the Jaccard similarity measure (presence/absence data) clear differences were seen between the acidic and neutral samples (Figure 10b), suggesting that differences between these two groups of samples is the result of difference in species composition between the acidic and neutral sites, not just differences in the abundances of

the taxa present. Differences in the community due to sediment pH were found to be statistically significant (Bray-Curtis: pseudo-*F* = 3.36, df = 1,49, p = 0.0164; Jaccard: pseudo-*F* = 3.01, df = 1,49, p = 0.0012).

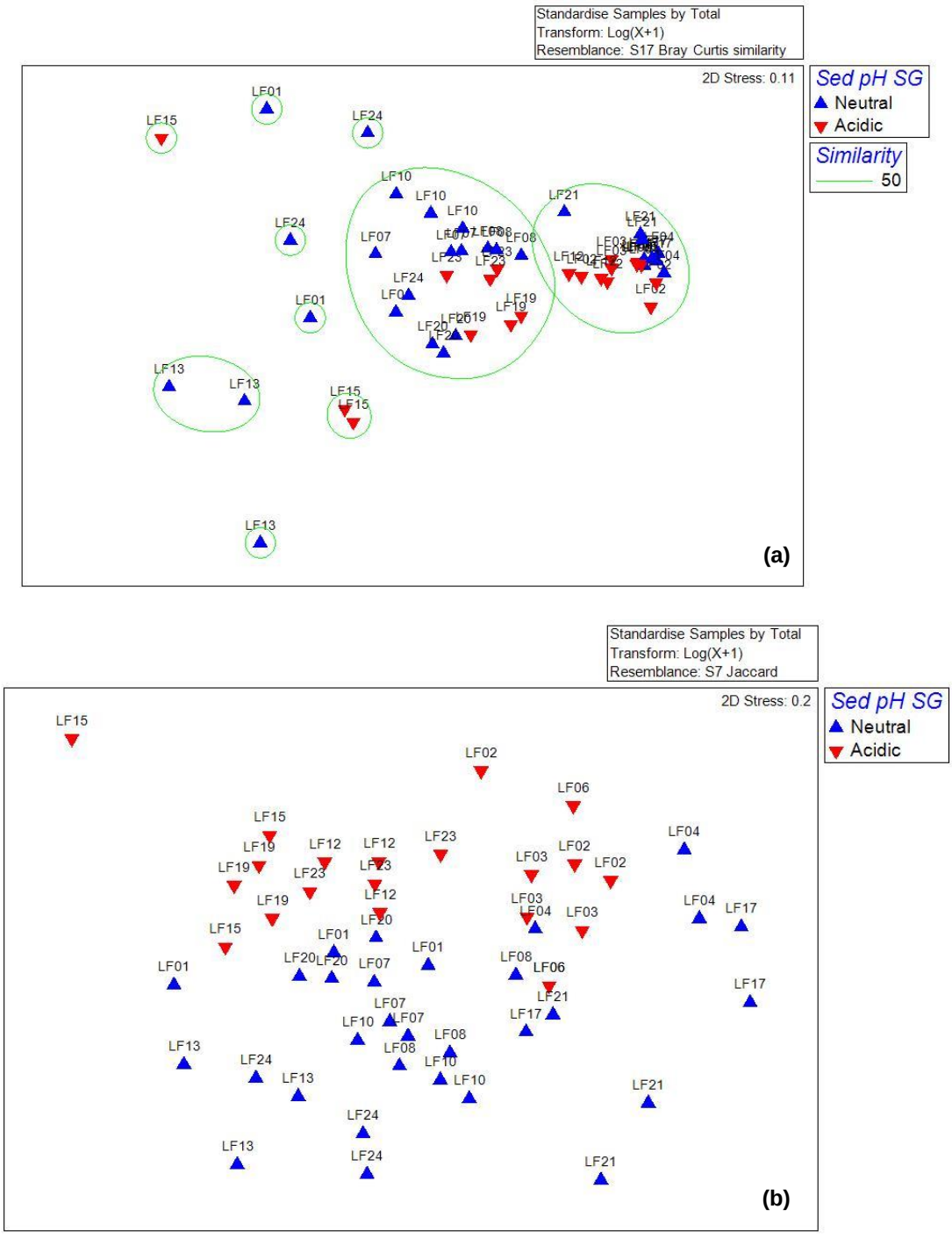

Figure 10 **Multi-Dimensional Scaling of March core samples using Bray-Curtis (a) and Jaccard (b) resemblance measures. Samples are colour coded according to acidity. The Bray-Curtis plot has been overlayed with the CLUSTER analysis.**

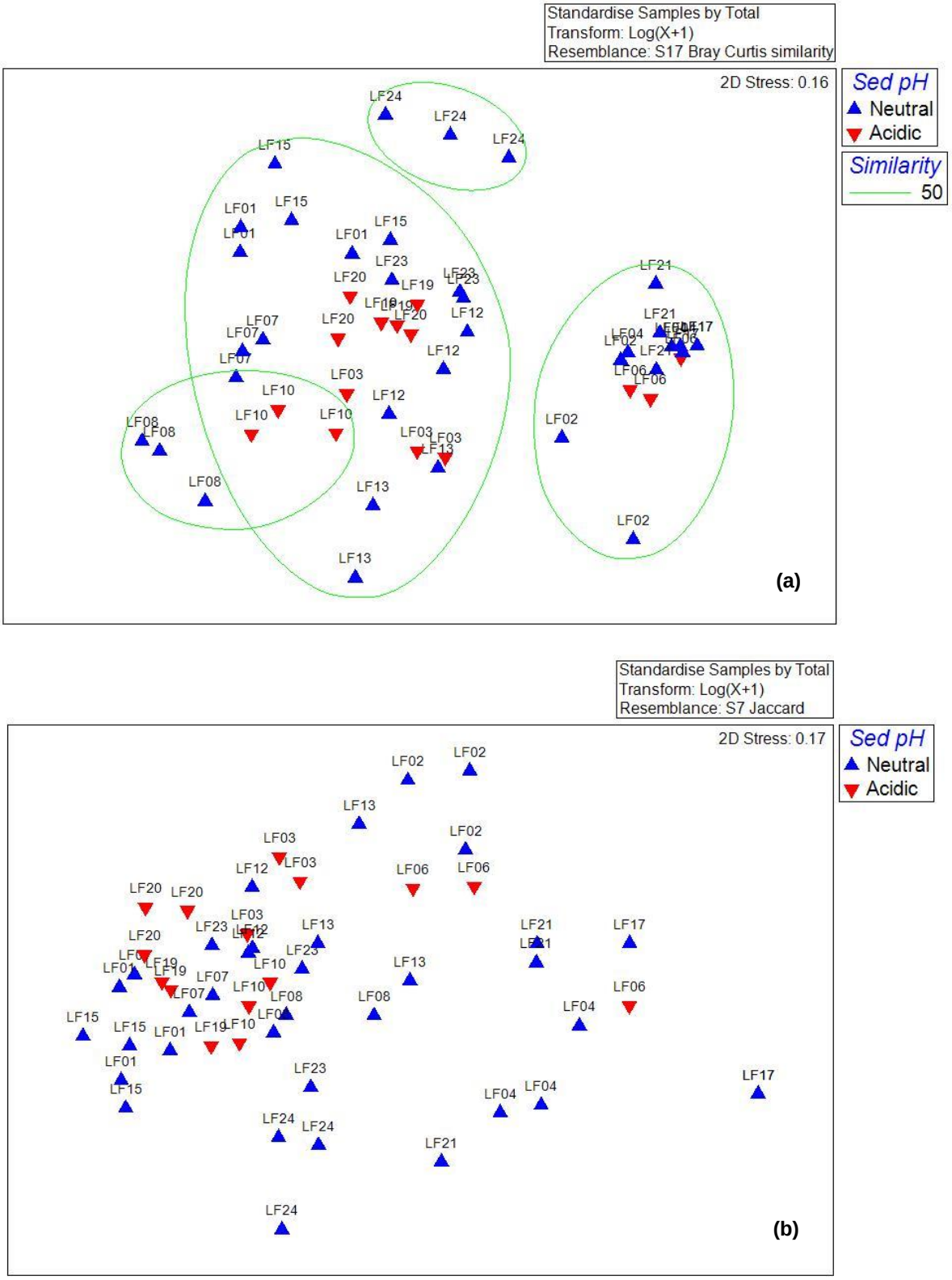

Figure 11 **Multi-Dimensional Scaling of November core samples using Bray-Curtis (a) and Jaccard (b) resemblance measures. Samples are colour coded according to acidity. The Bray-Curtis plot has been overlayed with CLUSTER analysis.**

Samples collected from acidic sites in November were mostly located in the centre of the Bray-Curtis MDS plot (Figure 11a). Dog Lake (LF19) and Boggy Lake (LF20) grouped together with a similarity of 70% and Campbell Park (LF10) and Milang (LF03) are also plotted as being spatially similar but were not clustered together in the

hierarchical cluster analysis, suggesting there are other sites to which they were more similar. The acidic sites on the far right-hand side of the plot are the samples from Poltalloch (LF06) which grouped with other coarse substrate sites rather than with other acidic sites. The MDS shows the samples from the acidic sites are plotted closer together than samples from the neutral sites, suggesting that there is more variability in the species abundance and diversity at the neutral sites. The difference in community composition (Jaccard plot) between the acidic and neutral sites, in November was less pronounced than occurred in March (Figure 11b vs Figure 10b) with samples from the acidic sites being located near the centre-left of the plot but not separated spatially from the samples from the neutral sites. Consequently, there was no significant difference between the acidic and neutral sites in November when using either the Bray-Curtis or Jaccard similarity measures.

Identification of acid-tolerant and acid-sensitive taxa

The relative abundances of each taxon present in the acidic versus the neutral sites was explored for both seasons (combined) to determine if certain taxa were more likely to be found in either acidic or neutral conditions. Abundances for each taxon were summed for all core samples collected from acidic sites and divided by the number of samples collected to produce a relative abundance; the same was then done for the samples from the neutral sites. As there were more neutral sites it was important to calculate relative abundance to provide a more accurate comparison between the two pH groups. An overall score was calculated for each taxon by dividing the relative abundance of that taxa at acidic sites by it's relative abundance at neutral sites. For the purposes of this report, a score greater than 2 indicated acid-tolerance and a score less than 0.5 indicated acid-sensitivity. Table 2 presents the list of taxa which could be considered to be either acid-tolerant taxa or acid-sensitive taxa as determined by the findings from this study.

Table 2 Acid-tolerant and acid-sensitive taxa as determined through this study.

Acid-tolerant taxa	Score	Acid-sensitive taxa	Score
Nematoda	2.07	Cnidaria, Clavidae *Cordylophora* sp.	0.37
Oligochaeta spp.	2.34	Playthelminthes, Turbellaria	0.16
Oligochaeta, Tubificidae - Group B	3.03	Oligochaeta, Tubificidae *Aulodrilus* sp.	0.23
Oligochaeta, Naididae *Nais* sp.	11.00	Oligochaeta, Enchytraeidae spp.	0.00
Mollusca, *Corbiculina* sp.	6.30	Copepoda, Harpacticoida spp.	0.20
Hydracarina, Oribatida	3.10	Amphipoda, Corophiidae Corophiidae SAsp1	0.35
Cladocera, Chydoridae *Alona* sp. sp.	7.33	Amphipoda, Eusiridae spp.	0.35
Cladocera Macrothricidae *Macrothrix* sp. sp.	10.35	Cladocera, Chydoridae *Chydorus* sp.	0.14
Cladocera Macrothricidae *Neothrix* sp.	2.11	Cladocera, Chydroidae *Leydigia* spp.	0.44
Chironominae *Cryptochironomus* sp.	2.21	Cladocera, Daphniidae *Daphnia lumholtzi*	0.06
Chironominae *Cladopelma* sp.	2.12	Cladocera, Daphniidae *Simocephalus* sp.	0.00
Chironominae *Cladotanytarsus* sp.	5.42	Cladocera, Sididae spp.	0.00
		Ostracoda, Cyprididae spp.	0.21
		Ostracoda, Candonidae, *Candonopsis* sp.	0.00
		Ostracoda, Limnocytheridae	0.32
		Mollusca, *Physa acuta*	0.00
		Diptera, Tanypodinae *Coelopynia pruinosa*	0.37
		Diptera, Chironominae *Paratanytarsus* sp.	0.09
		Diptera, Chironominae *Chironomus* sp.	0.15
		Diptera, Chironominae *Dicrotendipes* sp.	0.14
		Diptera, Chironominae *Kiefferulus* sp.	0.00
		Diptera, Chironominae *Parachironomus* sp.	0.00
		Diptera, Orthocladiinae *Cricotopus* sp.	0.42
		Diptera, Orthocladiinae *Paralimnophyes* sp.	0.00
		Ephemeroptera, Baetidae *Cloeon* sp.	0.00
		Ephemeroptera, Caenidae *Tasmanocoenis tillyardi*	0.00
		Trichoptera, Ecnomidae *Ecnomus* sp.	0.00
		Trichoptera, Ecnomidae *Ecnomus pansus*	0.00
		Trichoptera, Ecnomidae *Ecnomus turgidus*	0.00
		Trichoptera, Hydroptilidae *Hellyethira* sp.	0.00
		Trichoptera, Hydroptilidae *Orthotrichia* sp.	0.00
		Trichoptera, Leptoceridae *Oecetis* sp.	0.00
		Odonata, Coenagrionidae spp.	0.00

Relationship between the invertebrate core data and physical variables

The relationship between the invertebrate core data and both the substrate type (or grain size) and the location of each site within the Lower Lakes region was also explored. Two sites were located on the Finniss River, one on Currency Creek and four in Lake Albert. All other sites were considered to be within the Lake Alexandrina region.

Substrate type

Samples collected from coarse substrate sites in March (except Currency Creek, LF23) grouped together on the right side of the MDS plot (Figure 12a). Samples collected from medium substrate (from Waltowa (LF07), Campbell Park (LF10) and Meningie (LF08)) grouped together in the middle of the plot with Currency Creek (the yellow triangles), samples from Dog Lake (LF19) and Boggy Lake (LF20) (both fine substrate sites) and one sample from each of the Finniss River sites (LF01 and LF24) (also fine substrate). The Bray-Curtis plot displays a greater variability between the samples collected from the fine substrate sites compared to the coarse substrate samples, which through the CLUSTER analysis were shown to be very similar to each other. It may be that the high abundances of nematodes at these sites was the reason for the close groupings of these samples on the Bray-Curtis MDS plot, as when abundance information is removed (Jaccard plot, Figure 12b), greater dispersion between these samples results which suggests that the species composition found in these samples varied considerably.

A progressive shift in community composition with increasing grain size was apparent in both MDS plots (Figure 12a and b) with loose groupings based on substrate type, ordered from fine substrates sites on the left of the plot, through to coarse grain sizes on the right. These differences in community were found to be significantly different (Bray-Curtis: pseudo-F = 18.3, df =2,48, p=0.0001) with pair-wise tests identifying significant differences between each pair of substrates for both the Bray-Curtis measure (fine and coarse p = 0.0001, fine and medium p = 0.0029, medium and coarse p = 0.0001) and the Jaccard measure (fine and coarse p = 0.0001, fine and medium p = 0.0001, medium and coarse p = 0.0005).

