

Environment Protection Authority

Research into the macroinvertebrate community composition and chironomid deformity in the Lower Lakes

- Interim Report, June 2013

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Research into the macroinvertebrate community composition and chironomid deformity in the Lower Lakes

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1 Executive Summary

The recent drought in the Murray–Darling Basin resulted in extensive soil acidification within the region. When water-levels were reinstated to pre-drought levels, it was found that acidic conditions within the sediment profile still existed in some areas. The effect that these acidic sediments are having on the aquatic ecosystem is unknown. The Sediment Quality Triad (the Triad) approach is being used to assess the sediment quality in the Lower Lakes region by combining assessments of the sediment chemistry, sediment toxicity and the benthic community composition. The EPA is conducting the benthic community composition component of the Triad.

Sediment cores samples and sweep net samples were collected from 17 sites across the Lower Lakes region as a means of assessing the macroinvertebrate community. Core samples were collected in triplicate and divided into three horizons (0-2 cm, 2-5 cm and 5-10 cm). To date, only a portion of these samples have been processed with the remainder to be processed in the second phase of the research project. Chironomid specimens (from the subfamilies Chironominae and Tanypodinae) were also collected from nine sites to determine the rate of deformities within the region.

Most macroinvertebrates were found in the top 2 cm of sediment core samples, although many taxa types were found living in the 2-5 cm layer as well. Very few individuals were collected from the 5-10cm layer. More than 30 taxa and 9000 individuals were collected from the top 2 cm of sediment from the 17 sites sampled. The highest diversity of benthic macroinvertebrates was seen at Boggy Creek (LF15) and Waltowa (LF07), however, at other sites nematodes (round worms) made up a large portion (up to 99%) of the individuals collected. The high abundance of nematodes may be due to the samples being collected many metres away from the nearest aquatic macrophyte habitat. When investigating the similarities in community composition across the sites, no obvious groupings occurred when using either multi-dimensional scaling or hierarchical clustering that could be explained by the acidic conditions. Nor were there any clear groupings of sites when investigating substrate type (coarse versus fine). Deformity rates seen in the chironomid mouthparts and antennae ranged from 0% to 16.7% at the nine sites investigated. The highest deformity rate occurred at a site where only 6 specimens were collected and 1 was deformed. The deformity rate at all other sites was less than 10% and not suggestive of pollution induced impacts. An unusually high percentage (approximately 50% of 92 specimens) of broken teeth was noticed in one species of chironomid collected from Finnis River, south (LF24). The exact reason for this phenomenon is unknown.

A further survey is planned for October/November 2013 to collect additional samples to investigate the macroinvertebrates community composition of the Lakes region over a second season. A side study into the changes in the community structure in the sediment at increasing distances from shoreline and fringing habitat may also be investigated to determine if some of the sites in the main part of Lake Alexandrina are being sampled at an unsuitably large distance away from the shoreline.

2 Introduction

Acid Sulfate Soils in the Murray-Darling Basin

During the recent drought in the Murray-Darling Basin, flow to the Lower Lakes was severely restricted resulting in a reduction in the lake levels to as low as -1.0 m AHD. This led to exposure of sediments with pyritic minerals on the lake margins and the subsequent oxidation of these produced acidic sediments. Soil acidification was extensive within the region, with soil reducing to pH<4 at many sites. During late 2010, the drought was broken in the Murray-Darling Basin and water levels were reinstated to pre-drought levels. However, research by CSIRO 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 with only the top 5 cm or less of the sediment profile being neutralised to a pH>4. (Fitzpatrick *et al.* 2011). High concentrations of various metals (e.g. aluminium, arsenic, boron, cadmium, chromium, copper, manganese, nickel, lead and zinc) in the pore water were also observed, with many concentrations exceeding the ANZECC trigger values (Fitzpatrick *et al.* 2011; Hicks *et al.* 2009). The presence of acid sulfate soils in the Lower Lakes presents a potential toxicity hazard to the aquatic ecosystem from the reduced sediment pH and the increased availability of metal ions.

Sediment Quality Triad Approach

Research trends in sediment quality assessment have demonstrated the need for an integrated approach that combines chemical characterisation with biological effects evaluation (Chapman 1990). The Sediment Quality Triad (the Triad) approach is a multiple lines of evidence approach used to determine the extent and significance of sediment pollution and pollution-induced degradation. The Triad includes an assessment of the sediment chemistry, sediment toxicity through bioassays and benthic community assemblage (Long and Chapman 1985, Figure 1). There are limitations when using each of these three approaches alone. For example, determining the concentrations of chemicals present within sediments does not tell us if those chemicals are bioavailable or not. There may also be other chemicals present in the sediment at concentrations that may be harmful to biota but are not tested for. Toxicity testing can provide useful information on the toxicity of the chemicals to biota, but results from these laboratory studies may not truly reflect the impacts that are occurring *in-situ*. The reasons for observed effects on the benthic community composition can be difficult to determine on their own as they may be the result of physical alterations or trophic level interactions rather than due to toxic impacts from sediment pollution. However, the combination of the potential cause (chemistry) and effect (biology) makes the Triad a powerful tool when investigating sediment pollution and potential impacts (Chapman 1990).

The Triad approach was first evaluated by Long and Chapman (1985) by using a series of datasets on the Puget Sound, an inland marine system in Washington, USA. This study proved that the Triad approach is a useful tool for assessing the sediment quality in benthic environments, with results indicating that the chemical levels measured were not always a reflection of the biological effects observed. Since then, many other studies have shown the importance of using multiple lines of evidence in the Triad approach, (Chapman *et al.* 1987, Canfield *et al.* 1994, Soucek *et al.* 2000, Riba *et al.* 2004, McDonald 2005, Pinkey *et al.* 2009, Crévecoeur *et al.* 2011, de Deckere *et al.* 2011). Guidelines for the use and interpretation of the Triad have been developed over time, with the most recent guidelines published by Chapman *et al.* (1997) and results from findings have been used for management and legislation purposes (e.g. de Deckere *et al.* 2011).

Benthic Community Structure

The aquatic benthic biological community is dominated by macroinvertebrates which play an important role in the functioning of aquatic systems, such as the processing of organic matter. They are also important in aquatic foodwebs, being the major link between organic materials and higher trophic levels, such as fish, birds, tortoises, water rats and frogs. Macroinvertebrates are most commonly used in biological monitoring studies designed to assess the ecological health of a water body because they are common, easily sampled, can be readily identified and are known to have a range of environmental tolerances with some able to survive in low oxygenated or

polluted waters, while others are sensitive and require well-oxygenated water and unpolluted waters. Macroinvertebrates have been well studied in South Australia and their presence, diversity and abundance can be used as indicators of aquatic ecosystem condition.

The affect of acid sulfate soils on aquatic invertebrates is largely unknown for the Lower Lakes but is crucial in understanding the ecological significance of the risks posed by acid sulfate soils in the region. Areas where sediments were previously exposed still contain acidic sediment and pore water which may be hindering the recovery of the macroinvertebrate community. Monitoring of the benthic community structure between 2008 and 2010 has found that while improvements in the health of the benthic community have occurred as water levels returned to normal, molluscs and some crustaceans had not returned to the system (Giglio 2011).

