Macroinvertebrate and Sediment Baseline Surveys of the Murray River at Albury for Norske Skog

Helen Gigney, Rochelle Petrie and John Pengelly

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The Murray-Darling Freshwater Research Centre

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For further information contact:

Helen Gigney or Rochelle Petrie
Murray-Darling Freshwater Research Centre
PO Box 991
Wodonga Vic 3689

Phone: (02) 60582300

Fax: (02) 60597531

e-mail: Helen.Gigney@csiro.au or Rochelle.Petrie@csiro.au

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Cover: Deploying artificial substrate samplers in the Murray. Photo by Chris Davey.

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Introduction

A long term river environment monitoring program was conducted by the Murray Darling Freshwater Research Centre (MDFRC) for Norske Skog (formerly, Australian Newsprint Mills) from 1992 to 1998 (King and Baldwin 1993, 1994, 1995, 1996 & 1997).

Norske Skog's effluent outfall diffuser is located in the Murray River at Albury, approximately 300m upstream of the Spirit of Progress Bridge (construction of the latter completed in 2007). In preparation for a proposed discharge of effluent via the diffuser, baseline surveys were conducted in 2008 to characterise benthic macroinvertebrate community structure and deposition zone sediments using the same methods and a subset of the sites that were employed during the surveys conducted previously by the MDFRC.

Objectives

1. To describe the deposition zone sediments and macroinvertebrate communities of the Murray River upstream and downstream of Norske Skog's effluent diffuser in autumn / winter 2008.
2. To provide the baseline data for the river in 2008 to permit comparisons[1] with historic monitoring data.

Site Description

The Murray River at Albury receives flow from the Kiewa River and is regulated by discharges from Hume Dam. The hydrograph at Albury (Figure 1) is usually characterised by flows of approximately 20 000 ML/day during irrigation season and periods of low flow in winter/spring when irrigation demand is low. Severe drought throughout the region has resulted in drastically reduced flows in 2007 and 2008 and extended periods of very low flow during winter/spring.

[1] Not included as part of this work

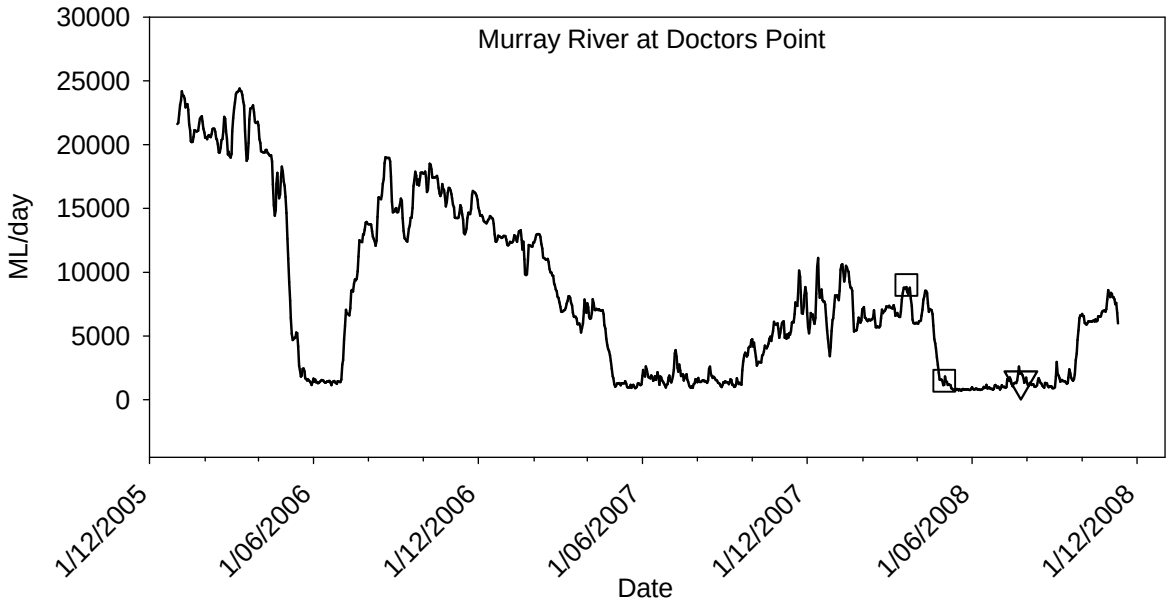

Figure 1: Discharge for the Murray River at Doctors Point from January 2006 to December 2008. Sediment sampling indicated by inverted triangle, deployment and retrieval of artificial substrates for macroinvertebrate sampling indicated by squares.

Methods

Surveys of sediment quality as well as macroinvertebrate community composition were conducted using the same methods and a subset of the sites (upstream and downstream of the diffuser) that were employed during the surveys conducted by MDFRC for Norske Skog (formerly, Australian Newsprint Mills) from 1992 to 1998 (King and Baldwin 1993, 1994, 1995, 1996 and 1997).

Sediment

Sample Collection and Handling

Sediment sampling was conducted at the two sites in winter when water levels were low as for previous surveys (King and Baldwin 1993, 1994, 1995, 1996 & 1997). A series of forty sediment samples were taken on the 25th of July 2008. Sediment samples were collected from two deposition zones on the River Murray, located approximately equidistant (ca 500 m upstream and ca 500 m downstream) of the Norske Skog diffuser. Samples were collected at 10 metre intervals

along the 60 cm depth contour (approximately 2 meters from, and parallel to, the river bank). A total of 20 samples were taken from each deposition zone.

Sediment to a depth of approximately 5 - 10 cm was scooped into 500 mL wide mouthed polyethylene bottles until filled. Bottles had previously been acid washed (5 % HC1) and repeatedly rinsed with Type 1 grade ultrapure water. The bottle was sealed while under water to minimise the loss of fine material. The samples were immediately returned to the laboratory and air dried. The air dried samples were sieved (2 mm) - the fraction passing through the sieve was thoroughly mixed and a representative portion of the sample.

A cone and quartering technique (Krumbein and Pettijohn, 1938) was used to split the sieved sediment material into representative aliquots of smaller volume. The material was piled into a cone and cut into quarters. Two alternate quarters were removed, and the remaining two were recombined, re-piled into a cone and quartered once again. This procedure was repeated until the desired mass of material was achieved.