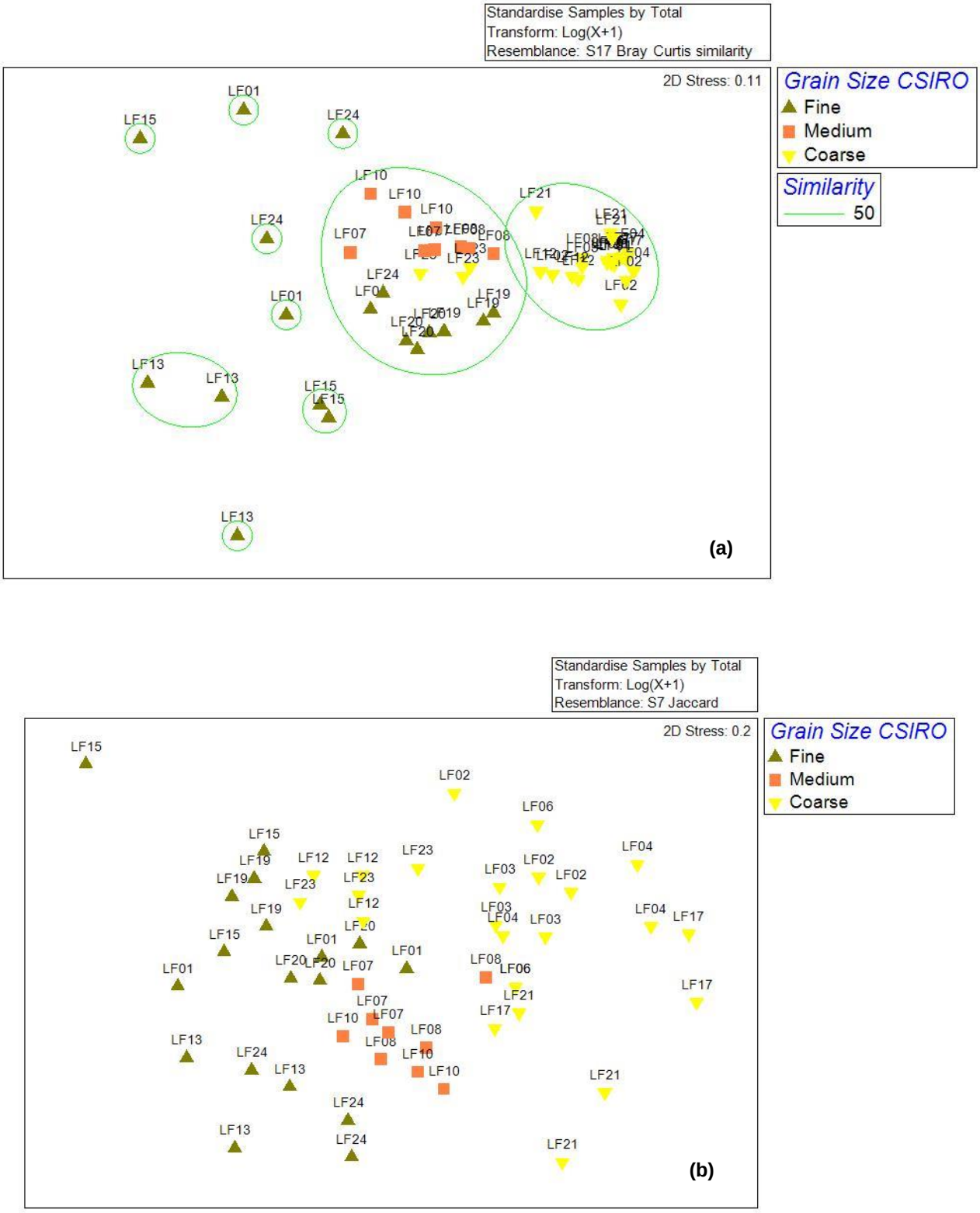

Figure 12 **Multi-Dimensional Scaling of March core samples using Bray Curtis (a) and Jaccard (b) resemblance measures. Samples are colour coded according to substrate type. The Bray-Curtis plot has been overlayed with the CLUSTER analysis.**

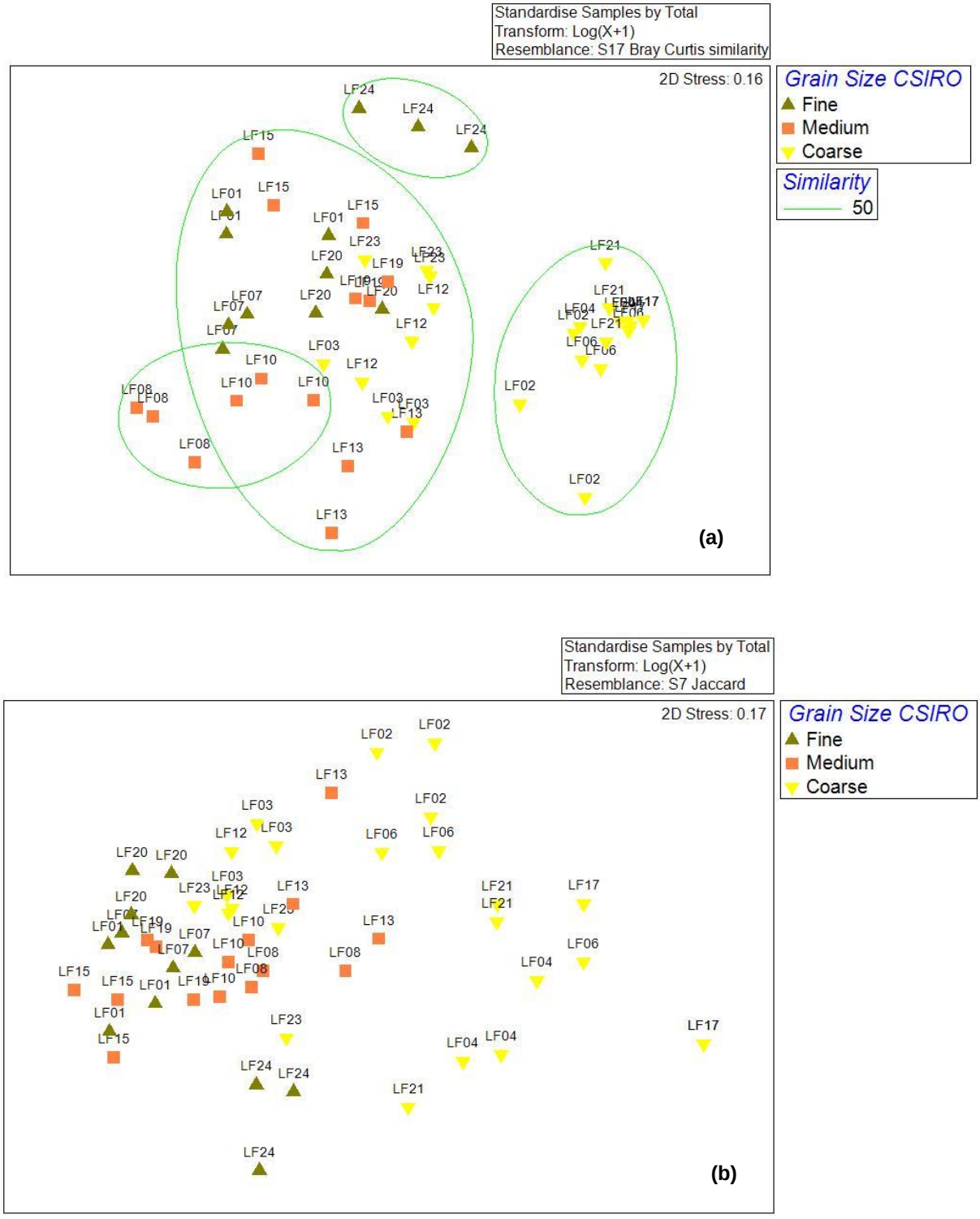

Figure 13 **Multi-Dimensional Scaling of November core samples using Bray Curtis (top) and Jaccard (bottom) resemblance measures. Samples are colour coded according to substrate type. The Bray-Curtis plot has been overlayed with the CLUSTER analysis.**

A similar pattern in the data occurred in November, with samples collected from coarse substrate sites generally plotted on the right side of the MDS and samples from the medium and fine substrate sites were located in the centre - left of the plot. Coarse substrate samples collected from Currency Creek (LF23), Loveday Bay (LF12) and Milang (LF03) were more centrally located, suggesting that their fauna had a greater similarity to that in the medium and fine substrate samples than to other coarse substrate sites. This similarity was confirmed by the

CLUSTER analysis, with the grouping of 50% similarity overlayed on the MDS plot (Figure 13a and Figure 13b). Significant differences due to substrate type were also seen in November (Bray-Curtis: pseudo F = 9.52, df = 2,48, p=0.0001) with significant differences occurring between all pairs of substrate type when using both the Bray-Curtis measure (fine and coarse, p = 0.0001, fine and medium. p = 0.0264 and medium and coarse p = 0.0001) and the Jaccard measure (fine and coarse, p = 0.0001, fine and medium. p = 0.0361 and medium and coarse p = 0.0002).

Species highly associated with coarse sediments across both seasons were found to include the midges *Cryptochironomus* sp. and *Tanytarsus barbitarsis*, the little basket shell *Corbiculina*, nematodes, the freshwater hydroid *Cordylophora,* enchytraeids worms, and the caddisfly *Ecnomus pansus*. Most of these taxa either burrow into the coarse sandy substrate or use the sand grains to construct cases. In contrast, worms, particularly those from the Tubificidae family, the non-biting midges *Chironomus* and *Cladopelma*, the amphipod family Corophiidae, the cladocerans *Ilyocryptus*, *Leydigia* and *Macrothrix*, the copepods Calanoida and Cyclopoida and the ostracod family Cyprididae were found in higher number at sites with fine substrate.

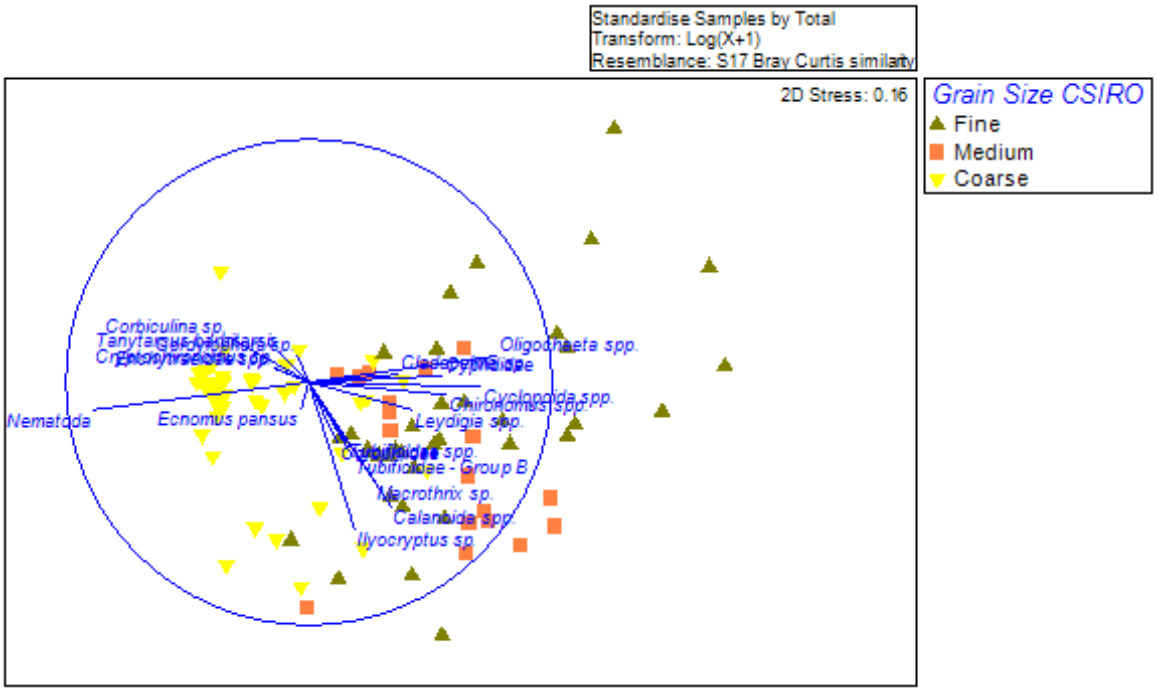

Figure 14 Species vectors overlayed on Bray-Curtis MDS showing samples according to sediment grain size.

Region

Samples collected from Lake Alexandrina sites in March were plotted on the bottom half of the MDS plot, with one sample collected from Boggy Creek (LF15) being the exception (Figure 15). Most of the sites in the Lake Alexandrina region show little dispersion across the plot, samples from Boggy Creek and Tauwitcherie (LF13) being the exception (Figure 15). Currency Creek (LF23) samples and Lake Albert samples were plotted in the centre - top of the plot and samples collected from the two Finniss River sites were highly variable and widely scattered in the top left corner.

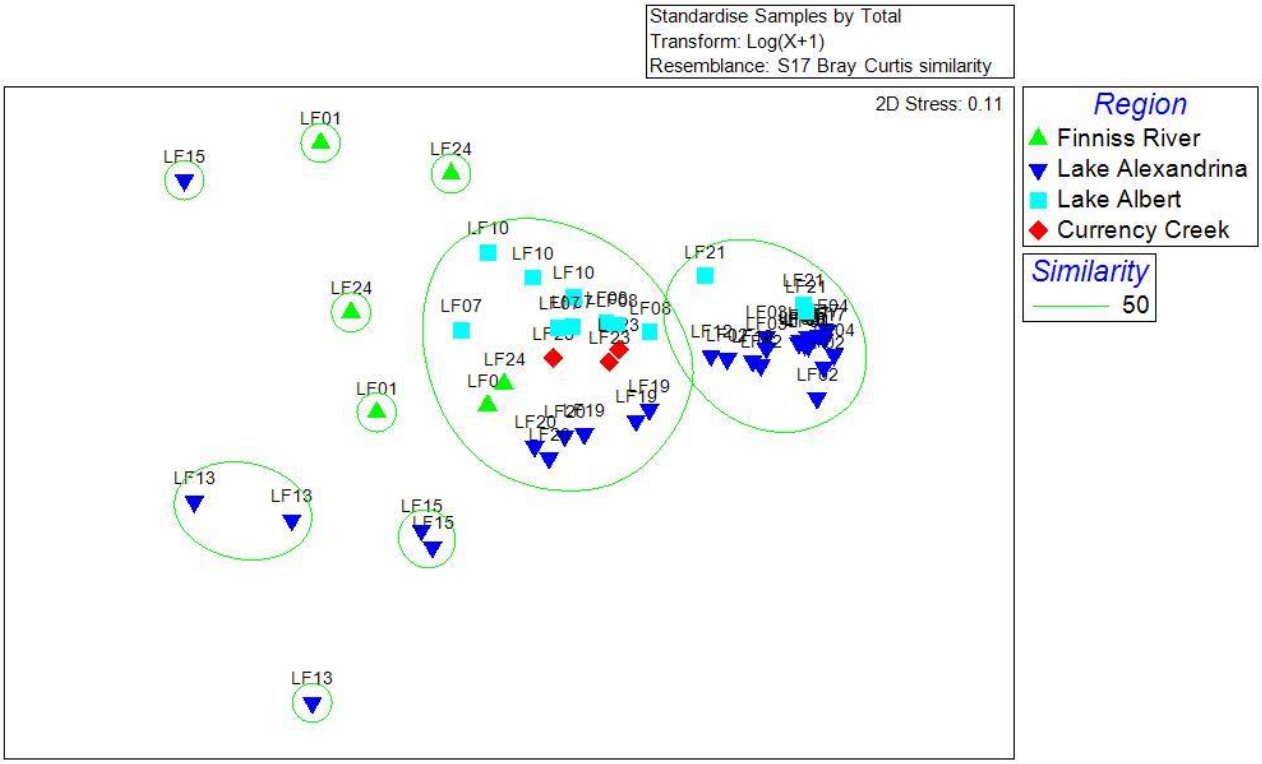

Figure 15 **Multi-Dimensional Scaling of March core samples using the Bray-Curtis similarity measure. Sample points are colour coded according to their respective region and overlayed with CLUSTER analysis.**

Significant differences occurred due to region in March (Bray Curtis: pseudo-*F* = 3.55, df = 3,47, p = 0.006) suggesting the invertebrate community at a site was strongly influenced by where it was located within the Lower Lakes region. Significant differences existed between Lake Alexandrina and both Finniss River (p = 0.0036) and Lake Albert (p = 0.0099), and between Lake Albert and Finniss River (p = 0.0014). However, no significant differences occurred between the samples collected from the Currency Creek site and any of the other three regions in March, possibly due to just the one site being sampled on Currency Creek.

Significant differences also occurred in November (Bray Curtis: pseudo-*F* = 4.20, df = 3,47, p=0.0001) between Finniss River sites and each of Lake Alexandrina, Lake Albert and Currency Creek regions (p = 0.0092, 0.018 and 0.0455 respectively) and between Lake Albert and both the Lake Alexandrina and Currency Creek regions (p = 0.0007 and 0.0173 respectively).

These differences were observed in the MDS plot with the Finniss River samples located near the top of the plot, and most Lake Albert samples grouped on the bottom-left of the plot. (Figure 16). One sample from Lake Albert; Windmill site (LF21) grouped with other coarse substrate sites on the left of the plot rather than with the other samples from Lake Albert, suggesting the substrate type had a greater influence on the community composition at that site than it's location.