This study encompasses the biological component of the sediment quality triad (see Figure 1) with the other two components being conducted by the CSIRO from 2013 to 2015. The overall objective of the benthic community composition component is to assess the macroinvertebrate community composition both in the water column and in the sediment, at sites throughout the Lower Lakes region. This report provides information on the study to date and, being an interim report, covers only a portion of the results that will be gained from this project.

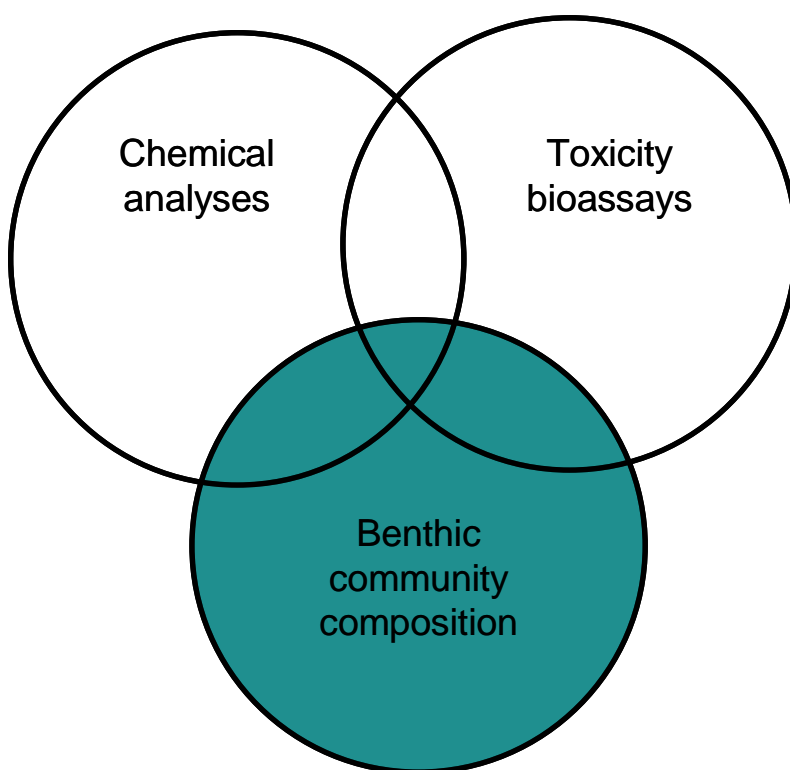


Figure 1 Illustration depicting the three lines of evidence providing the basis for a Sediment Quality Triad study. The section highlighted in green is the part of the Triad being investigated by the EPA.

3 Methods

Study Site

Seventeen sites in the Lower Lakes were monitored (Table 1, Figure 2), including sites in Lake Alexandrina, Lake Albert and the lower sections of Currency Creek and Finnis River. Each of these sites was visited in March 2013. Sites were categorised by the sediment characteristics noted whilst sampling (coarse vs. fine), where coarse substrate consisted of primarily sand and fine substrate consisted of predominantly silt and clay. Each site was also categorised as either acidic (pH<6.5) or neutral (pH 6.5 to 8) based on results from CSIRO's sampling in February/March 2013 (see Table 1).

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

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

^a = Sediment acidity was determined through sampling conducted by CSIRO in February/March 2013.

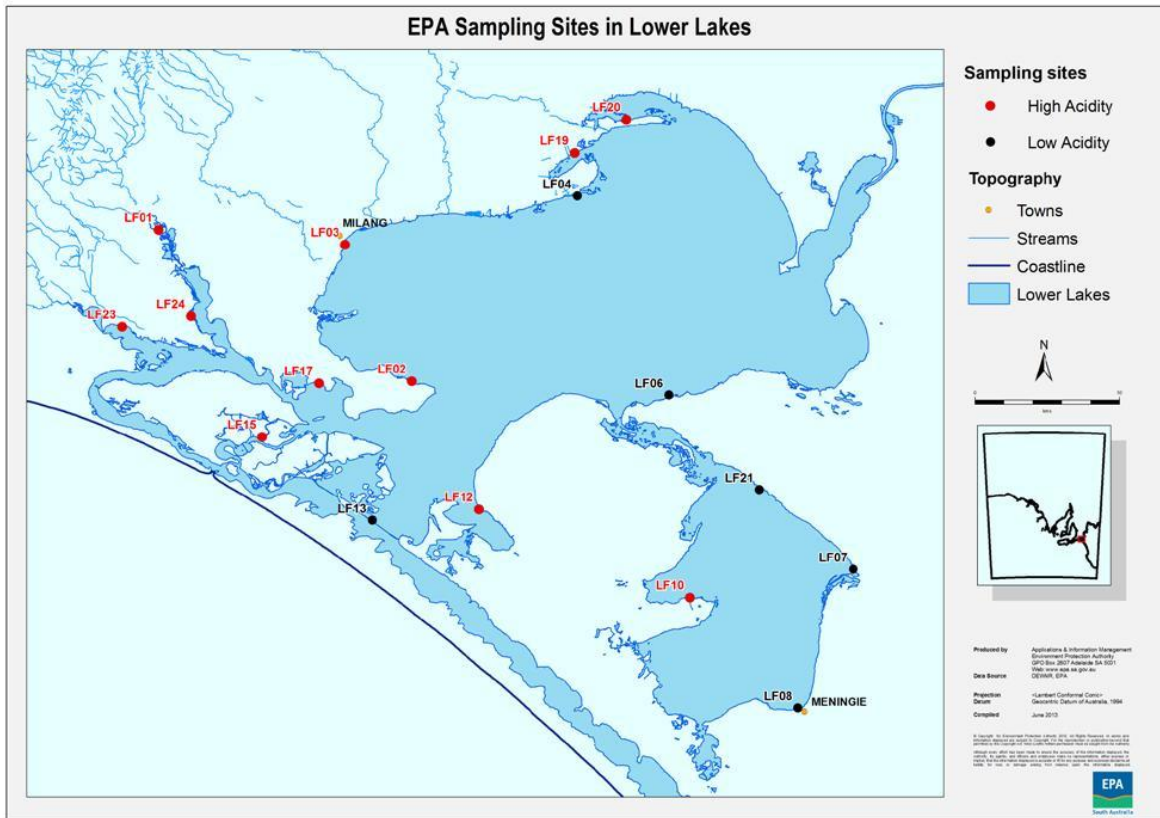


Figure 2 The locations of the acidic and neutral sampling sites across the Lower Lakes region.

Field Procedures

The macroinvertebrate assemblage at each site was investigated in two ways; sediment core samples and sweep net samples. To assess the possible direct impacts to an individual species, specimens of the non-biting midge subfamilies Chironominae and Tanypodinae were also collected and checked for deformities.