Composite samples for both upstream and downstream deposition zones were determined by grouping deposition sites 1-4, 5-8, 9-12, 13-16 and 17-20. Ten composite samples in all were obtained through the coning and quartering method, retaining approximately 20 g of sediment from the first quarter from each site. Approximately 80 g of sediment were retained for each composite sample. All subsequent analysis was performed only on the composite samples.

Particle Size Analysis and Loss on Ignition (LOI)

Approximate particle fractionation was carried out on all samples. Fractionation was by the method described by Grimshaw (1989). The portion of the sample that passed through a 2 mm sieve was considered a mixture of silt, clay and sand. The percentage of silt & clay in this fraction was determined by the 4 minute 48 second hydrometer method described by Grimshaw (1989).

Loss on ignition was determined gravimetrically after firing up to 20 g of dried sediment at 550 ^{o}C for 2 hours (Grimshaw 1989).

Analysis of Acid Extractable Metals.

The fraction of acid extractable metals in the samples was determined by a modification of the method of Blutstein (1981). Five grams of sediment was accurately weighed into 50 mL

polyethylene centrifuge tubes (which had previously been washed with 5% HCl and extensively rinsed with Type 1 grade ultrapure water). 25 mL of 0.1 M "ARISTAR" grade HC1 was subsequently added to the sediment. The tubes were then capped and placed on a "Ratek" orbital shaking table for one hour. The samples were allowed to settle overnight and, subsequently filtered through acid rinsed 0.45µm polyester membrane filters with 1.0µm prefilter. The filtrate was placed in 30 mL polycarbonate bottles (which had previously been washed with 5% HNO_3 and repeatedly rinsed with Type 1 grade ultrapure water) and dispatched to the National Measurement Institute for analysis by Inductively Couple Plasma Atomic Emission Spectroscopy (ICP-AES). The elements assayed for were cadmium, copper, iron, manganese and zinc. An extraction blank, acid blank and a standard reference material (CANMET River sediment - STSD-4) were processed in exactly the same manner as the samples.

Analysis for Total Nitrogen and Total Phosphorus

Total nitrogen and total phosphorus was determined by a modification of the technique of Hosmoi and Sudo (1986). Approximately 0.25 g of sediment was accurately weighed into a 30 mL vial, 10 mL of an alkaline persulfate digestion medium (0.9 % NaOH, 4.0% $K_2S_20_4$) and 20 mL of Type 1 grade ultrapure water was added to each vial. The vials were sealed and subsequently heated in an autoclave for one hour. The solution was the analysed for nitrate by an automated version of the cadmium reduction method (APHA 2005) and phosphorus by an automated version of the ascorbic acid molybdophosphate method (APHA 2005). All analyses were done at least in duplicate.

Macroinvertebrates

Aquatic macroinvertebrates were collected using artificial substrate samplers (ASS). Substrates were deployed for 6 weeks (23 March - 1 May 2008) at three paired sites (two sites 100 to 200m upstream, two sites 2 km downstream and 2 sites in the mixing zone (100 to 200m downstream)). Five ASS were deployed at each site to allow for loss. Three substrates from each site were randomly selected for processing (18 samples) and returned to the lab for processing.

Each of the artificial substrates was rinsed over a 250um mesh sieve. The material collected was preserved in 70% ethanol. Macroinvertebrates were sorted from the samples using a stereo microscope, then identified to either genus or species for the majority of taxonomic groups (as

per surveys 1993-1997) and counted so that taxonomic diversity and relative abundance could be calculated for each site (King and Baldwin 1993, 1994, 1995, 1996 & 1997).

Data Analysis

To comply with current best practice, data analyses included a range of bioassessment metrics, utilising either family or genus level resolution. Family level metrics were: SIGNAL2, Family Richness and EPT (Ephemeroptera, Plecoptera, Trichoptera); Genus level metrics were Taxonomic richness and % Abundance. The average abundance was also calculated for each site.

The SIGNAL2 (Stream Invertebrate Grade Number Average Level) biotic index (Chessman 2003a & b) assesses the relative sensitivity of different taxa to water quality parameters and was calculated and used to compare the macroinvertebrate communities from the different sites. The site SIGNAL2 score was calculated using only presence/absence data where the pollution sensitivity grades of the families collected are summed and then the total divided by the number of family units identified (Chessman 2006). ['Family unit' includes some higher taxonomic levels and the more common subfamilies of Diptera: Chironomidae). Coleoptera adults and larvae are combined]. The SIGNAL2 site scores calculated in this study cannot be compared with AUSRIVAS type assessments nor Victorian EPA biological objectives for rivers and streams as the sampling method used in this project (ASS) is not consistent with typical AUSRIVAS/SIGNAL2 edge, sweep and rifle kick methods; however they can be used to compare macroinvertebrate communities between sites and provide a baseline condition for each site.

The SIGNAL biotic index is most discriminating when used for assessment of point source pollution, such as discharge of effluent, but should not be used as the soul indicator for assessment (Chessman 2003). A low SIGNAL2 score may indicate poor water quality but can also be a result of the low habitat diversity that is natural in lowland rivers compared to upland rivers.

Family Richness (i.e. the number of families) is a measure of diversity that "can give a reasonable representation of the ecological health of a stream" (Metzeling *et al*. 2004). A low number of families may indicate poor water quality or degraded habitat but can also be a result of

natural variations in the quality of habitat or natural cycles of physical stress (Metzeling *et al*. 2004, Chessman 2006).

Taxonomic richness was calculated using the lowest possible taxonomic resolution (i.e. genus wherever possible). The accumulated number of distinct taxa identified across all replicates is reported.

The EPT index is the total number of families that belong to the order Ephemeroptera (Mayflies), Plecoptera (Stoneflies) and Tricoptera (Caddisflies). Most of the taxa in these groups are sensitive to organic pollution and any loss of families usually indicates disturbance. (Plafkin *et al.*, 1989). The EPT index is used in this baseline survey only to compare sites within this study and cannot be compared to Victorian EPA Biological Indicator objectives on the basis that the Victorian EPA does not use EPT for the Murray and Western Plains Bioregion (Metzeling *et al*. 2004).

Percentage abundance of each taxon and percentage composition of Function Feeding Groups (FFG) serves to characterize the site. For this report the five most abundant taxa at each site is reported as an average across replicates. Average abundances for each site were also calculated and reported.