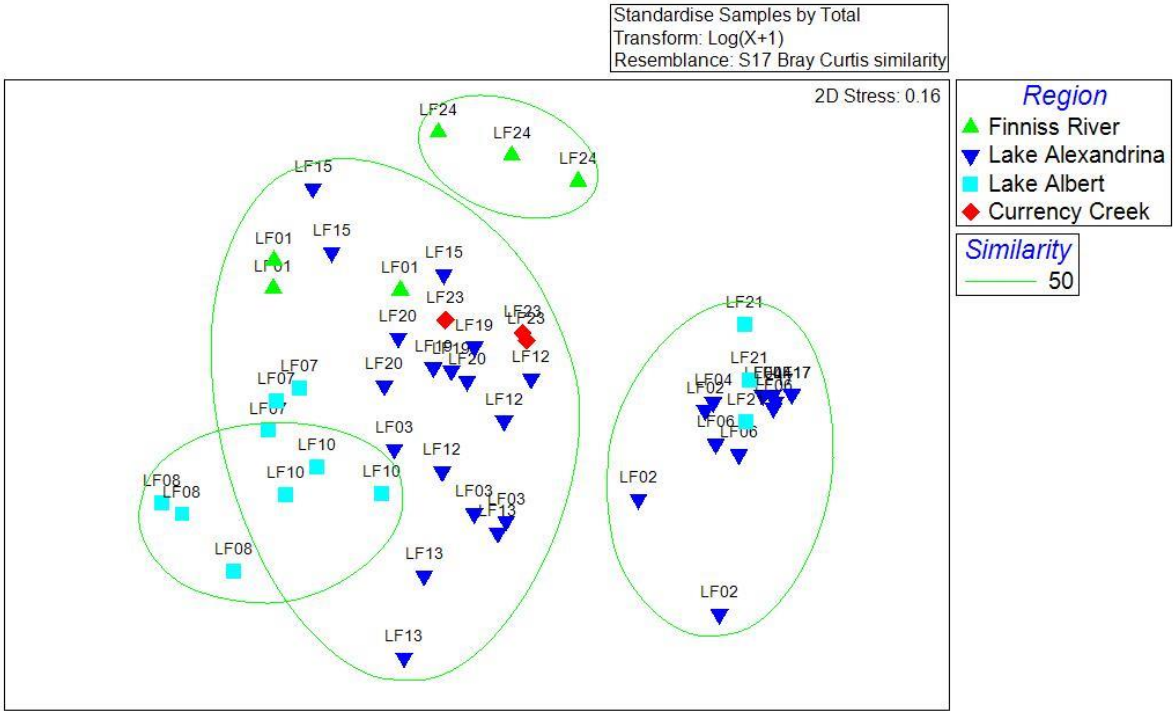

Figure 16 **Multi-Dimensional Scaling of November core samples using the Bray-Curtis similarity measure. Sample points are colour coded according to their respective region and overlayed with CLUSTER analysis.**

Relationship between the macroinvertebrate core data and chemical variables

BEST analysis was used to determine which environmental variables explained the most variation in the invertebrate data and was only used for numerical data, such as metal concentrations and water chemistry measurements. Categorical data, such as substrate type and regional categories (discussed above) were not included in the BEST analysis.

For the March cores samples (using the Bray-Curtis resemblance measure) BEST analysis showed that the correlation coefficient was optimised (at *Rho* = 0.796) for just one variable; reduced inorganic sulfur (RIS). When tested individually the environmental variables which best explained the community data were RIS with a correlation of 0.796, followed by sediment zinc concentrations (*Rho* = 0.709), TOC (*Rho* = 0.598), sediment lead concentrations (*Rho* = 0.592) and sediment potassium concentrations (*Rho* = 0.585).

The highest RIS concentrations in March occurred at sites with fine substrate (Figure 17), particularly the two Finniss River sites (LF01, LF24), Boggy Creek (LF15) and Tauwitcherie (LF13). These sites were also characterised by high TOC and high taxon richness. The high TOC suggests that these sites contain a high amount of organic matter and possibly also biofilm. This provides an abundant source of food for the invertebrates as well as increasing habitat complexity. The high TOC also promotes the production of RIS under low oxygen conditions. It is therefore likely that rather than the high RIS leading to high taxon richness both of these variables instead are related to the amount of TOC present at a site. The relationship between taxon richness and RIS and between RIS and TOC is presented in Appendix 5.

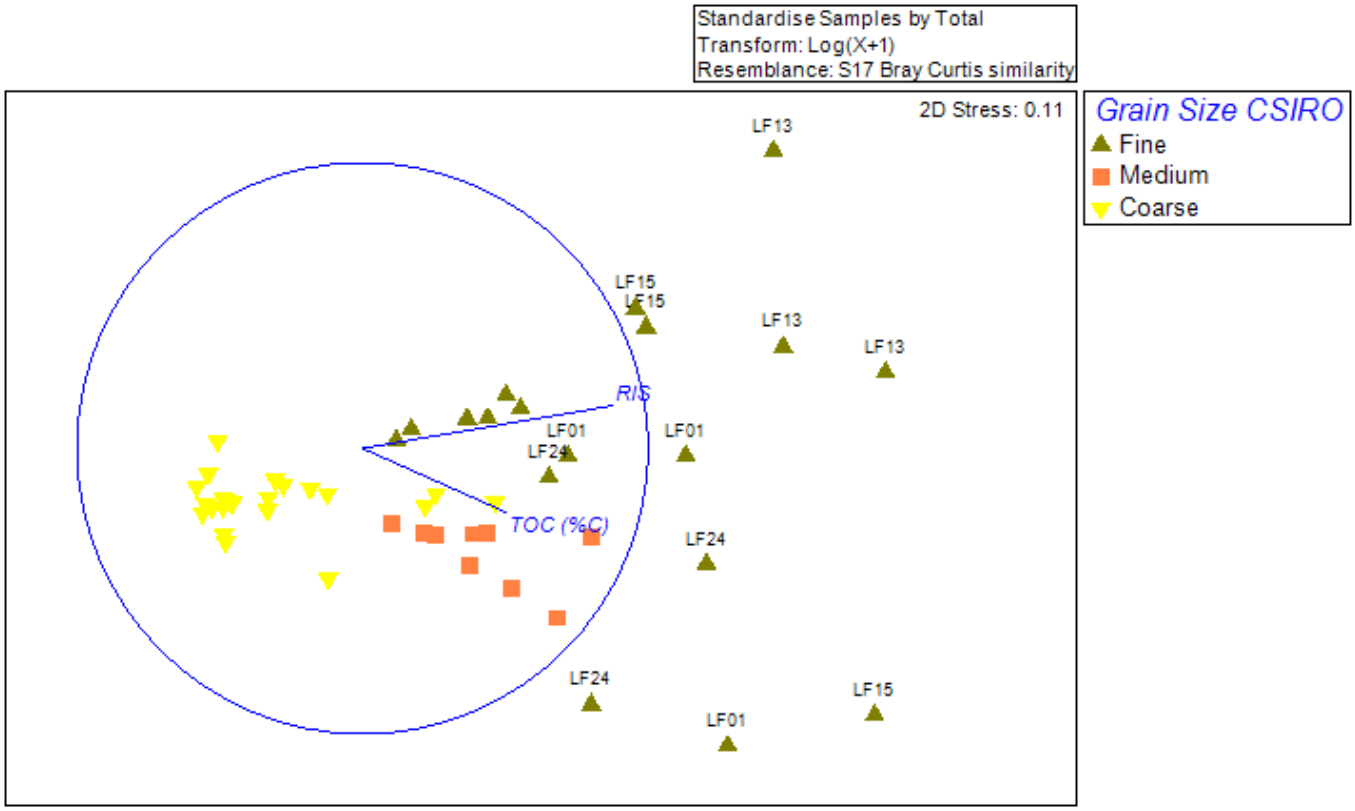

Figure 17 **Bray-Curtis Multi-Dimensional Scaling of March core samples colour coded by substrate type showing the relationship between the invertebrate community and RIS and TOC concentrations.**

For November cores, BEST analysis showed that the correlation coefficient was optimised (at *Rho* = 0.588) for five variables; sediment Eh, sediment vanadium concentrations, NH_4, pore water flouride concentrations and pore water sodium concentrations. However, the environmental variable that best explained the November invertebrate data, when the variables were assessed individually using the Bray-Curtis measure, was sediment Eh with a correlation of 0.448. All other variables showed only weak correlations. A positive redox potential was associated with coarse substrate sites (Figure 18) and recorded at only four sites. However, these four sites were also characterised by low invertebrate diversity and low TOC concentrations (Appendix 5).

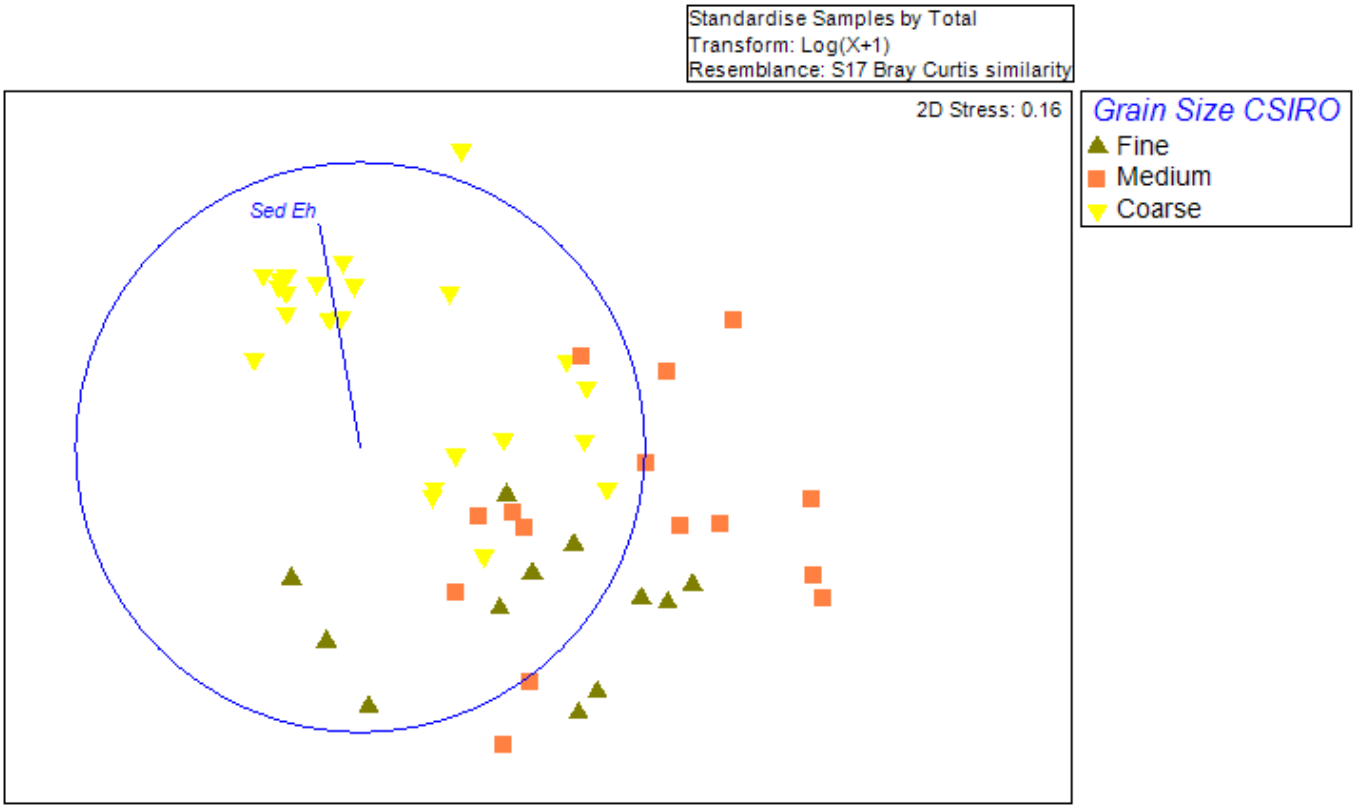

Figure 18 **Bray-Curtis Multi-Dimensional Scaling of November core samples colour coded by substrate type showing the relationship between the invertebrate community and sediment redox potential.**

Comparison of core samples between seasons

The MDS plot of core samples collected in both March and November show clear differences in the species composition using both the Bray-Curtis measure and the Jaccard measure (Figure 19) with November samples dominating the top half of the plot and the March samples dominating the bottom half. Figure 19a (Bray-Curtis measure) shows a very close clustering of samples from coarse substrate sites (Lake Alexandrina) on the right-hand side of the plot, most of which had very high abundances of nematodes and showed very little change in community composition between the two seasons. This plot also highlights the considerable variability in the samples collected from the fine substrate sites in March with those samples widely dispersed across the bottom–left of the plot. However, Figure 19b (Jaccard measure) shows a clear shift in species composition for all sites from March to November, indicating seasonal changes occurred in the species present at each site as well as the abundances. These differences were considered to be significant (Bray Curtis: pseudo-*F* = 5.24, df = 1,100, p=0.0009, Jaccard: pseudo-*F* = 7.42, df = 1,100, p=0.0001). Taxa such as tardigrads, the snail *Physa* sp., non-biting midges (*Tanytarsus barbitarsis* and *Paralimnophyes* sp.*)*, the mayfly *Cloeon* sp., the caddisflies *Ecnomus pansus* and *Oecetis* sp., the springtail Isotomidae, as well as many microcrustaceans such as the cladocerans *Pleuroxus* sp., *Chydorus* sp., *Daphnia lumholtzi*, *Daphnia carinata*, *Simocephalus* sp., *Ceriodaphnia* sp. and *Neothrix* sp. and the ostracod family Ilyocyprididae were only collected from core samples from the Lower Lakes in November. It should be noted however, that most of these taxa were present in the Lakes in March but were only collected in sweep net samples. The only taxa not recorded from any of the samples in March were tardigrads (which are minute invertebrates and easily missed or washed through the net/sieve during collection and processing) and the cladocerans *Ceriodaphnia* sp. and *Neothrix* sp. The cladoceran *Moina* sp., caddisflies *Hellyethira* sp., *Orthotrichia* sp., *Ecnomus cygnitus* and *Ecnomus turgidus*, the water bug *Micronecta* sp., non-biting midges *Nanocladius* sp. and *Parachironomus* sp.*,* the freshwater shrimp *Paratya australienses*, the springtail Sminthuridae, the polychaete Spionidae, *Hydra* sp., and the primitive worm Nemertea were collected in March but

not in November. Again, however, most of these taxa were collected in sweep net samples in November, *Moina* sp., *Orthotrichia* sp. and Spionidae being the only exceptions.

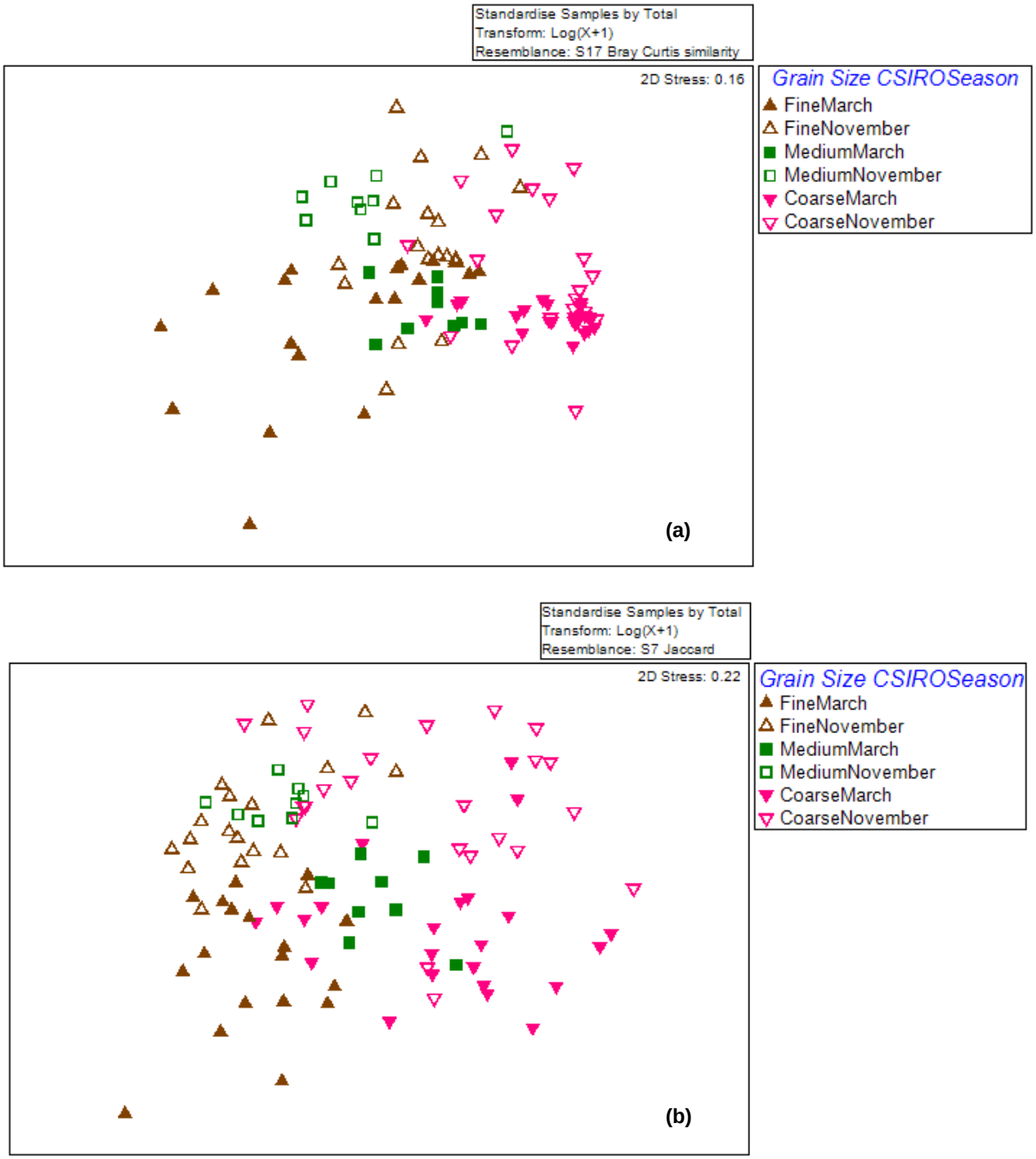

Figure 19 **Multi-Dimensional Scaling of November core samples using Bray Curtis (a) and Jaccard (b) resemblance measures. Samples are colour coded according to season.**

Sweep Net samples

The macroinvertebrate fauna found in the March sweep nets samples consisted of a total of 83 identified taxa; 20 species, identified from 58 genera and 42 families with an additional 9 taxa identified at a higher level. In the November sweep nets a total of 111 identified taxa; comprising of 25 species, identified from 79 genera and 51 families with an additional 10 taxa identified at a higher level.

