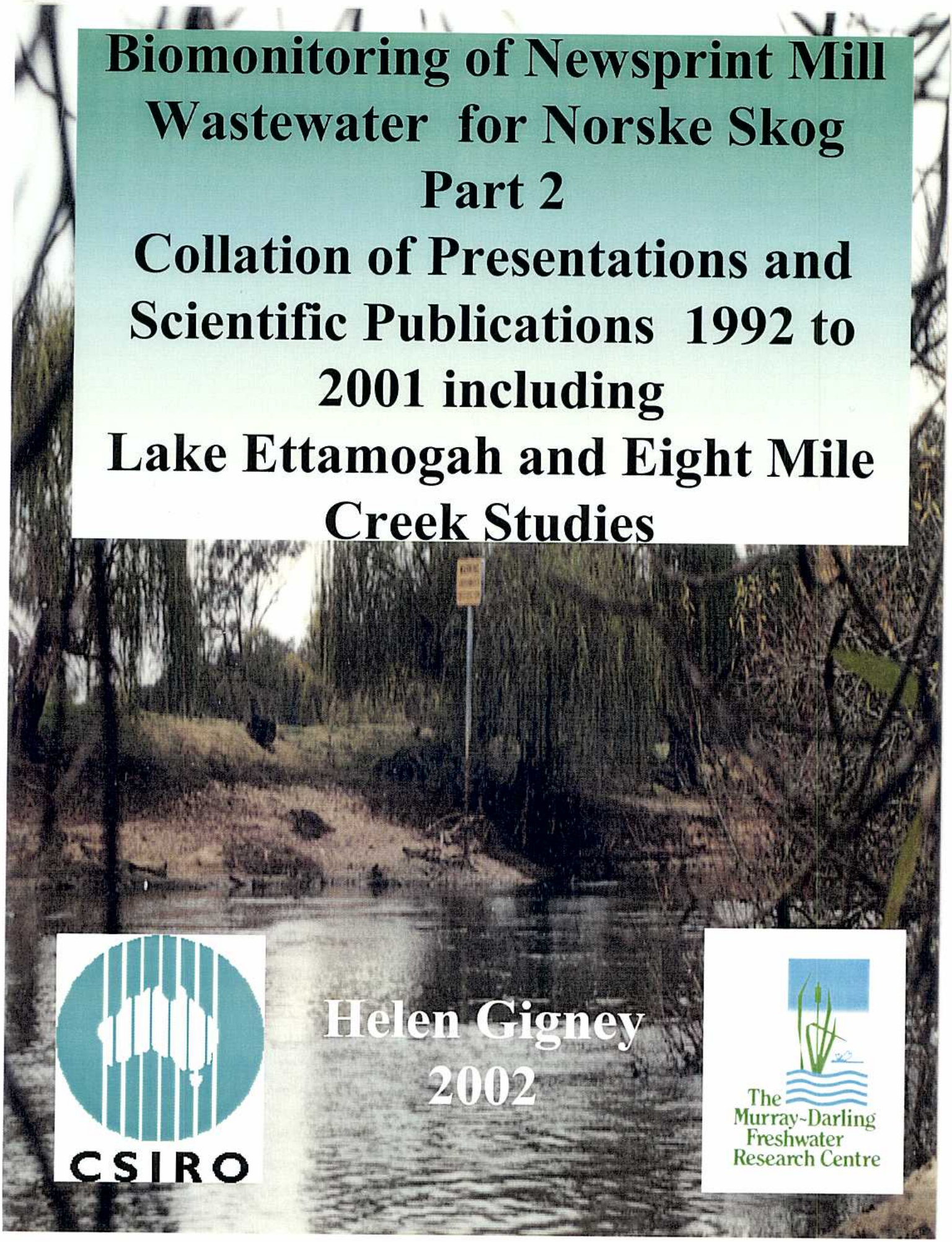
NORSKE SKOG ALBURY MILL

Revised Treated Process Water Management Strategies

STATEMENT OF ENVIRONMENTAL EFFECTS

APPENDIX 8

Biomonitoring of Newsprint Mill Wastewater for Norske Skog Part 2 Collation of Presentations and Scientific Publications 1992 to 2001 including Lake Ettamogah and Eight Mile Creek Studies



Helen Gigney

2002

CSIRO

The Murray-Darling Freshwater Research Centre

Biomonitoring of Newsprint Mill Wastewater for Norske Skog Part 2

Collation of Presentations and Scientific Publications - 1992 to 2001 including Lake Ettamogah and Eight Mile Creek Studies

Preface

The Murray Darling Freshwater Research Centre was contracted by Norske Skog (formerly, Fletcher Challenge Paper, and previous to that, Australian Newsprint Mills Ltd) to provide this collation of work completed by the Centre under contact to the Mill commencing in 1991.

This collation includes material presented at conferences and additional studies supported by Mill as well as an image library highlighting all aspects of the work. A copy of all of the material in this compilation is supplied on CD and the presentations and images may be reproduced with permission of MDFRC.

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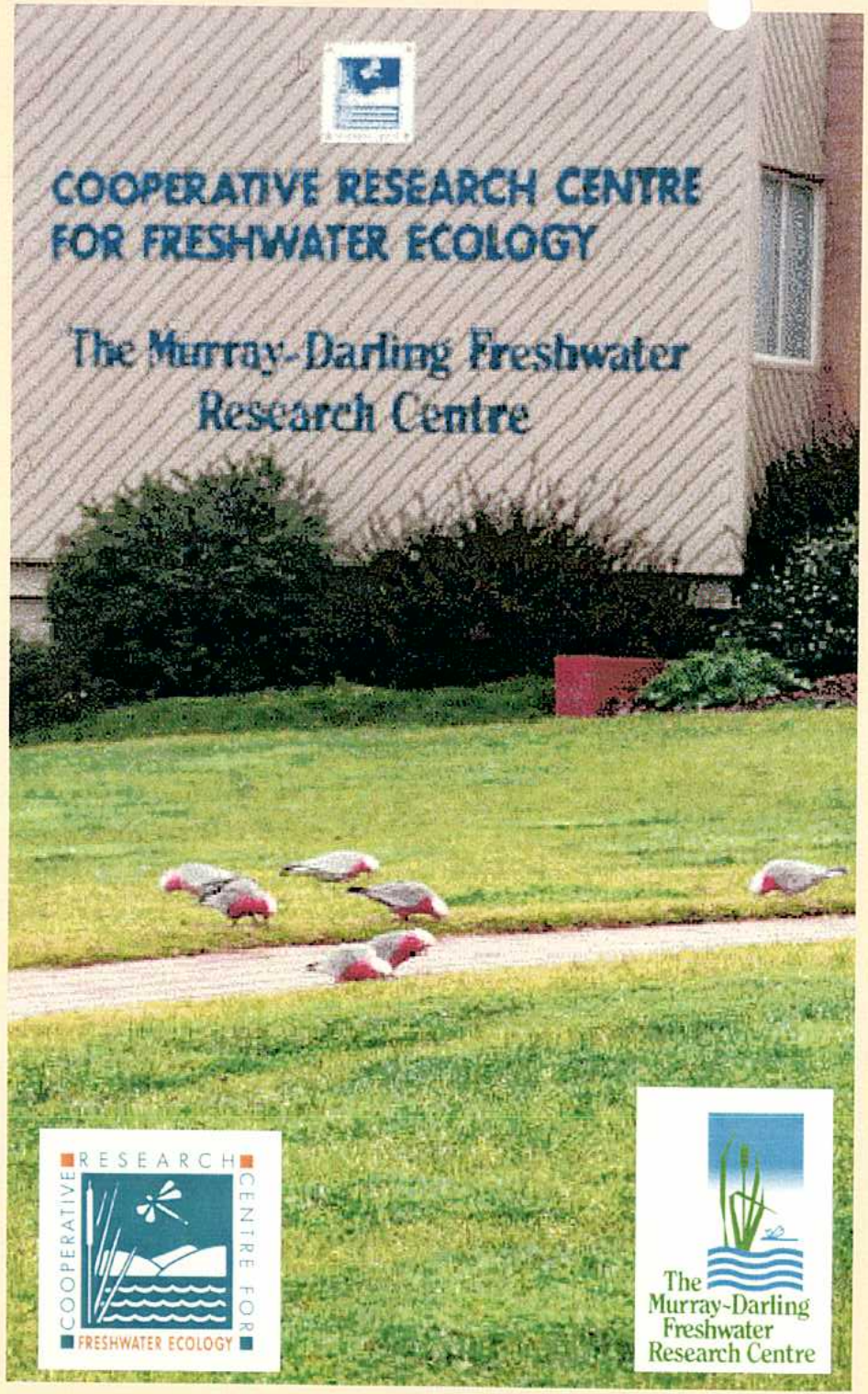
1

Ecological impacts of maintaining a constant flow regime in a temporary stream

ASL Presentation
Moama 2001

John Hawking
&
Robert Cook



This project was funded by Norske Skog Paper Mills (Australia) Limited and the following talk is based on the results published in their Annual Environmental Compliance Report, compiled by Stephen Dahl and Natalie Young, April 2001

Further Information

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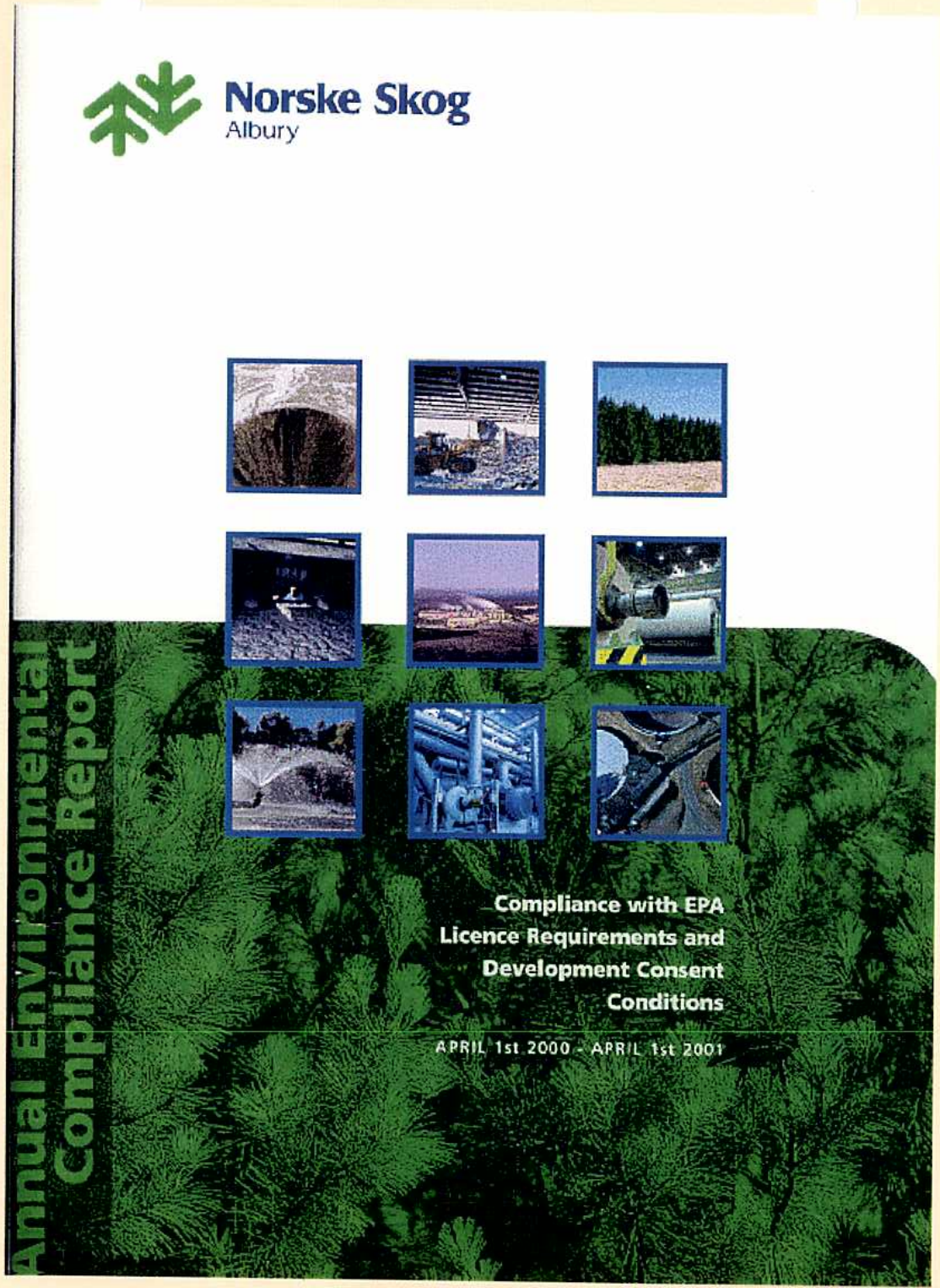
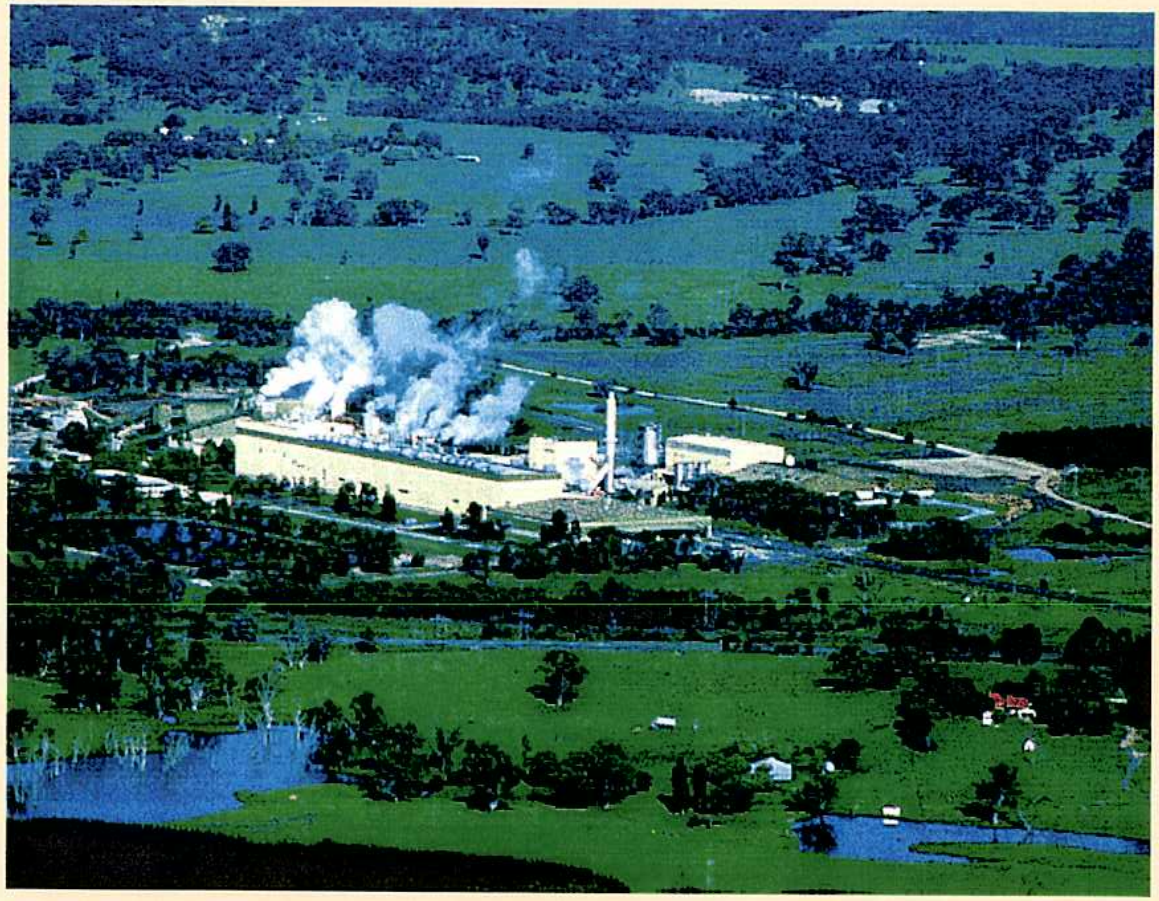
NSW 2641
Ph. 02-60583111
Attention: Stephen Dahl



Background to the Project

In the mid 1980's an arrangement was developed which saw the Norske Skog Paper Mill discharge treated processed water into Eight Mile Creek via a scour valve in its return water pipeline.



Background to the Project

This discharge was done in the summer time and the water was used by the Thurgoona Golf Club (downstream) to irrigate fairways and greens on its course. Prior to 1996 this water was a combination of treated effluent and cooling water. After 1996 only cooling water was discharged

Project requirements

As part of EPA licence negotiations in 1999, Norske Skog (NS) was required to examine the ecological impacts on EMC associated with the summer discharge.

The licence required an environmental survey of Eight Mile Creek to be conducted over a 12 month period.

Summer discharges of cooling water were to be ceased intermittently in order to attempt to simulate a more natural flow regime.

Projects Aims

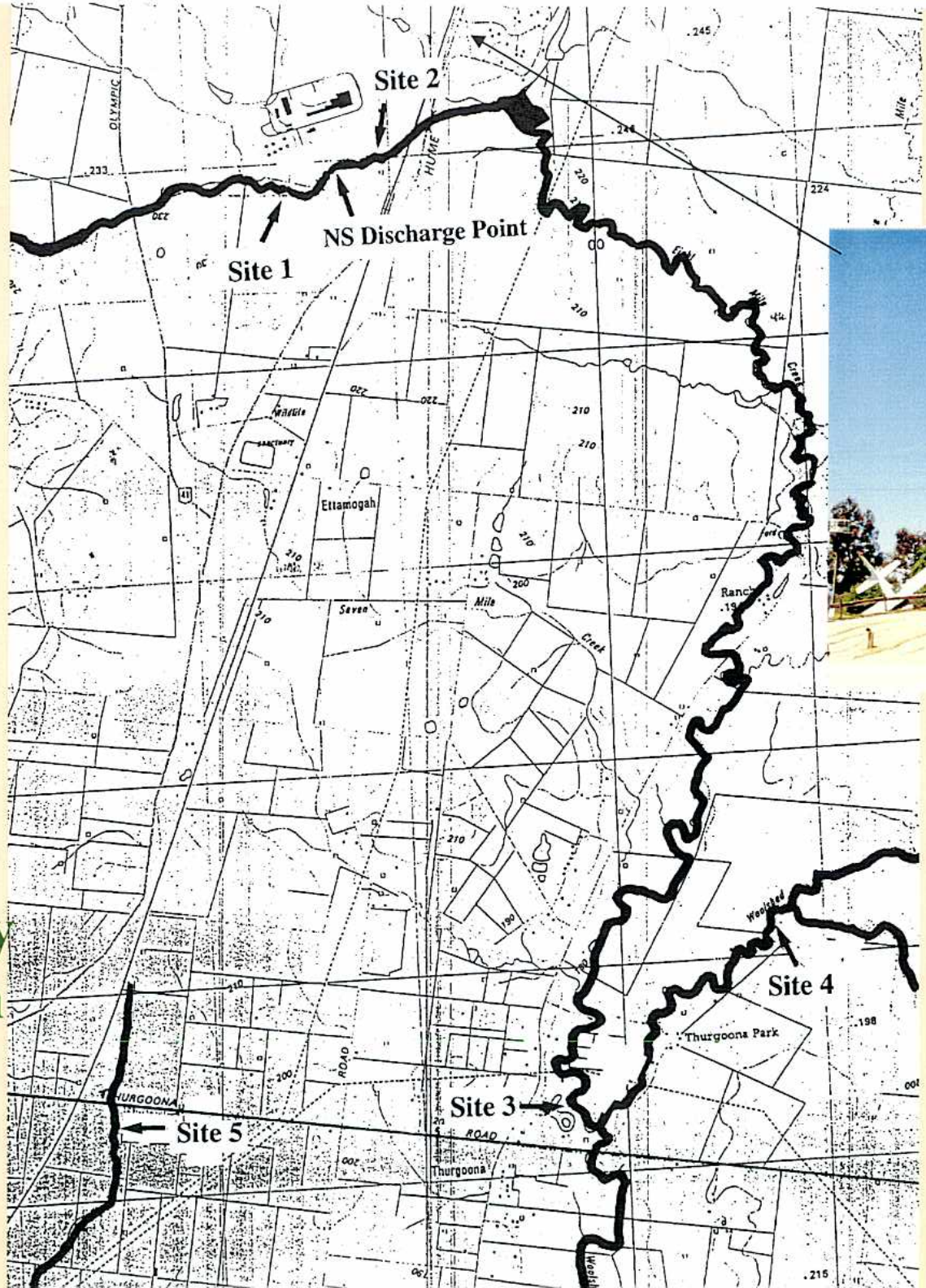
This study investigated the ecological effects of cooling water discharges on the natural environment of Eight Mile Creek

Project outline

- This was undertaken by monitoring the physical and chemical properties, and the microinvertebrate and macroinvertebrate community compositions at three sites on EMC and at a site on each of two nearby reference streams, Woolshed and Corrys Wood Creek.