Results and Discussion

Sediment

Overall, concentrations of analytes were similar to those reported from monitoring of river sediments in 1997 (King and Baldwin 1997). The concentrations of total persulfate nitrogen (N), total persulfate phosphorus (P), acid extractable cadmium, copper, iron, manganese, and zinc, as well as the % loss on ignition are summarised in Figure 2. For each analyte a box plot showing the analytes distribution for samples taken from the upstream and downstream of the outfall are presented. The solid horizontal lines of the box plot represent the 10^{th}, 25^{th}, 50^{th}, 75^{th} and, 90^{th} percentiles of the data - the box itself represents the 25^{th} to 75^{th} percentile. All data outside the 10^{th} and 90^{th} percentiles are shown as open circles on the plots. The mean of the data is

represented by a dotted line. The sediments from the upstream site tend to have a greater range of concentrations of analytes, compared with the sediments from the downstream site. This is particularly true for the concentrations of iron, zinc and nitrogen as well as % loss on ignition (Figure 2). Although there is overlap in the ranges of concentrations recorded from the two sites, the mean concentrations of iron, copper, zinc, cadmium, total nitrogen and total phosphorus for the upstream sediments was significantly lower ($p<0.05$) than those for the downstream sediments. There was no difference in the concentration of manganese between the two sites.

There was a strong positive correlation between LOI and Cu, Cd, Zn, TP and TN (Figure 3). This is consistent with previous finding reported by King and Baldwin (1996). This correlation between LOI and analyte concentration is a function of the geomorphology of the particular deposition. Higher concentrations of metals were measured in the downstream sites that contained finer grained material and organic matter than the upstream sites. The Murray River downstream of Lake Hume has been subject to extended periods of unusually low flow due to widespread drought, sediment sampling was conducted after approximately 3 months of very low flows and under these conditions there tends to be greater accumulation of fine material in deposition zones.

This point is further illustrated by Baldwin *et al* (2008) where, during periods of extreme drawdown of the Lake Hume reservoir water flows are significantly altered allowing sediment particles to settle out into deposition zones along the river continuum. This factor may have been further exacerbated due to the construction of a bridge across the River Murray during the Albury Freeway Bypass Project in 2006/2007, just downstream of the diffuser. Baldwin *et al* (2008) also noted that during these extreme drawdown events the biogeochemical process that occurred within the reservoir also influenced the water chemistry downstream of the dam wall. Lake Hume was found to be a net source of nitrogen, phosphorus, carbon and iron to the river and a net sink for manganese.

The relationship between LOI and Iron and Manganese was not as clear and may be influenced by the presence of clay particles and iron- and manganese-oxidising bacteria associated with biofilms growing on exposed surfaces in the water (King et al, 1999, van Hullebusch et al, 2003).

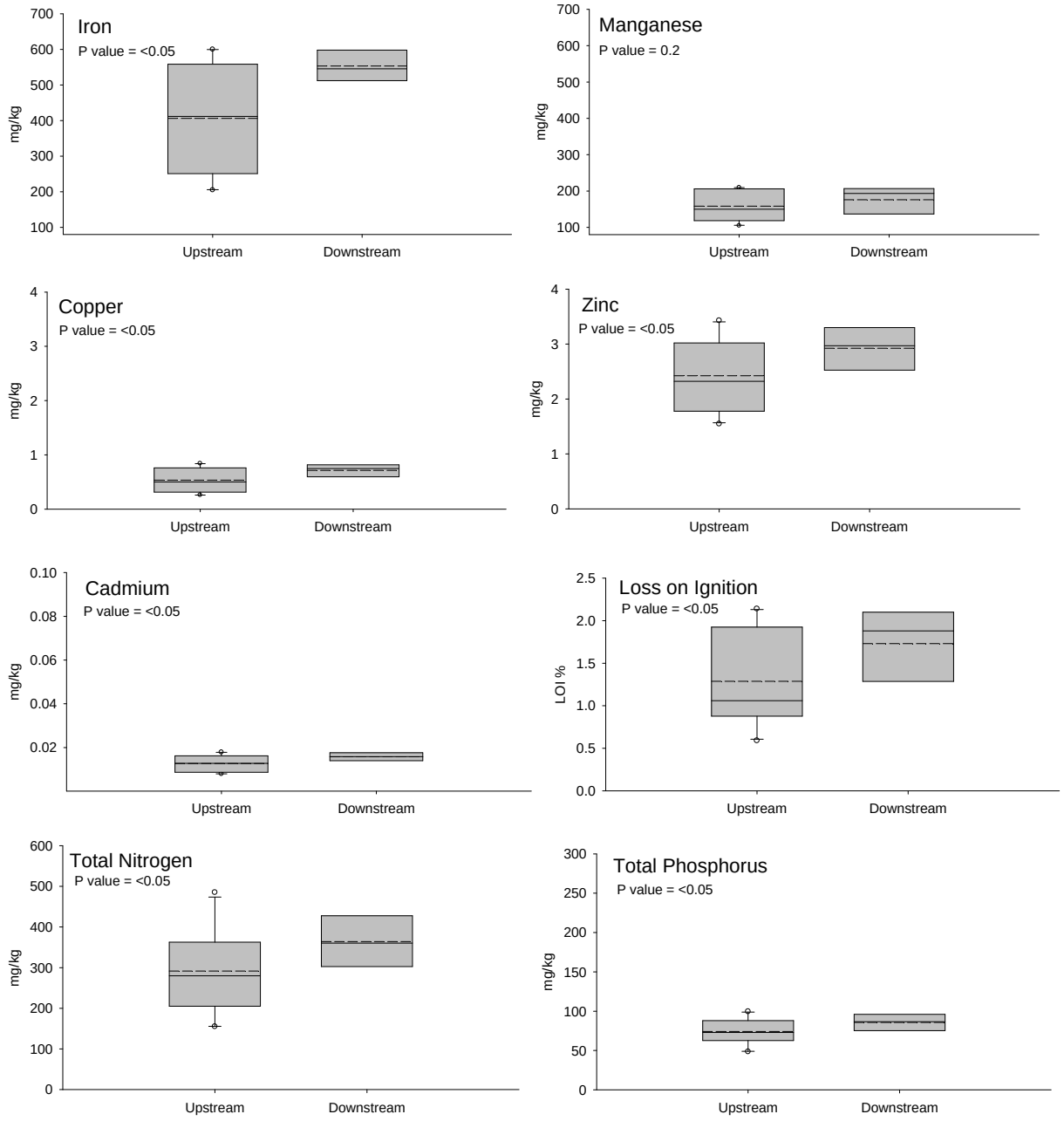

Figure 2: Box plots for the distribution of elements in sediments from deposition sites upstream and downstream of the diffuser.