Taxa collected in the sweep net samples included snails, limpets, bivalves, worms, mites, scuds, freshwater shrimp, freshwater prawns, springtails, beetles, biting midges, non-biting midges, craneflies, soldierflies, danceflies, mayflies, waterboatmen, backswimmers, damselflies, dragonflies, caddisflies, cladocerans, copepods and ostracods. Some freshwater fish were also collected in the sweep net samples, including common galaxias, flathead gudgeons, carp gudgeons and introduced mosquito fish and carp. A specimen of *Velesunio ambiguus* (the freshwater mussel) was seen at Milang and yabbies were also seen at Boggy Creek and Dog Lake.

March samples

The highest invertebrate richness occurred at LF24 (Finniss River, South) with 29 taxa identified (Figure 20). The next highest was LF12 (Loveday Bay) with 25 taxa and then LF03 (Milang), LF08 (Meningie) and LF23 (Currency Creek) with 24 taxa present. The lowest invertebrate richness was seen at the Windmill site in Lake Albert, where only hydras, nematodes, snails, scuds, shrimp and one type of midge was collected.

The greatest abundance was seen at LF15 (Boggy Creek) due to very high numbers of worms (83%) collected from this site (Figure 20). The lowest abundances occurred at three sites in Lake Alexandrina (LF02, Point Sturt, North; LF04, Tolderol and LF06, Poltalloch).

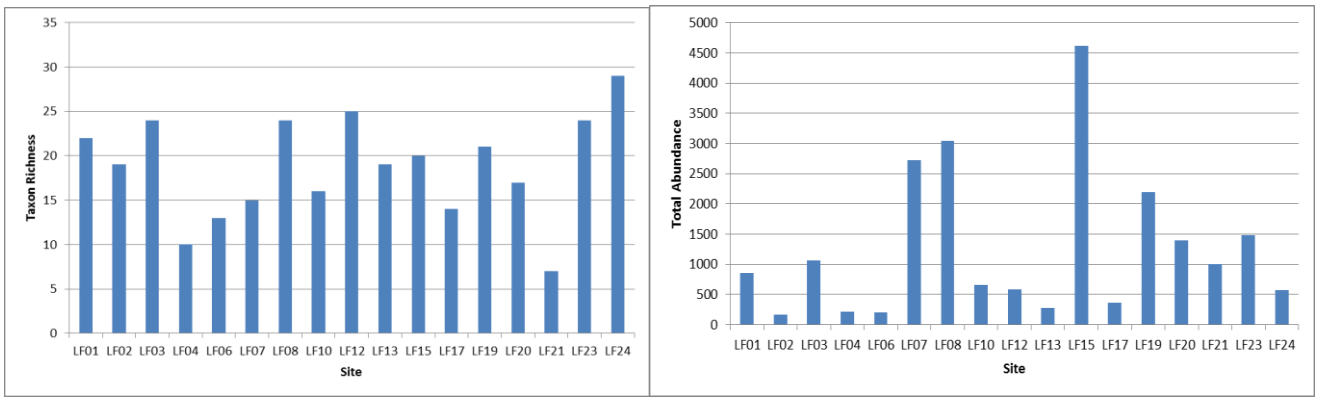

Figure 20 Taxon richness (left) and abundance (right) data for the sweep net samples collected in March.

The highest abundance of mayflies, caddisflies and odonates (the more acid sensitive taxa) occurred at LF01 (Finniss River, Wally's Landing) and LF19 (Dog Lake) (Figure 21), however, at Dog Lake only mayflies and caddisflies (no odonates) were collected. Sites LF08 (Meningie), LF19 (Dog Lake) and LF20 (Boggy Lake) had the highest abundances of nematodes.

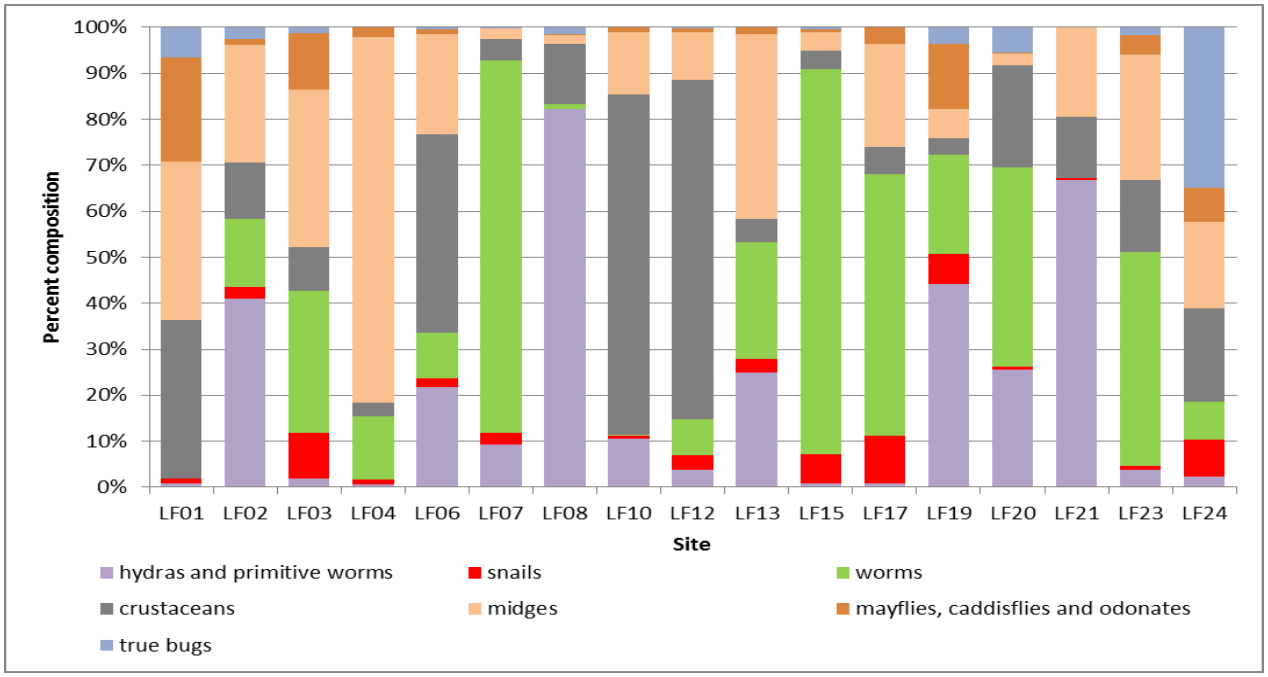

Figure 21 **Percentage community composition of sweep net samples collected from each site in March**

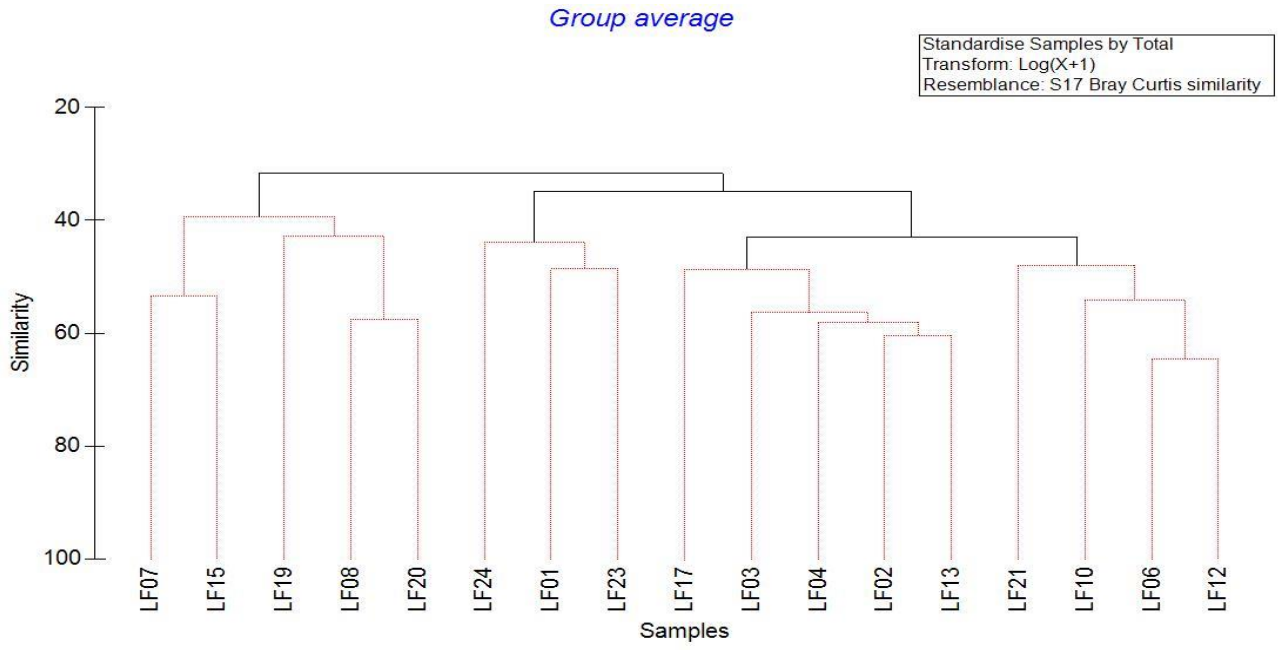

Figure 22 **Hierarchical cluster analysis of March sweep net samples. Black lines identify groups that are significantly different from each other (at p = 0.05), determined by the SIMPROF routine.**

Hierarchical cluster analysis produced four significantly different groups (Figure 22). The first group included Waltowa (LF07) and Meningie (LF08) in Lake Albert, Boggy Creek, on Hindmarsh Island (LF15) and Dog and Boggy Lake (LF19 and LF20) at the top of Lake Alexandrina. The second group consisted of the two sites on the Finniss River and the site on Currency Creek, indicating these two tributaries were more similar to each other than

to the other sites in the Lower Lakes region. The third group comprised of sites in Lake Alexandrina (Point Sturt, South (LF17) Milang (LF03) Tolderol (LF04) Point Sturt, North (LF02) and Tauwitcherie (LF13), and the Windmill site (LF21) and Campbell Park (LF10) in Lake Albert, and Poltalloch (LF06) and Loveday Bay (LF12) in Lake Alexandrina made up the last group of sites.

As with the core samples, sweep net sample sites were categorised as acidic (pH<6.5) or neutral (pH>6.5) according to the surface sediment pH measured at each site. This was done to determine if acidification in the sediment was impacting on the biota living within the water column and macrophytes in the littoral zone. No apparent groupings of samples occurred due to pH (Figure 23) and consequently no significant difference resulted due to acidity (Bray-Curtis: pseudo-*F* = 0.63, df = 2,15, p = 0.8372).

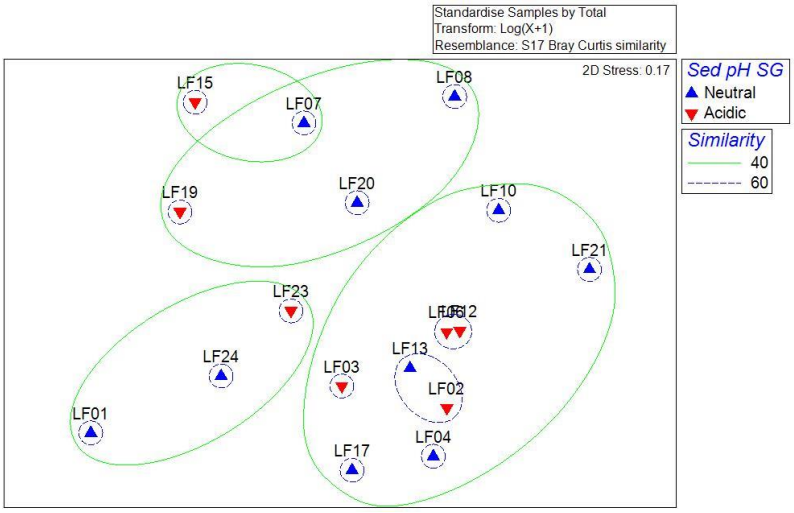

Figure 23 **Multi-Dimensional Scaling of March sweep net samples using the Bray-Curtis similarity measure. Sample points are colour coded according to acidity and overlayed with CLUSTER analysis.**

Samples collected from Lake Albert plotted at the top of the MDS and samples from the two Finniss River sites and the Currency Creek site grouped together (Figure 24). These three latter sites had amongst the highest taxon richness of all sites sampled. Significant differences due to region were apparent (Bray-Curtis: pseudo-*F* = 2.04, df = 3,14, p=0.0016), with samples collected from Lake Alexandrina significantly different from both Finniss River and Lake Albert samples (Figure 24) (p=0.0172 and p=0.0063 respectively). Sites with the highest abundances were grouped towards the top of the plot and samples with low abundances were generally located in the bottom half of the plot, however, the low diversity sites did not group together in the CLUSTER analysis.

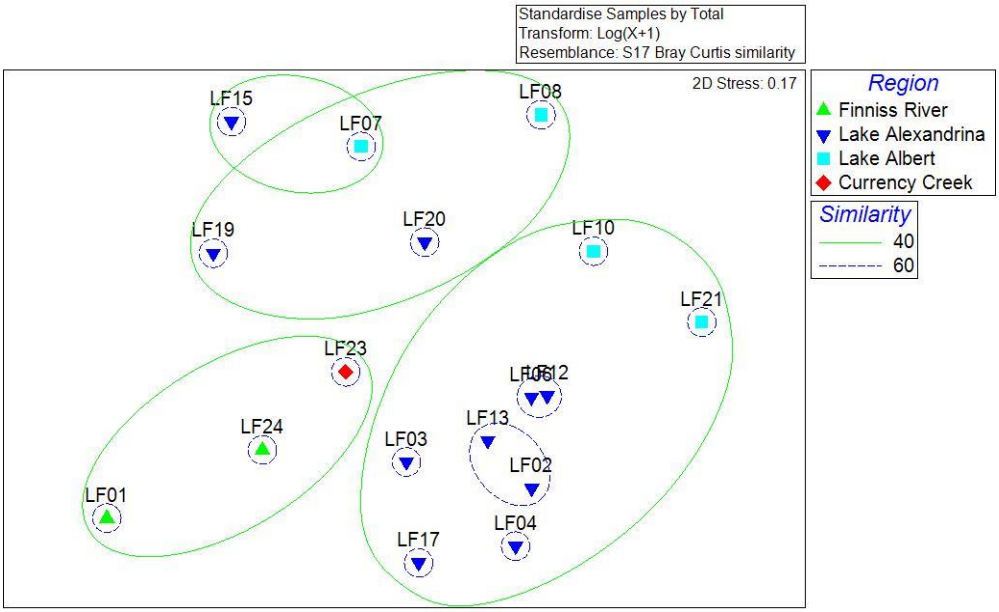

Figure 24 Multi-Dimensional Scaling of March sweep net samples using the Bray-Curtis similarity measure. Samples are colour coded according to their respective region and overlayed with CLUSTER analysis.