- The two reference streams were investigated to enable comparison between the receiving stream (EMC) and streams under a more natural wetting and drying regime.

- The survey was to be conducted between June 1999 and June 2000.



Albury
6 km

Study Area

Site 1

Eight Mile Creek immediately upstream of the discharge point

Norske Skog's Discharge Point

Site 2

Eight Mile Creek immediately downstream of the discharge point

Site 3

Eight Mile Creek at Thurgoona Drive, 10 km below the discharge point

Site 4

Woolshed Creek, Thurgoona

Site 5

Corrys Road Creek, Thurgoona

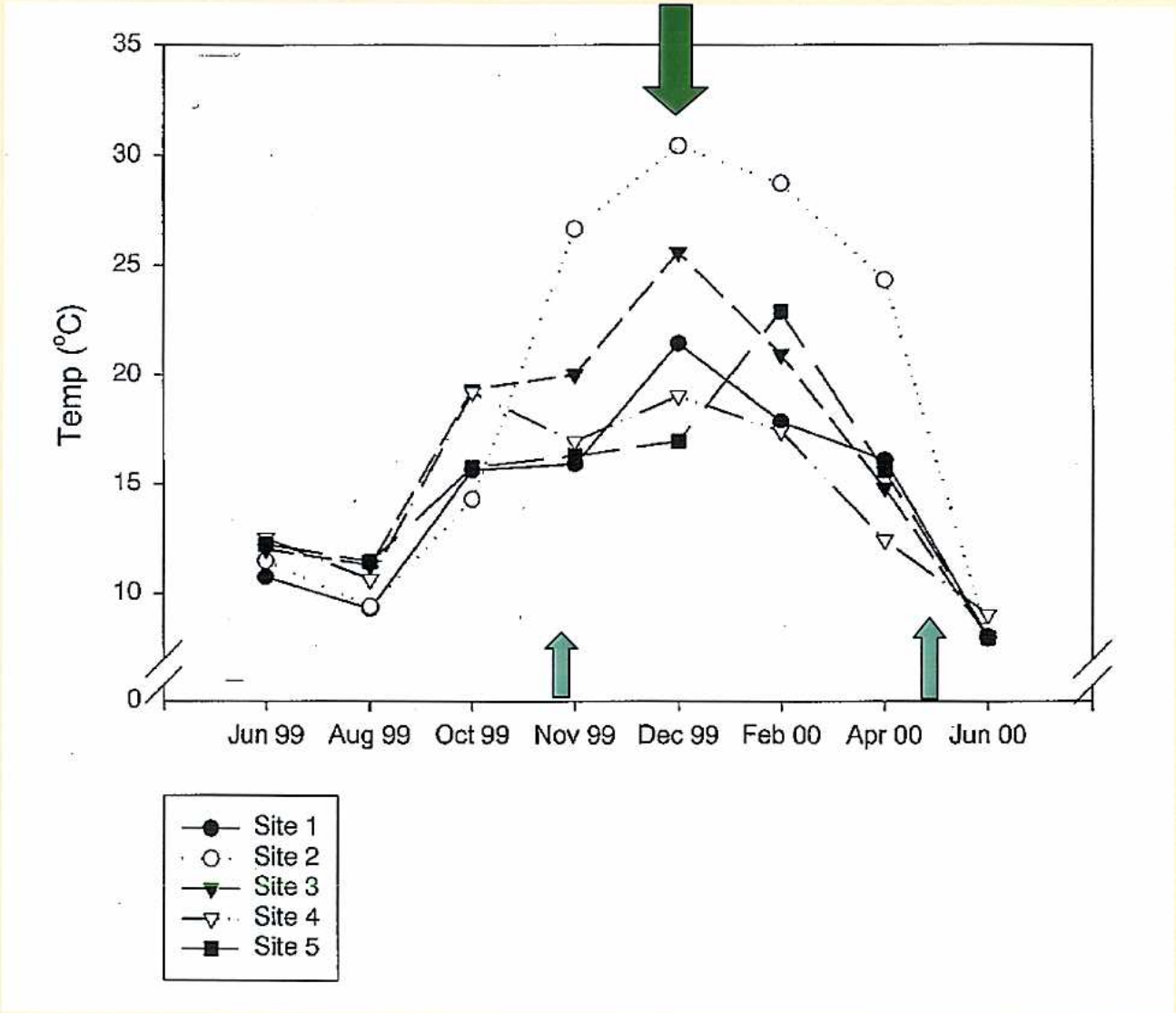
Sampling Regime

- Conducted at five site, EMC (3 sites), Woolshed and Corrys Creek
- Samples collected randomly from the site stretch
- Bimonthly, June 1999 to June 2000

Methods

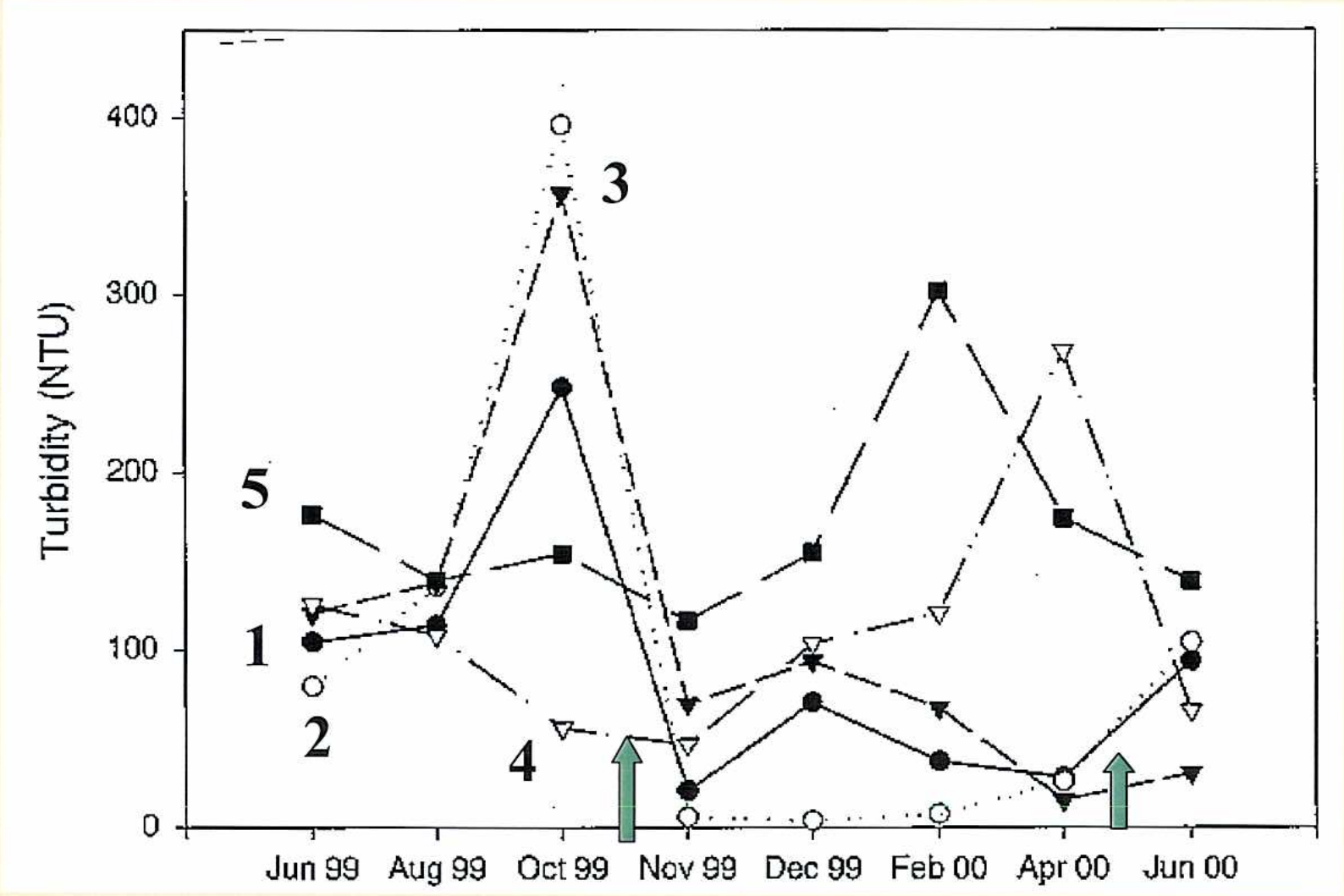
✡ Macroinvertebrates	1 metre Kick, 5 replicates/site
✠ Microinvertebrates	Schindler trap - volume 8 litres total
✸ Water Chemisty Physico-chem	Horiba U10 Water Quality Analyser (pH, TDS, Turbidity, DO, Temp)
Nutrients	200 ml sample, frozen, chem lab. (TN, TP, COD)

Results - Temperature



Plot of Water Temperature for Sites 1-5, June 99 - June 2000

Results - Turbidity



Macroinvertebrate Results

- ✡ 44,322 animals collected;
- ✡ 168 taxa;
- ✡ 64 families

No. of taxa at a site

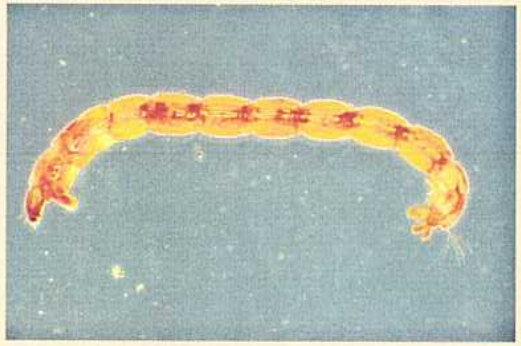
- ✠ Site 1 88 taxa
- ✠ Site 2 91 taxa
- ✠ Site 3 99 taxa
- ✠ Site 4 56 taxa
- ✠ Site 5 58 taxa

95% of abundance

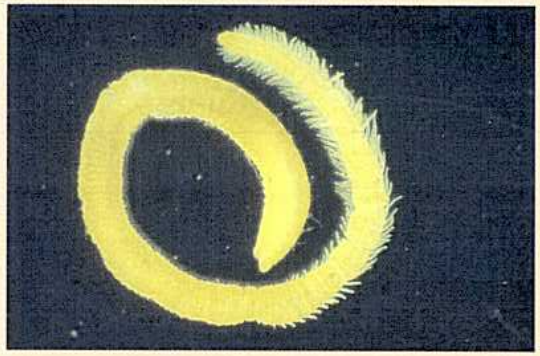
- ✠ Site 1 8 spp.
- ✠ Site 2 5 spp.
- ✠ Site 3 12 spp.