Sediment Cores

Sediment core samples were collected from each site with a shovel to a depth of 10 cm and divided into three horizons; 0–2 cm, 2–5 cm, and 5–10 cm (Figure 3). Three core samples were collected from each site, approximately 1-2 m away from each other. The dense stand of reeds at the site at Tauwitcherie (LF13) made sampling difficult and due to the high percentage of silt it was difficult to keep the core intact to then be able to differentiate between the horizons in the core profile. As such, the samples collected from this site did not reach a depth of 10 cm, instead only reaching 5 cm and they were not divided into horizons but instead preserved as one sample. At most sites each horizon was individually sieved through a 250 µm mesh to remove fine sediments whilst in the field. All samples were placed in separate plastic screw-topped jars and preserved with methylated spirits in the field.



Figure 3 Sediment core collected from LF20 Boggy Lake, showing distinct layers of sediment.

Sweep Net Samples

The sweep net samples were collected according to the Australian River Assessment System (AUSRIVAS) sampling protocol for South Australia. This involved sampling a 10 m section of edge habitat using a triangular dipnet with 30 cm sides and a 250 μm mesh (Anon 1997) (Figure 4). All available habitats (e.g. sandy bank, reed beds) were sampled and the sample placed in a plastic screw-topped jar and preserved with methylated spirits in the field.



Figure 4 Collecting a sweep net sample along the edge of the bank at LF21 Windmill Site, Lake Albert.

Chironomid Deformity Study

Specimens of non-biting midges (subfamilies; Chironominae and Tanypodinae) were collected from each site where possible. The methodology for chironomid collection varied at each site due to the range of habitats available and the differences in site characteristics. Specimens were collected by either using a sweep net to disturb the sediment, or shovelling sediment into a sieve. The sediment samples were then placed on a white field tray and picked through removing the Chironominae and Tanypodinae larvae as they were found (Figure 5). Third and fourth instar larvae were targeted with smaller specimens returned to the water. The third and fourth instar stages are more likely to exhibit deformities and, being larger, the deformities are more easily noticeable. A collection of at least 50 individuals was aimed for from each site. The low abundance of midges at some sites meant collecting a large number of individuals at those sites was impossible. To maximise productivity in the field, the number of individuals collected was assessed after half an hour of searching, and if the total number was below ten searching and collection was abandoned. Collected specimens were placed in a small plastic screw-topped jar and preserved with methylated spirits in the field.



Figure 5 Collection of chironomid individuals for deformity study.

Laboratory procedures

Sediment Cores

Rose Bengal solution (approximately 5-10 mL) was added to each sample jar to stain the fauna present and assist in picking out the specimens. To process the core samples, each sample was washed through a 250 μm sieve to remove fine sediments and then placed in a sorting tray. Under a dissecting microscope, specimens were picked out of the sample, counted and identified to the lowest practical level using available identification guides.

At the time this report was written, only a subset of the samples collected had been processed. To provide some basic information of the benthic community of the Lower Lakes in this report a set of samples were prioritised to ensure they were processed first. These samples included all nine samples (3 horizons from 3 core samples) from two sites Milang (LF03) and Loveday Bay (LF12) in Lake Alexandrina and the top horizon (0-2 cm) for one core sample from each of the other 15 sites monitored.

Sweep Net Samples

To date, all of the sweep net samples are yet to be processed. These samples will be processed as part of the second phase of the research.

Chironomid Deformity Study

All third and fourth instar larvae were identified and the head of each individual removed and mounted on a slide using Hoyer's medium. The slides were left for a couple of weeks to allow the head capsules to clear. This increases the visibility of the features of the head and allows for easy identification of deformities in the mouthparts

and antennae. The presence and type of deformity on each midge larvae were noted and the number of deformities at each site totalled.

Data Analyses

Abundance and richness information was calculated using an Excel spreadsheet. The remaining data analyses were performed in PRIMER v6 with the PERMANOVA+ add on. The amount of sediment collected for each horizon in each core varied which hinders direct comparison between the samples. Therefore the 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, to enable direct comparisons between the samples. The Simpson's Diversity Index ($1-\lambda'$) was calculated in PRIMER using the DIVERSE routine. Diversity indices take into account both the number of individuals identified and the number of taxa present at each site. The value of a diversity index is higher when evenness increases and is thus maximised when all taxa are equally abundant. Multi-dimensional scaling plots were produced from a Bray-Curtis resemblance matrix to display the relationships in community composition in a 2-dimensional format. A 2D stress value lower than 0.20 demonstrates the plot is adequately showing the relationships of the data in two dimensions without significant distortion. Hierarchical cluster analyses were undertaken to determine whether samples with high similarities grouped out by acidity or dominant substrate. The cluster analysis was undertaken using the group average linkage option and a SIMPROF (similarity profile) test which was conducted to determine which groupings of samples were statistically significant.

4 Results

Entire Core Sample

At the time of writing this report, all nine samples (3 horizons from 3 replicate samples) had been processed for two sites (Milang, LF03 and Loveday Bay, LF12). This enabled examination of the variation in taxon assemblage throughout the 10 cm sediment profile at these two sites. A moderate number of taxa were collected from the two sites with high abundances (Table 2) due to a large number of nematodes being collected. Other taxa found at these sites include aquatic worms, proboscis worms, flatworms, little basket shells (*Corbiculina*), the mite Oribatida, non-biting midge larvae (*Cryptochironomus*, *Cladopelma*, *Polypedilum*, *Dicrotendipes*, *Paratanytarsus*, *Cladotanytarsus*, *Procladius*, *Coelopynia pruinosa*) and zooplankton (Cyclopoida, Calanoida, Ostracoda (*Lymnocythere*), and cladocerans (*Ilyocryptus* and Chydoridae (*Leydigia*)). Most individuals were found in the top 2 cm of sediment, although many taxa types were found living in the 2-5 cm layer as well. Very few individuals were collected from the 5-10cm layer, which is consistent with the findings in Corbin *et al.* (2012).

Table 2 Taxa diversity (and abundance) through the sediment profile at Milang (LF03) and Loveday Bay (LF12) in the Lower Lakes.

Replicate	Milang (LF03)			Loveday Bay (LF12)		
	1	2	3	1	2	3
0-2 cm	5 (1682)	5 (613)	7 (2078)	6 (1154)	13 (2517)	8 (4401)
2-5 cm	2 (25)	1 (1)	7 (183)	9 (139)	4 (281)	6 (181)
5-10 cm	1 (2)	1 (1)	3 (4)	1 (6)	1 (12)	1 (6)

Top Horizon (0 – 2 cm)

Samples collected from the top horizon (0-2 cm layer) at each of the 17 sites were processed first to enable comparisons to be made between the sites. Samples from the lower horizons will be processed in phase 2 of the project. A total of 9195 individuals were identified in the top horizon across all sites in the Lower Lakes. The total abundance varied widely across the sites (Figure 6), with Milang (LF03) having the highest abundance of individuals recorded (1682 individuals), followed by Poltalloch (LF06) with 1386 individuals and Loveday Bay (LF12) with 1154 individuals. The high abundances at these sites was due to a large number of nematodes (round worms) being found. Nematodes made up 99% of the total abundance at Milang and Poltalloch and 94% at Loveday Bay. Tauwitchenie had the lowest abundance of all sites (only 33 individuals found), despite this sample being a 0-5 cm horizon, rather than a 0-2 cm horizon.