BEST analysis showed that the five environmental variables that best explained the community composition of the March sweep nets samples were sediment copper concentrations (*Rho* = 0.468), sediment sodium concentrations (*Rho* = 0.465), titratable actual acidity (*Rho* = 0.464), sediment lithium concentrations (*Rho* = 0.464) and sediment conductivity (*Rho* = 0.461). These are only moderate correlations and do not explain the patterns in the data particularly well.

November samples

There were 11 statistically significant groupings from the 42 sweep net samples collected in November (Figure 25). The distribution of these 42 samples among micro-habitat categories consisted of 13 *Phragmites*, nine *Typha*, eight bare bank, six *Schoenoplectus* and six submerged/marsh-like macrophytes. The submerged/marsh habitat included samples collected from *Berula*, *Bolboschoenus*, *Vallisneria*, *Ludwigia* and *Myriophyllum*. There were four sites with only one habitat available for sampling (Milang (LF03), Tolderol (LF04) and Tauwitcherie (LF13) where only *Phragmites* was sampled, and the Windmill site in Lake Albert (LF21) where only bank habitat was available), five sites had two habitat types present, four sites had three habitats and four sites had four habitats (Table 3).

Table 3 Micro-habitat samples at each site in the Lower Lakes in November, 2013.

Site Code	Site name	Bank	Typha sp.	Phragmites sp.	Schoenoplectus sp.	Marsh species
LF01	Finniss River, Wally's Landing	✓		✓	✓	✓ (*Myriophyllum* sp.)
LF02	Point Sturt, North	✓		✓	✓	
LF03	Milang			✓		
LF04	Tolderol			✓		
LF06	Poltalloch	✓		✓		
LF07	Waltowa		✓	✓		
LF08	Meninigie	✓		✓		
LF10	Campbell Park		✓	✓		
LF12	Loveday Bay		✓	✓	✓	
LF13	Tauwitcherie			✓		
LF15	Boggy Creek		✓			✓ (*Bolboschoenus* sp. and *Myriophyllum* sp.)
LF17	Point Sturt, South	✓	✓	✓	✓	
LF19	Dog Lake		✓		✓	✓ (*Ludwigia* sp. and *Vallisneria* sp.)
LF20	Boggy Lake	✓	✓			
LF21	Windmill site	✓				
LF23	Currency Creek		✓	✓	✓	✓ (*Berula* sp.)
LF24	Finniss River, South	✓	✓	✓		

The CLUSTER analysis showed that within site variability (comparing the different micro-habitats at a site) was usually less than between site variability (comparing the same habitat types across different sites) with samples collected at a single site often grouping together within one of the 11 significant groupings.

Where differences were seen between micro-habitats within a site it was usually the sample collected from the *Typha* which differed the most. This occurred at Loveday Bay (LF12), Point Sturt, South (LF17), Boggy Lake (LF20) and Currency Creek (LF23) where the *Typha* samples were placed into a different significant grouping than the other micro-habitats from the same sites. The reason for this difference, however, is unclear.

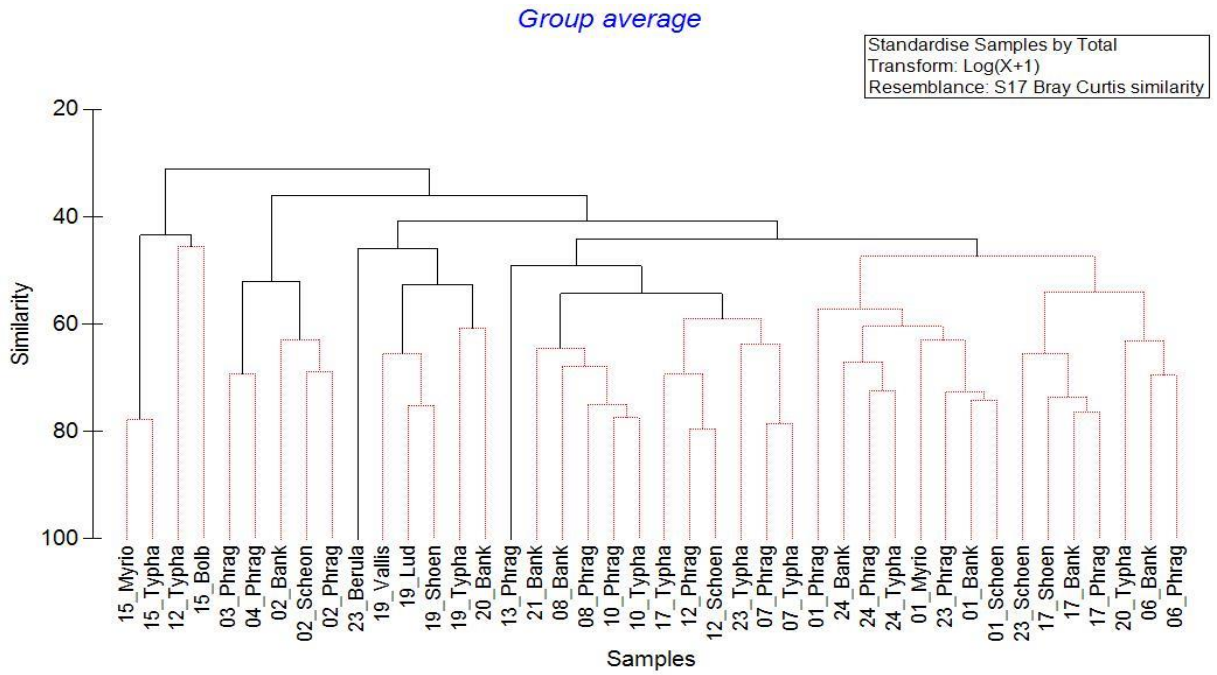

Figure 25 **Hierarchical cluster analysis of November sweep net samples. (Myrio = *Myriophyllum*, Bolbo = *Bolboschoenus*, Phrag = *Phragmites*, Schoen = *Schoenoplectus*, Vallis = *Vallisneria*, Lud = *Ludwigia*). Black lines identify groups that are significantly different from each other (at *p* = 0.05), determined by the SIMPROF routine.**

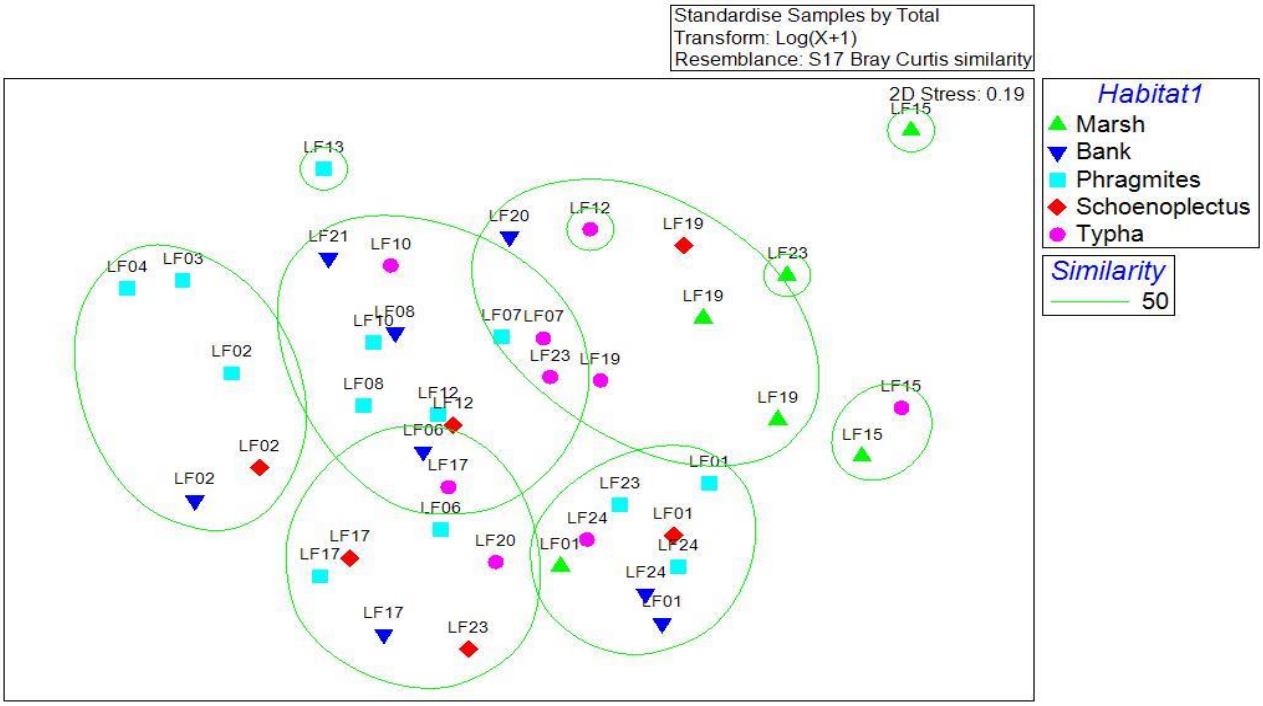

Figure 26 **Multi-Dimensional Scaling of November sweep net samples using the Bray-Curtis similarity measure. Samples are colour coded according to their respective habitat and overlayed with CLUSTER analysis.**

There were no obvious grouping of micro-habitats (Figure 26), although samples collected from marsh habitats were all plotted on the left of the MDS. Significant differences due to micro-habitat were found (Bray-Curtis: pseudo-*F* = 1.78, df = 4,37, p = 0.0082), with pair-wise tests identifying the marsh habitat type to be significantly different from samples collected from the bare bank (p = 0.0022), *Phragmites* (p = 0.002) and *Schoenoplectus* (p =

0.0162) micro-habitats across the Lower Lakes but not with *Typha* (p = 0.1022). No significant differences were found between the different types of emergent plants (*Typha*, *Schoenoplectus* and *Phragmites*) or between any of the emergent plants and the bare bank habitat.

Significant difference due to sediment pH (Bray-Curtis: pseudo-*F* = 2.93, df = 1,40, p = 0.006) and region (Bray-Curtis: pseudo-*F* = 3.31, df = 3,38, p = 0.0001), with pair-wise tests showing differences between Finniss River and both Lake Alexandrina (p = 0.0006) and Lake Albert (p = 0.0006), between Lake Albert and both Lake Alexandrina (p = 0.0069) and Currency Creek (p = 0.0135) (Figure 27).

There were no environmental variables that explained the patterns in the data well when using BEST analysis (all correlations with *Rho* < 0.4).

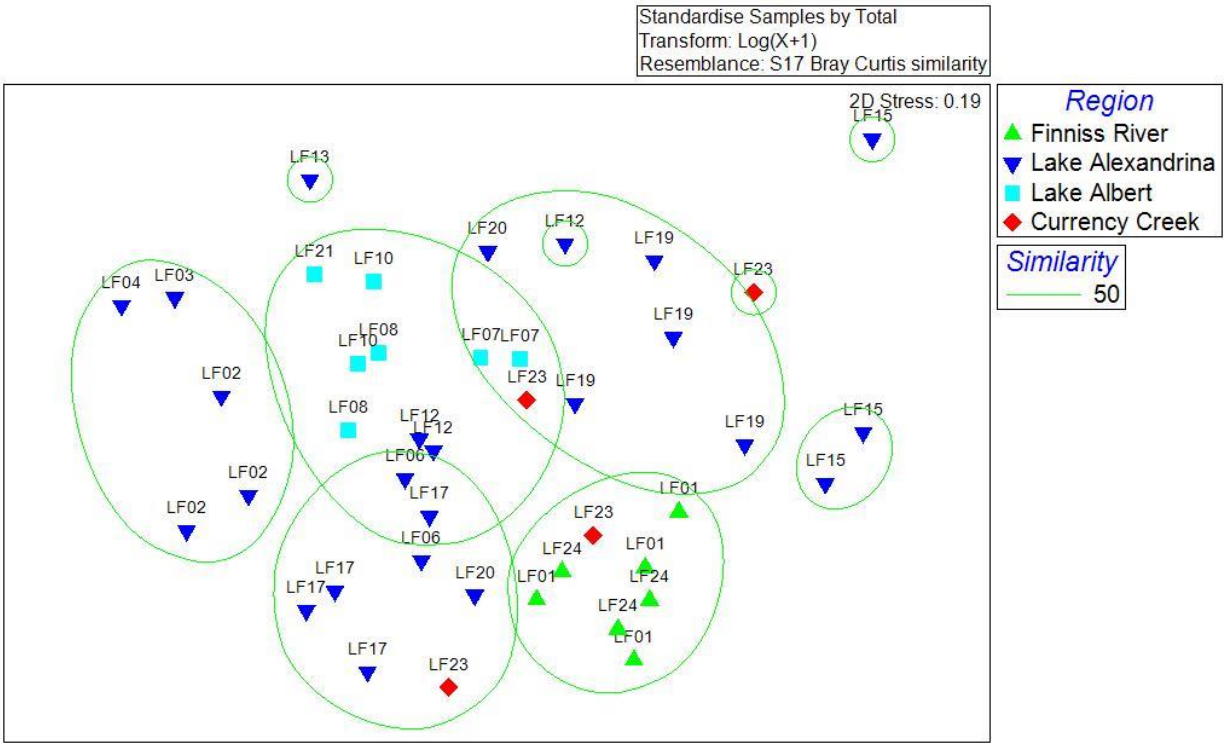

Figure 27 **Multi-Dimensional Scaling of November sweep net samples using the Bray-Curtis similarity measure. Samples are colour coded according to their respective region and overlayed with CLUSTER analysis results.**

5 Discussion

The drought between 2007 and 2010 resulted in greatly reduced water levels in the Lower Lakes and consequently increased levels of salinity and nutrients (Mosley *et al.* 2013). The low water levels also exposed sediments containing pyritic minerals on the lake margins, resulting in acidic sediments. Since the drought broke in late 2010/early 2011, and water levels were reinstated to pre-drought levels, acidified sediments have persisted at some locations in the region.

The degree to which acid sulfate soils are impacting on the Lower Lakes ecosystem, three years after the drought was broken, was assessed by sampling the aquatic invertebrate community during two seasons in 2013. Both core samples and sweep net samples were collected and both physical and chemical variables as well as acidity have been explored to determine which variables are having the greatest influence on the invertebrate community structure.

Influence of acidity on the invertebrate community

Sampling conducted in March and November 2013 in the Lower Lakes at 17 sites found that the surface sediment was acidic ($6.0 < pH < 6.5$) at seven sites in March and five sites in November. As only three sites (Milang, Poltalloch and Dog Lake) had acidic sediment during both seasons, and no measurement was recorded less than 6.0, the results suggest that acidity fluctuated across the region throughout the year but when acidic, the sites could be considered to be only weakly acidic. The lack of prolonged acidic conditions may be the reason for the weak relationships seen between invertebrate community structure and pH, and why sediment pH was not one of the environmental variables that best explained the community structure across the Lower Lakes. As these pH measurements were only collected on two occasions; once in March and once in November, it is not known how these pH values may have fluctuated in the months in between the two sampling events or for how long the low pH values had persisted prior to sample collection.

While sediment acidification was found to have influenced the benthic invertebrate community in March, the influence was less apparent in November, perhaps due to an increase in pH across the region resulting in fewer sites being classified as acidic, or due to the higher taxon richness seen in that season, increasing the number of acid sensitive taxa present. As the increase in taxon richness occurred at both acidic and neutral sites, it is unlikely then that the lower taxon richness in March was a result of acidification, instead being associated with a seasonal effect. This may suggest that in the summer/autumn months when taxon richness is lowest, the impact of acidification may be more pronounced.

Other studies have shown that quite pronounced changes occur to the invertebrate community structure with low pH levels, where communities shift from one dominated by acid-sensitive taxa to one dominated by acid-tolerant taxa (Tripole *et al*. 2008, Orendt 1999, Sommer and Horwitz 2009). This change, however, may not result in a reduction in invertebrate richness but may instead be associated with a change in the types of species found (Sommer and Horwitz 2009). The acid tolerance of individual species varies greatly with some able to withstand only small declines in pH, while others have a much greater acid tolerance range, and different species within a genus may exhibit markedly different tolerance levels. Orendt (1999) studied acidified streams in Germany and suggested changes in chironomid populations may occur at pH 6.0, pH 5.5 and again at pH 4.0 according to the acid tolerance of different species in the community. Gerhardt *et al*. (2004) found that some hemipterans and beetles could be found in water with a pH as low as 3. Monitoring in the Lower Lakes in 2013 has shown some modifications in the invertebrate community structure due to acidification, however, these changes appeared to be minor, as they were only significant in autumn. The fluctuating pH levels in the region would allow times of reprieve from the acidic condtions and allow for recovery of the community, when pH measurements exceed 6.5, including the return of acid-sensitive taxa. Should acidic conditions worsen, either through lower pH levels or through more

prolonged acidification events, the change in community structure is likely to be more apparent, resulting in a shift towards a community dominated by acid tolerant taxa.