Macroinvertebrates

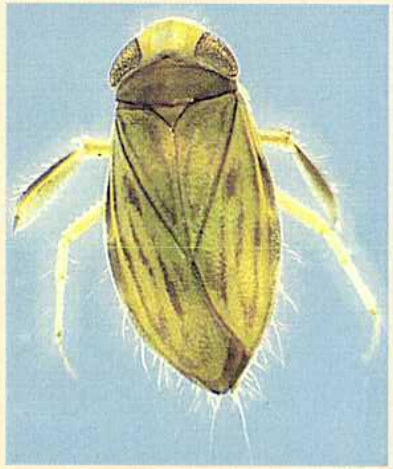
40% Chironomidae



28% Oligochaeta



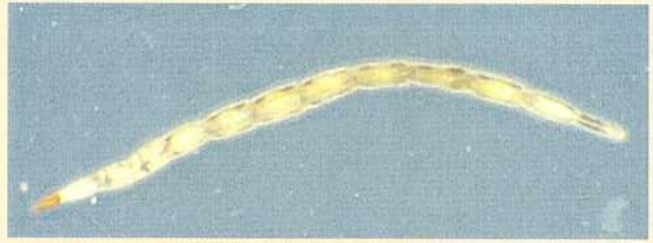
4% *Micronecta*



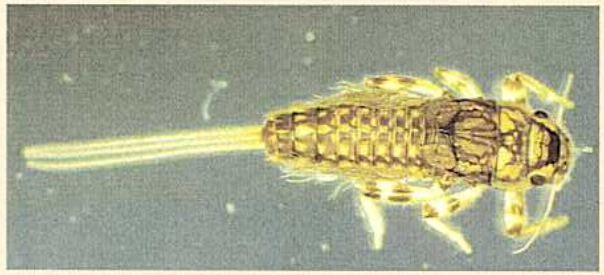
3% *Anisops*

Other dominant macroinvertebrates

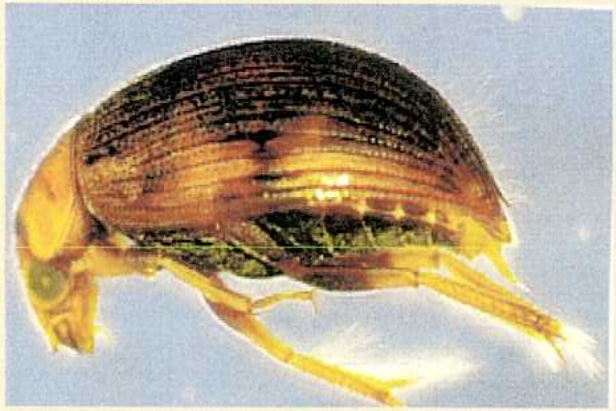
Caenidae, *Tasmanocoenis*



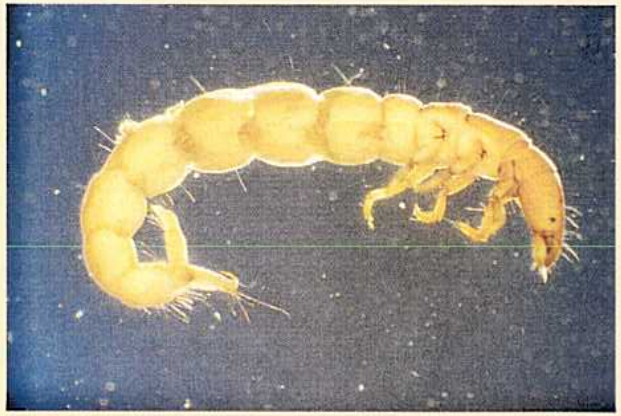
Ceratopogonidae



Leptophlebiidae, *Atalophlebia*



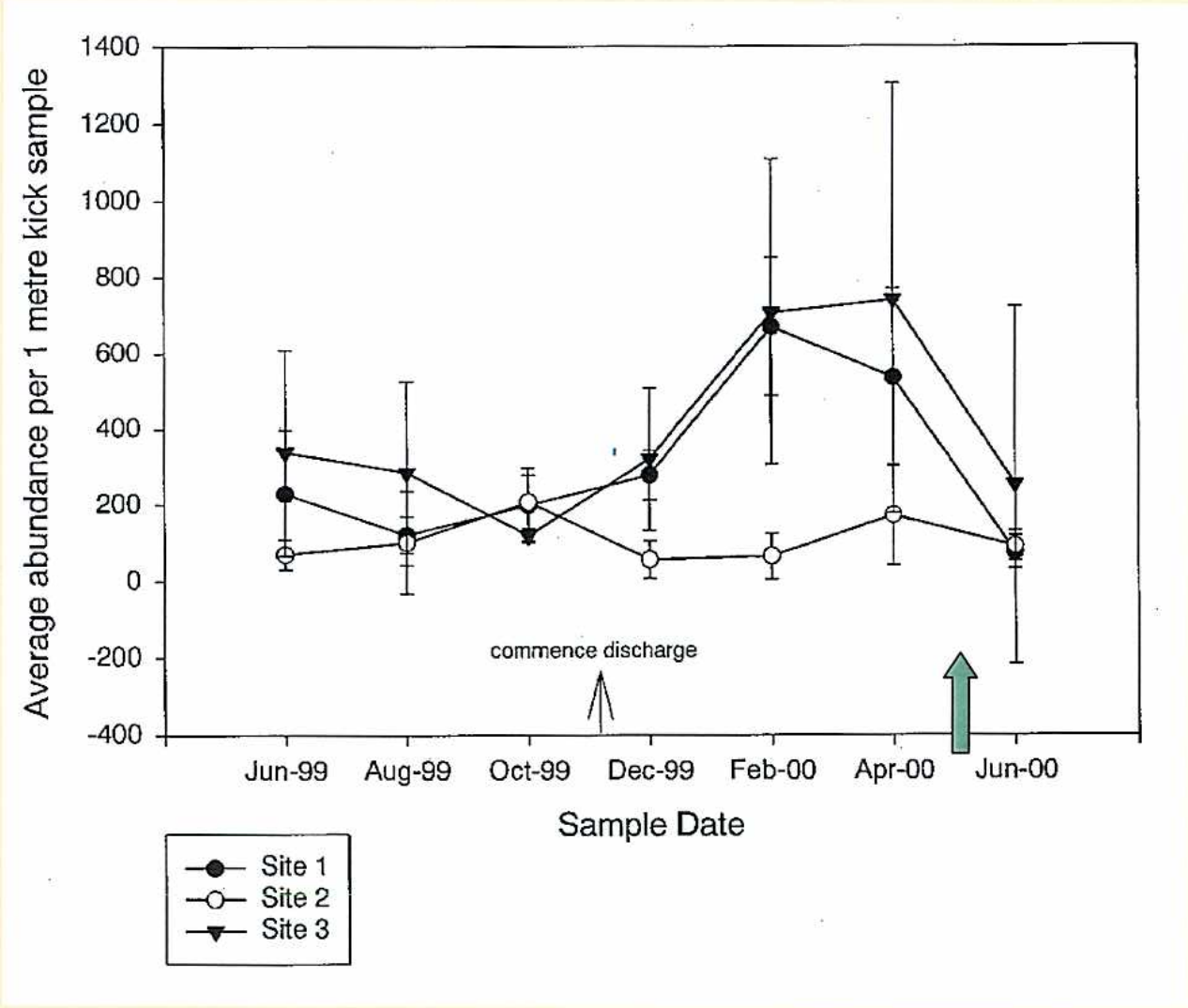
Hydrophilidae, *Berosus*



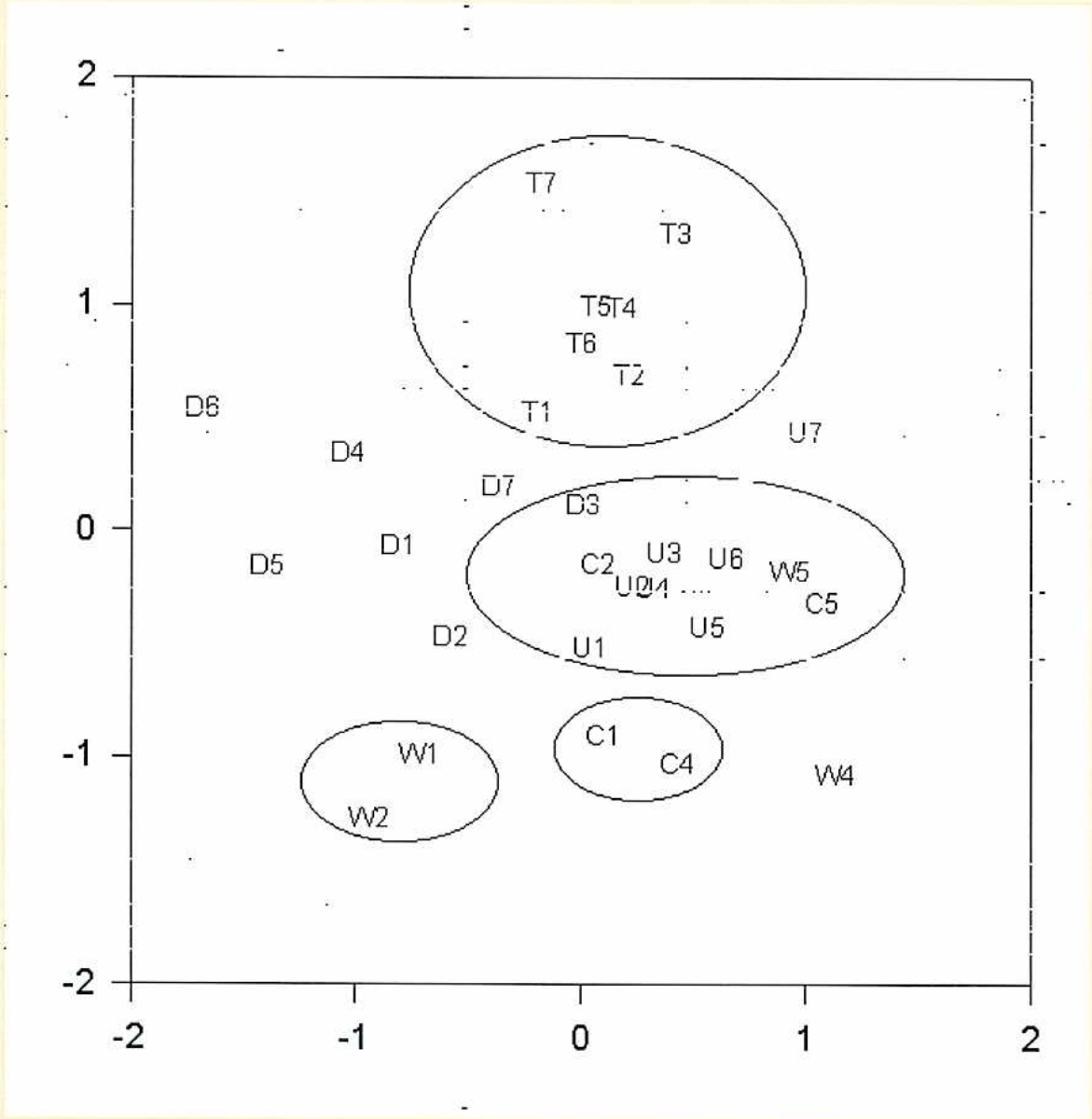
Ecnomidae, *Ecnomus*

Physidae, *Physella acuta*

Macroinvertebrate Abundances Sites 1-3

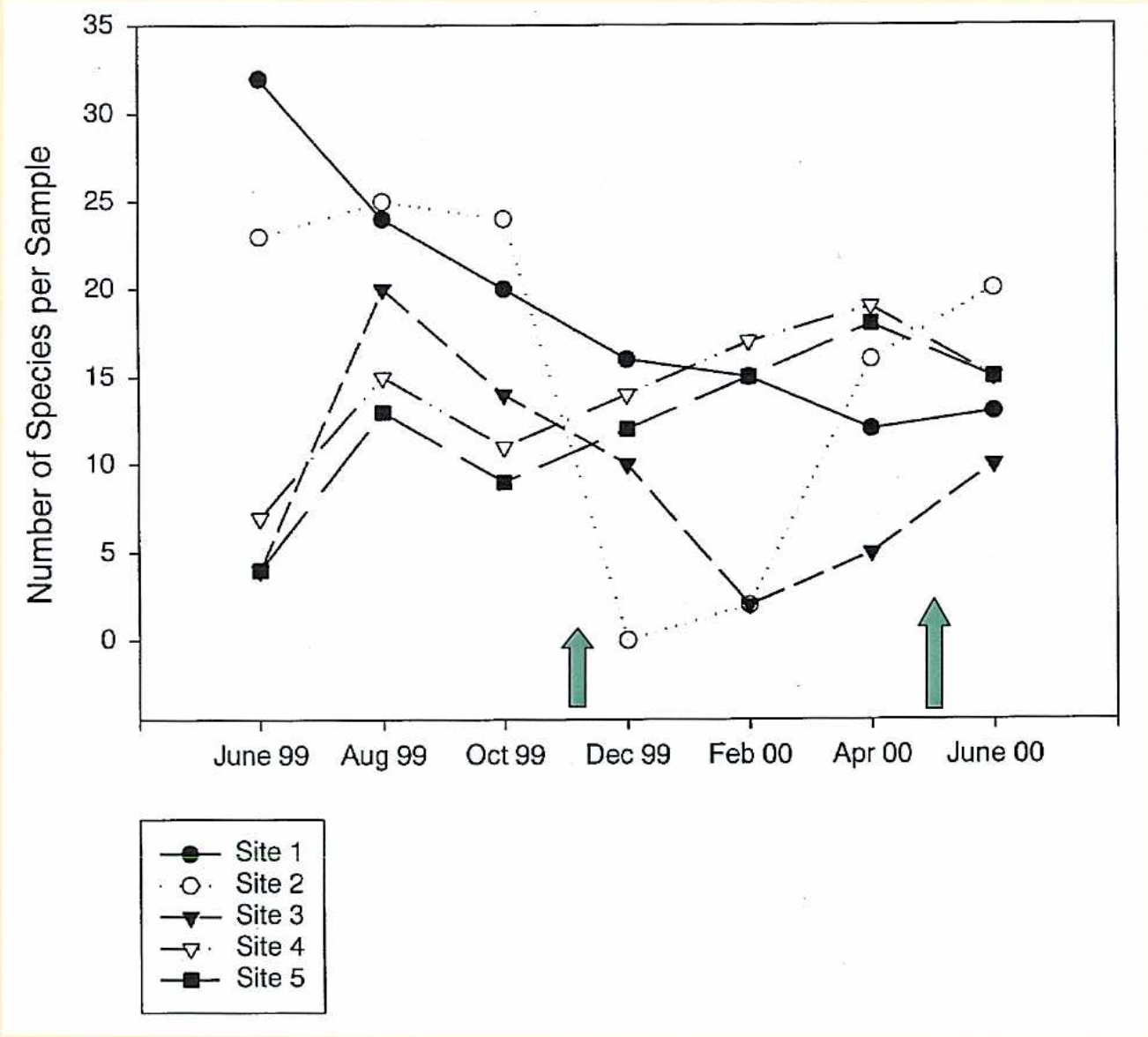


MDS Ordination of the Macroinvertebrate Data



Site 1, U
Site 2, D
Site 3, T
Site 4, W
Site 5, C

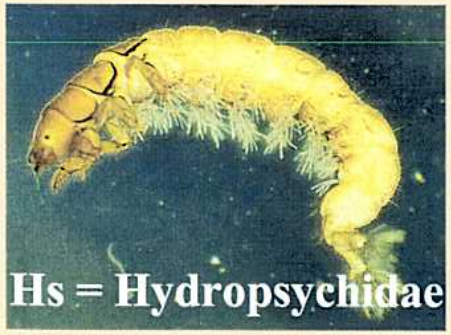
Plot of Macroinvertebrate Species Richness

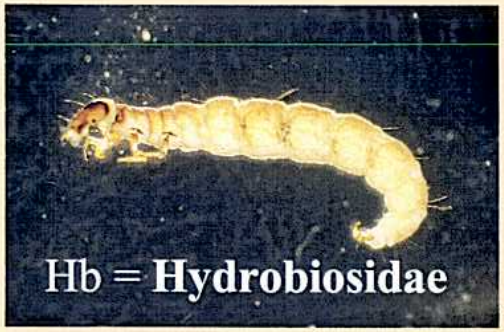


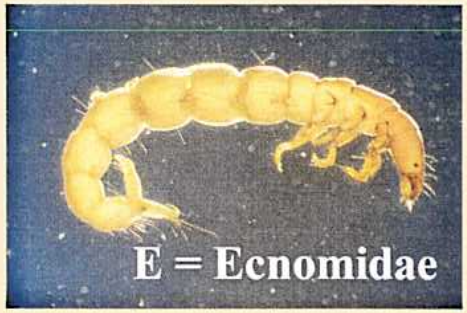
Trichoptera (Caddisflies)

	10/6/99	10/8/99	6/10/99	6/12/99	15/2/00	6/4/00	17/6/00
Site 1		L	L	L	L		
Site 2		H	H Hb			E	E Hs
Site 3	L E H	L E Hs		L E Hs	L E H	L E Hs	L E Hs
Site 4				L	E		
Site 5							


L = Leptoceridae


Hs = Hydropsychidae


Hb = Hydrobiosidae

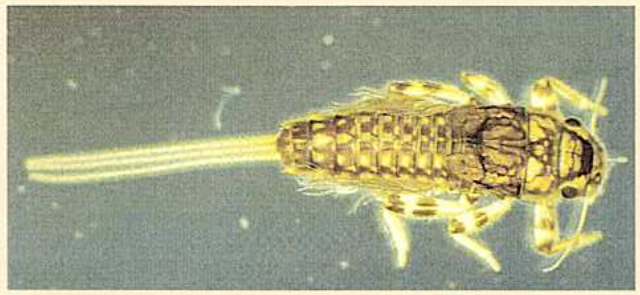

E = Ecnomidae


H = Hydroptilidae

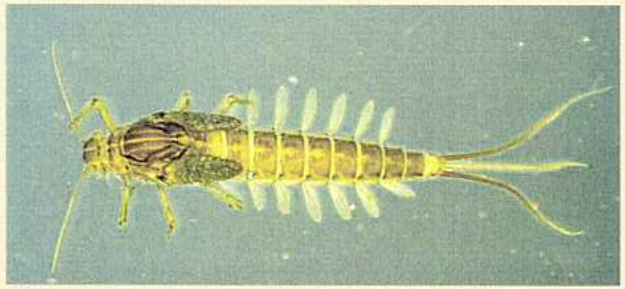
Ephemeroptera

	10/6/99	10/8/99	6/10/99	6/12/99	15/2/00	6/4/00	17/6/00
Site 1		B	L B C	B C	B	L B	L B C
Site 2	C						L B
Site 3	B	L B C		B C	L B C	L B C	L B C
Site 4		L		L	L B		
Site 5		B			C		

C Caenidae

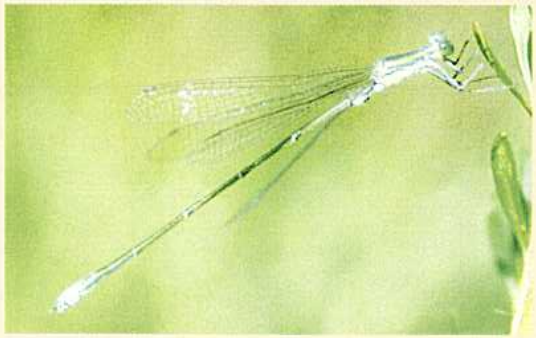


L Leptophlebiidae



B Baetidae

Damselflies



	10/6/99	10/8/99	6/10/99	6/12/99	15/2/00	6/4/00	17/6/00
Site 1	Aa	Aa	Aa	Aa	Aa		
Site 2		Aa					Aa
Site 3	Rs			Rs			Rs
Site 4	Aa			Aa			
Site 5	Aa	Aa		Aa			

Aa *Austrolestes analis*

Rs *Rhadinosticta simplex*

Dragonflies

	10/6/99	10/8/99	6/10/99	6/12/99	15/2/00	6/4/00	17/6/00
Site 1		Ps	Ps		Ps	Ps	Ps
Site 2							
Site 3		Ac		Ac			
Site 4							
Site 5				Ps	Ps		

Ac ***Austrogomphus cornutus***

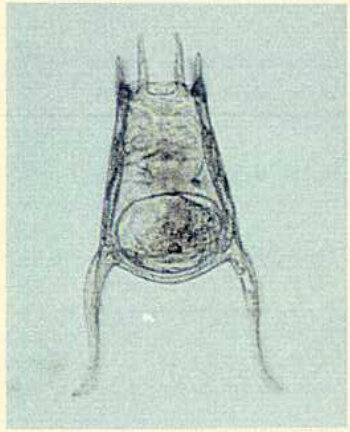
Ps ***Parasynthemis regina***

Plecoptera

	10/6/99	10/8/99	6/10/99	6/12/99	15/2/00	6/4/00	17/6/00
Site 1							
Site 2	G	G	G				
Site 3							
Site 4							
Site 5							

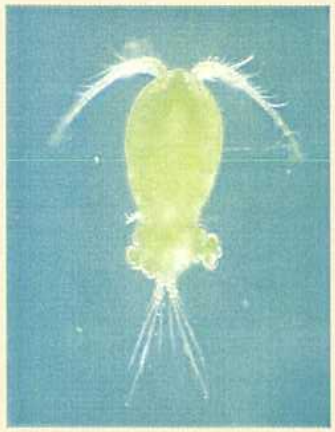
Microinvertebrates

A total of 74 microinvertebrate taxa were collected

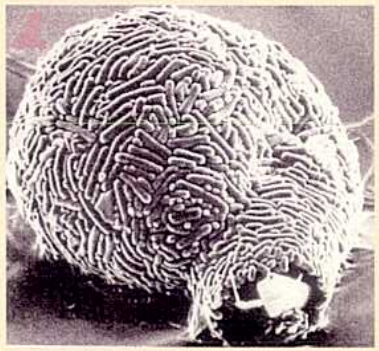


Rotifera
50 spp.

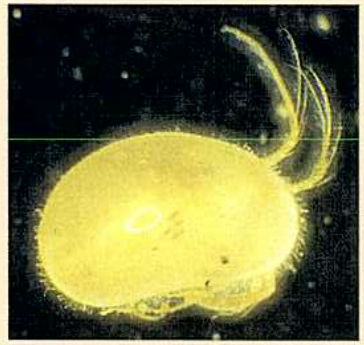
Cladocera
10 spp.



Copepoda
9 spp.

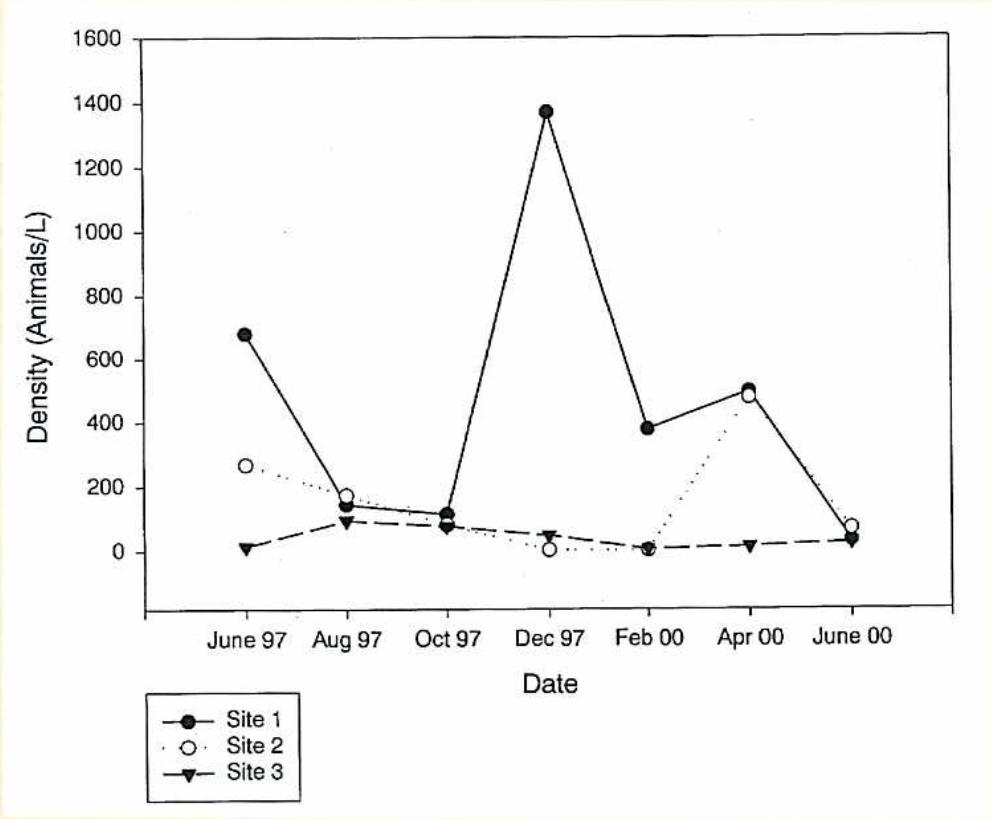


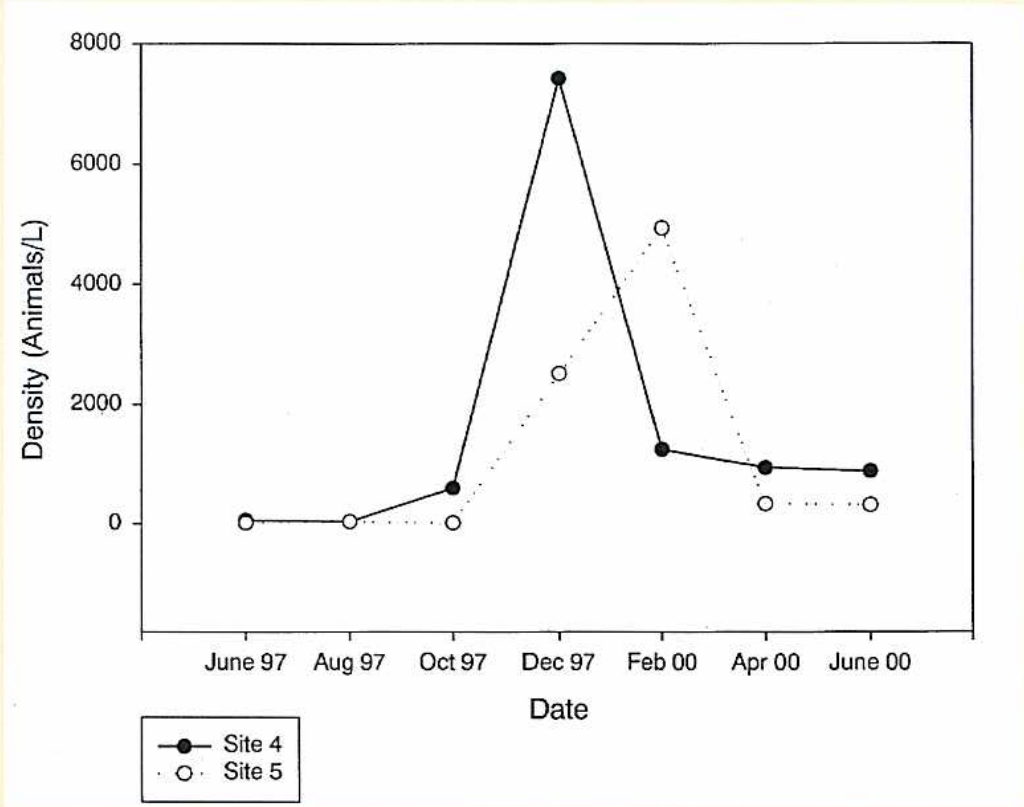
Testate
Amoebae
4 spp.



Ostracoda
1 sp.

Microinvertebrates (Densities)





Summary Points

1. **Temperature** - Reached a substancially higher level at Site 2 after discharge commenced

2. **Turbidity** - Remained very low throughout discharge period at Site 2

3. **Macroinvertbrates** - Site 2, many species disappeared and abundances dropped
   - Site 3, faunal composition changed to a more riverine community

4. **Microinvertebrates** - Species richness and abundance dropped substancially at both Sites 2 and 3 during the period of flow

CONCLUSIONS

(1) A rapid increase in water temperature at Site 2 had an impact on the macroinvertebrate communities in the section immediately below the discharge point

(2) An alteration of the hydrological regime of the creek downstream of the discharge point from an intermittent temporary stream to a permanent riverine environment, prevented the substantial microinvertebrate populations from developing.

(3) Altering the temporary stream macroinvertebrate fauna by providing non-seasonal conditions at Sites 2 and 3, changed the stream habitats that favour riverine taxa.


The End

2

Monitoring Norske Skog

Cooling Water Discharge

to Eight Mile Creek

Robert Cook and John Hawking

Murray Darling Freshwater Research Centre
Cooperative Research Centre for Freshwater Ecology

November 2000

This report prepared for Norske Skog as fulfilment of Contract dated 26 May 1999, issued to John hawking, CRC for Freshwater Ecology,
Murray Darling Freshwater Research Centre
PO Box 921, Albury, NSW, 2640, Ph. 02-60582300

Cover: *Diplacodes bipunctata*
Photograph: Karlie Hawking

INTRODUCTION

Eight Mile Creek is an ephemeral stream that dries to pools over the summer period. The catchment drains the hills to the north of Albury, flowing through grazing land before joining the River Murray, upstream of Albury. The Norske Skog newsprint mill is situated to the north-west of Eight Mile Creek (EMC), at Ettamogah, N.S.W.

In the mid nineteen eighties an arrangement was developed which saw the mill discharge treated processed water into EMC via a scour valve in its return water pipeline. This discharge was done in the summer time and the water was used by the Thurgoona Golf Club (downstream) to irrigate fairways and greens on its course. Prior to 1996 this water was a combination of treated effluent and cooling water. After 1996 only cooling water was discharged.

As part of EPA licence negotiations in 1999, Norske Skog (NS) was required to examine the ecological impacts on EMC associated with the summer discharge. The licence required an environmental survey of EMC to be conducted over a 12 month period. Summer discharges of cooling water were to be ceased intermittently in order to attempt to simulate a more natural flow regime.

This study investigated the ecological effects of cooling water discharges on the natural environment of EMC. This was undertaken by monitoring the physical and chemical properties, and the microinvertebrate and macroinvertebrate community compositions at three sites on EMC and at a site on each of two nearby reference streams, Woolshed and Corrys Wood Creek. The two reference streams were investigated to enable comparison between the receiving stream (EMC) and streams under a more natural wetting and drying regime. The survey was to be conducted between June 1999 and June 2000.

SITES

Norske Skog's Discharge Point

Norske Skog's discharge pipe (Fig. 2) is located on the eastern bank of EMC, approximately 400 metres upstream of the Hume Highway road-bridge (Fig. 1). The pipe allows cooling water to be discharged into the creek by manually operating a release valve. The discharge was metered and the gauge readings recorded.