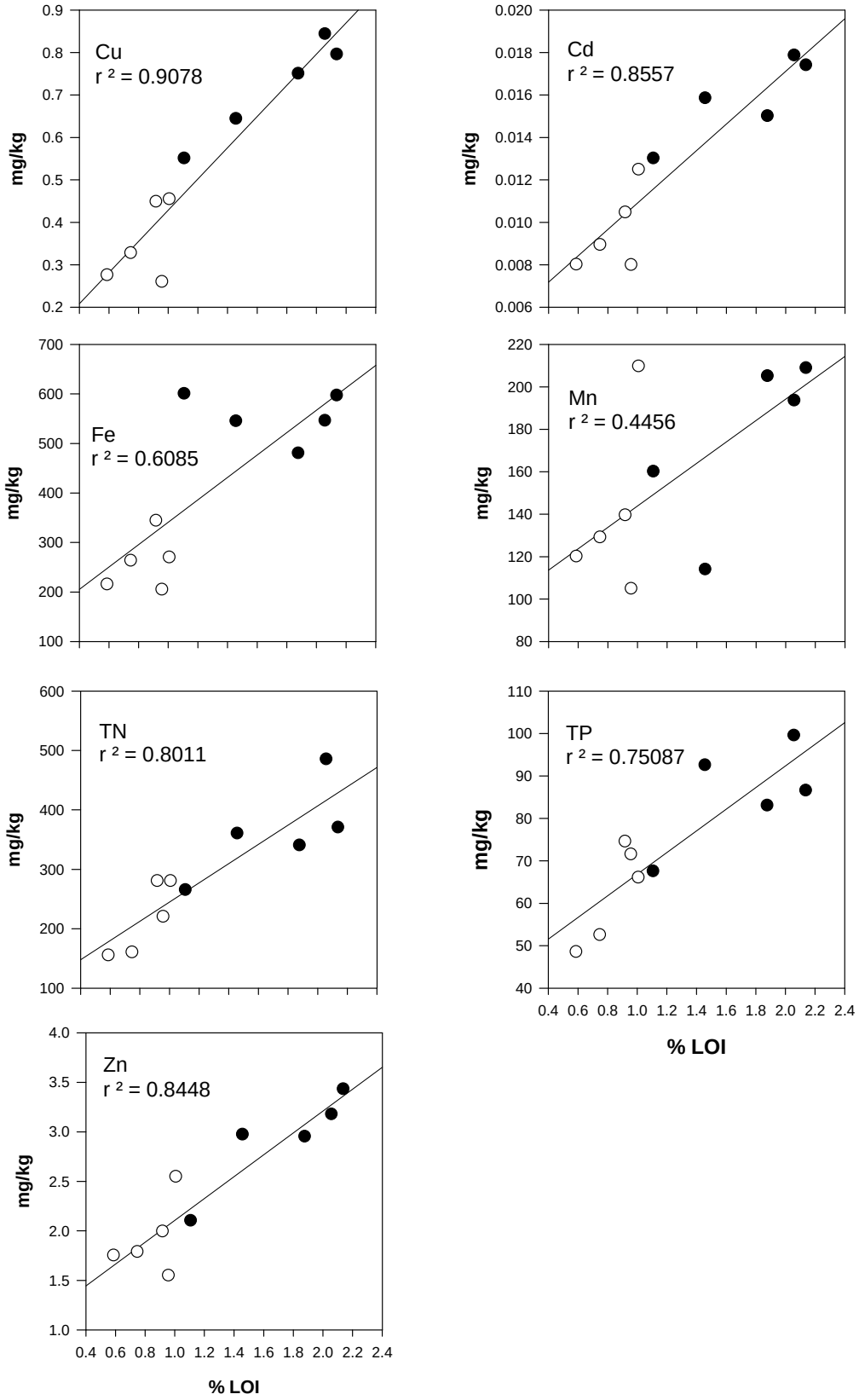

Figure 3: Element versus loss on ignition sediments upstream (open circles) and downstream (closed circles) of the diffuser.

Macroinvertebrates

Macroinvertebrates representing 84 taxonomic groups were collected and analysed as part of this baseline study on the Murray River at Albury. A range of bioassessment metrics were used to describe the current condition of the macroinvertebrate community.

SIGNAL2

SIGNAL-2 (Stream Invertebrate Grade Number Average Level) biotic index (Chessman 2003a & b) was used to calculate the relative sensitivity of different taxa to water quality parameters for each of the sites.

There was little difference in SIGNAL2 scores between sites with all sites scoring between 3.7 and 4.4 (Figure 4). The two downstream sites and mixing zone 2 recorded slightly lower scores than upstream sites 1 and 2 and mixing zone 1. The low SIGNAL2 scores are attributed to 'very tolerant' and 'tolerant' taxa dominating the community (Figure 4; Figure 5). A dominance of 'tolerant' animals is characteristic in the naturally variable flows of an inland lowland river system because of the natural stresses that occur (Chessman 2006) aside from any anthropogenic influences.