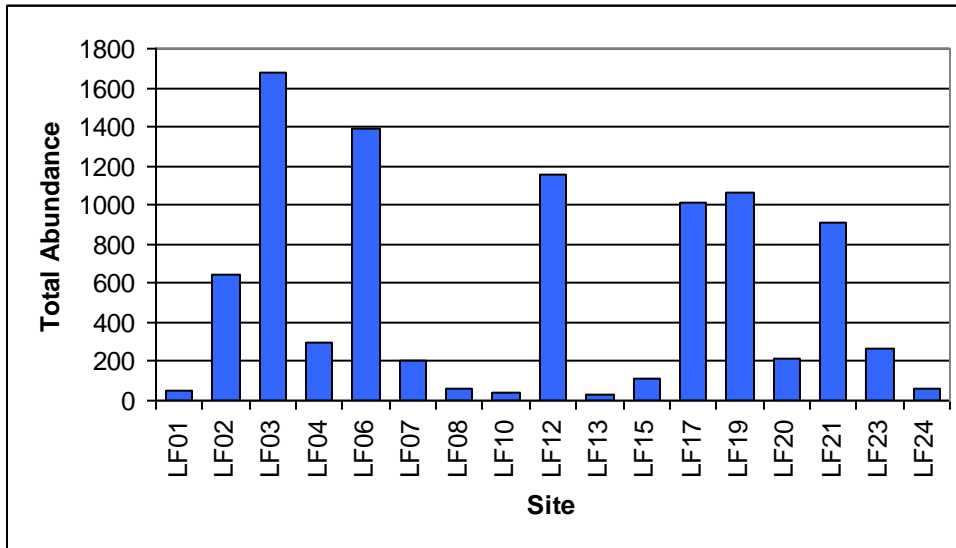


Figure 6 Total abundance for the 0-2 cm horizon of the Lower Lakes benthic samples. Refer to Table 1 for site names.

A total of 37 individual taxa were identified from the benthic samples, with Boggy Creek (LF15) having the highest richness value of 17, followed by Waltowa (LF07) with 11 taxa identified (Figure 7). Taxon richness was lowest at Meningie (LF08), Point Sturt South (LF17) and Poltalloch (LF06) with just 2, 3 and 4 taxa found respectively.

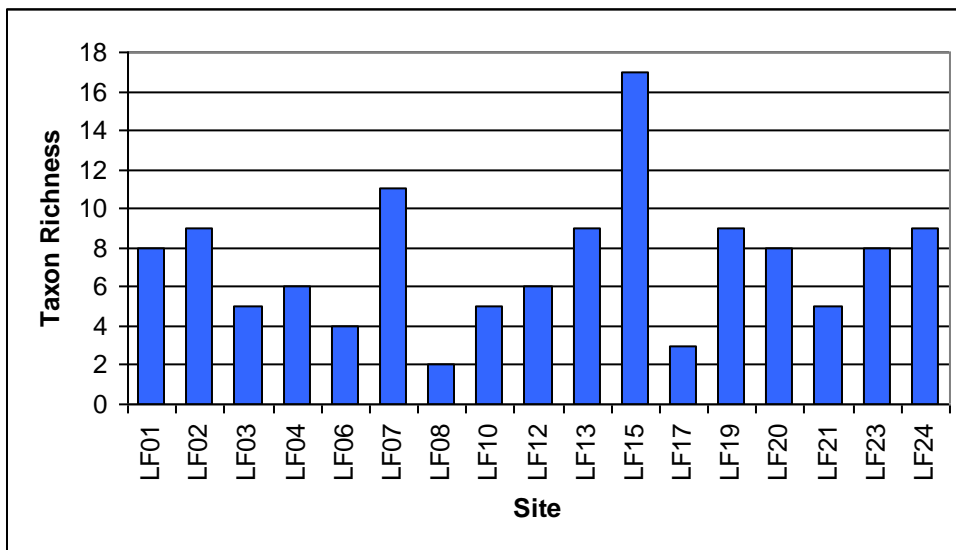


Figure 7 Taxon richness for the 0-2 cm horizon of the Lower Lakes benthic samples. Refer to Table 1 for site names.

The Simpson’s diversity index is a measure of the evenness of the data. A high Simpson’s diversity index suggest good evenness of taxa at the site with all taxa present in near equal abundance, a low Simpson’s diversity index suggest dominance by one or two taxa and very low numbers of all other taxa present at the site. Boggy Creek (LF15), Lower Finniss (LF24) and Tauwitcherie (LF13) all had a high diversity index (Figure 8). Point Sturt South (LF17), Poltalloch (LF06) and Milang (LF03) had the lowest diversity indices, due to the high number of nematodes collected from these sites.

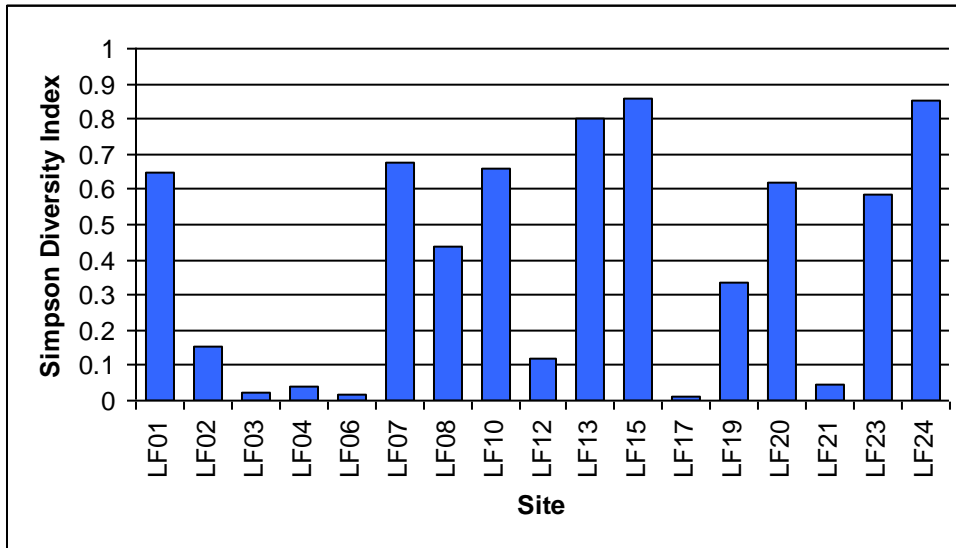


Figure 8 Simpson Diversity Indices for the 0-2 cm horizon of the Lower Lakes benthic samples. Refer to Table 1 for site names.

When the data was analysed using multi-dimensional scaling (MDS) the sites at Tauwitcherie (LF13), Boggy Creek (LF15) and Lower Finniss (LF24) were separated from the remaining sites in the MDS plot (Figure 9), corresponding with higher Simpson Diversity Indices. No obvious groupings by sediment acidity were observed amongst the sites. However, the sites with fine sediment (silt and clay) tended to group above the sites with coarse sediment (sand) suggesting there could be some slight differences in the macroinvertebrate community structure at sites with these sediment types.