Sommer and Horwitz (2009) considered acid-sensitive taxa to include amphipods, isopods, ostracods, chydorids, daphnids, mayflies, oligochaetes, clam and snails and acid tolerant taxa to comprise sandflies, macrothricids and waterboatmen. We found a very similar assemblage with crustaceans, some chydorids, daphnids, ostracods, non-biting midges, mayflies, caddisflies and odonates more likely to be collected from the neutral sites and nematodes, some worms, the cladoceran family Macrothricidae and the midges *Cryptochironomus, Cladopelma* and *Cladotanytarsus* more likely to be found at the acidic sites.

Giglio (2011) reported that certain families of snails (Hydrobiidae, Ancylidae, Planorbidae and Corbiculidae) and crustaceans (the yabby *Cherax destructor* and the freshwater crab *Amarinus lacustris*) had not been collected from the Lower Lakes since the drought broke in 2010 but had been previously collected from the Lower Lakes in 2003-2004. The patchy acidic conditions across the Lower Lakes compromising the survival of these species is one of the possible reasons for the reduced diversity of these taxa. Both snail shells and the carapaces of crustaceans are made of calcium carbonate making these taxa sensitive to low pH. However, all of these taxa were either collected from the Lower Lakes or noticed in the field during 2013. Other taxa that were also reported as 'missing' by Giglio (2011) such as the true bugs *Mesovelia*, *Hebrus*, *Naucoris* and Pleidae and the mayfly family Baetidae have also been collected during this study. The true bugs were all collected in November from Finniss River, Wally's Landing and Currency Creek and tend to be more associated with marshy habitat, which is only present in limited parts of the Lower Lakes. It is possible that these taxa have returned to the Lower Lakes from the upper sections of these tributaries through drift or aerial dispersion. *Cloeon*, from the Baetidae family of mayflies, were also collected in this study from Dog Lake, Currency Creek and Finniss River, South in March and Dog Lake, Boggy Creek and Wally's Landing in November. As all of these sites are fine substrate sites, it is possible the habitat requirements of this mayfly also restricts its distribution in the region.

Vertical distribution of aquatic invertebrates

Sediment core samples collected in March aimed to determine how deep invertebrate taxa were present and where in the sediment profile they were most abundant. Across the Lakes, the highest abundances and richness occurred in the top 2 cm of sediment. The community composition in the top horizon was considered to be significantly different from the deeper horizons, and the differences between the horizons was not influenced by sediment pH of the site or the substrate type (coarse, medium or fine grain size). Some taxa were found further down the sediment profile but those taxa were not unique to the lower layers of the sediment, and were always present in low abundances. Few individuals were collected from the 5 – 10 cm layer, which is consistent with previous studies in this region (e.g. Dittmann *et al.* 2011 and Corbin *et al.* 2012).

Bergtold and Traunspurger (2004) also found similar results, with benthic organisms being found up to a depth of 4 cm in a lake but with most organisms in the uppermost centimetre of sediment. They often found copepods and mites in the deeper layers, between 1 and 4 cm deep. We also occasionally found copepods, particularly harpacticoids, or mites in the deeper layers but more commonly found nematodes and worms. There were no overall patterns to suggest that certain taxa were more likely to be found living deeper down the sediment profile, suggesting that some taxa, can on occasions tolerate the deeper layers, but will usually be found living in the top most layers of sediment. The surface layers of the sediment are more oxygenated and contain more organic matter and biofilms which provide a food source for the invertebrates. As the reduced community in the deeper layers appeared to be unrelated to the issue of acidity and other studies have also found a reduced community in the deeper layers of sediment it seems likely that this is unrelated to the issue of acid sulfate soils.

The influence of the physical habitat on benthic invertebrates

The abundance and richness of taxa found across the 17 sites varied substantially between sites, with very high abundances seen at some sites, particularly those with coarse substrate. This was usually due to the presence of large numbers of nematodes and sometimes worms. In March, 93% of the benthic community across the Lower Lakes was comprised of worms and nematodes, but this reduced to 76% in November due to an increase in the abundance of microcrustaceans. For some sites the nematode population accounted for more than 99% of the individuals found.

Worms and nematodes have also been found to be the most dominant taxa living in sediment of other lake environments (Fletcher *et al.* 2001, Mastrantuono 1995 and Canfield *et al*. 1994, Bergtold and Traunspurger 2004). Mastrantuono (1995) found a benthic community dominated by worms, nematodes, crustaceans and chironomids in a sandy substrate lake in Italy, although other groups were also collected in lower numbers. Both Mastrantuono (1995) and Bergtold and Traunspurger (2004) found similar abundances of nematodes in their study as in this Lower Lakes study with nematodes comprising about 76% of the fauna. Fletcher *et al.* (2001) also found an abundance of ostracods in their samples and Canfield *et al*. 1994 recorded high numbers of chironomids (usually in fine sediment). Ostracod and chironomid abundances in the Lower Lakes were much lower than the abundance of worms and nematodes.

Interestingly, we found the worm community in the core samples were dominated by tubificids, however the worms collected in the sweep nets samples (closer to shore) were almost exclusively naidids. Manstrantuono (1995) found a similar pattern of distribution in a sandy lake in Italy with naidids more prevalent in the littoral zone (edge) and tubificids occurring in greater abundance in the profundal zone (open water).

Substrate types appears to have a considerable influence on the benthic invertebrate community with coarse substrate types characterised by low diversity and a high abundance of nematodes, with higher taxon richness occurring at the fine substrate sites. Significant differences in community composition were found between the different substrate types (coarse, medium and fine substrate) for both the March and November core samples. Species commonly found in the coarse substrate sites included species likely to burrow into the sandy substrate or use the sand particles to construct cases. These taxa included some non-biting midges, two families of worms, nematodes, the little basket shell and a caddisfly.

Regional and seasonal effects on the aquatic invertebrate communities

Significant differences due to region were apparent, particularly between the three regions of the Finniss River, Lake Alexandrina and Lake Albert. These differences have also been noted in previous research in the region (Dittmann *et al.* 2011). The invertebrate community in Lake Albert is likely to continue to be different from communities seen elsewhere in the Lower Lakes due to its higher salinity levels. Salinity levels in Lake Albert post-drought (approximately 3000 µS/cm) have remained well above those measured pre-drought (on average less than 1600 µS/cm) and while these salinity levels remain elevated the community composition in Lake Albert will be expected to remain different from the rest of the Lower Lakes.

The differences observed between the biota in the Finniss River and Currency Creek, and the biota seen in Lake Alexandrina may suggest that there is more reliance on invertebrate drift from the upper catchments rather than cross-colonisation occurring within the Lower Lakes region. As there is little pre-drought data available for comparison for our study it is difficult to determine if these differences are natural or if the differences suggest that the Lakes have not yet fully recovered from the effects of the drought. Future monitoring (in 3-5 years) in the Lower Lakes at these same sites will help to determine if the variability between the different regions within the Lakes persist, or if, as expected, they become more similar as cross-colonisation occurs throughout the region and there becomes more reliance on the within system community rather than on drift from sites further upstream. Another possible reason for the differences in community structure between the regions may be due to the degree of

sheltering at a site. The lacustrine environment of Lake Alexandrina and parts of Lake Albert are generally open to wind and wave action whereas the more riverine areas of the Lower Lakes are more sheltered from these disturbances. This issue is discussed in more detail later.

An obvious shift in the invertebrate community structure was seen from March to November with greater taxa richness occurring in November. Seasonal changes in community structure are common due to different lifecycles (both length of lifecycle and number of lifecycles per year) and due to differing rainfall patterns throughout the year resulting in invertebrate drift downstream during times of higher flow. This often results in more diverse communities occurring in spring, following the winter rains.

Influence of sediment chemistry on the aquatic invertebrate community

The variation in the invertebrate community in the Lakes in March was best explained by the RIS concentrations, and explained a considerably high percentage of the variation (80%). RIS concentrations were highest at the two Finniss River sites, Boggy Creek and Tauwitcherie, all of which grouped together in the CLUSTER analysis, and three of which, were also characterised by high taxon richness. As these four sites also had high concentrations of TOC (indicative of decaying vegetation, biofilm and algae in the sediments), it is likely the high TOC concentrations is suggestive of an abundance of food for grazers, shredders and detritivores, leading to a higher diversity at these sites. The high RIS is due to sulfate reductions occurring due to the low redox potentials (anoxic conditions) and these reductions are also being promoted by the high organic matter. Therefore, while the high organic matter is probably driving a higher diversity at these sites it is also promoting the formation of RIS, and therefore pyrite formation in the sediment.

We also considered whether sulfate reduction (producing RIS) may have neutralised acidity as this process generates alkalinity (HCO_3^-) via the reaction (from Di Toro 2001):

$$CH_2O + 4/9\ FeOOH_{(s)} + 4/9\ SO_4^{-2} \rightarrow 1/9\ CO_2 + 8/9\ HCO_3^- + 4/9\ FeS_2 + 7/9\ H_2O$$

Where CH_2O represents organic carbon, the diagenesis of which reduces sulfate (SO_4^{-2}) to hydrogen sulfide (H_2S), and assuming iron sulfide (FeS_2) is the final repository of the sulfide following reduction of Fe^{III} oxides ($FeOOH_{(s)}$) to Fe^{II} (which then reacts with the sulfide) in the sediment. The alkalinity produced could potentially assist in neutralising any H^+ acidity present in the sediment, raising the pH. However, when we plotted RIS vs pH (not shown) there was no clear relationship to suggest sulfate reduction has significantly influenced the pH of the surface sediment.

In November, sediment Eh (oxidation-reduction potential) best explained the patterns in the invertebrate community, explaining 45% of the variation. High taxon richness occurred at the four sites with the most negative Eh values (most reducing environments; the two Finniss River sites, Boggy Creek and Tauwitcherie). These sites also had high TOC indicating the presence of decaying vegetation in the sediment, leading to the reducing environment. Conversely, the only four sites where a positive Eh was measured recorded low TOC concentrations, and were characterised by coarse sediment and low taxon richness. These results suggest the degree of organic enrichment in the sediment has a strong influence on the benthic invertebrate community at a site but while it may result in “poor” sediment quality does not appear to adversely affect the invertebrate community present, instead higher diversity is more likely to result. This finding again supports the “food” hypothesis outlined for the relationship between RIS and invertebrate diversity.

The sites where higher taxon richness occurred were generally located in more protected areas of the Lower Lakes, whereas the main lacustrine areas of Lake Alexandrina and parts of Lake Albert are open to wind and wave action. These open sites experience a considerable amount of seiching where the top layers of sediment are regularly re-suspended. This results in limited settlement of fine sediment particles such as silts, clays and fine organic material and the constant re-suspension would also limit the growth of biofilm on the sediment surface,

resulting in the low TOC concentrations measured in this study. The combination of the re-suspension of sediment particles and the low TOC concentrations (representing amount of food available) make these open areas of the Lakes less inhabitable for many invertebrates than the more sheltered areas across the region. However, these less inhabitable areas are providing an ideal ecological niche for nematodes, which are present in very high numbers at these sites. Nematodes can be found in almost all inland waters, and are highly tolerant of degraded water quality conditions. While the presence of such high numbers of nematodes at a site would normally be indicative of degraded water quality or sediment quality, it is believed that the high number of nematodes in Lake Alexandrina is instead related to the low TOC concentrations and therefore reduced presence of other invertebrates.

Influence of micro-habitat on aquatic invertebrate communities

In November, the sweep net samples were collected from different micro-habitats. Significant differences were seen between the submerged/marshy habitat and other micro-habitats sampled, which included three types of emergent macrophytes and bare bank habitat. There were no significant differences seen between the other micro-habitat when assessing the Lower Lakes region as a whole. However, when looking at the micro-habitats within each site it was often the *Typha* sample that was significantly different from the other micro-habitats sampled at the same site, suggesting the invertebrate community in that type of macrophyte may be different from the other emergent plant types (*Phragmites* and *Schoenoplectus*) at some sites. The reason for this is unclear.

Conclusions

The surface sediment at all sites monitored in the Lower Lakes were either neutral or weakly acidic during 2013 with only three sites considered to be acidic during both seasons. All other sites that exhibited acidity were only acidic for one season, suggesting fluctuating pH levels across the Lower Lakes region rather than prolonged acidification. This allows the residing biota with extended periods of reprieve from acidification events and the opportunity for acid-sensitive taxa to recolonise during times of improved water and sediment quality. Prolonged acidification is more likely to result in on-going alterations to the community structure from one comprising mostly acid sensitive taxa to one dominated by taxa tolerant of low pH levels. The weak acidity noted in the Lower Lakes in 2013 did result in changes to the invertebrate community in the sediment at some sites during March but not in November.

The substrate type (grain size) was likely the biggest driver of community composition in 2013 with the highest species richness occurring at sites with fine substrate, and low species richness but high abundances of nematodes occurring at coarse substrate sites. The substrate type is related to the degree of sheltering at a site with the lacustrine environment of Lake Alexandrina comprises mainly coarse substrate and the more sheltered areas of the Lower Lakes, such as the riverine environments of Finniss River, Currency Creek and Boggy Creek and the adjacent wetland areas of Dog Lake and Boggy Lake, consisting of fine substrate types such as silts and clays. The degree of sheltering also influences the amount of fine organics and biofilm growth, which, in turn, drives the species richness of a site. A brief summary of the general characteristics of the two different environments, as determined through this study, has been presented in Table 4.

Table 4 Summary table of characteristics of lacustrine versus riverine/wetland environments in the Lower Lakes.

Lacustrine environment	Riverine environment and adjacent wetlands
Coarse substrate (coarse sand)	Fine substrate (silts and clays)
Low total organic carbon	High total organic carbon
Low Reduced Inorganic Sulfur	High Reduced Inorganic Sulfur
Oxidising environment	Reducing environment
Low invertebrate richness. Taxa may include: • Cnidaria; *Cordylophora* sp. • Nematoda spp. • Mollusca; *Corbiculina australis* • Oligochaeta; Enchytraeidae spp. • Chironomidae; *Cryptochironomus* sp., *Tanytarsus barbitarsis*, • Trichoptera; *Ecnomus pansus*	High invertebrate richness. Taxa may include: • Oligochaeta spp., Tubificidae, Tubificidae Group B • Amphipoda; Corophiidae sp. • Chironomidae; *Chironomus* sp., *Cladopelma* sp. • Cladocera; *Ilyocryptus* sp., *Macrothrix* sp., *Leydigia* sp., • Copepod; Calanoida spp., Cyclopoida spp. • Ostracoda; Cyprididae spp.

Higher species richness and abundances occurred in the top horizon (top 2 cm) of the sediment core sample, with significantly fewer taxa present in the deeper horizons, to a depth of 10 cm. Neither the sediment pH, nor the substrate type, influenced the species richness in these deeper horizons.

The invertebrate communities did not differ significantly between different types of emergent macrophytes or bank habitat. However, different communities were found in marshy or submerged macrophyte types in comparison to the other micro-habitats investigated. The marsh and submerged macrophyte types provide a unique habitat for a range of species, such as some beetles and hemipterans, not found in the emergent of bank habitats.

It is recommended that periodic monitoring of surface sediment pH occur at some sites across the Lower Lakes to monitor for changing and seasonal pH conditions. pH levels declining below 6.0 would suggesting worsening conditions from those observed in 2013 and this may result in further changes to the aquatic communities. Sampling the invertebrate community at some of these sites in 3 – 5 years should provide additional information about the recovery of the Lakes from drought and acidification issues and perhaps provide more baseline information about the natural state of the system.

6 References

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Appendix 1 – List of biota identified in the sediment core and sweep net samples

Table 5 Total abundances of taxa in sediment core and sweep net samples collected March and November 2013.