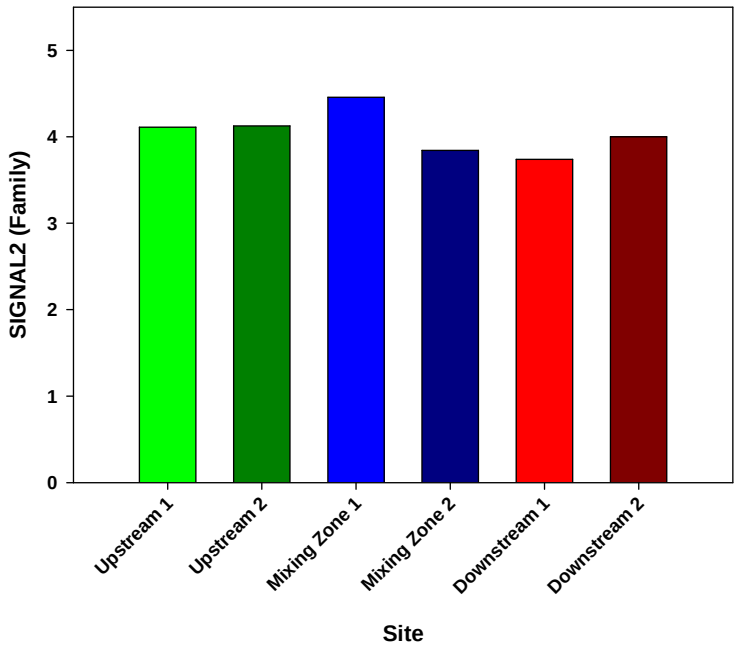

Figure 4: SIGNAL2 site scores for the six sampling sites

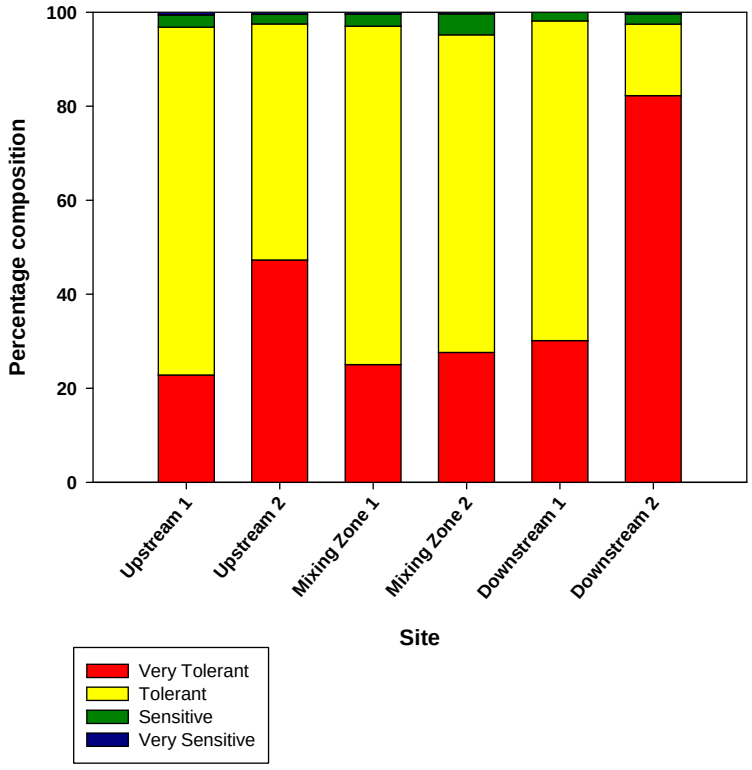

Figure 5: Percentage composition of macroinvertebrate sensitivity groups at each site

Family Richness

The overall number of families is an indicator of macroinvertebrate diversity.

The total number of families recorded at each site varies across all sites (Table1), with site mixing zone 2 recording the lowest number of families (19 families) and site downstream 2 recording the highest (32 families).

EPT Biotic Index

Macroinvertebrates from the Orders of Ephemeroptera, Plecoptera and Trichoptera are known to be quite sensitive to poor water. The number of EPT was consistent for all sites (Table 1).

Table 1: Number of Families (Total and EPT) at each site

Site	Upstream 1	Upstream 2	Mixing Zone 1	Mixing Zone 2	Downstream 1	Downstream 2
Total Families	27	24	24	19	23	32
EPT Families	6	6	7	5	5	7

Taxonomic Richness

The number of taxa is an indicator of macroinvertebrate community diversity at the lowest level of resolution identified; in this case genus.

There was little difference in the number of taxa between sites (Figure 6). Site downstream 2 recorded the highest number of taxa across all sites (54 taxa), with mixing zone 2 recording the lowest number (approx. 39 taxa). For a complete taxonomic list see Appendix 1.

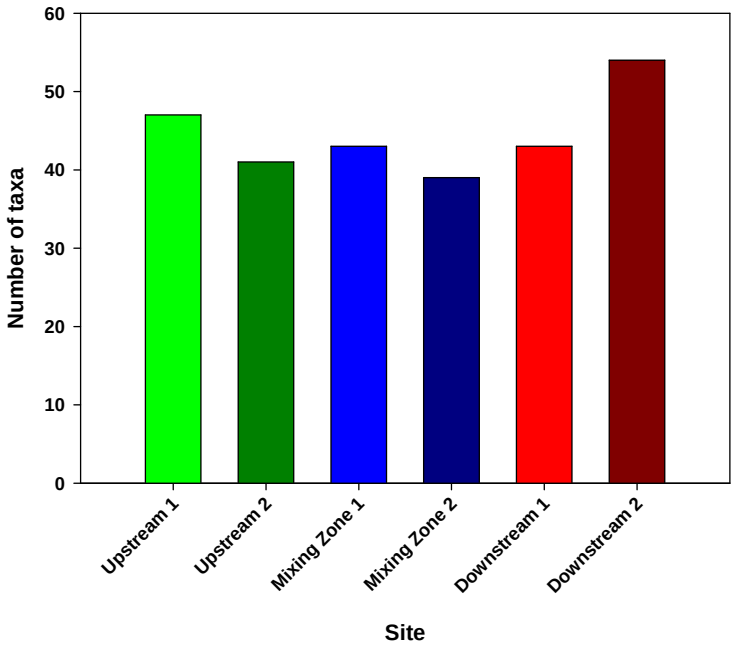

Figure 6: Macroinvertebrate taxonomic richness for each site

Percentage Abundance

Percentage abundance measures the relative abundance of the macroinvertebrates collected from each site. Differences in the proportions of the common taxa may indicate subtle differences in the environment at the site. There were 6 dominant taxa which comprised ~80% of the

macroinvertebrates collected at each site (Figure 7). All of the most dominant taxa are considered 'Tolerant' with an average SIGNAL2 score of 3 (Appendix 2). In all sites except sites upstream 2 and downstream 2 the mayfly *Tasmanocoenis* (Caenidae) was the most dominant taxa accounting for 25% to 50% of the total community. The segmented worm Oligochaeta was the second most dominant taxa making up 10% to 25% of the community, with the exception of site upstream 2 where it dominates the community at 40%. The flat worm Tricladida is present at all sites, however accounts for 63% of the community in site downstream 2.