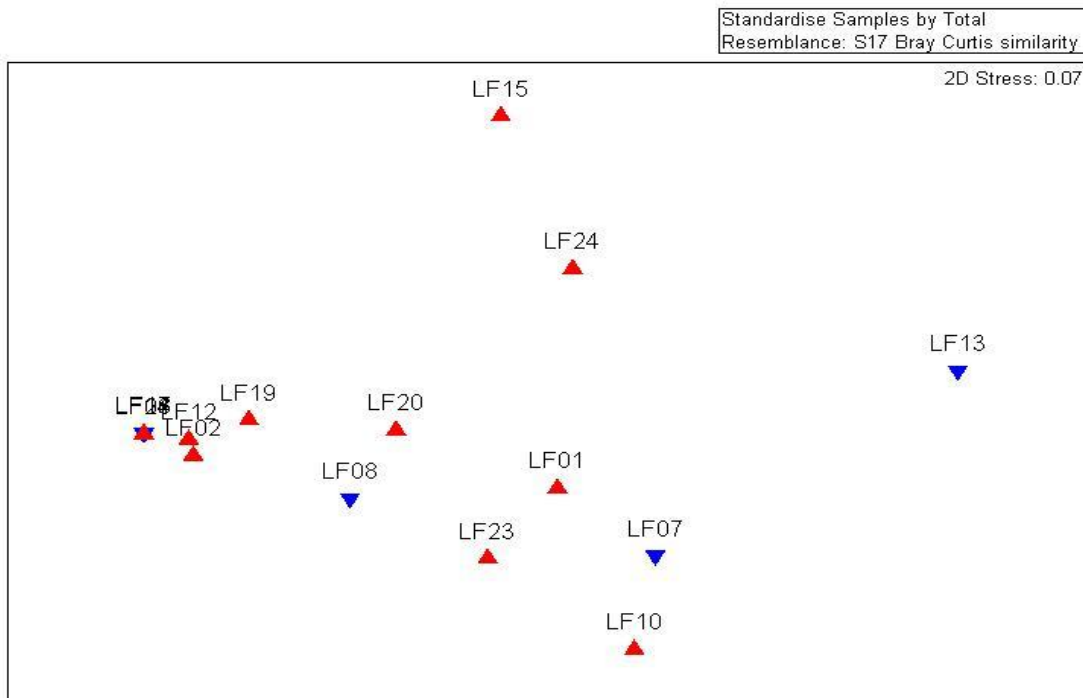


Figure 9 MDS plot showing the similarity in community composition between each of the samples. Red triangles are sites with sediment pH < 6.5 (acidic) and blue triangles are sites with sediment pH > 6.5 (neutral).

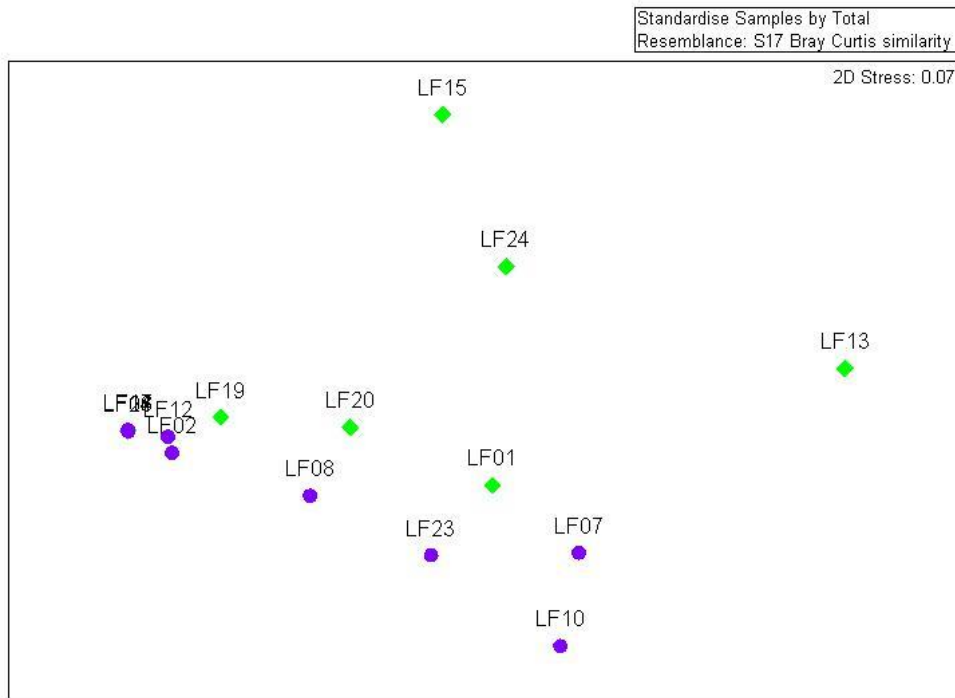


Figure 10 MDS plot showing the similarity in community composition between each of the samples. Green diamonds are sites that had fine substrates as the dominant substrate type and purple circles are sites with predominantly coarse substrates.

The cluster analysis showed a similar pattern as the MDS plot, with no clear separation of sites by sediment acidity (Figure 11) or substrate type (Figure 12). Again LF13, LF15 and LF 24 are the sites that are least similar to the other sites sampled. There is a some indication that sites may be grouped together based of their geographical location within the Lower Lakes region with Wally’s Landing on the Finnis River (LF01) and Currency Creek (LF23) grouping together with a similarity of approximately 85% and the sites LF02, LF03, LF04, LF06, LF12, LF17 and LF21 forming two groups (one with LF21, LF04, LF17, LF03, LF06 and the other with LF02 and LF12) with high similarity, of more than 90%. These sites are all located in the central section of Lake Alexandrina, the upper site on Lake Albert and the site near Clayton. These sites were seven of the top eight sites with the highest abundance of nematodes with between 280 and 1664 nematodes collected from each site. The other site in this group of eight sites was Dog Lake (LF19) where 859 nematodes were collected. These seven sites were also sampled a considerable distance away from shore, for example, the site at Milang (LF03) was sampled almost 200 m from shore (according to GIS calculations). The distance from the shoreline, or more importantly the dense reed beds fringing the shoreline, may be playing a significant role in determining the diversity of species seen in the sediment.