Sample Collection Sites

Site 1. A 500 metre section of EMC, immediately upstream of the NS discharge pipe (Fig. 3). This section was generally steep sided and sparsely vegetated with common rush (*Juncus* sp.) and *Phalaris* (*Phalaris* sp.). In parts the section is shaded by large river red gums (*Eucalyptus camaldulensis*). A natural flow regime occurred at this site, with the flow ceasing in November, drying to a series of isolated pools.

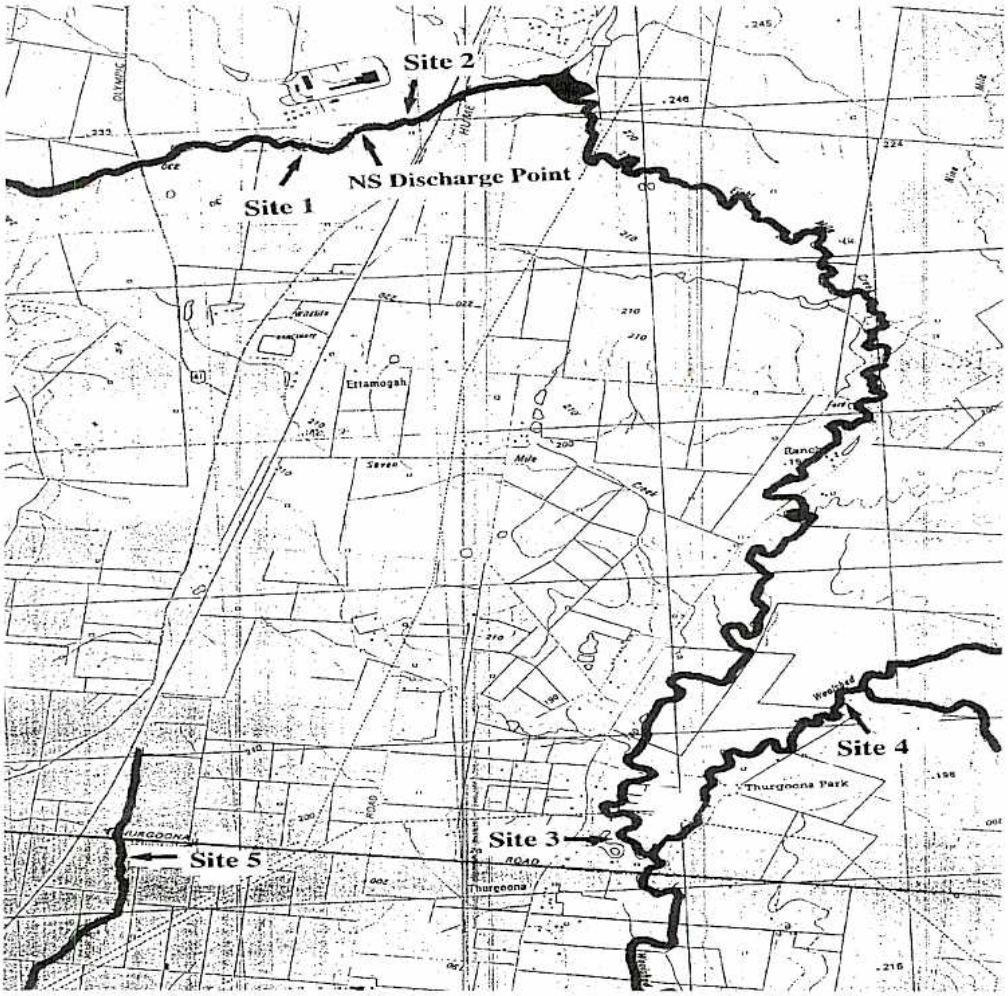


Fig. 1. Map of the study area, showing the sample sites on Eight Mile Creek, Woolshed Creek and Corrys Wood Creek.

Fig. 2. Norske Skog discharge point on Eight Mile Creek, Ettamogah.

Fig. 3. Sample Site 1, above the Norske Skog discharge point on Eight Mile Creek, Ettamogah.

Site 2. A 500 metre section of EMC, immediately downstream of the NS discharge pipe (Fig. 4). The character of this section changes along its length. Initially it is steep sided, vegetated mostly with water couch (*Paspalum distichum*) and *Phalaris*. The section to the crossing of the railway line contains a dense continuous stand of the common reed (*Phragmites australis)*. The remaining section below the railway line contains a dense stand of cumbungi (*Thypha* sp.). The natural flow regime was changed at this site with discharge from NS causing the stream to unnaturally flow throughout the study period.

Fig. 4. Sample Site 2, below the Norske Skog discharge point on Eight Mile Creek, Ettamogah.

Site 3. A 500 metre section of EMC, at the rear of Thurgoona Anglican Church, Thurgoona Park, Old Sydney Road, approximately 10 kilometres downstream of the NS discharge pipe. The upper section flows mostly through open grazed pasture and the riparian vegetation consisted mostly of water couch (Fig. 5a). The lower part of this site consisted mostly of large pools bounded by dense stands of common reed, and fringed by river red gums and silver wattles (Acacia dealbata) (Fig. 5b). The natural flow regime was altered at this site, with EMC flowing throughout the study period, because of NS discharges.

Site 4. A 500 metre section on Woolshed Creek at Thurgoona Park, downstream of the Kerrs Road crossing (Fig. 6). The banks are mostly steep-sided, with fringing vegetation of mainly common rush and the sedge (*Carex* sp.), with pasture grass *Phalaris* on the upper portion. A large portion of the stream is heavily shaded by large river red gums. The stream flow was intermittent and it never flowed during the samplings occasions, but due to storm events flowing periods were experienced between samplings.

Fig. 5. Sample Site 3, Eight Mile Creek, Thurgoona Park, Thurgoona. (a) pool conditions, no flow, May 1999; (b) stream conditions, flowing, February 2000.

Fig. 6. Sample Site 4, Woolshed Creek, Kerrs Road, Thurgoona.

Site 5. A 500 metre section of Corrys Wood Creek, immediately downstream of the Thurgoona road crossing, Corrys Wood (Fig. 7). This was a narrow section of stream vegetated with mostly common rush, sedge and *Phalaris*, and was heavily shaded in parts by river red gums. The stream ceased to flow in October and dried to a series of isolated pools.

Fig. 7. Sample Site 5, Corrys Wood Creek, Thurgoona Drive, Thurgoona.

Flow Regime

All three streams under natural conditions are ephemeral streams, which typically dry to pools over the summer period. Woolshed and Corrys Wood Creek maintained their natural flow regimes, whereas the flow regime in EMC was altered at Sites 2 and 3 by releases from NS. Site 1 was above the release point, therefore a natural flow regime existed.

Discharge commenced into EMC at 11am Monday 1st November 1999, and ceased on the 17th April 2000, with a total discharge of 367 megalitres over this period. In november, prior to NS discharge, the creek flow had decreased to a trickle and the release of water started EMC flowing again. A discharge pattern of cooling water releases operated on a cycle of 12 days discharge, followed by two days of no discharge.

METHODS

Sample Collection
Samples were collected bimonthly for twelve months, from all sites from June 1999 to June 2000. At each site samples were randomly collected, from within the designated 500 m sections, on each collation date. Physico-chemical, nutrient, chemical oxygen demand (COD), macroinvertebrate and microinvertebrate samples were taken at each site.

Macroinvertebrates – Macroinvertebrate samples were collected by taking a benthic kick sample, over a distance of 1 m, using a 500 µm FBA "A" frame net. Five replicates were randomly collected from each site. Samples were immediately transferred to a 500 ml sample jars and preserved using 95 % ethanol. These were later sorted using a Wild M8 stereo microscope at the MDFRC laboratory and identified using the following taxonomic keys: Cranston (1995) [Diptera], Cartwright (1997) [Trichoptera], Dean (1997, 1999) [Trichoptera], Dean & Suter (1996) [Ephemeroptera], Hawking (1986), Hawking & Theischinger (1999) [Odonata], Horwitz (1995) [Crustacea], St Clair (1997, 2000) [Trichoptera], Suter (1997, 1999) [Ephemeroptera], Watts (1998) [Coleoptera] or from voucher specimens held in the MDFRC Taxonomy Reference Collection, which are keyed from references in Hawking (2000).

Microinvertebrates –Replicates were collected using a 4 litre box Schindler trap. Two traps were collected and mixed to make one replicate giving a total volume of 8 litres. These samples were reduced in volume using a 53 µm mesh sieve and immediately preserved in 70 % ethanol. Five replicates were collected from each site with only one replicate being used in the analysis. Sorting and identification was carried out under a Wild M8 stereomicroscope, using dark field illumination, on one replicate. Specimens that had to be slide mounted were identified under a Ziess Axioskop 2 compound microscope. All identifications were made using the keys of Shiel (1995) and Koste (1978).

Physico-chemical Parameters – Measurements of six parameters (pH, conductivity, turbidity, dissolved oxygen and temperature) were taken insitu using a Horiba U10 water quality analyser.

Nutrients –A 200 ml water sample was collected from each site, immediately placed on ice and returned to the laboratory, frozen and stored for analysis. The samples were analysed for total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand by the MDFRC analytical chemistry laboratory. TN & TP samples were digested simultaneously by the alkaline/persulfate autoclave technique (Hosomi & Sudo 1986) and the digested samples were analysed using the Lachat Flow Injection analyser. The method for TN is the cadmium reduction of nitrate to nitrite and the colorimetric determination of the pink azo dye complex at 520 nm and the phosphorus method is based on the standard Murphy and Riley molybdate/ascorbic method where the blue phosphomolybdate complex was measured colorimetrically at 880 nm. Chemical Oxygen Demand was determined by the digestion and oxidation of samples at 150° C with acidic potassium chromate. Unreduced potassium chromate is measured spectrophotometrically using a Varian Cary 1 UV-VIS spectrophotometer.

Statistical Methods

Presence–absence data for the macroinvertebrates for each site was analysed using a Bray-Curtis Similarity matrix (Clarke 1993, Clarke and Warwick 1994). Analysis was conducted at the lowest taxonomic level available, generic level, except for the Diptera (family), Oligochaeta (order). This analysis was used to create a Multi-dimensional Scaling (MDS) ordination to display the similarity between sites. An ANOSIM (one way) was used to test for homogeneity of sites including Site 2 before, during and after discharge from NS.

RESULTS

Water Quality

Nutrients and COD

The nutrient data is presented in appendix 1 and the bimonthly values graphed to show trends (Fig. 8). The levels of total nitrogen at each site initially increased, followed by a decrease in October (Fig. 8a). At Sites 2 and 3 the levels continued to decrease through to February. At site 1 concentrations decreased in November, then increased steadily over the next four months and decreasing to a value in June, which was similar to the previous June figure. Sites 4 and Site 5 followed a similar trend to Site 1.

Total phosphorus followed similar trends to the other nutrients at all sites, increasing and decreasing over the study period, with the concentration at the sites attaining a similar result, near 0.1 mg/l in June 2000. Total phosphorus concentrations at Site 1 decreased in November to 0.06 mg/l, then increased dramatically over the next four months to slightly >0.4 mg/l, then decreasing to approximately 0.1 mg/l (Fig. 8b). A reduction in total phosphorous occurred at Site 2 in November with levels remaining low through to April (0.02mg/l). A similar reduction occurred at Site 3 but was not apparent until December.

The COD followed similar trends to nitrogen. Levels at Sites 2 and 3 fell dramatically in November and December respectively, with both attaining a level <20 mg/l in February (Fig. 8c), maintaining this low level till April and increasing to 50-60 mg/l in June. In contrast the level at Site 1, 4 and 5 increased substantially by February, to 79 mg/l, 135 mg/l and 105 mg/l respectively, then decreasing in April to a 60- 80 mg/l concentration range in June.

In general, nutrient levels and COD fell substantially at Sites 2 and 3 following the commencement of the discharge. At Site 1, Site 4 and Site 5, TN and COD concentrations increased over the spring/summer period as the pools continued to dry. However, by June 2000 the nutrient level were all roughly similar, with the three streams now flowing due to heavy rains.

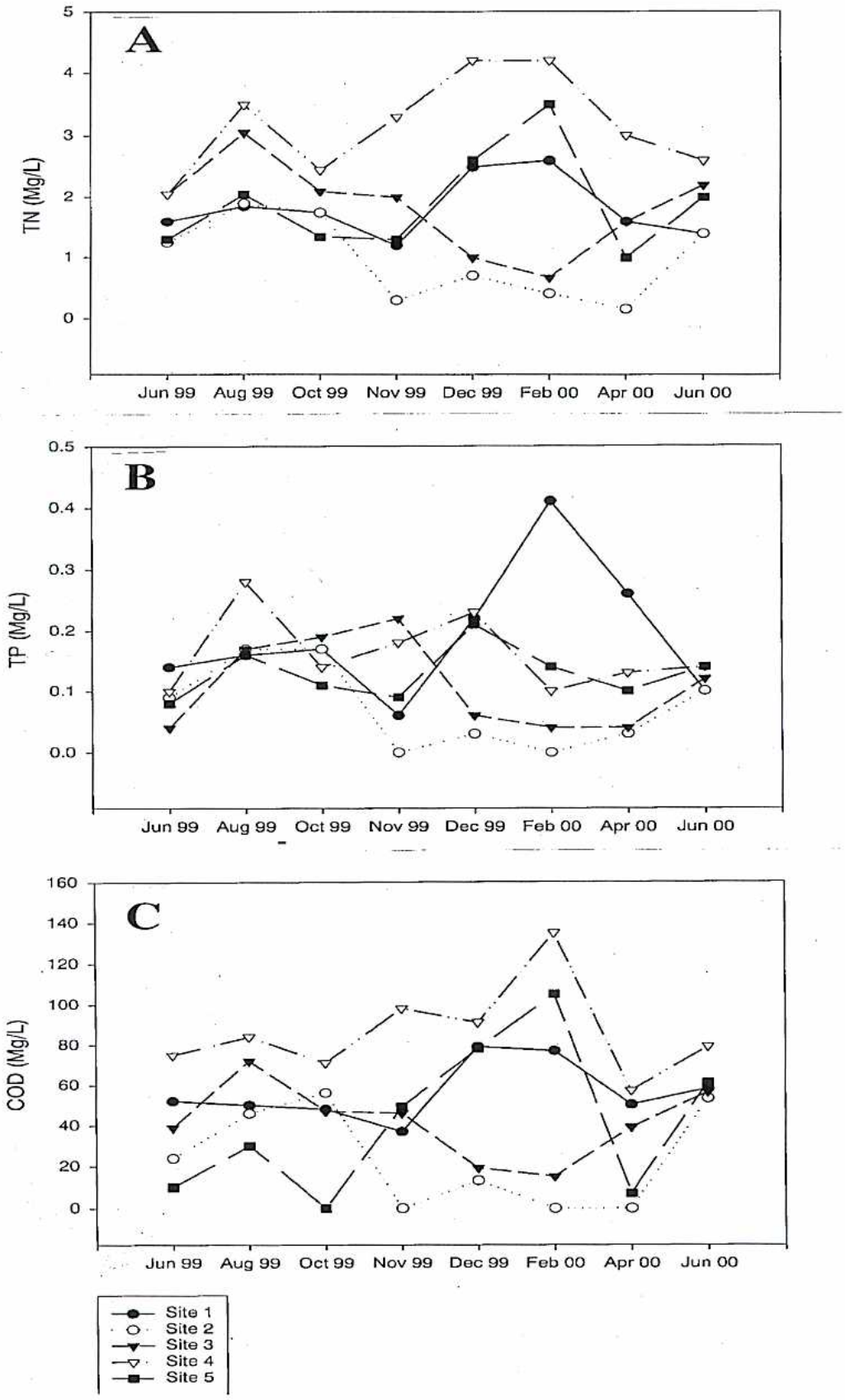


Fig. 8A-C. Plots of Nutrients and COD from the five sampling sites on Eight Mile Creek, Woolshed Creek and Corrys Road Creek, (A) Total Nitrogen, (B) Total Phosphorous and (C) COD, June 1999 to June 2000.

Physico-chemical

The physico-chemical data is presented in appendix 2 and the bimonthly values graphed to show trends (Figs 9, 10). The pH at all sites was generally between 7 to 8, although Site 3 was increasing to approximately 8.0 early in the year, then decreasing to 6.8 in February (Fig. 9A). Like-wise the conductivity values for all sites were similar, except at Site 3 which initially was 1.0 µS/cm in June but had dropped to the level of the other sites by October (Fig. 9C). It increased rapidly to 1.8 µS/cm in April only to decrease to 0.25 µS/cm by June 2000.

Turbidity at Site 1 increased from 100 NTU in June to 250 in October, decreasing dramatically to 21 NTU in November and rising slightly to 37 NTU in February (Fig. 9B). Site 2 followed a similar trend, but increased significantly to 396 NTU in October, also decreasing dramatically to 6 NTU in November, then remaining low, December (4 NTU) and February (7 NTU). After the commencement of releases the clarity of the water at Site 2 became crystal clear, with a slight bluish hue, and the organic matter on the creek bed became clearly visible. Site 3 was similar to Site 1, but was slightly higher at all times. Site 4 was between 100 -126 NTU in June/August period, decreased to 45 – 56 NTU in the October/November period and then back to 100 -120 NTU in the December/February period. Site was consistently between 117 - 176 NTU over the period, except in February it increased to 301 NTU.

The dissolved oxygen at all sites attained a maximum in August and dropped substantially by February at Sites 1, 4 and 5 (<4.0 mg/l), whereas the levels at Sites 2 and 3 remained higher (6 mg/l)(Fig. 10A). The levels at all sites increased to greater than 8.0 % by June 2000.

Temperature at all sites increased over the Spring/Summer period (Fig. 10B). However, a rapid increase in temperature occurred at Site 2 following the release of cooling water, from 14° C to 27° C. All other sites maintained similar temperatures over the same period.

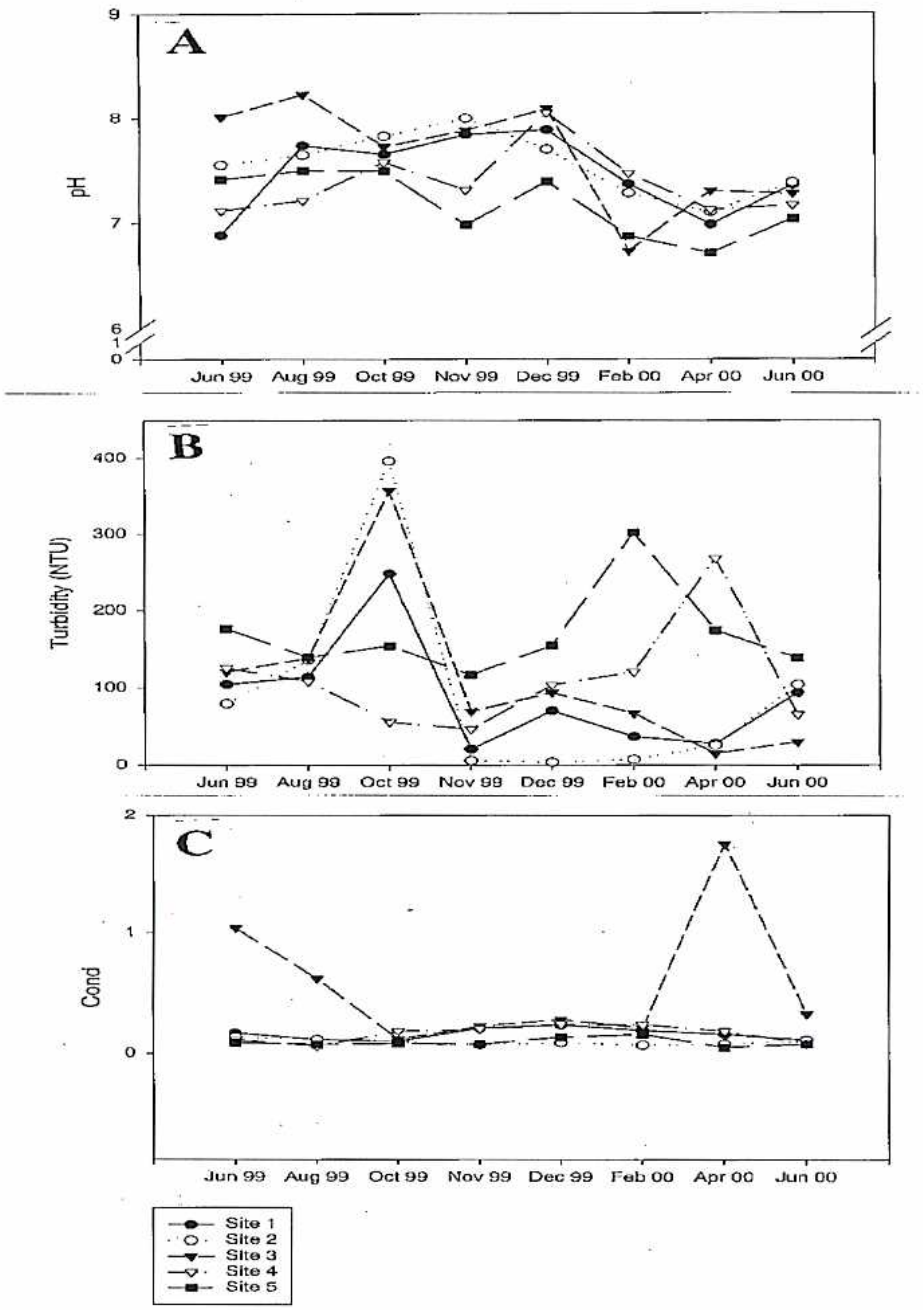


Fig. 9A-C. Plots of Physico-chemical parameters from the five sampling sites on Eight Mile Creek, Woolshed Creek and Corrys Road Creek, (A) pH, (B) Turbidity, (C) Conductivity, June 1999 to June 2000.