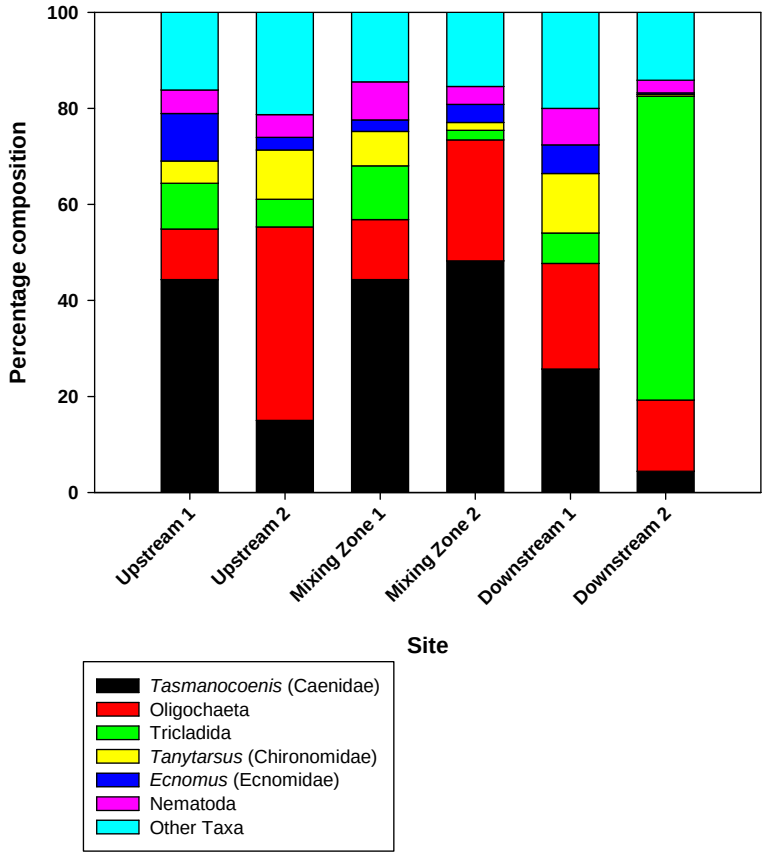

Figure 7: Percentage composition of dominant macroinvertebrate taxa at each site

Functional Feeding Groups

Functional feeding groups provide an assessment of the roles or trophic status of a group of animals, which may vary quite significantly within a family or even a genus. The percentage composition of functional feeding groups (FFG) (Figure 8) is characteristic of a lowland river (Cummins et al. 2005). Across all sites there tends to be a dominance of 'gathering collectors', 'scrapers' and 'predators'. 'Scrapers' graze rock and wood surfaces or stems of aquatic vegetation and feed on attached non-filamentous algae and associated detritus. 'Gathering

collectors feed on decomposing detrital particles; algae and bacteria by ingesting sediment or gathering loose particles in depositional areas. And 'predators' capture and ingest living animals. Site downstream 2 is dominated by predators; this is mainly driven by an abundance of the flat worm Tricladida.

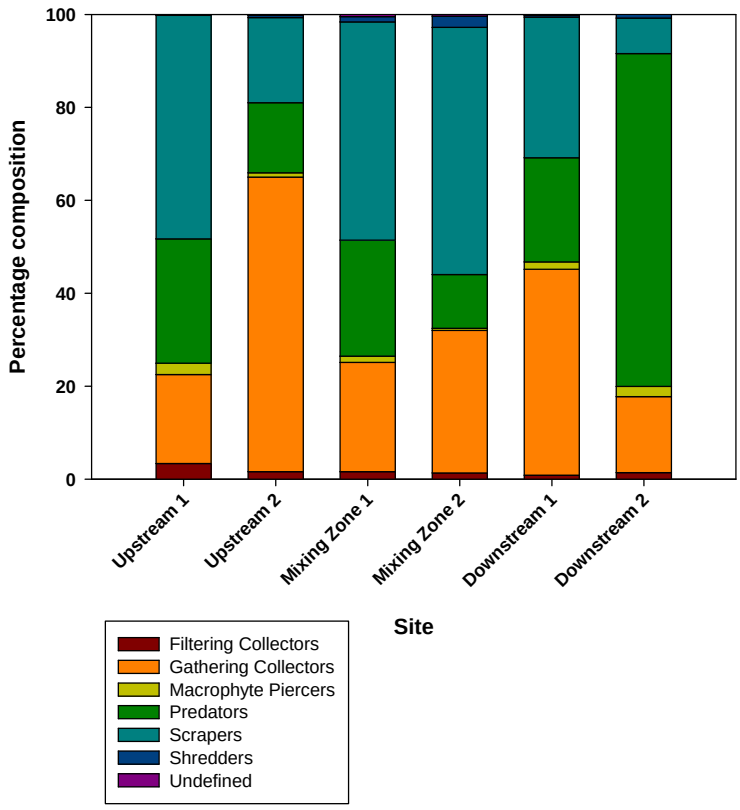

Figure 8: Percentage composition of functional feeding groups of macroinvertebrates at each site

Average Abundance

The average abundance of animals collected gives an indication of the carrying capacity of the habitat. Each of the sites from both upstream and mixing zone recorded a higher average abundance per substrate than the two downstream sites, where upstream 1 recorded the highest number of individuals (2228 individuals) and the downstream 1 recording the lowest (761 individuals) (Figure 9). Further statistical analysis is required to determine if there is any significant difference between sites.

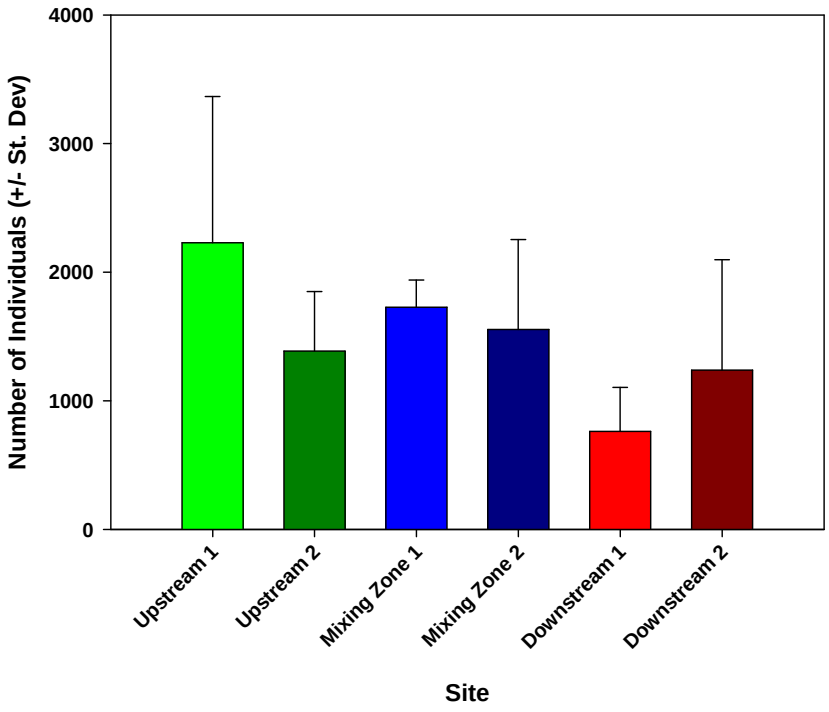

Figure 9: Average abundance of macroinvertebrates at each site.