Species Name	March	November	March	November
	Core	Core	Sweep net	Sweep net
Cnidaria, Hydridae Hydra sp.	1	0	12	8
Cnidaria, Clavidae Cordylophora sp.	2	110	839	2003
Tardigrada spp.	0	3	0	0
Playthelminthes, Temnocephalidae Temnocephala sp.	0	0	0	5
Playthelminthes, Turbellaria spp.	5	107	0	133
Nematoda spp.	33124	26533	4294	271
Nemertea spp.	2	0	5	1
Mollusca, Hydrobiidae Posticobia sp.	0	0	14	171
Mollusca, Hydrobiidae spp.	0	0	3	0
Mollusca, Ancylidae Ferrissia sp.	1	2	352	247
Mollusca, Planorbidae Glyptophysa sp.	0	0	0	129
Mollusca, Physidae Physa acuta	0	2	365	949
Mollusca, Corbiculidae Corbiculina australis	65	10	32	0
Mollusca, Gastropoda sp.	0	0	2	16
Oligochaeta, Tubificidae spp.	13	686	0	0
Oligochaeta, Tubificidae Aulodrilus sp.	11	3	0	0
Oligochaeta, Tubificidae - Group B spp.	61	307	0	0
Oligochaeta, Naididae Nais sp.	8	0	1	14
Oligochaeta, Naididae Dero sp.	2	1	2	15
Oligochaeta, Naididae Pristina longiseta	0	0	1	0
Oligochaeta, Naididae Pristina sp.	1	0	2	11
Oligochaeta, Naididae Chaetogaster	0	0	0	17
Oligochaeta, Naididae Slavina sp.	0	1	0	0
Oligochaeta, Naididae spp.	16	7	15	4454
Oligochaeta, Enchytraeidae spp.	0	15	0	0
Oligochaeta, Lumbriculidae spp.	2	1	0	0
Oligochaeta spp.	3451	4938	8560	5358
Polychaeta, Spionidae spp.	1	0	0	0
Polychaeta, Syllidae spp.	0	1	0	0
Hydracarina, Oribatida spp.	35	15	43	374
Hydracarina, Mesostigmata spp.	0	0	10	43
Hydracarina, Astigmata spp.	0	0	4	44
Hydracarina spp.	0	1	3	3
Amphipoda, Ceinidae Austrochiltonia sp.	4	12	866	11753
Amphipoda, Eusiridae spp.	48	334	1080	48921
Amphipoda, Corophiidae SAsp1	174	649	450	7494
Decapoda, Aytidae Paratya australiensis	3	0	549	547
Decapoda, Aytidae spp.	0	0	67	105

Species Name	March	November	March	November
	Core	Core	Sweep net	Sweep net
Decapoda, Palaemonidae Macrobrachium sp.	0	0	20	70
Decapoda, Hymenosomatidae Amarinus lacustris	0	0	0	10
Collembola, Hypogasturidae spp.	0	0	11	38
Collembola, Isotomidae spp.	0	1	4	232
Collembola, Sminthuridae spp.	1	0	5	300
Coleoptera, Haliplidae Haliplus gibbus	0	0	0	1
Coleoptera, Dytiscidae Liodessus sp. (Adult)	0	0	0	50
Coleoptera, Dytiscidae Chostonectes sp. (Larva)	0	0	0	40
Coleoptera, Dytiscidae Necterosoma sp. (Adult)	0	0	0	10
Coleoptera, Dytiscidae Lancetes lanceolatus (Adult)	0	0	0	10
Coleoptera, Hydrophilidae Berosus majusculus (Adult)	0	0	0	7
Coleoptera, Hydrophilidae Enochrus sp. (Adult)	0	0	0	10
Coleoptera, Hydrophilidae Helochares sp. (Adult)	0	0	0	10
Coleoptera, Hydrophilidae Limnoxenus zealandicus (Adult)	0	0	1	91
Coleoptera, Hydrophilidae Paracymus pygmaeus	0	0	0	20
Coleoptera, Hydrophilidae spp. (larvae)	0	0	0	10
Coleoptera, Hydrophilidae spp. (Adult)	0	0	2	0
Coleoptera, Ptiliidae spp.	0	0	1	1
Coleoptera, Staphylinidae spp.	0	0	0	3
Coleoptera, Curculionidae spp.	0	0	0	1
Coleoptera, Nanophyidae Austronanodes sp. (adult)	0	0	1	0
Diptera, Tipulidae Tipulidae EWS sp1	0	0	0	2
Diptera, Tipulidae Tipulidae EWS sp7	0	0	0	33
Diptera, Tipulidae Tipulidae EWS sp13	0	0	0	30
Diptera, Ceratopogonidae Bezzia sp.	0	0	40	30
Diptera, Ceratopogonidae Culicoides sp.	7	1	10	4
Diptera, Ceratopogonidae Forcipomyia sp.	0	0	0	5
Diptera, Ceratopogonidae Dasyhelea sp.	0	0	0	10
Diptera, Ceratopogonidae spp.	0	0	12	5
Diptera, Psychodidae spp.	0	0	0	4
Diptera, Stratiomyidae spp.	0	0	1	1
Diptera, Empididae spp.	0	0	19	61
Diptera, Muscidae spp.	0	0	0	15
Diptera, Tanypodinae Coelopynia pruinosa	5	2	0	0
Diptera, Tanypodinae Procladius sp.	116	114	33	92
Diptera, Tanypodinae Monopelopia	0	0	50	90
Diptera, Tanypodinae sp.	1	0	2	0
Diptera, Orthocladiinae Nanocladius sp.	1	0	58	63
Diptera, Orthocladiinae Corynoneura sp.	0	0	0	979
Diptera, Orthocladiinae Paralimnophyes (light sp.)	0	4	37	1246
Diptera, Orthocladiinae Cricotopus sp.	2	14	1049	1915
Diptera, Orthocladiinae spp.	2	1	6	15
Diptera, Chironominae Stempellina sp.	0	0	0	15

Species Name	March	November	March	November
	Core	Core	Sweep net	Sweep net
Diptera, Chironominae Cladotanytarsus sp.	50	48	120	139
Diptera, Chironominae Tanytarsus barbitarsis	0	9	8	1
Diptera, Chironominae Tanytarsus sp.	3	4	29	17
Diptera, Chironominae Paratanytarsus sp.	235	7	306	2161
Diptera, Chironominae Tanytarsini sp.	1	1	0	17
Diptera, Chironominae Chironomus spp.	32	148	13	32
Diptera, Chironominae Dicrotendipes sp.	37	2	249	666
Diptera, Chironominae Kiefferulus sp.	21	3	5	0
Diptera, Chironominae Polypedilum sp.	22	2	112	2633
Diptera, Chironominae Cryptochironomus sp.	30	50	8	31
Diptera, Chironominae Cladopelma sp.	95	76	87	25
Diptera, Chironominae Paracladopelma sp.	0	0	0	20
Diptera, Chironominae Parachironomus sp.	14	0	45	38
Diptera, Chironominae Microchironomus sp.	12	8	0	0
Diptera, Chironominae Chironomini sp.	0	0	45	10
Diptera, Chironomidae Chironominae spp.	37	41	91	474
Chironomidae spp.	0	0	11	12
Diptera spp.	0	0	0	3
Ephemeroptera, Baetidae Cloeon sp.	0	3	18	30
Ephemeroptera, Baetidae spp.	0	0	80	0
Ephemeroptera, Caenidae Tasmanocoenis tillyardi	2	1	143	1
Ephmeroptera, Caenidae Tasmanocoenis sp.	0	0	66	7
Hemiptera, Mesoveliidae Mesovelia sp.	0	0	47	2
Hemiptera, Hebridae spp.	0	0	0	61
Hemiptera, Veliidae Microvelia oceanica	0	0	0	93
Hemiptera, Veliidae Microvelia sp.	0	0	0	83
Hemiptera, Corixidae Sigara sp.	0	0	1	20
Hemiptera, Corixidae Agraptocorixa eurynome	0	0	1	0
Hemiptera, Corixidae Agraptocorixa sp.	0	0	57	4
Hemiptera, Corixidae Micronecta robusta	0	0	1	80
Hemiptera, Corixidae Micronecta sp.	3	0	419	1159
Hemiptera, Corixidae spp.	2	0	0	9
Hemiptera, Naucoridae Naucoris congrex	0	0	0	3
Hemiptera, Notonectidae Anisops thienemanni	0	0	1	20
Hemiptera, Notonectidae Anisops sp.	0	0	7	23
Hemiptera, Pleidae Paraplea sp.	0	0	0	7
Lepidoptera, Pyralidae Nymphulinae spp.	0	0	10	2
Odonata, Coenagrionidae Ischnura heterosticta	0	0	48	306
Odonata, Coenagrionidae Ischnura sp.	0	0	120	10
Odonata, Coenagrionidae spp.	8	1	116	102
Odonata, Aeschnidae Hemianax papuensis	0	0	0	148
Odonata, Aeschnidae sp.	0	0	20	15
Trichoptera, Hydroptilidae Hellyethira malleoforma	0	0	20	52

Species Name	March	November	March	November
	Core	Core	Sweep net	Sweep net
Trichoptera, Hydroptilidae Hellyethira sp.	2	0	32	55
Trichoptera, Hydroptilidae Hydroptila losida	0	0	32	50
Trichoptera, Hydroptilidae Hydroptila sp.	0	0	0	7
Trichoptera, Hydroptilidae Orthotrichia sp.	1	0	7	0
Trichoptera, Ecnomidae Ecnomus pansus	0	1	8	27
Trichoptera, Ecnomidae Ecnomus cygnitus	1	0	30	4
Trichoptera, Ecnomidae Ecnomus turgidus	4	0	0	2
Trichoptera, Ecnomidae Ecnomus sp.	10	1	31	39
Trichoptera, Leptoceridae Notolina spira	0	0	3	0
Trichoptera, Leptoceridae Oecetis sp.	0	1	20	31
Trichoptera, Leptoceridae Triplectides australis	0	0	7	41
Trichoptera, Leptoceridae Triplectides ciuskus	0	0	0	7
Trichoptera, Leptoceridae Triplectides sp.	0	0	7	40
Trichoptera, Leptoceridae (juveniles)	1	0	2	0
Pisces, Undifferentiated fishes (Larval fish)	0	0	2	248
Pisces, Galaxiidae Undifferentiated galaxias (Juvenille galaxias)	0	0	1	0
Pisces, Galaxiidae Galaxias maculatus (Common galaxias)	0	0	2	0
Pisces, Poeciliidae Gambusia	0	0	26	40
Pisces, Eleotridae (Juvenille gudgeons)	0	0	3	0
Pisces, Eleotridae Philypnodon grandiceps (Flathead gudgeon)	0	0	4	0
Pisces, Eleotridae Hypseleotris sp 3 (Murray-Darling carp gudgeon)	0	0	0	10
Ostracoda Cyprididae Cypridopsis sp.	0	0	0	18598
Ostracoda, Cyprididae Bennelongia sp.	0	0	Present	0
Ostracoda, Cyprididae spp.	71	118	Present	3514
Ostracoda, Candonidae Candonopsis sp.	0	1	0	140
Ostracoda Newnhamia sp.	0	0	0	1312
Ostracoda, Limnocytheridae Limnocythere sp.	25	764	Present	1557
Ostracoda, Ilyocyprididae spp.	0	57	Present	1852
Ostracoda spp.	2	11	Present	0
Copepoda, Calanoida spp.	152	530	Present	175352
Copepoda, Cyclopoida spp.	144	283	Present	9265
Copepoda, Harpacticoida spp.	60	562	Present	200
Cladocera, Chydoridae Alona sp.	3	7	0	40787
Cladocera, Chydoridae Camptocercus sp.	0	0	0	1365
Cladocera, Chydroidae Chydorus sp.	0	14	Present	120258
Cladocera, Chydroidae Leydigia sp.	97	447	Present	1431
Cladocera, Chydroidae Pleuroxus sp.	0	218	Present	23466
Cladocera, Chydoridae spp.	6	4	Present	0
Cladocera, Daphniidae Daphnia sp.	0	0	0	2
Cladocera, Daphniidae Daphnia carinata	0	43	Present	3458
Cladocera, Daphniidae Daphnia lumholtzi/projecta	0	63	Present	15971
Cladocera, Daphniidae Simocephalus sp.	0	1	Present	8041
Cladocera, Daphniidae Ceriodaphnia sp.	0	169	0	36843

Species Name	March	November	March	November
	Core	Core	Sweep net	Sweep net
Cladocera, Daphniidae spp.	0	0	Present	0
Cladocera, Sididae spp.	0	3	0	0
Cladocera, Moinidae Moina sp.	7	0	Present	0
Cladocera, Macrothricidae Macrothrix sp.	0	480	Present	3377
Cladocera, Macrothricidae Neothrix sp.	0	535	0	130
Cladocera, Macrothricidae spp.	7	0	0	0
Cladocera, Ilyocryptidae Ilyocryptus sp.	764	3852	Present	2983
Cladocera, Bosmina meridionalis	117	61	Present	19075
Cladocera spp.	2	0	0	0

Appendix 2 – Analytes used to investigate relationships with invertebrates

Environmental variables	March		November	
	Cores	Sweeps	Cores	Sweeps
Water quality/Field Observations[a]				
Water temperature (degrees Celcius)	✓	✓	✓	✓
Specific conductivity (µS/cm°C)	✓	✓	✓	✓
Dissolved Oxygen (% saturation)	✓	✓	✓	✓
pH (water)	✓	✓	✓	✓
Macrophyte diversity	✓	✓	✓	✓
%coverage by biofilm/detritus	✓	✓	×	×
%coverage by macrophytes	✓	✓	×	×
%coverage by filamentous algae	✓	✓	×	×
%boulder	×	×	×	✓
%algae	×	×	×	✓
%gravel	×	×	×	✓
%sand	×	✓	×	✓
%silt	×	✓	×	✓
%clay	×	✓	×	✓
%detritus (living)	×	✓	×	✓
%detritus (dead)	×	✓	×	✓
%detritus (total)	×	✓	×	✓
Sediment measurements[b]				
Sediment pH (Water 1:1 (moist))	✓	✓	✓	✓
EC (ds/m) 1:5 (40°C)	✓	✓	×	×
OX 1:1 (moist)	✓	✓	×	×
Sediment Eh	×	×	✓	✓
Depth of oxic layer (cm)	×	×	×	✓
Ions/Metals in sediment[b] (HCl extracted)				
Al (mg/kg)	✓	✓	✓	✓
Ca (mg/kg)	✓	✓	✓	✓
Fe (mg/kg)	✓	✓	✓	✓
K (mg/kg)	✓	✓	✓	✓
Mg (mg/kg)	✓	✓	✓	✓
Na (mg/kg)	✓	✓	✓	✓
P (mg/kg)	✓	✓	✓	✓
S (mg/kg)	✓	✓	✓	✓
Li (mg/kg)	✓	✓	×	×
Be (mg/kg)	✓	✓	✓	✓
Sc (mg/kg)	✓	✓	×	×
Ti (mg/kg)	✓	✓	✓	✓
V (mg/kg)	✓	✓	✓	✓
Cr (mg/kg)	✓	✓	✓	✓
Mn (mg/kg)	✓	✓	✓	✓
Co (mg/kg)	✓	✓	✓	✓
Ni (mg/kg)	✓	✓	✓	✓
Cu (mg/kg)	✓	✓	✓	✓
Zn (mg/kg)	✓	✓	✓	✓
As (mg/kg)	✓	✓	✓	✓
Se (mg/kg)	✓	✓	✓	✓
Mo (mg/kg)	✓	✓	✓	✓
Ag (mg/kg)	✓	✓	×	×

Cd (mg/kg)	✓	✓	×	×
Sn (mg/kg)	✓	✓	✓	✓
Cs (mg/kg)	✓	✓	×	×
Ba (mg/kg)	✓	✓	×	×
Pb (mg/kg)	✓	✓	✓	✓
U (mg/kg)	✓	✓	✓	✓
Ga (mg/kg)	×	×	✓	✓
Sr (mg/kg)	×	×	✓	✓
La (mg/kg)	×	×	✓	✓
Bi (mg/kg)	×	×	✓	✓
Ions/Metals in Pore Water[b]				
NH4-N (µg/L)	×	×	✓	✓
NOx-N (µg/L)	×	×	✓	✓
F- (µg/L)	×	×	✓	✓
Cl- (µg/L)	×	×	✓	✓
Br- (µg/L)	×	×	✓	✓
NO3- (µg/L)	×	×	✓	✓
SO4= (µg/L)	×	×	✓	✓
Ca (µg/L)	✓	×	✓	✓
K (µg/L)	✓	×	✓	✓
Mg (µg/L)	✓	×	✓	✓
Na (µg/L)	✓	×	✓	✓
S (µg/L)	✓	×	✓	✓
Al (µg/L)	✓	×	✓	✓
As (µg/L)	✓	×	✓	✓
Cd (µg/L)	✓	×	✓	✓
Co (µg/L)	✓	×	✓	✓
Cu (µg/L)	✓	×	✓	✓
Fe (µg/L)	✓	×	✓	✓
Mn (µg/L)	✓	×	✓	✓
Mo (µg/L)	✓	×	✓	✓
Ni (µg/L)	✓	×	✓	✓
Pb (µg/L)	✓	×	✓	✓
U (µg/L)	✓	×	✓	✓
V (µg/L)	✓	×	✓	✓
Zn (µg/L)	✓	×	✓	✓
Acid Base Accounting[c]				
TAA (to pH6.5 mole H^+/tonne)	✓	✓	✓	✓
RIS (mole H^+/tonne)	✓	✓	✓	✓
TOC (%C)	✓	✓	✓	✓
ANCBT (mole H^+/tonne)	✓	✓	✓	✓
Net Acidity (chromium suite mole H^+/tonne)	✓	✓	✓	✓
AVS (%S_{av} WW)	×	×	✓	✓
AVS(%S_{av} DW)	×	×	✓	✓

[a] = variables recorded by SA EPA, [b] = variables measured by CSIRO, [c] = variables measured by Southern Cross University

Appendix 3 – SIMPER analysis

Table 6 SIMPER analysis results showing differences in community structure between significantly different groupings of sediment core samples.