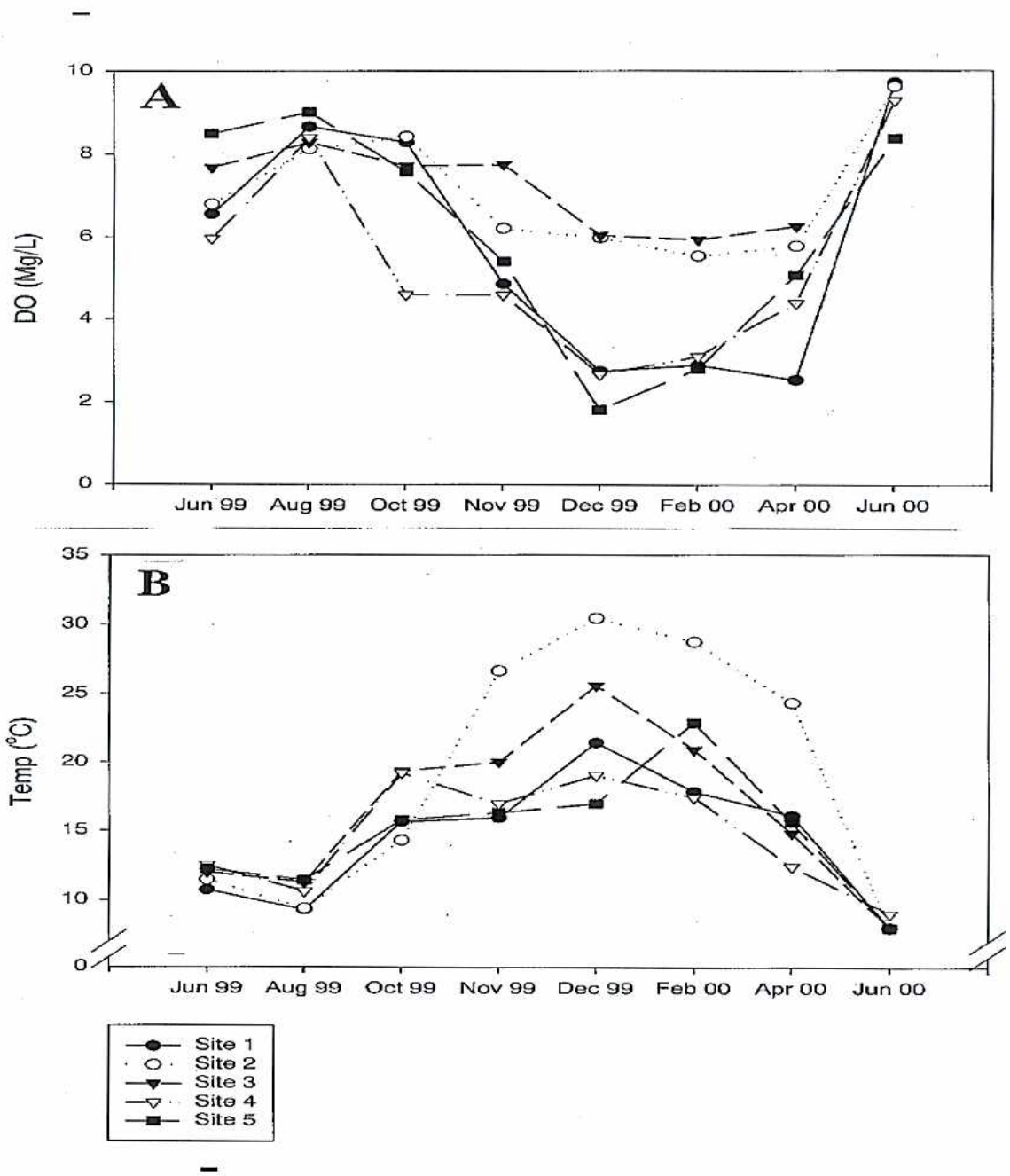


Fig. 10A-B. Plots of Physico-chemical parameters from the five sampling sites on Eight Mile Creek, Woolshed Creek and Corrys Road Creek, (A) Dissolved Oxygen, (B) Temperature, June 1999 to June 2000.

Macroinvertebrates

A total of 44,322 animals were identified, represented by 168 taxa from 64 families (Appendix 3). Site 3 was the most speciose site with 99 taxa followed by Site 2 having 91 taxa occurring, and Site 1, 88 taxa. The reference sites 4 and 5 had a much lower diversity with 58 and 56 taxa, respectively. Ninety five percent of the abundance at each of the three sites on EMC was attributed to a small number of dominant taxa: Site 1, 8 spp.; Site 2, 5 spp.; and Site 3, 12 spp. (Fig. 11). The dominant taxa across all sites were the chironomids (40%) and the oligochaetes (28%). Other taxa that were common at different times or sites were *Physa acuta* (Mollusca), *Atalophlebia* sp. (Ephemeroptera), *Tasmanocoenis* spp. (Ephemeroptera), Ceratopogonidae (Diptera), *Berosus* spp. (Coleoptera), *Ecnomus* spp. (Trichoptera), *Anisops* spp. (Hemiptera) and *Micronecta* (Hemiptera) (Appendix 4). The numbers of taxa present at Site 1, Site 4 and Site 5 generally increased over the Spring/Summer period and then decreased by June to a level similar to the previous June (Figs 12A, B). Site 2 increased to 37 species in October, only to decline rapidly to 18 species after the commencement of the discharge. The species richness remained constant during the release period only to increase after the release period ceased to a level slightly higher than Sites 1 and 3. Overall trends for Sites 4 and 5 could not be presented due to some samples not being processed and only four dates being used in the analysis.

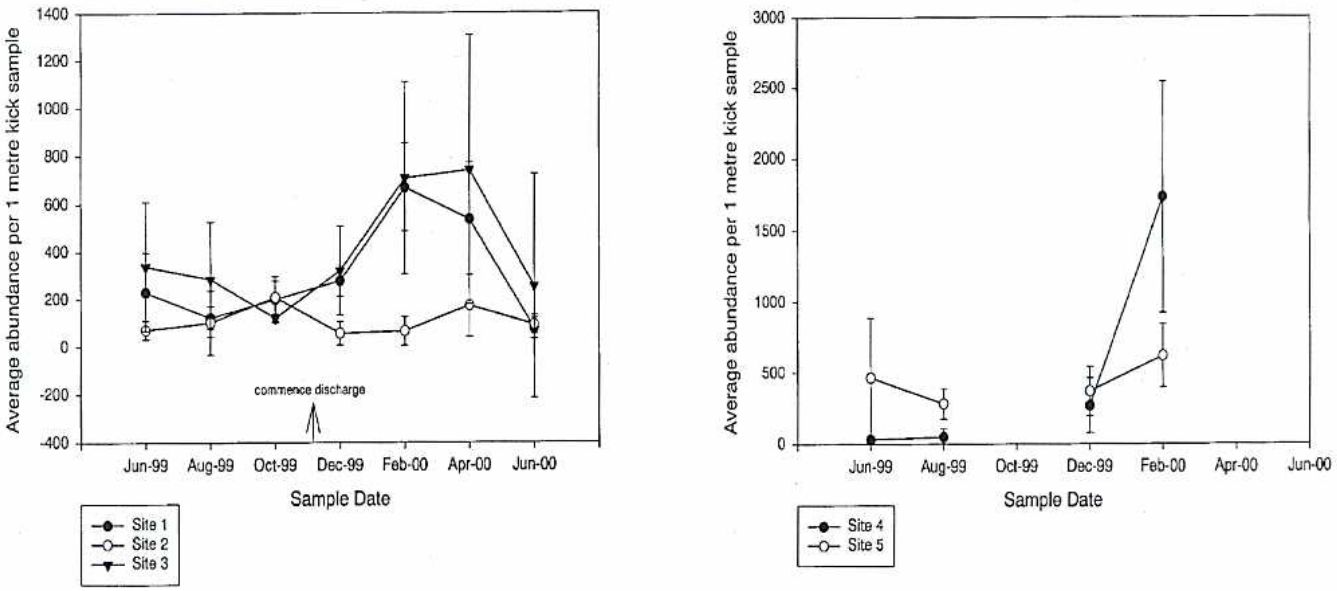


Fig. 11(A, B). Plot of macroinvertebrate abundance from Sites 1-3 (11A), Sites 4,5 (11B) from June 1999 to June 2000.

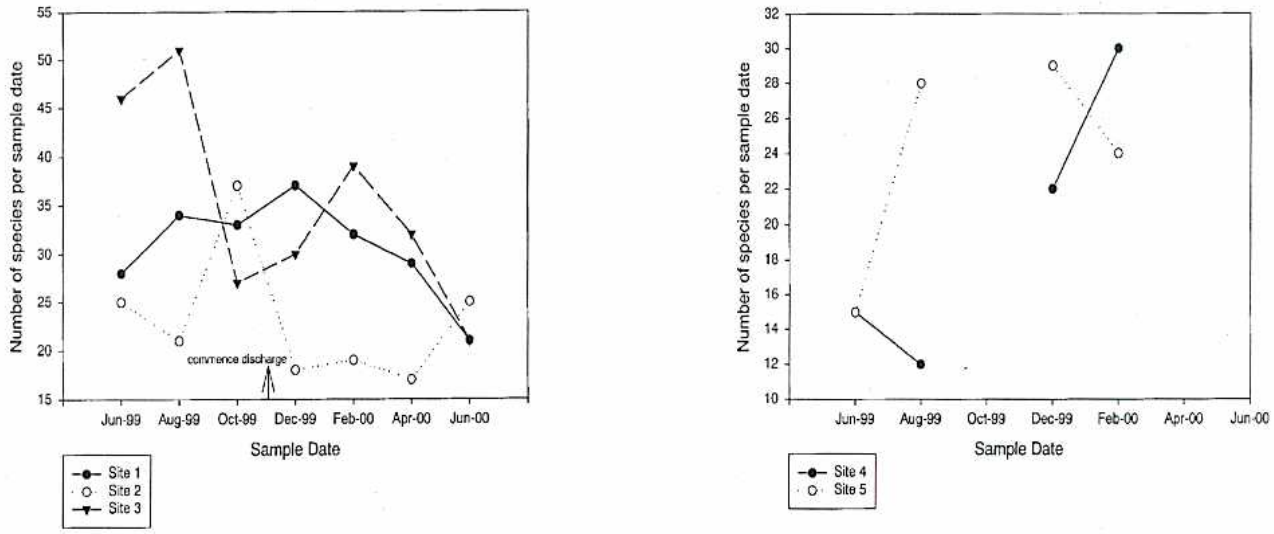


Fig. 12(A,B). Plot of macroinvertebrate species diversity from Sites 1-3 (12A), Sites 4,5 (12B), from June 1999 to June 2000.

The sampling method used was semi-quantitative, consequently no density data can be generated and no statistical analysis was performed on abundance data. However, an indication of macroinvertebrate abundances can be inferred as a standard 1 metre kick sample collected for each replicate. Site 1 and 3 increased substantially over the Spring/Summer period (Fig. 11. A). Sites 4 and 5 increased moderately whereas Site 2 increased in abundance up to October but declined again following the release of the cooling water (Fig. 11. B).

The MDS groupings show that Site 1 (U1 – U6) are equiv-distant from Site 2 (D1 – D3) post discharge and after the discharge commenced the distance increased (D4 – D6) (Fig. 13). After the discharge ceased the position of D7 moved back to the vicinity of pre-release position between D1 and D3. In contrast Site 1 (U7) has moved further away from the cluster, suggesting a changed fauna. The samples collected from Site 3 (T1 – T7) were grouped, but away from the other sites. The two reference streams, Site 4 (W1 – W3) and Site 5 (C1 – C3), showed no distinct grouping but were generally separate from the 8 Mile Creek sites, other than a single sample, Site 5, (C2). All of the site 1 samples were grouped together, except for sample U7 (Fig 13). The pre-cooling water release samples (D1-D3) from Site 2 were close to the Site 1 samples (D1-D6). However, after the release of the cooling water the MDS plot showed a shift in the D4-D6 samples which moved further away. After the release finished and conditions returned to normal seasonal regime (winter runoff flows) as shown by sample D7 moving back to a position Site 2 had per-release. The community abundance at Site 3 followed the trend as Site 1 (Fig. 11A) with an increase in abundance.

A homogeneity ANOSIM (one-way) test revealed that Site 2 site was not significantly different before, during or after the discharge (Table 1). Site 1 was significantly different from Site 2 prior to the discharge and remained different following the commencement of the discharge.

Site 2 was not significantly different from Site 4 throughout the study but was significantly different from the other reference Site 5 before and during the discharge period. Site 3 was significantly different from all other sites.

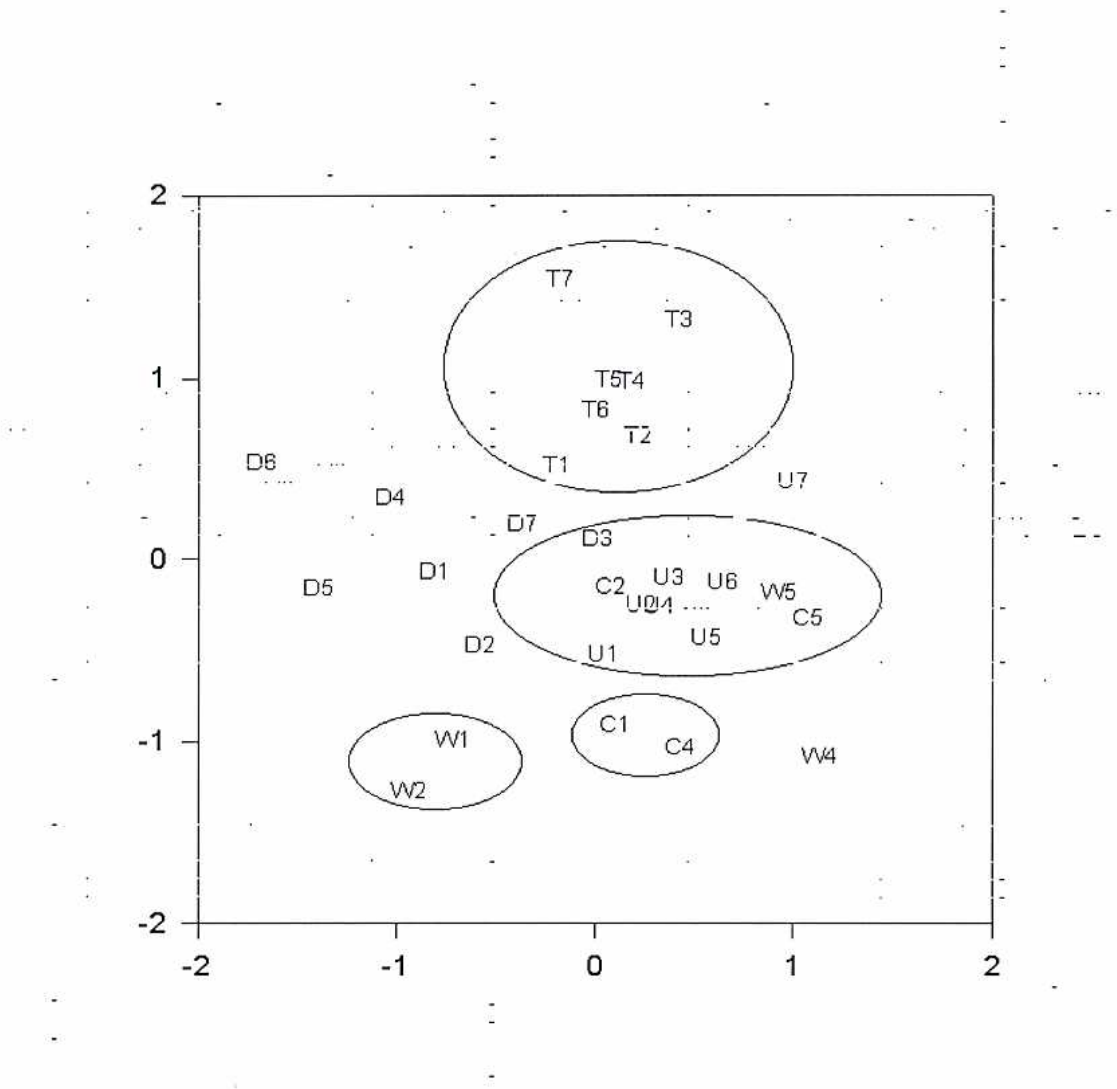


Fig. 13. MDS ordination of the macroinvertebrate data, analysis performed on presence/absence data at mostly the generic level.

Anosim values. Global R = 0.690

	Site 1	Site 2 (B)	Site 2 (D)	Site 2 (A)	Site 3	Site 4	Site 5
Site 1	1						
Site 2 (Before)		0.02*	1				
Site 2 (During)		0.008**	0.1	1			
Site 2 (After)	0.25	1	1	1			
Site 3	0.01**	0.008**	0.008**	0.13	1		
Site 4	0.009**	0.23	0.06	0.6	0.003**	1	
Site 5	0.03*	0.03*	0.03*	0.2	0.003**	0.29	1

Table 1. Table of significant values indicating significant differences between sites. (* significance at p = 0.05, ** significance at p = 0.01; Site 2 – (B) = before, (D) = during, (A) = after).

Microinvertebrates

A total of 74 microinvertebrate taxa were collected, including 46 spp. of Rotifera, 9 spp. of Copepoda, 11 spp. of Cladocera, 4 spp. of Testate Amoebae and one species of Ostracoda (Appendix 5). Site 5 was the most diverse site with 50 spp., followed by Site 2, 43 spp., Site 4, 40 spp., Site 1, 33 spp. and Site 3, 27 spp. The Rotifera and Copepoda were the dominant groups at all sites (Table 2, Appendix 6). As only one replicate was processed from each sampling occasion no statistical analysis has been carried out on the microinvertebrate data. Presence and absence of the taxa at each of the sites, separating the before and after discharge period is presented in Appendix 7. The density at Site 1 steadily declined until October, then drastically increased to approximately 1400 in December, followed by a gradual decline over the next six months to approx. 50 in June. A reduction in density also occurred at site 3, from 174 to 2 animals per litre. The Thurgoona Park site also showed a decrease in density, 62 to 26 animals/litre. All the other sites that did not receive the cooling water increased in density (Figure 14, 15). Site 2 remained steady from June to October (25 –30 taxa) followed by a sudden fall to 2 species in December and February (post release), with a rise in April, only to decrease again in June. Site 3 was consistently low, with 7 species in June to 23 species in August, then decreased to 5 species by February. Site 4 decreased in species number to October (18 to 12 species), then increased over the Spring/Summer period to 18 species. Site 5 increased between June and August (6 to 15 species), fell in October then gradually increased through to February (17 species).

Site	8 Mile Creek US	8 Mile Creek DS - Before	8 Mile Creek DS - After	8 Mile Creek ThP	Corry Wood CW	Woolshed ThP
Rotifera	55.66	61.96	33.33	25.34	45.45	75.40
Copepoda	35.75	30.37	66.67	67.91	44.16	18.72
Ostracod	2.56	0.00	0.00	1.16	4.71	0.61
Cladocera	0.84	1.81	0.00	2.42	3.86	0.66
Testates	5.19	5.86	0.00	3.16	1.81	4.63

Table 2. Proportions of the major microinvertebrate groups for Sites 1 to 5, from June 1999 to February 2000.