Macroinvertebrate Summary

There was little difference between all sites for SIGNAL2 site scores and percentage composition of sensitivity groups, with the exception of site downstream 2 which recorded the highest amount of 'very tolerant' taxa. There was some variation of total number of families recorded across all sites and little difference in the number of taxa between sites. All sites had similar percentage composition of dominant taxa with the exception of site downstream 2 which was dominated by flat worms (Tricladida). There was no loss of EPT families from the mixing zone or downstream sites. All sites had a similar composition of functional feeding groups which were characteristic of a lowland river. Although all upstream and mixing zone sites recorded higher abundances than both downstream sites, a significant difference cannot be assumed without further statistical analysis.

Conclusions

This report describes the deposition zone sediments and macroinvertebrate communities of the Murray River upstream and downstream of Norske Skog's effluent diffuser in autumn / winter 2008. Sampling was conducted using the same methods as those used in the long term monitoring program (1993 to 1997) conducted when the Mill was discharging treated effluent to the river. It provides baseline data against which future monitoring of data collected in a similar manner may be compared.

Acknowledgments

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Appendices

Appendix 1. ***Complete Taxonomic list (Genus)***

Higher Taxa	Family/subfamily	Genus	SIGNAL Score	Sensitivity Band	FFG	US 1	US 2	MZ 1	MZ 2	DS 1	DS 2
Acarina	Halacaroidea	Pezidae	6	none	predators				●		●
Acarina	Oribatida	Hydrozetar	6	none	predators	●			●	●	●
Acarina	Oribatida	T. longisetosus	6	none	predators	●			●	●	●
Acarina	Oribatida		6	none	predators	●	●	●	●		
Acarina	Oxidae	Flabellifrontipoda	6	Sensitive	predators						●
Bivalva	Corbiculidae	larvae	4	tolerant	filtering collectors	●	●	●	●	●	●
Bivalva	Corbiculidae		4	tolerant	filtering collectors	●			●	●	
Bryozoa			4	tolerant	x	●	●	●		●	●
Cnidaria			3	tolerant	x	●	●	●		●	●
Coleoptera	Dytiscidae	Antiporus	2	very tolerant	predators						●
Coleoptera	Dytiscidae	Necterosoma	2	very tolerant	predators						●
Coleoptera	Dytiscidae	Sternopriscus sp. Adult	2	very tolerant	predators	●					●
Coleoptera	Dytiscidae	Sternopriscus sp. Larvae	2	very tolerant	predators	●					
Coleoptera	Elmidae	Austrolimnius	7	very sensitive	scrapers	●	●				
Coleoptera	Elmidae	Notriolus	7	very sensitive	shredders				●		●
Coleoptera	Gyrinidae	Aulonogyrus	4	tolerant	predators		●				
Coleoptera	Scirtidae		6	sensitive	filtering collectors						●
Collembola			1	very tolerant	gathering collectors				●		●
Crustacea	Corallanidae	Tachaea	2	very tolerant	Parasite (predator)						●
Crustacea	Neoniphargidae		2	very tolerant	shredders					●	
Crustacea	Parastacidae	cherax	4	tolerant	gathering collectors						●
Diptera	Aphroteniinae	Aphroteniella tenuicornis	8	very sensitive	shredders			●			
Diptera	Ceratopogonidae	Ceratopogoninae	4	tolerant	predators	●	●	●	●	●	●
Diptera	Chironominae	Chironominae	3	tolerant	gathering collectors		●	●		●	
Diptera	Chironominae	Chironomus	3	tolerant	gathering collectors		●	●			●
Diptera	Chironominae	Cladopelma	3	tolerant	gathering collectors		●				
Diptera	Chironominae	Cladotanytarsus	3	tolerant	gathering collectors	●	●	●	●	●	●

Diptera	Chironominae	Cryptochironomus	3	tolerant	predators		●	●	●		●
Diptera	Chironominae	Dicrotendipes	3	tolerant	gathering collectors		●			●	●
Diptera	Chironominae	Dicrotendipes larval spA	3	tolerant	gathering collectors			●		●	●
Diptera	Chironominae	Harrisius	3	tolerant	gathering collectors	●					
Diptera	Chironominae	Kiefferulus	3	tolerant	scrapers	●	●	●	●	●	●
Diptera	Chironominae	Microchironomus	3	tolerant	gathering collectors					●	
Diptera	Chironominae	Parachironomus	3	tolerant	gathering collectors	●	●	●	●	●	●
Diptera	Chironominae	Paratanytarsus	3	tolerant	gathering collectors						●
Diptera	Chironominae	Polypedilum	3	tolerant	gathering collectors	●	●	●		●	●
Diptera	Chironominae	Polypedilum leei	3	tolerant	gathering collectors					●	
Diptera	Chironominae	Polypedilum watsoni	3	tolerant	gathering collectors	●	●	●	●	●	●
Diptera	Chironominae	Rheotanytarsus	3	tolerant	gathering collectors		●		●	●	●
Diptera	Chironominae	Riethia	3	tolerant	scrapers	●	●	●	●	●	●
Diptera	Chironominae	Tanytarsus	3	tolerant	gathering collectors	●	●	●	●	●	●
Diptera	Empididae	NMV sp2	5	sensitive	predators			●			●
Diptera	Empididae	NMV sp3	5	sensitive	predators	●	●	●	●	●	●
Diptera	Ephydridae		2	very tolerant	gathering collectors		●				
Diptera	Muscidae		1	very tolerant	predators	●					
Diptera	Orthocladiinae	Botryocladius	4	tolerant	scrapers				●		
Diptera	Orthocladiinae	Cricotopus	4	tolerant	gathering collectors	●	●	●	●	●	
Diptera	Orthocladiinae	Nanocladius	4	tolerant	gathering collectors	●	●	●	●	●	●
Diptera	Orthocladiinae	Orthocladiinae	4	tolerant	gathering collectors	●	●	●	●	●	
Diptera	Orthocladiinae	Parakiefferiella	4	tolerant	scrapers	●	●	●	●	●	●
Diptera	Orthocladiinae	Paratrichocladius	4	tolerant	gathering collectors			●		●	
Diptera	Orthocladiinae	Rheocricotopus	4	tolerant	gathering collectors	●	●		●	●	
Diptera	Orthocladiinae	Thienemanniella	4	tolerant	gathering collectors				●		
Diptera	Simuliidae	Austrosimulium	5	sensitive	filtering collectors						●
Diptera	Tanypodinae	Ablabesmyia	4	tolerant	predators	●		●		●	●
Diptera	Tanypodinae	Djalmabatista	4	tolerant	predators	●	●	●			
Ephemeroptera	Baetidae	Cloeon sp.	5	sensitive	gathering collectors					●	
Ephemeroptera	Baetidae		5	sensitive	gathering collectors						●
Ephemeroptera	Caenidae	Irpacaenis	4	tolerant	scrapers	●			●		●