Group average

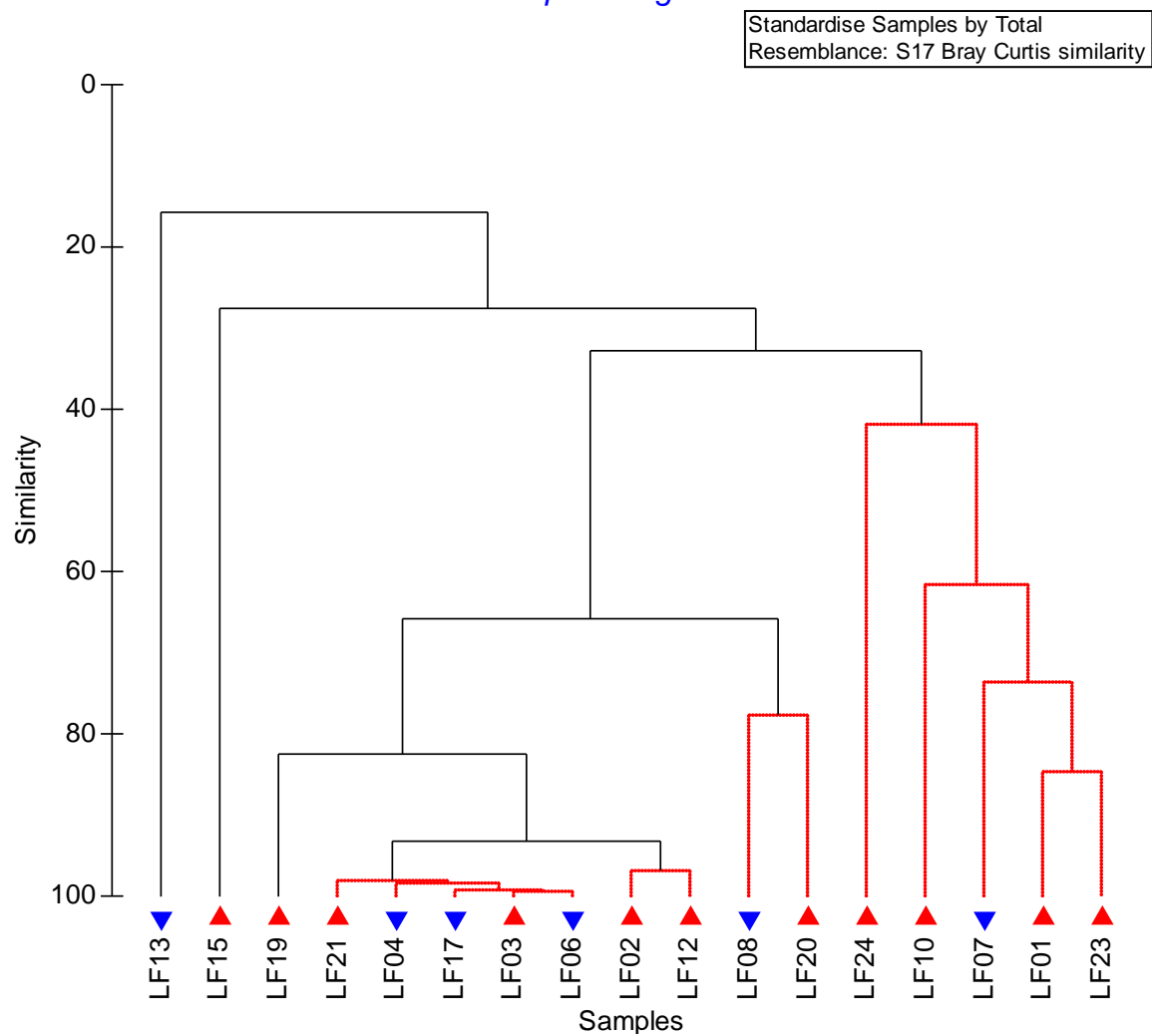


Figure 11 Cluster analysis showing seven statistically significant groupings, as identified by the SIMPROF routine in PRIMER (displayed by the solid red lines). Red triangles are site with sediment pH <6.5 (acidic) and blue triangles are sites with sediment pH > 6.5 (neutral).

Group average

Standardise Samples by Total
Resemblance: S17 Bray Curtis similarity

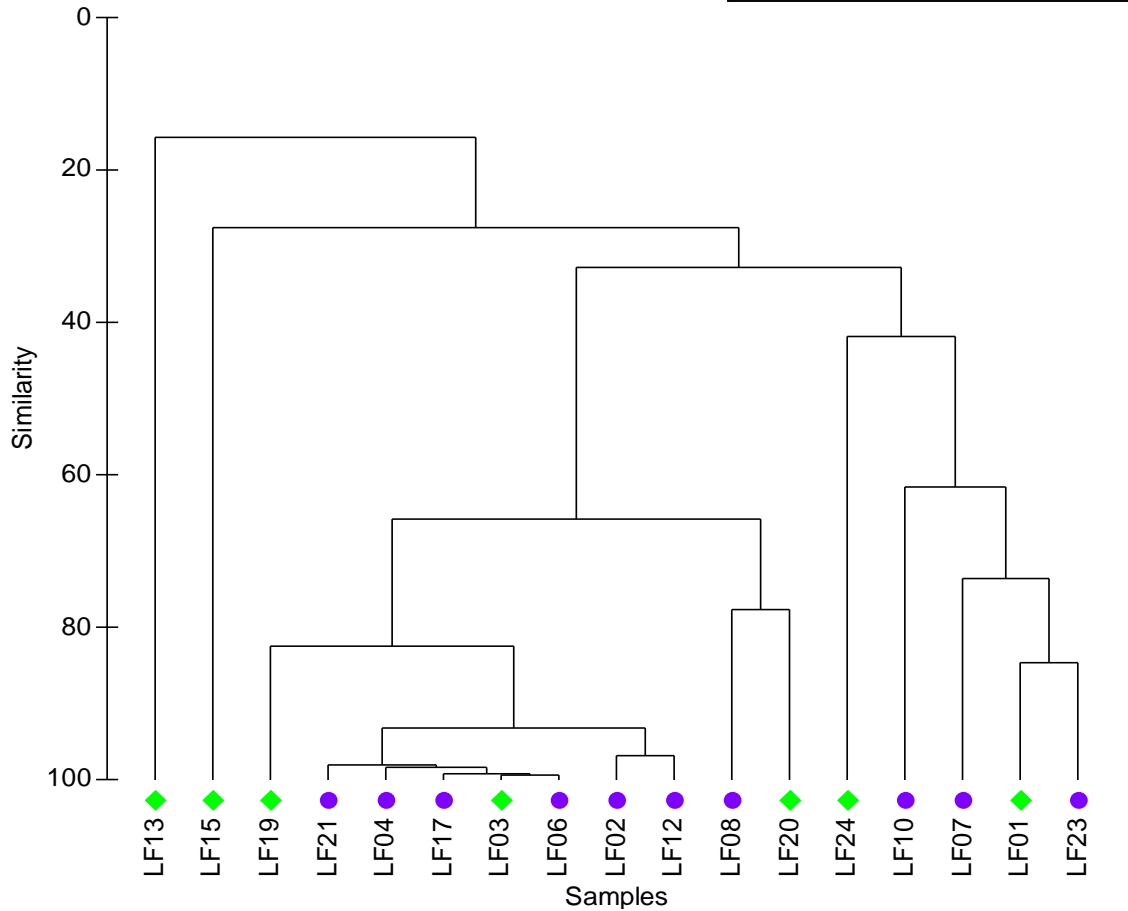


Figure 12 Cluster analysis showing seven statistically significant groupings, as identified by the SIMPROF routine in PRIMER. Green diamonds are sites that had fine substrates as the dominant substrate type and purple circles are sites with predominantly coarse substrates.