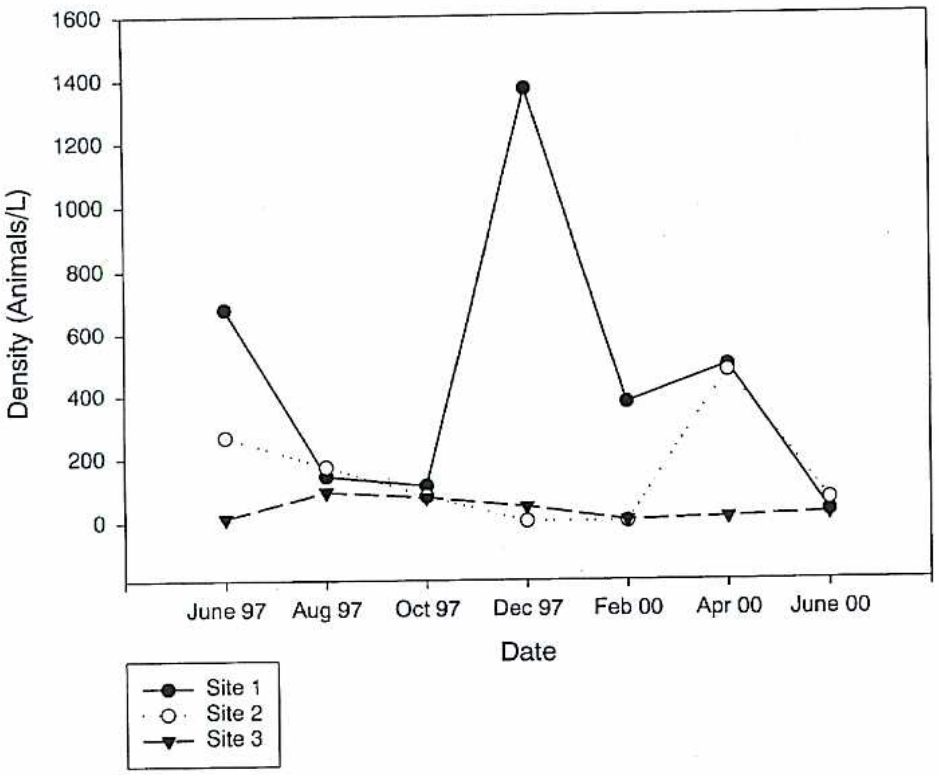


Fig. 14. Plot of average microinvertebrate density for Eight Mile Creek (Sites 1-3), from June 1999 to June 2000.

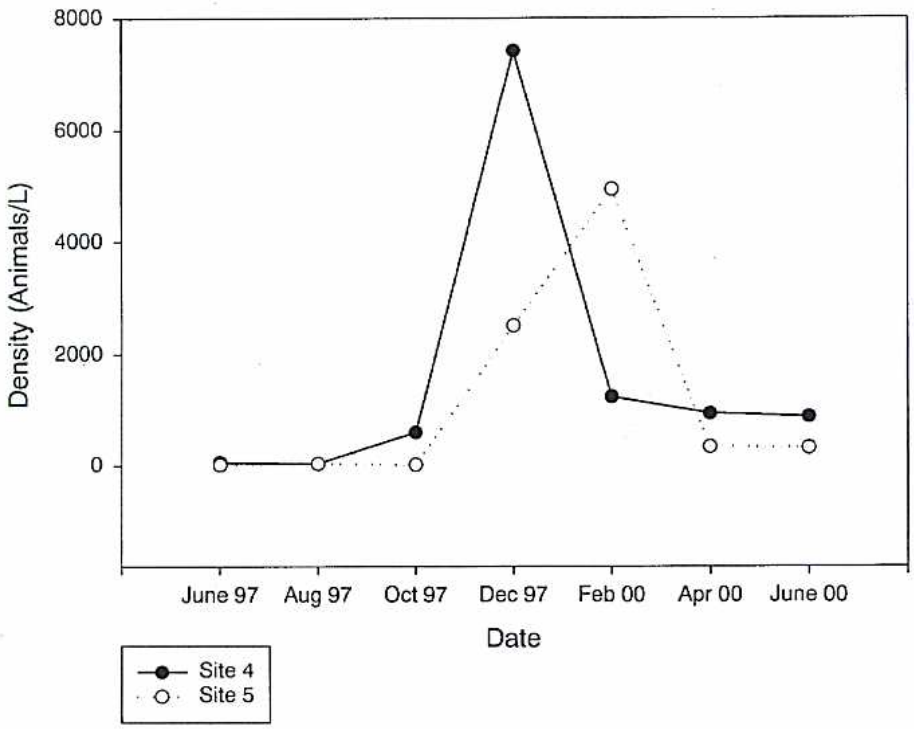


Fig. 15. Plot of microinvertebrate density for Sites 4, 5, on Woolshed and Corrys Wood Creeks, from June 1999 to June 2000

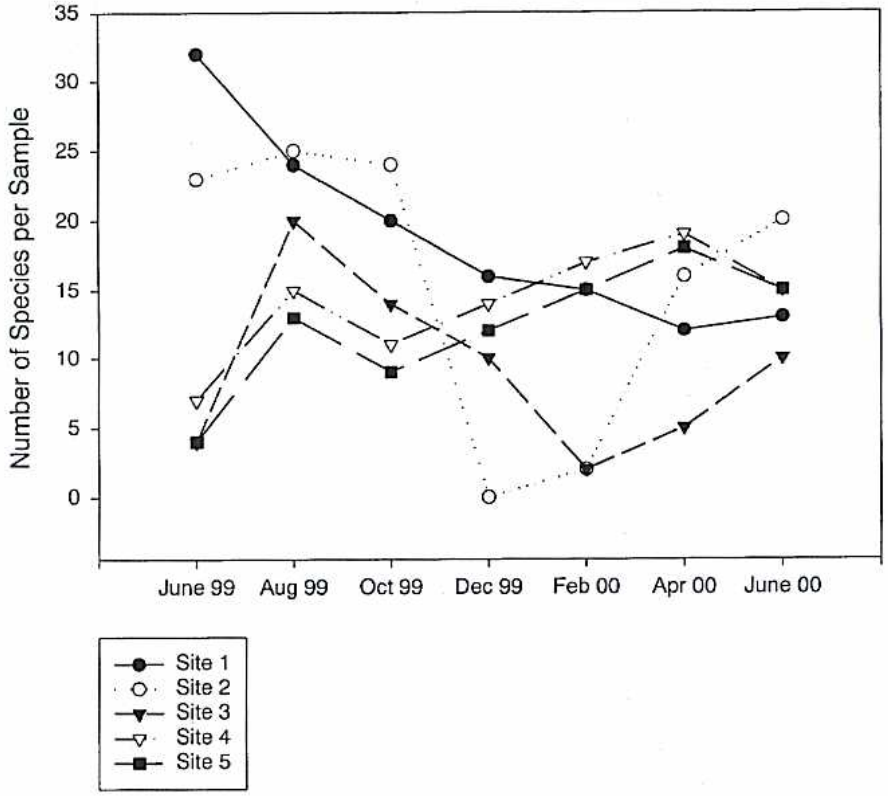


Fig. 16. Plot of microinvertebrate species richness from Sites 1-5, from June 1999 to June 2000

DISCUSSION

Eight Mile Creek, Corrys Wood Creek and Woolshed Creek flow seasonally and can be regarded as intermittent temporary streams as outlined in Boulton & Suter (1986). Site 1, 4 and 5 continued to show characteristics of a temporary stream throughout the study period. However, after NS cooling water releases, sites downstream of the discharge have had their flow regime altered and show characteristics of a permanent stream.

The processes outlined in Boulton & Suter (1986) were evident at the three sites that remained under the natural summer drying regime (Site 1, 4 and 5) where TN and COD steadily rose over the spring/summer period. Total phosphorous concentrations also rose throughout the summer at Site 1, displaying a very different nutrient profile when compared with Sites 2 and 3. A reduction in TN, TP and COD during the cooling water discharges and extended flow regime were resultant at sites 2 and 3. This may be attributed to the change to flow conditions, as Boulton & Suter (1986) report that the nutrient dynamics alters when a water body changes from a flowing stream to a series of pools and vise-versa.

Flow effects virtually every ecological process in streams. Changes to the flow regime of running waters result in altered physical, chemical and biological attributes

(Boulton and Brock 1999). Site 2 had reduced conductivity over the summer period and both Sites 2 and 3 had a higher dissolved oxygen level than the three non-flowing sites. This is most likely to have been a direct result of maintaining flow.

Over a spring/summer period water temperature will typically rise in response to the warmer day temperatures, this was apparent at all five sites. However, following the release of cooling water a rapid increase in water temperature (14°C to 27°C) occurred at Site 2, a rise which was not apparent at any other sites and was due to the warmer NS discharges. ANZECC (1992) suggests that an increase of no more than 2° C to maintain the health of aquatic ecosystems. The rise in temperature at Site 2 must be considered as an important factor in determining the faunal composition of the stream at Site 2, as indicated by the depressed macroinvertebrate species diversity and microinvertebrate and macroinvertebrate numbers. However it appears that temperature has a different effect on community structure at Site 3 and that flow is probably the dominant factor affecting the macroinvertebrate community structure at Site 3.

The water released from NS into EMC contains low concentrations of suspended particles resulting in low turbidity. A rapid decline in turbidity occurred at Site 2 following the release of the cooling water. A decrease also occurred at Sites 1 and 3 but not to the same degree as Site 2 and these sites increased again over the summer period, whereas Site 2 remained consistently low. The visual clarity of the water at Site 2 was noticeably crystal clear, and may be attributed to the cooling water floccing out the clay particles suspended in the water column. Reduced turbidity has been shown to have a positive effect on the primary productivity of water bodies and consequently the water body can sustain higher faunal populations (Cook 1999; Lloyd *et al.* 1987).

Graphically (refer to Fig. 11A, 13), there appeared to be changes in the macroinvertebrate community structure between Site 1 and 2 in the December to April period following the release of cooling water. Figure 11A clearly shows differences in the abundance of macroinvertebrates between Site 1 and Site 2, with the major difference occurring in February, the peak of summer and greatest reduction in the size of the drying pools. The spring and summer period is a time of maximum growth and reproduction for most aquatic fauna, with species richness and abundance increasing over the period at all sites, except Site 2. The rapid increase in temperature that occurred following the cooling water discharge may have been responsible for this decline in abundance.

It appeared that the cooling water was having a different impact on Site 3, than it had on Site 2. Any adverse impacts, like reduction in community abundance and diversity that the cooling water may have had at Site 2 are less apparent at Site 3. The macroinvertebrate community structure is now more diverse with the inclusion of several additional taxa indicative of a riverine environment. This is most likely due to the continuous flow, turning this section of EMC into a permanent stream environment. Barmuta (1990) demonstrated that flow had a positive effect on both species diversity and abundance in the Acheron River. In contrast the macroinvertebrate community structure at Site 1 developed aspects typical of a temporary stream, with the presence of mobile species (hemipteran spp.), desiccation

resistant species (*Parasynthemis regina*) and stagnant water/rapid life cycle species (mosquito larvae).

The process of drying is typical of temporary streams creating different habitats and generally a community structure different to that of a flowing environment. *Parasynthemis regina,* a dragonfly that can withstand desiccation was common at Site 1, but was not found at Site 2, although it was reported from Site 2 by Hawking (1999) prior to releases in November 1998. However maintaining the flow over this period has potentially altered the community structure of EMC. Barmuta (1990) found that pool areas and riffle sites within the Acheron River contained different sets of species and attributed this to the flow regime. Maintaining the flow in Eight Mile Creek is likely to be responsible for the presence of the *Tasmanocoenis* spp. (Ephemeroptera), *Ecnomus* spp. (Trichoptera) and *Rhadinosticta simplex* (Odonata) at Site 3, as these taxa are reported from lotic environment, with good water quality (Cartwright 1997, Suter 1999, Hawking 1986). In contrast the two reference streams and EMC at site 1, contained a number of taxa that are typical of pool environments, Mosquitos (Culicidae, Diptera), backswimmers (*Anisops* spp., Hemiptera) and other true flies (Diptera).

The microinvertebrate densities generally increased over the summer period at the sites that dried to pools (Sites 1,4 and 5). The discharge from NS appeared to have altered the community structure and density of the microinvertebrates. The microinvertebrate populations at Sites 2 and 3 decreased following discharges from NS most likely in response to maintaining a riverine environment. Flowing waters generally have different microinvertebrate populations to standing water bodies such as billabongs (Hillman 1986).

CONCLUSIONS

The study suggests that the main effects of the cooling water discharges are:

(1) A rapid increase in water temperature at Site 2 impacting on the macroinvertebrate communities in the section immediately below the discharge point;
(2) An alteration of the hydrological regime of the creek downstream of the discharge point from an intermittent temporary stream to a permanent riverine environment, preventing the substantial microinvertebrate populations from developing.
(3) Altering the temporary stream macroinvertebrate fauna by providing non-seasonal conditions at Site 2 and 3, which changed the stream habitats which favour riverine taxa.

ACKNOWLEDGMENTS

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Appendix 1. Results of the nutrients (Total Nitrogen and Total Phosphorus) and Chemical Oxygen Demand for Sites 1 to 5, June 1999 to June 2000.

	Date	TN (mg/L)	TP (mg/L)	COD (mg/L)
Site 1	Jun-99	1.6	0.14	52
Site 1	Aug-99	1.85	0.16	50
Site 1	Oct-99	1.75	0.17	48
Site 1	Nov-99	1.2	0.06	37
Site 1	Dec-99	2.5	0.22	79
Site 1	Feb-00	2.6	0.41	77
Site 1	Apr-00	1.6	0.26	50
Site 1	Jun-00	1.4	0.1	58
Site 2	Jun-99	1.25	0.09	24
Site 2	Aug-99	1.9	0.17	46
Site 2	Oct-99	1.75	0.17	56
Site 2	Nov-99	0.3	0	0
Site 2	Dec-99	0.7	0.03	13
Site 2	Feb-00	0.41	0	0
Site 2	Apr-00	0.16	0.03	0
Site 2	Jun-00	1.4	0.1	53
Site 3	Jun-99	2.05	0.04	39
Site 3	Aug-99	3.05	0.17	72
Site 3	Oct-99	2.1	0.19	47
Site 3	Nov-99	2	0.22	46
Site 3	Dec-99	1	0.06	19
Site 3	Feb-00	0.67	0.04	15
Site 3	Apr-00	1.6	0.04	39
Site 3	Jun-00	2.2	0.12	56
Site 4	Jun-99	2.05	0.1	75
Site 4	Aug-99	3.5	0.28	84
Site 4	Oct-99	2.45	0.14	71
Site 4	Nov-99	3.3	0.18	98
Site 4	Dec-99	4.2	0.23	91
Site 4	Feb-00	4.2	0.1	135
Site 4	Apr-00	3	0.13	57
Site 4	Jun-00	2.6	0.14	79
Site 5	Jun-99	1.3	0.08	10
Site 5	Aug-99	2.05	0.16	30
Site 5	Oct-99	1.35	0.11	0
Site 5	Nov-99	1.3	0.09	49
Site 5	Dec-99	2.6	0.21	78
Site 5	Feb-00	3.5	0.14	105
Site 5	Apr-00	1	0.1	7
Site 5	Jun-00	2	0.14	61

Appendix 2. Results of the physicochemical parameters (pH, conductivity and Dissolved Oxygen) from Sites 1 to 5, June 1999 to June 2000.

Site	Date	pH	Cond	Turb	DO	Temp	Salinity
Site 1	Jun-99	6.89	0.1742	104.6	6.564	10.76	0
Site 1	Aug-99	7.74	0.1178	114	8.658	9.32	0
Site 1	Oct-99	7.654	0.1042	248	8.286	15.66	0
Site 1	Nov-99	7.842	0.2158	21	4.86	15.94	0
Site 1	Dec-99	7.886	0.2412	70.4	2.748	21.44	0
Site 1	Feb-00	7.368	0.1954	37.2	2.89	17.86	0
Site 1	Apr-00	6.984	0.1642	28.2	2.538	16.12	0
Site 1	Jun-00	7.356	0.1144	93.6	9.71	8.02	0
Site 2	Jun-99	7.554	0.138	79.6	6.804	11.48	0
Site 2	Aug-99	7.65	0.1166	136	8.124	9.42	0
Site 2	Oct-99	7.828	0.0998	396.2	8.42	14.3	0
Site 2	Nov-99	7.998	0.0756	6	6.216	26.66	0
Site 2	Dec-99	7.702	0.0926	3.8	5.986	30.44	0
Site 2	Feb-00	7.282	0.072	7.4	5.54	28.72	0
Site 2	Apr-00	7.1	0.0796	25.6	5.788	24.32	0
Site 2	Jun-00	7.382	0.11	104.2	9.618	7.98	0
Site 3	Jun-99	8.012	1.0408	120.6	7.682	12.02	0.04
Site 3	Aug-99	8.23	0.62	138.2	8.286	11.3	0.02
Site 3	Oct-99	7.728	0.122	357.4	7.72	19.36	0
Site 3	Nov-99	7.88	0.2306	69.6	7.756	20.02	0
Site 3	Dec-99	8.09	0.2848	93.6	6.046	25.6	0.01
Site 3	Feb-00	6.736	0.2158	67.2	5.94	20.94	0
Site 3	Apr-00	7.302	1.756	15.2	6.268	14.84	0.08
Site 3	Jun-00	7.274	0.3294	30.4		8.02	0.002
Site 4	Jun-99	7.118	0.1164	125.6	5.96	12.52	0
Site 4	Aug-99	7.218	0.064	108.2	8.396	10.66	0
Site 4	Oct-99	7.58	0.1876	56	4.598	19.22	0
Site 4	Nov-99	7.314	0.2076	46.6	4.592	16.98	0.002
Site 4	Dec-99	8.05	0.245	103.6	2.668	19.06	0
Site 4	Feb-00	7.47	0.243	120.6	3.096	17.44	0.008
Site 4	Apr-00	7.122	0.1902	268	4.396	12.44	0.004
Site 4	Jun-00	7.166	0.086	65.8	9.278	9.04	0
Site 5	Jun-99	7.416	0.0932	176.4	8.49	12.24	0
Site 5	Aug-99	7.5	0.0782	139.2	9.006	11.46	0
Site 5	Oct-99	7.494	0.0838	154.4	7.58	15.78	0
Site 5	Nov-99	6.9802	0.0802	116.6	5.406	16.32	0
Site 5	Dec-99	7.394	0.1392	154.8	1.822	16.98	0
Site 5	Feb-00	6.876	0.1612	301.8	2.8	22.88	0
Site 5	Apr-00	6.716	0.0532	174.2	5.066	15.62	0
Site 5	Jun-00	7.036	0.0818	138.6	8.376	8	0.002

Appendix 3. Macroinvertebrate fauna list showing presence of species at Sites 1-5, from June 1999 to June 2000

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Hydrozoa	Hydridae	Hydra			*		
Turbellaria		*Temnocephala*	*			*	*
Turbellaria		*Craspedella spenceri*					*
Platyhelminthes	Dugesiidae	TC 0152		*	*		
Nemertea				*			
Nematoda			*	*	*	*	*
Oligochaeta			*	*	*	*	*
Hirudinea	Richardsonianidae	Richardsonianidae	*	*			*
Gastropoda	Lymnaeidae	*Austropeplea tomentosa*		*		*	
Gastropoda	Lymnaeidae	*Austropeplea lessoni*		*			
Gastropoda	Lymnaeidae	*Austropeplea* sp.		*		*	
Gastropoda	Ancylidae	*Ferrissia petterdi*	*	*	*	*	*
Gastropoda	Planorbidae	*Bayardella cosmeta*	*	*	*		
Gastropoda	Planorbidae	*Glyptophysa gibbosa*	*			*	*
Gastropoda	Planorbidae	*Glyptophysa* sp	*				
Gastropoda	Planorbidae	*Glyptophysa* TC 0164					*
Gastropoda	Planorbidae	*Isidorella* sp.	*	*			
Gastropoda	Planorbidae	Immature				*	
Gastropoda	Physidae	*Physa acuta*	*	*	*	*	*
Gastropoda		TC 0155	*	*	*	*	
Gastropoda	Planorb/Physidae		*				
Bivalvia	Sphaeridae	*Sphaerium*		*	*		
Bivalvia	Sphaeridae	TC 0132	*		*		
Bivalvia	Sphaeridae	immature			*		
Bivalvia	Sphae/Corbic	immature	*				
Acarina			*	*	*	*	*
Isopoda	Janiridae	Janiridae				*	
Isopoda	Oniscidae	*Haloniscus* sp.	*	*			
Isopoda	Oniscidae	*Haloniscus* TC 0153	*	*			
Decapoda	Atyidae	*Paratya australiensis*	*	*	*	*	*
Decapoda	Atyidae	immature	*				
Decapoda	Atyidae	*Caradina mccollochi*			*		
Decapoda	Parasticidae	*Cherax destructor*	*	*	*	*	*
Collembola			*	*	*	*	*
Ephemeroptera	Leptophlebiidae	*Atalophlebia* sp AV12	*		*		
Ephemeroptera	Leptophlebiidae	*Atalophlebia*	*		*	*	
Ephemeroptera	Leptophlebiidae		*	*	*	*	
Ephemeroptera	Baetidae	*Cloeon*	*	*	*		
Ephemeroptera	Baetidae	*Offadens* spMVsp4			*		
Ephemeroptera	Baetidae	*Offadens* sp7			*		
Ephemeroptera	Baetidae	*Offadens* sp.			*		
Ephemeroptera	Baetidae	*Centroptilum* sp.			*		
Ephemeroptera	Baetidae		*	*	*	*	*
Ephemeroptera	Caenidae	*Tasmanocoenis arcuata*			*		
Ephemeroptera	Caenidae	*Tasmanocoenis tillyardi*	*	*	*		
Ephemeroptera	Caenidae	*Tasmanocoenis rieki*			*		
Ephemeroptera	Caenidae	*Tasmanocoenis* spB			*		
Ephemeroptera	Caenidae	*Tasmanocoenis* spJ			*		