Month	Variable (%similarity within group)	% dissimilarity between groups	Descriminating taxa
March	Acidic (56) vs neutral (46)	52	Acidic – nematodes Neutral – worms, cyclopoida
March	Fine (43) vs coarse (74)	68	Fine – worms, cyclopoida Coarse - nematodes
	Fine (43) vs medium (70)	52	Fine – *Ilyocryptus*, cyclopoida Medium – nematodes, corophiidae
	Medium (70) vs coarse (74)	50	Medium – worms, corophiidae, cyclpoids, tubificid Group B worms Coarse - nematodes
November	Fine (54) vs coarse (54)	58	Fine – *Ilyocryptus*, calanoids Coarse - nematodes
	Fine (54) vs medium (59)	49	Fine – *Ilyocryptus*, calanoids, *Chironomus* Medium - *Macrothrix*, cyclopoids, *Procladius*
	Medium (59) vs coarse (54)	58	Medium – *Ilyocryptus*, *Macrothrix*, *Ceriodaphnia*, calanoida Coarse - nematodes
March	Finniss River (44) vs Lake Alexandrina (51)	67	Finniss – worms, cyclopoids, *Leydigia*, Cyprinidae, *Cladopelma* Lake Alexandrina - nematodes
	Finniss River (44) vs Lake Albert (63)	55	Finniss – cyclopoids, *Leydigia*, *Cladopelma*, Cyprinidae, tubficid Group B Lake Albert – nematodes, Corophiidae
	Lake Alexandrina (51) vs Lake Albert (63)	51	Lake Alexandrina – no taxa Lake Albert – worms, Corophiidae, tubificid Group B
November	Finniss River (52) vs Lake Alexandrina (54)	57	Finniss – *Ilyocryptus*, *Leydigia*, calanoida, *Chironomus*, *Procladius*, *Cladopelma* Lake Alexandrina – worms, nematodes
	Finniss River (52) vs Lake Albert (51)	56	Finniss – *Ilyocryptus*, *Leydigia*, calanoida, *Chironomus* Lake Albert – Corophiidae, worms, *Macrothrix*
	Lake Alexandrina (54) vs Lake Albert (51)	56	Lake Alexandrina – *Ilyocryptus*, nematodes Lake Albert – worms, Corophiidae, *Macrothrix*, harpacticoida, calanoida
	Finniss River (52) vs Currency Creek (82)	47	Finniss – *Chironomus*, *Procladius*, *Cladopelma*, calanoida Currency – *Ilyocryptus*, worms, harpacticoida

	Lake Albert (51) vs Currency Creek (82)	54	Lake Albert – Corophiidae, worms, *Macrothrix*, *Ceriodaphnia*, harpacticoida, calanoida, *Limnocythere* Currency Creek - nematodes
Both seasons	March (48) vs November (46)	55	March – worms November - *Ilyocryptus*

Appendix 4 – CLUSTER analysis plots

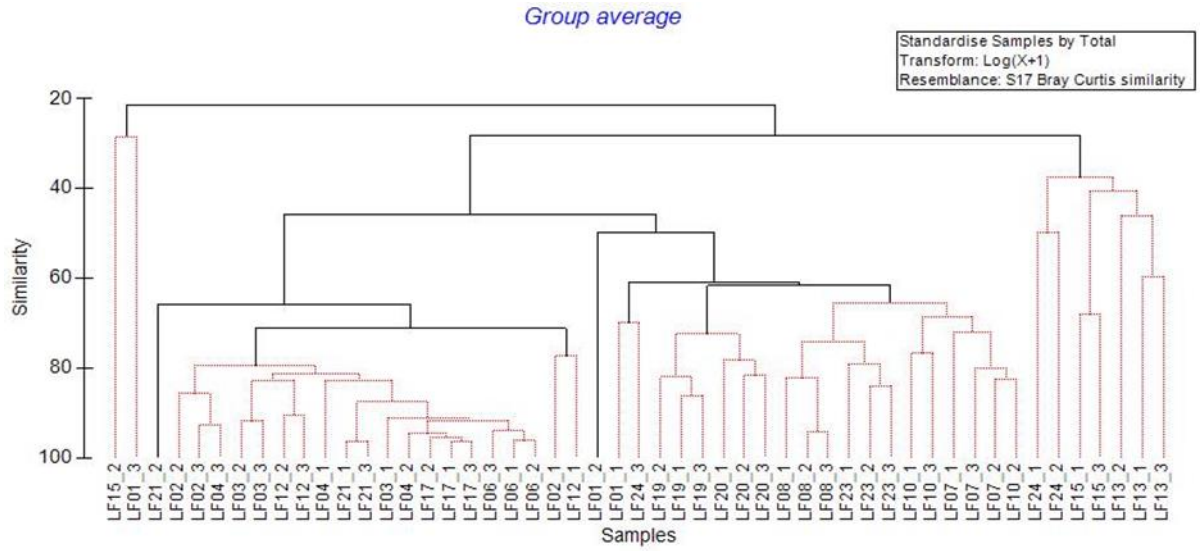

Figure 28 **Hierarchical cluster analysis of the March core data. Samples have been labelled by their site number followed by a 1, 2 or 3 identifying each replicate. Black vertical lines identify groups that are significantly different from each other (at *p* = 0.05), determined by the SIMPROF routine.**

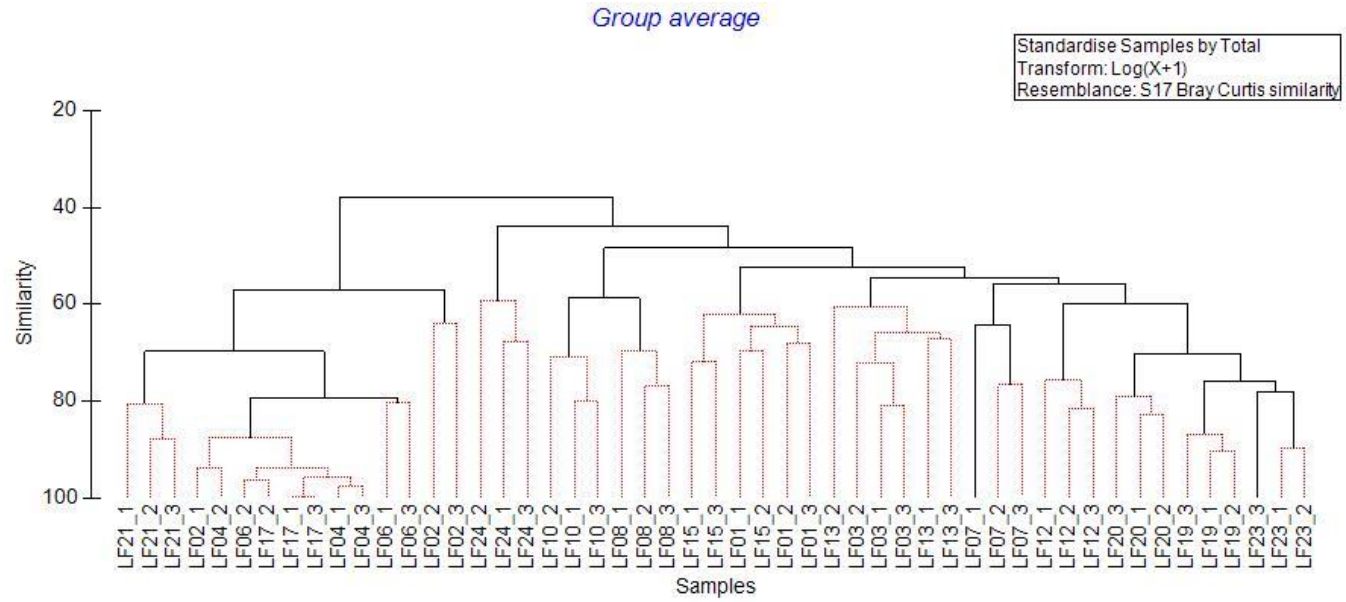

Figure 29 **Hierarchical cluster analysis of the November core data. Samples have been labelled by their site number followed by a 1, 2 or 3 identifying each replicate. Black vertical lines identify groups that are significantly different from each other (at *p* = 0.05), determined by the SIMPROF routine.**

Appendix 5 – Relationship between variables that best explained the community structure and taxon richness and TOC

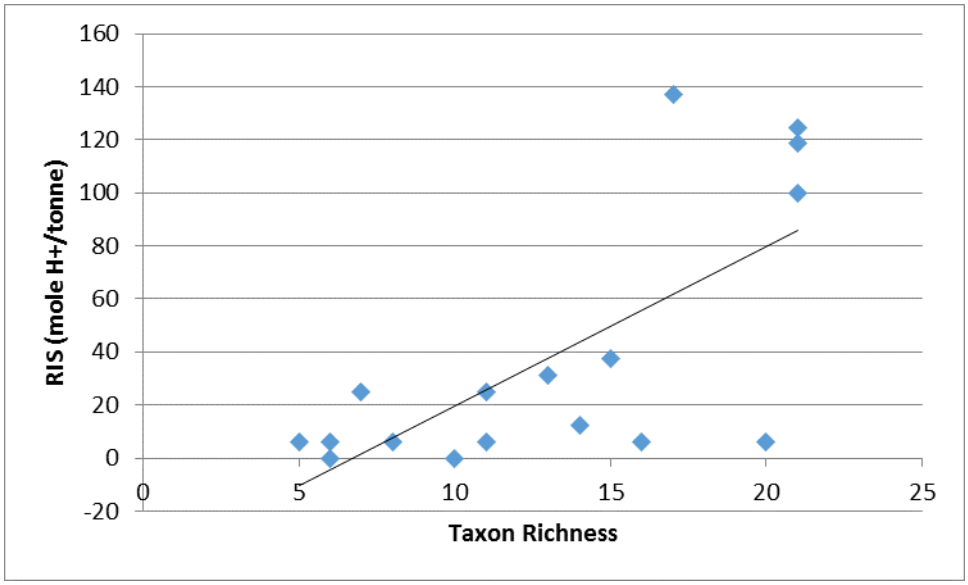

Figure 30 **Relationship between Reduced Inorganic Sulfur concentrations and taxon richness at the 17 sites in March.**

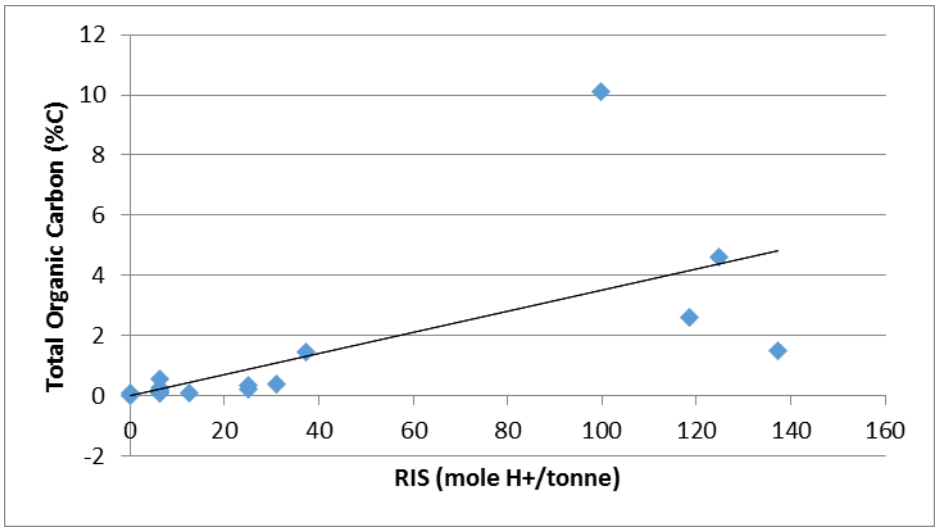

Figure 31 **Relationship between Reduced Inorganic Sulfur and total organic carbon content of the sediment at each of the 17 sites in March.**

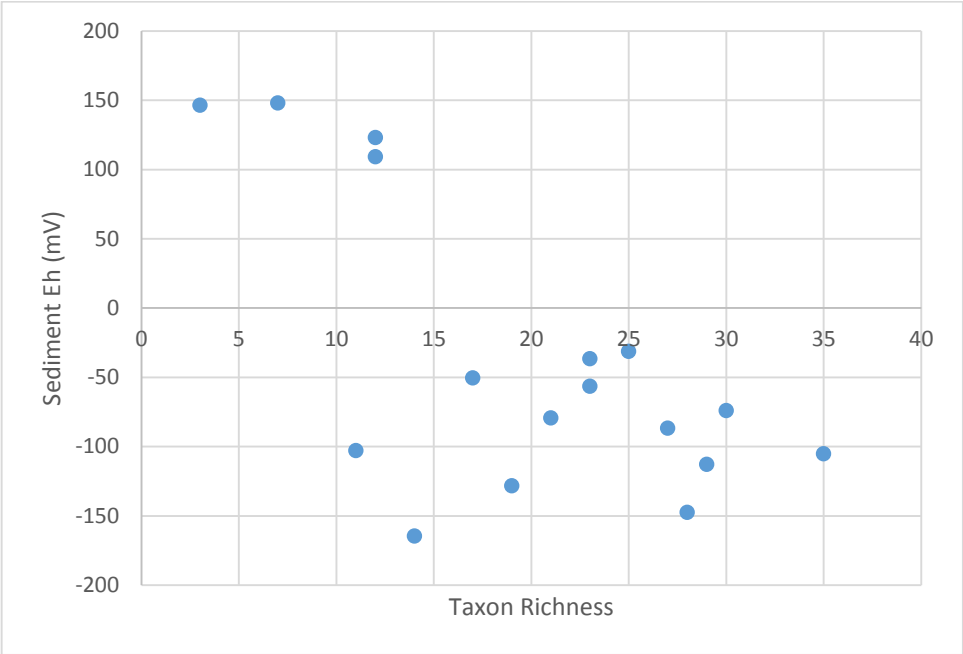

Figure 32 **Relationship between sediment Eh and taxon richness of the 17 sites sampled in November.**

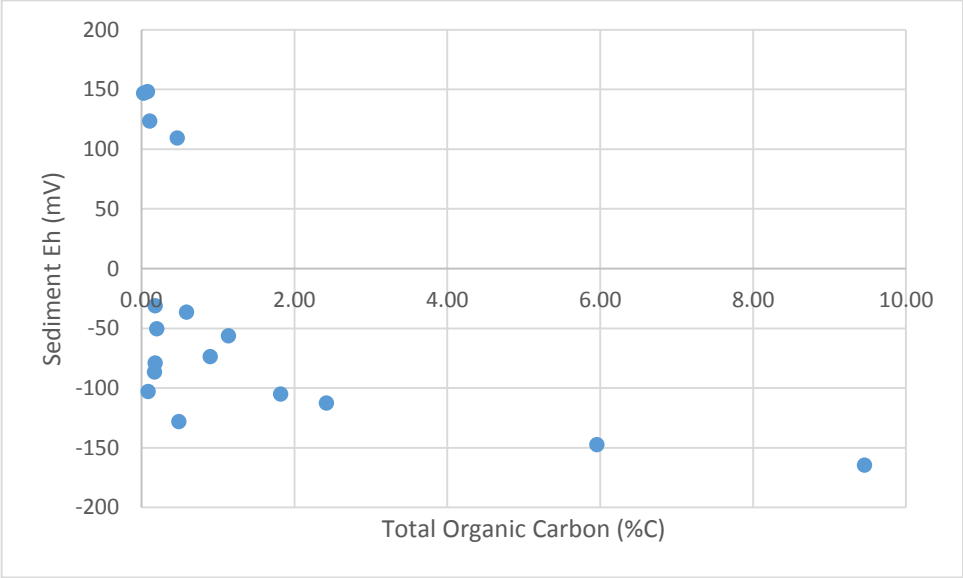

Figure 33 **Relationship between sediment Eh and total organic carbon.**