Chironomid Deformity Study

Specimens from the midge subfamilies Chironominae and Tanypodinae were collected from nine sites. At most sites the percentage of deformed individuals was less than 10% (Table 3). The exception was at Point Sturt North (LF02) where a deformity percentage of 16.67% resulted, however, this was due to just one deformed individual in only 6 specimens collected. Most of the deformities noticed occurred in mouthparts, either on the mentum, the paraligula, the mandibles, the premandibles or the epipharyngis. Some antennal deformities were also noted. Very few specimens exhibited more than one type of deformity.

Of the nine sites from where chironomids were collected, only one of these sites has neutral acidity. That site was Tauwitcherie, where 18 midge larvae were collected, none of which were deformed. From the other sites (acidic sites) a total of 435 midge larvae were collected and examined and 27 were deformed (or 6.2%). The deformity rate at each of these eight acidic sites ranged from 0% to 16.7%. At Finnis River, south (LF24) approximately 50% of the 92 *Polypedilum nubifer* collected had broken teeth on the mentum. Broken teeth are not considered to be a deformity, instead being the result of wear and tear through eating and gnawing. However, this is an unusually high percentage of broken teeth and the only site (of the nine investigated) to show this phenomenon, with other sites showing the percentage of broken teeth to be approximately 5% (similar to the rate of deformities). The exact reason for the high percentage at Finnis River, south (LF24) is unknown.

Table 3 Deformities in chironomid specimens from the subfamilies Chironominae and Tanypodinae at 9 sites in the Lower Lakes region.

Site Name	No. of Chironominae collected	No. of Tanypodinae collected	No. of deformities (percentage of individuals with deformities)	Types of deformity
LF01	57	7	5 (7.81%)	1 <i>Procladius</i> with short paralgula on one side 1 <i>Paratanytarsus</i> with slightly split median tooth 1 <i>Microchironomus</i> with missing tooth on mandible 1 <i>Dicrotendipes</i> with missing distal antennal segments 1 <i>Kiefferulus</i> with missing tooth on mentum
LF02	6	0	1 (16.7%)	1 <i>Cladotanytarsus</i> with short segment on one antenna and other antennae missing distal segments
LF03	54	8	0 (0%)	n/a
LF12	23	0	1 (4.35%)	1. <i>Polypedilum nubifer</i> with asymmetrical epipharyngeal pecten
LF13	18	0	0 (0%)	n/a
LF15	67	1	5 (7.35%)	2 <i>Chironomus</i> with split median tooth on mentum, 1 <i>Chironomus</i> with “rotted” antennae 1 <i>Kiefferulus</i> with distorted median tooth on mentum 1 <i>Kiefferulus</i> with missing tooth on mentum on one side, extra teeth on the other and one antenna with only one segment
LF19	51	0	4 (7.84%)	1 <i>Chironomus</i> with extra tooth on one side of the mentum but missing a tooth on the other side 1 <i>Chironomus</i> with an asymmetrical epipharyngeal pecten 1 <i>Chironomus</i> with four-toothed premandible 1 <i>Chironomus</i> with missing tooth on mentum
LF23	60	0	5 (8.33%)	1 <i>Kiefferulus</i> with missing tooth on mentum 1 <i>Chironomus</i> with extra tooth on epipharyngeal pecten 1 <i>Chironomus</i> with an extra tooth on the mentum 1 <i>Chironomus</i> with split median tooth on the mentum 1 <i>Chironomus</i> with missing antennal segment
LF24	101	0	6 (5.94%)	1 <i>Cryptochironomus</i> with missing tooth on mandible 1 <i>Cryptochironomus</i> with missing tooth on mentum 1 <i>Polypedilum nubifer</i> with antennal segment misshapen 2 <i>Polypedilum nubifer</i> with missing lateral tooth on the mentum 1 <i>Polypedilum nubifer</i> with asymmetrical epipharyngeal pecten

5 Discussion

The preliminary results from this study suggest:

- The abundance and diversity of taxa found across the 17 sites sampled in the Lower Lakes varied substantially between sites. The high abundance seen at some sites was usually due to a large number of nematodes being collected. From some sites the nematode population accounted for 99% of the individuals found.
- Based on data from two of the sites (Milang and Loveday Bay in Lake Alexandrina) it seems likely that very few taxa can be found below 5 cm down the sediment profile, probably due to lack of oxygen and food.
- The results gathered to date show no obvious differences in the biota in the sediment between those sites believed to be impacted by acidic sediments and those that have been found to have neutral pH. However, the MDS plot suggests the possibility of differences between sites being related to dominant substrate type (i.e. sandy sites versus clay/silt sites).
- The sites that were most similar to each other were located in the main part of the Lake Alexandrina, the top of Lake Albert, and near Clayton. This grouping of sites occurred primarily because of high numbers of nematodes being found. This may be related to these sites being located some distance from the shoreline.
- Most of the sites sampled for this study had a considerable amount of vegetation growing along the bank. This vegetation usually consisted of *Phragmites* and *Typha*, although other emergent macrophytes such as *Bolboschoenus* and *Schoenoplectus* as well as some submerged plants (*Myriophyllum*, *Chara*) are also present throughout the region. This vegetation provides a food source for the macroinvertebrates, can provide shelter from predators and from high waves and water movement and, in the case of non-biting midges, suitable egg-laying sites. It seems likely that the further away the core samples are collected, the fewer taxa there are in the sediment and the higher the abundance of nematodes, although further work is required to confirm this. This variable (distance from suitable habitat) maybe a variable that is masking the impact of the acid sulfate soils. That is, it may be having a larger influence on the presence and diversity of macroinvertebrates in the sediment than the acidic conditions.
- Processing the remaining samples will demonstrate the depths benthic invertebrates are inhabiting and whether this differs between sites. Comparing the results from the three replicate core samples will also provide an indication of how variable the benthic invertebrates are at each site.

A further survey is planned for October/November 2013 to collect additional samples and investigate the differences in the macroinvertebrate community composition over the two seasons (autumn and spring). As it seems likely that some sites are being sampled at an unsuitably large distance away a further study is likely to occur to investigate changes in macroinvertebrate composition with distance from macrophyte beds. .

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Appendix 1 Taxa List

Table A1 List of taxa identified from the top (0-2 cm) horizon of the first replicate core sample collected from the Lower Lakes in March 2013.