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Ephemeroptera	Caenidae	*Tasmanocoenis* sp L			*		
Ephemeroptera	Caenidae				*		
Ephemeroptera	Caenidae	*Tasmanocoenis*	*		*		*
Ephemeroptera	Caenidae	*Irpacaenis* sp.		*			
Ephemeroptera			*				
Odonata	Coenagrionidae	*Ischnura aurora*	*		*		*
Odonata	Coenagrionidae	*Ischnura heterosticta*	*	*	*		
Odonata	Coenagrionidae	*Ischnura* sp	*	*	*	*	*
Odonata	Coenagrionidae	immature	*	*			
Odonata	Isostictidae	*Rhadinosticta simplex*			*		
Odonata	Lestidae	*Austrolestes analis*	*			*	*
Odonata	Lestidae	*Austrolestes leda/analis*	*				*
Odonata	Lestidae	*Austrolestes*	*	*		*	*
Odonata	Aeshnidae	*Aeshna brevistyla*		*			
Odonata	Aeshnidae	immature		*			
Odonata	Gomphidae	*Austrogomphus cornutus*			*		
Odonata	Corduliidae	*Hemicordulia tau*	*	*	*		*
Odonata	Corduliidae	*Hemicordulia australiae*		*			
Odonata	Corduliidae	*Hemicordulia*	*	*			
Odonata	Synthemistidae	*Parasynthemis regina*	*				*
Odonata	Libellulidae	*Orthetrum caledonicum*		*			
Odonata	Anispotera immature		*				
Plecoptera	Gryptopterygidae	*Leptoperla*	*	*			
Plecoptera	Gryptopterygidae	*Dinotoperla*	*				
Plecoptera		immature		*			
Hemiptera	Notonectidae	*Anisops*	*	*	*	*	*
Hemiptera	Notonectidae	*Enithares*	*		*	*	*
Hemiptera	Corixidae	*Agraptocorixa*	*	*		*	*
Hemiptera	Corixidae	*Sigara*	*	*		*	
Hemiptera	Corixidae	*Agraptocorixa/Sigara*	*	*	*	*	*
Hemiptera	Corixidae	*Micronecta*	*	*	*	*	*
Hemiptera	Corixidae		*	*	*	*	*
Hemiptera	Hydrometridae	*Hydrometra*	*			*	*
Hemiptera	Veliidae	*Microvelia*	*			*	*
Hemiptera	Veliidae		*	*			*
Hemiptera	Mesoveliidae	*Mesovelia*					*
Hemiptera	Nepidae	*Ranatra dispar*	*			*	*
Neuroptera	Sisyridae	*Sisyra* TC 0001 FCP			*		
Neuroptera	Sisyridae	TC 0126	*				
Coleoptera	Carabidae (L)				*		
Coleoptera	Elmidae	*Coxelmis v.fasciata* (L)			*		
Coleoptera	Elmidae	*Coxelmis* (A)				*	
Coleoptera	Hydrophilidae	*Coelosoma* sp. (TC0212)		*			
Coleoptera	Hydrophilidae(A)	TC213	*				
Coleoptera	Hydrophilidae	*Hydrochus* (A)	*			*	
Coleoptera	Hydrophilidae	*Heleochares* (A)	*			*	*
Coleoptera	Hydrophilidae	*Enochrus* (A)			*	*	*
Coleoptera	Hydrophilidae	*Berosus* (L)	*	*		*	*
Coleoptera	Hydrophilidae	*Berosus* (A)	*	*	*	*	*
Coleoptera	Hydrophilidae	*Laccophilus* (A)			*		
Coleoptera	Hydrophilidae	*Paracymus* (A)			*	*	*

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Coleoptera	Hydrophilidae (L)		*	*	*	*	*
Coleoptera	Hydrophilidae	*Sternolophus* TC210				*	
Coleoptera	Hydrophilidae	TC0211 (A)	*				
Coleoptera	Gyrinidae (L)				*		
Coleoptera	Hydraenidae	*Hydraena* (L)	*	*	*	*	
Coleoptera	Hydraenidae	*Hydraena* (A)	*	*	*	*	*
Coleoptera	Hydraenidae	Octhebiinae(A)		*	*	*	*
Coleoptera	Staphylinidae (L)		*				
Coleoptera	Scirtidae (L)		*		*	*	
Coleoptera	Carabidae (L)				*		
Coleoptera	Dytiscidae	*Allodesus* (A)	*	*	*		*
Coleoptera	Dytiscidae	*Liodessus* (A)				*	*
Coleoptera	Dytiscidae	*Rhantus* (L)	*	*			
Coleoptera	Dytiscidae	*Rhantus* (A)	*	*		*	*
Coleoptera	Dytiscidae	*Antiporus* (L)	*				
Coleoptera	Dytiscidae	*Antiporus femoralis* (A)		*		*	
Coleoptera	Dytiscidae	*Necterosoma regulare* (A)	*			*	*
Coleoptera	Dytiscidae	*Necterosoma* (L)	*	*			*
Coleoptera	Dytiscidae	*Necterosoma* (A)	*			*	*
Coleoptera	Dytiscidae	*Paroster/Necterosoma* (L)		*			
Coleoptera	Dytiscidae	*Chostonectes* (L)	*		*	*	*
Coleoptera	Dytiscidae	*Chostonectes* (A)	*			*	*
Coleoptera	Dytiscidae	*Chostonectes gigas* (A)	*			*	
Coleoptera	Dytiscidae	*Copelatus* (L)	*				
Coleoptera	Dytiscidae	*Megaporus howitti* (A)	*				
Coleoptera	Dytiscidae	*Lancetes* sp(A)		*			
Coleoptera	Dytiscidae	*Lancetes* sp(L)	*		*		
Coleoptera	Dytiscidae	*Eretes* sp (A)	*				
Coleoptera	Dytiscidae	damaged (L)	*		*		
Coleoptera	Dytiscidae	Unknown #1	*		*		
Coleoptera	Dytiscidae	Unknown (A)		*			*
Coleoptera	Curculionidae				*		
Coleoptera		Unknown					*
Diptera	Simulidae	*Simulium nicholsoni*			*		
Diptera	Simulidae	*Simulium ornatipes*	*	*	*		
Diptera	Simulidae	*Austrosimulium banerofti*			*		
Diptera	Simulidae	*Austrosimulium furiosum*	*		*		
Diptera	Simulidae	Immature		*	*		
Diptera	Simulidae	Simulid (P)	*		*		
Diptera	Tipulidae	TC 046	*	*	*		
Diptera	Tipulidae	TC 0081	*	*	*		*
Diptera	Tipulidae	TC 0086	*	*	*		*
Diptera	Tipulidae	TC 0125	*				
Diptera	Tipulidae	TC 0143	*				*
Diptera	Tipulidae	cf TC 046	*	*	*		
Diptera	Tipulidae	TC 0156		*			
Diptera	Tipulidae	TC 0167		*			
Diptera	Tipulidae	TC 0168		*			
Diptera	Tipulidae	immatures		*	*		
Diptera	Stratiomyidae	Stratiomyidae	*		*		
Diptera	Stratiomyidae	TC0136		*			*

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Diptera	Empididae	Empididae sp2			*		
Diptera	Empididae	Empididae sp3			*		
Diptera	Empididae	TC0013			*		
Diptera	Empididae	TC 0012 sp2 NMV		*	*		
Diptera	Empididae	TC 0135			*		
Diptera	Empididae	Empididae pupae			*		
Diptera	Ceratopogonidae	*Alluardingi* nmv sp3		*			
Diptera	Ceratopogonidae	*Dasyhelea* TC 014		*	*		
Diptera	Ceratopogonidae	*Atrichopogon* TC 0041	*	*	*		
Diptera	Ceratopogonidae	TC 0002 fcp			*		
Diptera	Ceratopogonidae	TC 0003 fcp			*		
Diptera	Ceratopogonidae	TC 0004 fcp			*		
Diptera	Ceratopogonidae	TC 0005 fcp			*		
Diptera	Ceratopogonidae	TC012		*			
Diptera	Ceratopogonidae	TC046	*	*			
Diptera	Ceratopogonidae	TC050			*		
Diptera	Ceratopogonidae	TC 080	*		*	*	
Diptera	Ceratopogonidae	TC 081	*	*			
Diptera	Ceratopogonidae	TC 0089					*
Diptera	Ceratopogonidae	TC0090	*				
Diptera	Ceratopogonidae	TC 0119		*			
Diptera	Ceratopogonidae	TC 0127	*	*	*	*	*
Diptera	Ceratopogonidae	TC 0128			*		*
Diptera	Ceratopogonidae	TC 0129	*				
Diptera	Ceratopogonidae	TC 0131	*	*	*	*	*
Diptera	Ceratopogonidae	TC 0138	*	*	*	*	*
Diptera	Ceratopogonidae	TC 0143					*
Diptera	Ceratopogonidae	TC 0160			*		
Diptera	Ceratopogonidae	TC 0161			*		
Diptera	Ceratopogonidae (P)		*		*		*
Diptera	Psychodidae	TC 0129	*	*	*	*	*
Diptera	Sciomyzidae	TC 0145		*			
Diptera	Sciomyzidae	TC 0146	*				
Diptera	Stratiomyidae			*			
Diptera	Stratiomyidae	TC 0136	*	*	*		*
Diptera	Syrphidae			*			
Diptera	Culicidae	Culicinae	*				
Diptera	Culicidae	TC 0140	*			*	
Diptera	Culicidae	TC 0144	*	*	*	*	*
Diptera	Culicidae	TC 0163	*	*			*
Diptera	Culicidae (P)		*			*	*
Diptera	Chironomidae (L)		*	*	*	*	*
Diptera	Chironomidae (P)		*	*	*	*	
Diptera	Muscidae	TC 0134	*	*	*		
Diptera	Muscidae	TC 0137	*	*	*	*	
Diptera	Muscidae	TC 0139		*			
Diptera	Muscidae	TC 142	*	*			
Diptera	Muscidae	TC 145	*	*	*	*	*
Diptera	Muscidae	TC 147		*			
Diptera	Muscidae	TC 0151		*			*
Diptera	Muscidae	TC 0154	*	*			

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Diptera	Muscidae	TC 0157	*	*	*	*	
Diptera	Muscidae	TC 0159			*		
Diptera	Muscidae	TC 0165		*			*
Diptera	Muscidae (P)			*			
Diptera	Muscidae				*		*
Diptera	Muscidae/Ephrididae	TC 098	*			*	*
Diptera		TC 0145	*		*		
Diptera		TC 0149	*				
Diptera		TC 0151		*	*		
Diptera		TC 0161			*		
Diptera		TC 0165				*	
Diptera		TC 0166				*	
Diptera	Diptera (P)	TC 0147	*				
Diptera	Diptera (P)	TC 0148	*				
Diptera	Diptera (P)		*	*	*		*
Trichoptera	Ecnomidae	*Ecnomus continentalis*		*	*	*	
Trichoptera	Ecnomidae	*Ecnomus cygnitus*			*		
Trichoptera	Ecnomidae	*Ecnomus pansus*		*	*		
Trichoptera	Ecnomidae	*Ecnomus turgidus*			*	*	
Trichoptera	Ecnomidae	*Ecnomus*		*	*		
Trichoptera	Hydropsychidae	*Cheumatopsyche*		*	*		
Trichoptera	Hydropsychidae	*Cheumatopsyche* sp AV 2			*		
Trichoptera	Hydropsychidae	*Cheumatopsyche* sp AV 4			*		
Trichoptera	Hydrobiosidae	*Ulmerochorema onychion*		*			
Trichoptera	Leptoceridae	*Triplectides australis*	*	*	*	*	
Trichoptera	Leptoceridae	*Triplectides australicus*	*		*		
Trichoptera	Leptoceridae	*Triplectides ciuskus*			*		
Trichoptera	Leptoceridae	*Triplectides*	*		*	*	
Trichoptera	Leptoceridae	*Oecetis*			*		
Trichoptera	Leptoceridae	immature	*		*		
Trichoptera	Leptoceridae	Leptoceridae pupae			*		
Trichoptera	Hydroptilidae	*Hellyethira simplex*		*	*		
Trichoptera	Hydroptilidae	*Helyethira*		*	*		
Lepidoptera	Pyralidae	Nymphulinae			*		

Appendix 4. Relative proportions of macroinvertebrate taxa from Sites 1 to 5 from June 1999 to June 2000.

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Hydrozoa	Hydridae	Hydra	0.00	0.00	0.02	0.00	0.00
Turbellaria		*Temnocephala*	0.02	0.00	0.00	0.01	0.09
Turbellaria		*Craspedella spenceri*	0.00	0.00	0.00	0.00	0.12
Platyhelminthes	Dugesiidae	TC 0152	0.00	0.04	0.01	0.00	0.00
Nemertea			0.00	0.07	0.00	0.00	0.00
Nematoda			1.06	0.51	1.55	0.93	1.30
Oligochaeta			34.81	58.55	16.96	21.93	8.79
Hirudinea	Richardsonianidae	Richardsonianidae	0.06	0.04	0.00	0.00	0.01
Gastropoda	Lymnaeidae	*Austropeplea tomentosa*	0.00	0.95	0.00	0.10	0.00
Gastropoda	Lymnaeidae	*Austropeplea lessoni*	0.00	0.04	0.00	0.00	0.00
Gastropoda	Lymnaeidae	*Austropeplea* sp.	0.00	0.06	0.00	0.10	0.00
Gastropoda	Ancylidae	*Ferrissia petterdi*	0.12	0.47	0.37	0.38	0.19
Gastropoda	Planorbidae	*Bayardella cosmeta*	0.24	0.04	0.02	0.00	0.00
Gastropoda	Planorbidae	*Glyptophysa gibbosa*	0.06	0.00	0.00	0.31	0.01
Gastropoda	Planorbidae	*Glyptophysa* sp	0.01	0.00	0.00	0.00	0.00
Gastropoda	Planorbidae	*Glyptophysa* TC 0164	0.00	0.00	0.00	0.00	0.01
Gastropoda	Planorbidae	*Isidorella* sp.	0.02	0.08	0.00	0.00	0.00
Gastropoda	Planorbidae	Immature	0.00	0.00	0.00	0.02	0.00
Gastropoda	Physidae	*Physa acuta*	0.47	4.03	0.67	0.15	0.29
Gastropoda		TC 0155	0.02	0.07	0.02	0.15	0.00
Gastropoda	Planorb/Physidae		0.01	0.00	0.00	0.00	0.00
Bivalvia	Sphaeridae	*Sphaerium*	0.00	0.04	0.02	0.00	0.00
Bivalvia	Sphaeridae	TC 0132	0.01	0.00	0.14	0.00	0.00
Bivalvia	Sphaeridae	immature	0.00	0.00	1.42	0.00	0.00
Bivalvia	Sphae/Corbic	immature	0.01	0.00	0.00	0.00	0.00
Acarina			0.16	0.32	0.22	1.04	0.34
Isopoda	Janiridae	Janiridae	0.00	0.00	0.00	0.10	0.00
Isopoda	Oniscidae	*Haloniscus* sp.	0.01	0.10	0.00	0.00	0.00
Isopoda	Oniscidae	*Haloniscus* TC 0153	0.01	0.16	0.00	0.00	0.00
Decapoda	Atyidae	*Paratya australiensis*	5.86	1.46	0.12	0.01	0.02
Decapoda	Atyidae	immature	0.65	0.00	0.00	0.00	0.00
Decapoda	Atyidae	*Caradina mccollochi*	0.00	0.00	0.06	0.00	0.00
Decapoda	Parasticidae	*Cherax destructor*	0.46	0.37	0.06	0.06	0.45
Collembola			0.43	0.14	0.91	4.60	0.09
Ephemeroptera	Leptophlebiidae	*Atalophlebia* sp AV12	0.01	0.00	0.00	0.00	0.00
Ephemeroptera	Leptophlebiidae	*Atalophlebia*	0.01	0.00	0.05	1.04	0.00
Ephemeroptera	Leptophlebiidae		0.05	0.03	0.08	0.97	0.00
Ephemeroptera	Baetidae	*Cloeon*	0.33	0.09	0.31	0.00	0.00
Ephemeroptera	Baetidae	*Offadens* spMVsp4	0.00	0.00	0.15	0.00	0.00
Ephemeroptera	Baetidae	*Offadens* sp7	0.00	0.00	0.02	0.00	0.00
Ephemeroptera	Baetidae	*Offadens* sp.	0.00	0.00	0.40	0.00	0.00
Ephemeroptera	Baetidae	*Centroptilum* sp.	0.00	0.00	0.14	0.00	0.00
Ephemeroptera	Baetidae		0.34	0.09	0.61	0.01	0.02
Ephemeroptera	Caenidae	*Tasmanocoenis arcuata*	0.00	0.00	0.01	0.00	0.00
Ephemeroptera	Caenidae	*Tasmanocoenis tillyardi*	0.03	0.08	1.93	0.00	0.00
Ephemeroptera	Caenidae	*Tasmanocoenis rieki*	0.00	0.00	0.08	0.00	0.00
Ephemeroptera	Caenidae	*Tasmanocoenis* spB	0.00	0.00	1.33	0.00	0.00
Ephemeroptera	Caenidae	*Tasmanocoenis* spJ	0.00	0.00	2.19	0.00	0.00

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Ephemeroptera	Caenidae	*Tasmanocoenis* sp L	0.00	0.00	0.05	0.00	0.00
Ephemeroptera	Caenidae		0.00	0.00	0.17	0.00	0.00
Ephemeroptera	Caenidae	*Tasmanocoenis*	0.04	0.00	3.28	0.00	0.01
Ephemeroptera	Caenidae	*Irpacaenis* sp.	0.00	0.01	0.00	0.00	0.00
Ephemeroptera			0.01	0.00	0.00	0.00	0.00
Odonata	Coenagrionidae	*Ischnura aurora*	0.08	0.00	0.01	0.00	0.01
Odonata	Coenagrionidae	*Ischnura heterosticta*	0.02	0.33	0.14	0.00	0.00
Odonata	Coenagrionidae	*Ischnura* sp	0.16	0.85	0.22	0.00	0.22
Odonata	Coenagrionidae	immature	0.01	0.42	0.00	0.00	0.00
Odonata	Isostictidae	*Rhadinosticta simplex*	0.00	0.00	0.12	0.00	0.00
Odonata	Lestidae	*Austrolestes analis*	0.06	0.00	0.00	0.06	0.29
Odonata	Lestidae	*Austrolestes leda/analis*	0.20	0.00	0.00	0.00	0.01
Odonata	Lestidae	*Austrolestes*	0.18	0.03	0.00	0.18	0.20
Odonata	Aeshnidae	*Aeshna brevistyla*	0.00	0.08	0.00	0.00	0.00
Odonata	Aeshnidae	immature	0.00	0.05	0.00	0.00	0.00
Odonata	Gomphidae	*Austrogomphus cornutus*	0.00	0.00	0.02	0.00	0.00
Odonata	Corduliidae	*Hemicordulia tau*	0.02	0.26	0.03	0.00	0.01
Odonata	Corduliidae	*Hemicordulia australiae*	0.00	0.48	0.00	0.00	0.00
Odonata	Corduliidae	*Hemicordulia*	0.01	0.09	0.00	0.00	0.00
Odonata	Synthemistidae	*Parasynthemis regina*	0.14	0.00	0.00	0.00	0.24
Odonata	Libellulidae	*Orthetrum caledonicum*	0.00	0.73	0.00	0.00	0.00
Odonata	Anispotera immature		0.00	0.00	0.00	0.00	0.00
Plecoptera	Gryptopterygidae	*Leptoperla*	0.01	0.15	0.00	0.00	0.00
Plecoptera	Gryptopterygidae	*Dinotoperla*	0.03	0.00	0.00	0.00	0.00
Plecoptera		immature	0.00	0.13	0.00	0.00	0.00
Hemiptera	Notonectidae	*Anisops*	2.35	2.10	0.01	4.22	4.14
Hemiptera	Notonectidae	*Enithares*	0.35	0.00	0.01	0.07	0.33
Hemiptera	Corixidae	*Agraptocorixa*	0.14	0.03	0.00	0.09	0.03
Hemiptera	Corixidae	*Sigara*	0.06	0.03	0.00	0.11	0.00
Hemiptera	Corixidae	*Agraptocorixa/Sigara*	0.12	0.23	0.00	0.07	0.05
Hemiptera	Corixidae	*Micronecta*	1.39	0.59	8.35	0.30	0.27
Hemiptera	Corixidae		0.07	0.01	0.02	0.04	0.02
Hemiptera	Hydrometridae	*Hydrometra*	0.05	0.00	0.00	0.01	0.02
Hemiptera	Veliidae	*Microvelia*	0.01	0.00	0.00	0.18	0.51
Hemiptera	Veliidae		0.10	0.13	0.00	0.00	0.01
Hemiptera	Mesoveliidae	*Mesovelia*	0.00	0.00	0.00	0.00	0.09
Hemiptera	Nepidae	*Ranatra dispar*	0.01	0.00	0.00	0.00	0.11
Neuroptera	Sisyridae	*Sisyra* TC 0001 FCP	0.00	0.00	0.01	0.00	0.00
Neuroptera	Sisyridae	TC 0126	0.00	0.00	0.00	0.00	0.00
Coleoptera	Carabidae (L)		0.00	0.00	0.02	0.00	0.00
Coleoptera	Elmidae	*Coxelmis v.fasciata* (L)	0.00	0.00	0.03	0.00	0.00
Coleoptera	Elmidae	*Coxelmis* (A)	0.00	0.00	0.00	0.15	0.00
Coleoptera	Hydrophilidae	*Coelosoma* sp. (TC0212)	0.00	0.02	0.00	0.00	0.00
Coleoptera	Hydrophilidae(A)	TC213	0.01	0.00	0.00	0.00	0.00
Coleoptera	Hydrophilidae	*Hydrochus* (A)	0.06	0.00	0.00	0.01	0.00
Coleoptera	Hydrophilidae	*Heleochares* (A)	0.01	0.00	0.00	0.00	0.01
Coleoptera	Hydrophilidae	*Enochrus* (A)	0.00	0.00	0.01	0.01	0.05
Coleoptera	Hydrophilidae	*Berosus* (L)	1.25	0.09	0.00	0.12	0.03
Coleoptera	Hydrophilidae	*Berosus* (A)	1.66	0.04	0.01	0.16	0.02
Coleoptera	Hydrophilidae	*Laccophilus* (A)	0.00	0.00	0.01	0.00	0.00
Coleoptera	Hydrophilidae	*Paracymus* (A)	0.00	0.00	0.00	0.09	0.09