Ephemeroptera	Caenidae	Tasmanocoenis	4	tolerant	scrapers	●	●	●	●	●	●
Ephemeroptera	Leptophlebidae	Atalophlebia	8	very sensitive	shredders	●	●	●			●
Ephemeroptera	Leptophlebidae		8	very sensitive	scrapers					●	
Gastropoda	Physidae	Physa	1	very tolerant	scrapers						●
Gastropoda	Physidae		1	very tolerant	scrapers	●	●	●		●	
Hemiptera	Corixidae	Micronecta	2	very tolerant	macrophyte piercers	●	●	●	●	●	●
Hemiptera	Veliidae	Drepanovelia	3	tolerant	predators	●					
Hemiptera	Veliidae	Microvelia	3	tolerant	predators	●			●		●
Nematoda	Nematoda	Nematoda	3	tolerant	predators	●	●	●	●	●	●
Nemertea	Tetrastemmatidae	prostoma	3	tolerant	predators	●			●	●	●
Odonata	Austrocorduliidae	Apocordulia	10	very sensitive	predators	●		●			
Odonata	Austrocorduliidae		10	very sensitive	predators			●			
Odonata	Protoneuridae	Nosoticta	4	Tolerant	predators					●	●
Odonata	Zygoptera		3	Tolerant	predators	●	●	●		●	●
Oligochaeta	Oligochaeta	Oligochaeta	2	very tolerant	gathering collectors	●	●	●	●	●	●
Plecoptera	Gripopterygidae		8	very sensitive	scrapers		●				●
Trichoptera	Calamoceratidae	Anisocentropus sp.	7	very sensitive	shredders	●		●			
Trichoptera	Ecnomidae	Ecnomus	4	tolerant	predators	●	●	●	●	●	●
Trichoptera	Hydropsychidae	Cheumatopsyche	6	sensitive	filtering collectors	●	●	●	●		●
Trichoptera	Hydropsychidae		6	sensitive	filtering collectors				●		
Trichoptera	Hydroptilidae	Orthotrichia	4	tolerant	macrophyte piercers			●	●		
Trichoptera	Leptoceridae	Oecetis sp.	6	sensitive	predators	●	●	●	●	●	●
Trichoptera	Leptoceridae	Triaenodes sp.	6	sensitive	shredders	●	●	●	●	●	●
Trichoptera	Leptoceridae	Triplectides	6	sensitive	shredders	●		●			●
Tricladida			2	very tolerant	predators	●	●	●	●	●	●

Appendix 2: Presence/Absence of families at each site and their signal 2 scores

Higher Taxa	Family	SIGNAL2 Score	SIGNAL2 Band	US1	US2	MZ1	MZ2	DS1	DS2
Collembola		1	very tolerant				●		●
Diptera	Muscidae	1	very tolerant	●					
Gastropoda	Physidae	1	very tolerant	●	●	●		●	●
Coleoptera	Dytiscidae	2	very tolerant	●					●
Crustacea	Corallanidae	2	very tolerant						●
Crustacea	Neoniphargidae	2	very tolerant					●	
Diptera	Ephydridae	2	very tolerant		●				
Hemiptera	Corixidae	2	very tolerant	●	●	●	●	●	●
Oligochaeta		2	very tolerant	●	●	●	●	●	●
Tricladida		2	very tolerant	●	●	●	●	●	●
Cnidaria		3	tolerant	●	●	●		●	●
Diptera	Chironominae	3	tolerant	●	●	●	●	●	●
Hemiptera	Veliidae	3	tolerant	●			●		●
Nematoda		3	tolerant	●	●	●	●	●	●
Nemertea		3	tolerant	●			●	●	●
Odonata	Zygoptera	3	tolerant	●	●	●		●	●
Bivalva	Corbiculidae	4	tolerant	●	●	●	●	●	●
Bryozoa		4	tolerant	●	●	●		●	●
Coleoptera	Gyrinidae	4	tolerant		●				
Crustacea	Parastacidae	4	tolerant						●
Diptera	Ceratopogonidae	4	tolerant	●	●	●	●	●	●
Diptera	Orthocladiinae	4	tolerant	●	●	●	●	●	●
Diptera	Tanypodinae	4	tolerant	●	●	●		●	●
Ephemeroptera	Caenidae	4	tolerant	●	●	●	●	●	●
Odonata	Protoneuridae	4	tolerant					●	●
Trichoptera	Ecnomidae	4	tolerant	●	●	●	●	●	●
Trichoptera	Hydroptilidae	4	tolerant			●	●		
Diptera	Empididae	5	sensitive	●	●	●	●	●	●
Diptera	Simuliidae	5	sensitive						●
Ephemeroptera	Baetidae	5	sensitive					●	●
Acarina		6	sensitive	●	●	●	●	●	●
Coleoptera	Scirtidae	6	sensitive						●
Trichoptera	Hydropsychidae	6	sensitive	●	●	●	●		●
Trichoptera	Leptoceridae	6	sensitive	●	●	●	●	●	●
Coleoptera	Elmidae	7	very sensitive	●	●		●		●
Trichoptera	Calamoceratidae	7	very sensitive	●		●			
Diptera	Aphroteniinae	8	very sensitive			●			
Ephemeroptera	Leptophlebidae	8	very sensitive	●	●	●		●	●
Plecoptera	Gripopterygidae	8	very sensitive		●				●
Odonata	Austrocorduliidae	10	very sensitive	●		●			