Site	LF01	LF02	LF03	LF04	LF06	LF07	LF08	LF10	LF12	LF13	LF15	LF17	LF19	LF20	LF21	LF23	LF24
Taxon Richness	8	9	5	6	4	11	2	5	6	9	17	3	9	8	5	8	9
Total Abundance	47	647	1682	292	1386	209	57	46	1154	33	111	1011	1063	219	914	267	57
Hydridae <i>Hydra</i> sp.											1						
Nemertea spp.															5		
Nematoda spp.	14	595	1664	286	1373	32	39	7	1082	1	27	1006	859	125	894	99	15
Oligochaeta spp.	24	32	6		5	110	18	19	53	7	17		90	45		141	8
Copepoda Calanoida spp.						1		1			2		3		1		
Copepoda Cyclopoida spp.	1			1		2		1		9	3		4	6			5
Copepoda Harpacticoida spp.																	2
Amphipoda				1	2	20		18			1				13		7
Corbiculidae <i>Corbiculina</i> spp.		5	1	1	6							2					
Oribatida spp.		4							1		5						
Cladocera <i>Bosmina meridionalis</i>													3	7			
Cladocera Ilyocryptidae <i>Ilyocryptus</i> sp.	3					30			1		5		89	27			3
Cladocera Chydoridae spp.										2	1						
Cladocera Chydoridae <i>Alonella</i> sp.											1						
Cladocera Chydoridae <i>Leydigia</i> spp.	2			2		5				1	11						
Cladocera Macrothricidae											3						
Cladocera spp.																	
Ostracoda Cyprididae	1									9	1		2	5			2
Ostracoda Limnocytheridae		2															1
Ceratopogonidae Ceratopogoninae spp.																	
Tanypodinae <i>Coelopynia pruinosa</i>																	
Tanypodinae <i>Procladius</i> sp.		1				1			8					3			1

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Site	LF01	LF02	LF03	LF04	LF06	LF07	LF08	LF10	LF12	LF13	LF15	LF17	LF19	LF20	LF21	LF23	LF24
Tanypodinae sp.										1			7				
Chironominae <i>Paratanytarsus</i> sp.			9							1							
Chironominae <i>Cryptochironomus</i> sp.		1	2	1								3			1		
Chironominae <i>Cladopelma</i> sp.	1								9		25		6			2	7
Chironominae <i>Chironomus</i> spp.										2	6						2
Chironominae <i>Dicrotendipes</i> sp.																	
Chironominae <i>Kiefferulus</i> sp.																	6
Chironominae <i>Microchironomus</i> sp.	1																
Chironominae <i>Cladotanytarsus</i> sp.		6				2										14	
Chironomidae Chironominae spp.		1				5					2						
Chironominae <i>Polypedilum</i> sp.						1											9
Orthocladiinae <i>Cricotopus</i> sp.											1						
Caenidae <i>Tasmanocoenis tillyardi</i>															1		

Appendix 2 Chironomid Deformities

Table A2 The number of each chironomid genera collected and found to be deformed from nine sites across the Lower Lakes region in March 2013.

Site	Genus	Number of individuals	Number deformed	Deformity type
LF01	<i>Procladius</i>	5	1	Short paralingula on one side
	<i>Coelopynia pruinosa</i>	2	0	
	<i>Cladotanytarsus</i>	2	0	
	<i>Paratanytarsus</i>	6	1	Slightly split media tooth
	<i>Cladopelma</i>	3	0	
	<i>Microchironomus</i>	18	1	Missing tooth on mandible
	<i>Polypedilum nubifer</i>	2	0	
	<i>Dicrotendipes</i>	8	1	Missing distal antennal segments
	<i>Chironomus</i>	5	0	
	<i>Kiefferulus</i>	13	1	Missing tooth on mentum
Total		64	5 (7.81%)	
LF02	<i>Cladotanytarsus</i>	6	1	One antennae with short segment and the antennae missing distal segments
Total		6	1 (16.7%)	
LF03	<i>Procladius</i>	6	0	
	<i>Coelopynia pruinosa</i>	2	0	
	<i>Cladotanytarsus</i>	5	0	
	<i>Paratanytarsus</i>	1	0	
	<i>Cladopelma</i>	10	0	
	<i>Microchironomus</i>	2	0	
	<i>Cryptochironomus</i>	10	0	
	<i>Polypedilum nubifer</i>	7	0	
	<i>Chironomus</i>	19	0	
Total		62	0 (0%)	
LF08	<i>Coelopynia pruinosa</i>	1	0	
Total		1	0 (0%)	
LF12	<i>Cladotanytarsus</i>	4	0	
	<i>Cryptochironomus</i>	5	0	
	<i>Polypedilum nubifer</i>	14	1	Asymmetrical epipharyngeal pecten
Total		23	1 (4.35%)	

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LF13	<i>Chironomus</i>	13	0	
	<i>Kiefferulus</i>	5	0	
Total		18	0 (0%)	
LF15	<i>Monopelopia</i>	1	0	
	<i>Paratanytarsus</i>	1	0	
	<i>Polypedilum</i>	1	0	
	<i>Chironomus</i>	44	3	2 with split median tooth on mentum and 1 with “rotted” antenna
	<i>Kiefferulus</i>	21	2	1 with distorted median tooth on mentum, 1 missing a tooth on mentum on one side, extra teeth on the other side and one antenna with only one segment
Total		68	5 (7.35%)	
LF17	<i>Cryptochironomus</i>	1	0	
	<i>Polypedilum nubifer</i>	1	0	
	<i>Kiefferulus</i>	1	0	
Total		3	0 (0%)	
LF19	<i>Cladopelma</i>	1	0	
	<i>Dicrotendipes</i>	1	0	
	<i>Chironomus</i>	49	4	1 with extra tooth on one side of mentum and missing tooth on the other side, 1 with asymmetrical epipharyngeal pecten, 1 had a four-toothed mandible, 1 was missing a tooth on the mentum
Total		51	4 (7.84%)	
LF23	<i>Cladotanytarsus</i>	1	0	
	<i>Paratanytarsus</i>	4	0	
	<i>Polypedilum nubifer</i>	2	0	
	<i>Chironomus</i>	33	4	1 with extra tooth on epipharyngeal pecten, 1 with extra tooth on mentum, 1 with a split median tooth on the mentum, 1 missing antennal segments
	<i>Kiefferulus</i>	20	1	Missing tooth on mentum
Total		60	5 (8.33%)	
LF24	<i>Cladotanytarsus</i>	2	0	
	<i>Paratanytarsus</i>	1	0	
	<i>Cryptochironomus</i>	5	2	1 missing tooth on mandible, 1 missing a tooth on the mentum
	<i>Polypedilum nubifer</i>	92	4	1 with antennal segment misshapen, 2 with missing lateral tooth from mentum, 1 with asymmetrical epipharyngeal pecten
	<i>Kiefferulus</i>	1	0	
Total		101	6 (5.94%)	

Appendix 3 Site Photographs

Please note: there are no available photos for Loveday Bay (LF12) or Tauwitcheria (LF13).



Figure A3.1 LF01 Wallys Landing



Figure A3.2 LF02 Point Sturt North



Figure A3.3 LF03 Milang



Figure A3.4 LF04 Tolderol



Figure A3.5 LF06 Poltalloch



Figure A3.6 LF07 Waltowa



Figure A3.7 LF08 Meningie



Figure A3.8 LF10 Campbell Park



Figure A3.9 LF15 Boggy Creek



Figure A3.10 LF17 Point Sturt South



Figure A3.11 LF19 Dog Lake



Figure A3.12 LF20 Boggy Lake



Figure A3.13 LF21 Windmill Site



Figure A3.14 LF23 Lower Currency



Figure A3.15 LF24 Lower Finniss