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Coleoptera	Hydrophilidae (L)		0.02	0.03	0.02	0.14	0.04
Coleoptera	Hydrophilidae	*Sternolophus* TC210	0.00	0.00	0.00	0.01	0.00
Coleoptera	Hydrophilidae	TC0211 (A)	0.04	0.00	0.00	0.00	0.00
Coleoptera	Gyrinidae (L)		0.00	0.00	0.27	0.00	0.00
Coleoptera	Hydraenidae	*Hydraena* (L)	0.03	0.12	0.07	0.03	0.00
Coleoptera	Hydraenidae	*Hydraena* (A)	0.19	0.04	0.05	0.61	0.21
Coleoptera	Hydraenidae	Octhebiinae(A)	0.00	0.05	0.00	0.01	0.14
Coleoptera	Staphylinidae (L)		0.01	0.00	0.00	0.00	0.00
Coleoptera	Scirtidae (L)		0.31	0.00	0.01	0.15	0.00
Coleoptera	Carabidae (L)		0.00	0.00	0.01	0.00	0.00
Coleoptera	Dytiscidae	*Allodesus* (A)	0.05	0.14	0.01	0.00	0.02
Coleoptera	Dytiscidae	*Liodessus* (A)	0.00	0.00	0.00	0.02	0.01
Coleoptera	Dytiscidae	*Rhantus* (L)	0.01	0.01	0.00	0.00	0.00
Coleoptera	Dytiscidae	*Rhantus* (A)	0.05	0.03	0.00	0.13	0.13
Coleoptera	Dytiscidae	*Antiporus* (L)	0.02	0.00	0.00	0.00	0.00
Coleoptera	Dytiscidae	*Antiporus femoralis* (A)	0.00	0.03	0.00	0.02	0.00
Coleoptera	Dytiscidae	*Necterosoma regulare* (A)	0.01	0.00	0.00	0.03	0.04
Coleoptera	Dytiscidae	*Necterosoma* (L)	0.11	0.10	0.00	0.00	0.02
Coleoptera	Dytiscidae	*Necterosoma* (A)	0.21	0.00	0.00	0.38	0.13
Coleoptera	Dytiscidae	*Paroster/Necterosoma* (L)	0.00	0.03	0.00	0.00	0.00
Coleoptera	Dytiscidae	*Chostonectes* (L)	0.30	0.00	0.01	0.17	0.46
Coleoptera	Dytiscidae	*Chostonectes* (A)	0.30	0.00	0.00	0.35	1.33
Coleoptera	Dytiscidae	*Chostonectes gigas* (A)	0.06	0.00	0.00	0.04	0.00
Coleoptera	Dytiscidae	*Copelatus* (L)	0.04	0.00	0.00	0.00	0.00
Coleoptera	Dytiscidae	*Megaporus howitti* (A)	0.01	0.00	0.00	0.00	0.00
Coleoptera	Dytiscidae	*Lancetes* sp(A)	0.00	0.03	0.00	0.00	0.00
Coleoptera	Dytiscidae	*Lancetes* sp(L)	0.01	0.00	0.02	0.00	0.00
Coleoptera	Dytiscidae	*Eretes* sp (A)	0.02	0.00	0.00	0.00	0.00
Coleoptera	Dytiscidae	damaged (L)	0.06	0.00	0.00	0.00	0.00
Coleoptera	Dytiscidae	Unknown #1	0.05	0.00	0.01	0.00	0.00
Coleoptera	Dytiscidae	Unknown (A)	0.00	0.14	0.00	0.00	0.03
Coleoptera	Curculionidae		0.00	0.00	0.01	0.00	0.00
Coleoptera		Unknown	0.00	0.00	0.00	0.00	0.01
Diptera	Simulidae	*Simulium nicholsoni*	0.00	0.00	0.24	0.00	0.00
Diptera	Simulidae	*Simulium ornatipes*	0.03	0.12	5.06	0.00	0.00
Diptera	Simulidae	*Austrosimulium banerofti*	0.00	0.00	0.09	0.00	0.00
Diptera	Simulidae	*Austrosimulium furiosum*	0.04	0.00	0.26	0.00	0.00
Diptera	Simulidae	Immature	0.00	0.14	3.07	0.00	0.00
Diptera	Simulidae	Simulid (P)	0.01	0.00	0.01	0.00	0.00
Diptera	Tipulidae	TC 046	0.25	0.37	0.11	0.00	0.00
Diptera	Tipulidae	TC 0081	0.08	0.05	0.89	0.00	0.06
Diptera	Tipulidae	TC 0086	0.02	0.25	0.51	0.00	0.04
Diptera	Tipulidae	TC 0125	0.01	0.00	0.00	0.00	0.00
Diptera	Tipulidae	TC 0143	0.13	0.00	0.00	0.00	0.02
Diptera	Tipulidae	cf TC 046	0.01	0.08	0.00	0.00	0.00
Diptera	Tipulidae	TC 0156	0.00	0.05	0.00	0.00	0.00
Diptera	Tipulidae	TC 0167	0.00	0.04	0.00	0.00	0.00
Diptera	Tipulidae	TC 0168	0.00	0.01	0.00	0.00	0.00
Diptera	Tipulidae	immatures	0.00	0.03	0.04	0.00	0.00
Diptera	Stratiomyidae	Stratiomyidae	0.04	0.00	0.01	0.00	0.00
Diptera	Stratiomyidae	TC0136	0.00	0.05	0.00	0.00	0.01

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Diptera	Empididae	Empididae sp2	0.00	0.00	0.03	0.00	0.00
Diptera	Empididae	Empididae sp3	0.00	0.00	0.01	0.00	0.00
Diptera	Empididae	TC0013	0.00	0.00	0.02	0.00	0.00
Diptera	Empididae	TC 0012 sp2 NMV	0.00	0.01	0.06	0.00	0.00
Diptera	Empididae	TC 0135	0.00	0.00	0.33	0.00	0.00
Diptera	Empididae	Empididae pupae	0.00	0.00	0.02	0.00	0.00
Diptera	Ceratopogonidae	*Alluardingi* nmv sp3	0.00	0.04	0.00	0.00	0.00
Diptera	Ceratopogonidae	*Dasyhelea* TC 014	0.00	0.04	0.01	0.00	0.00
Diptera	Ceratopogonidae	*Atrichopogon* TC 0041	0.02	0.03	0.01	0.00	0.00
Diptera	Ceratopogonidae	TC 0002 fcp	0.00	0.00	0.01	0.00	0.00
Diptera	Ceratopogonidae	TC 0003 fcp	0.00	0.00	0.01	0.00	0.00
Diptera	Ceratopogonidae	TC 0004 fcp	0.00	0.00	0.01	0.00	0.00
Diptera	Ceratopogonidae	TC 0005 fcp	0.00	0.00	0.01	0.00	0.00
Diptera	Ceratopogonidae	TC012	0.00	0.08	0.00	0.00	0.00
Diptera	Ceratopogonidae	TC046	0.01	0.03	0.00	0.00	0.00
Diptera	Ceratopogonidae	TC050	0.00	0.00	0.00	0.00	0.00
Diptera	Ceratopogonidae	TC 080	0.03	0.00	0.02	0.47	0.00
Diptera	Ceratopogonidae	TC 081	1.93	0.03	0.00	0.00	0.00
Diptera	Ceratopogonidae	TC 0089	0.00	0.00	0.00	0.00	0.02
Diptera	Ceratopogonidae	TC0090	0.04	0.00	0.00	0.00	0.00
Diptera	Ceratopogonidae	TC 0119	0.00	0.03	0.00	0.00	0.00
Diptera	Ceratopogonidae	TC 0127	0.30	0.16	0.33	0.02	2.40
Diptera	Ceratopogonidae	TC 0128	0.00	0.00	0.03	0.00	0.02
Diptera	Ceratopogonidae	TC 0129	0.00	0.00	0.00	0.00	0.00
Diptera	Ceratopogonidae	TC 0131	0.34	0.16	1.04	0.01	0.12
Diptera	Ceratopogonidae	TC 0138	0.02	0.21	0.13	7.63	0.02
Diptera	Ceratopogonidae	TC 0143	0.00	0.00	0.00	0.00	0.09
Diptera	Ceratopogonidae	TC 0160	0.00	0.00	0.01	0.00	0.00
Diptera	Ceratopogonidae	TC 0161	0.00	0.00	0.01	0.00	0.00
Diptera	Ceratopogonidae (P)		0.00	0.00	0.00	0.00	0.02
Diptera	Psychodidae	TC 0129	0.28	0.50	0.13	1.88	0.08
Diptera	Sciomyzidae	TC 0145	0.00	0.05	0.00	0.00	0.00
Diptera	Sciomyzidae	TC 0146	0.01	0.00	0.00	0.00	0.00
Diptera	Stratiomyidae		0.00	0.03	0.00	0.00	0.00
Diptera	Stratiomyidae	TC 0136	0.05	0.15	0.04	0.00	0.01
Diptera	Syrphidae		0.00	0.03	0.00	0.00	0.00
Diptera	Culicidae	Culicinae	0.06	0.00	0.00	0.00	0.00
Diptera	Culicidae	TC 0140	0.12	0.00	0.00	0.02	0.00
Diptera	Culicidae	TC 0144	0.38	0.25	0.01	3.74	2.67
Diptera	Culicidae	TC 0163	0.01	0.03	0.00	0.00	0.03
Diptera	Culicidae (P)		0.01	0.00	0.00	0.37	0.13
Diptera	Chironomidae (L)		38.95	19.91	30.61	38.64	72.57
Diptera	Chironomidae (P)		0.31	0.18	0.57	0.01	0.00
Diptera	Muscidae	TC 0134	0.07	0.01	0.01	0.00	0.00
Diptera	Muscidae	TC 0137	0.07	0.09	0.01	6.13	0.00
Diptera	Muscidae	TC 0139	0.00	0.13	0.00	0.00	0.00
Diptera	Muscidae	TC 142	0.00	0.05	0.00	0.00	0.00
Diptera	Muscidae	TC 145	0.11	0.04	0.07	0.28	0.05
Diptera	Muscidae	TC 147	0.00	0.05	0.00	0.00	0.00
Diptera	Muscidae	TC 0151	0.00	0.13	0.00	0.00	0.03
Diptera	Muscidae	TC 0154	0.01	0.10	0.00	0.00	0.00

Order	Family	Genus species	Site 1	Site 2	Site 3	Site 4	Site 5
Diptera	Muscidae	TC 0157	0.01	0.07	0.00	0.10	0.00
Diptera	Muscidae	TC 0159	0.00	0.00	0.03	0.00	0.00
Diptera	Muscidae	TC 0165	0.00	0.03	0.00	0.00	0.02
Diptera	Muscidae (P)		0.00	0.05	0.00	0.00	0.00
Diptera	Muscidae		0.00	0.00	0.00	0.00	0.01
Diptera	Muscidae/Ephrididae	TC 098	0.02	0.00	0.00	0.04	0.03
Diptera		TC 0145	0.10	0.00	0.01	0.00	0.00
Diptera		TC 0149	0.02	0.00	0.00	0.00	0.00
Diptera		TC 0151	0.00	0.03	0.01	0.00	0.00
Diptera		TC 0161	0.00	0.00	0.01	0.00	0.00
Diptera		TC 0165	0.00	0.00	0.00	0.61	0.00
Diptera		TC 0166	0.00	0.00	0.00	0.15	0.00
Diptera	Diptera (P)	TC 0147	0.02	0.00	0.00	0.00	0.00
Diptera	Diptera (P)	TC 0148	0.01	0.00	0.00	0.00	0.00
Diptera	Diptera (P)		0.14	0.19	0.34	0.00	0.53
Trichoptera	Ecnomidae	*Ecnomus continentalis*	0.00	0.02	0.29	0.01	0.00
Trichoptera	Ecnomidae	*Ecnomus cygnitus*	0.00	0.00	1.41	0.00	0.00
Trichoptera	Ecnomidae	*Ecnomus pansus*	0.00	0.02	0.23	0.00	0.00
Trichoptera	Ecnomidae	*Ecnomus turgidus*	0.00	0.00	0.81	0.03	0.00
Trichoptera	Ecnomidae	*Ecnomus*	0.00	0.06	3.10	0.00	0.00
Trichoptera	Hydropsychidae	*Cheumatopsyche*	0.00	0.06	4.88	0.00	0.00
Trichoptera	Hydropsychidae	*Cheumatopsyche* sp AV 2	0.00	0.00	1.25	0.00	0.00
Trichoptera	Hydropsychidae	*Cheumatopsyche* sp AV 4	0.00	0.00	0.27	0.00	0.00
Trichoptera	Hydrobiosidae	*Ulmerochorema onychion*	0.00	0.01	0.00	0.00	0.00
Trichoptera	Leptoceridae	*Triplectides australis*	0.04	0.06	0.06	0.09	0.00
Trichoptera	Leptoceridae	*Triplectides australicus*	0.02	0.00	0.19	0.00	0.00
Trichoptera	Leptoceridae	*Triplectides ciuskus*	0.00	0.00	0.04	0.00	0.00
Trichoptera	Leptoceridae	*Triplectides*	0.03	0.00	0.09	0.00	0.00
Trichoptera	Leptoceridae	*Oecetis*	0.00	0.00	0.06	0.00	0.00
Trichoptera	Leptoceridae	immature	0.04	0.00	0.03	0.00	0.00
Trichoptera	Leptoceridae	Leptoceridae pupae	0.00	0.00	0.01	0.00	0.00
Trichoptera	Hydroptilidae	*Hellyethira simplex*	0.00	0.01	0.03	0.00	0.00
Trichoptera	Hydroptilidae	*Helyethira*	0.00	0.03	0.01	0.00	0.00
Lepidoptera	Pyralidae	Nymphulinae	0.00	0.00	0.01	0.00	0.00

Appendix 5. Microinvertebrate fauna list (Presence*) from Sites 1 to 5, June 1999 to June 2000.

Taxa		Site 1	Site 2	Site 3	Site 4	Site 5
Rotifera	*Asplanchna brightwelli*	*	*	*	*	
	Asplanchna girodi		*	*		
	Asplanchna sieboldi				*	
	Anuraeopsis	*	*	*	*	*
	Bdelloid	*	*	*	*	*
	Brachionus angularis	*	*	*		
	Brachionus bidentatus					*
	Brachionus budapestinensis				*	
	Brachionus calyciflorus					*
	Brachionus lyratus	*	*		*	
	Brachionus leydigii					*
	Brachionus quadridentatus	*	*	*	*	*
	Cephalodella	*	*	*	*	*
	Cephalodella forficula	*	*	*	*	
	Colurella	*	*	*		*
	Conochilus	*	*			
	Dicranophorus			*	*	*
	Encentrum	*		*	*	*
	Epiphanes	*	*	*	*	*
	Euchlanis	*	*		*	
	Filinia	*	*	*	*	*
	Gastropus	*	*	*	*	
	Hexarthra		*			
	Keratella australis	*	*	*	*	*
	Keratella cochlearis	*	*	*	*	*
	Keratella procurva	*	*	*	*	*
	Keratella slacki	*	*	*	*	*
	Keratella tropica	*				
	Itura				*	
	Lecane	*	*	*	*	*
	Lepadella	*	*		*	*
	Lindia		*	*	*	*
	Lophocharis	*			*	
	Monommata	*	*			
	Monostyla	*				*
	Monostyla bulla		*			*
	Monostyla hamata	*	*		*	
	Notholca squamata		*			
	Polyarthra	*	*	*	*	*
	Pompholyx	*	*	*		*
	Platinonus patulus					*
	Synchaeta	*	*	*	*	*
	Taphrocampa cf selenura		*			
	Testudinella patina	*	*			*
	Trichotria tetractis	*	*		*	*
	Trichocerca rattus	*	*			
	Trichocerca tigris					*
	Trichocerca	*				

Taxa		Site 1	Site 2	Site 3	Site 4	Site 5
	Trichocerca similis	*	*	*	*	*
	Trichocerca similis	*	*	*	*	*
	Australocyclops	*	*			
Copepoda	*Boeckella*	*				
	Boeckella fluvialis	*	*	*	*	*
	calanoid copepodite	*	*	*	*	*
	Calamoecia	*	*	*		
	Calamoecia lucasi	*	*			
	Cyclopoid	*		*		
	cyclopoid copepodite	*	*	*	*	*
	Eucyclops				*	
	Harpacticoid	*	*			
	Macrocyclops	*				
	Mixocyclops				*	
	Mesocyclops					*
	nauplii	*	*	*	*	*
Ostracoda	Ostracod	*	*	*	*	*
Cladocera	*Alona*		*	*	*	*
	Bosmina meridionalis	*	*	*		*
	Ceriodaphnia	*	*		*	*
	Chydorid					*
	Chydorus	*	*	*	*	*
	Daphnia carinata	*	*		*	
	Daphnia cephalata	*				
	Daphnia lumholtzi		*			
	Ilyocyrptus		*			
	Macrothrix	*				
	Moina	*	*		*	*
	Scapholeberis kingii	*	*		*	
	Simocephalus	*	*			*
	Bosminopsis dietersi				*	
Testate amoeba	*Arcella*	*	*	*	*	*
	Difflugia	*	*	*	*	*
	Difflugia corona	*			*	*
	Difflugia varians	*	*		*	*

Appendix 6. Relative proportions of microinvertebrate taxa from Sites 1 to 5 from June 1999 to June 2000.

Taxa		Site 1	Site 2	Site 3	Site 4	Site 5
Rotifera	*Asplanchna brightwelli*	0.93	0.12	0.73	0.05	0.00
	Asplanchna girodi	0.00	0.06	0.54	0.00	0.00
	Asplanchna sieboldi	0.00	0.00	0.00	1.56	0.00
	Anuraeopsis	1.46	0.58	0.63	0.40	0.02
	Bdelloid	0.39	0.78	2.27	0.34	0.03
	Brachionus angularis	0.35	0.25	0.59	0.00	0.00
	Brachionus bidentatus	0.00	0.00	0.00	0.00	0.20
	Brachionus budapestinensis	0.00	0.00	0.00	1.68	0.00
	Brachionus calyciflorus	0.00	0.00	0.00	0.00	0.46
	Brachionus lyratus	0.19	0.34	0.00	0.22	0.00
	Brachionus leydigii	0.00	0.00	0.00	0.00	0.01
	Brachionus quadridentatus	0.10	0.03	0.23	0.96	0.48
	Cephalodella	0.23	0.52	0.23	0.04	0.60
	Cephalodella forficula	0.08	0.18	0.36	0.00	0.00
	Colurella	0.08	0.05	0.41	0.00	0.05
	Conochilus	0.10	0.12	0.00	0.00	0.00
	Dicranophorus	0.00	0.00	0.45	0.07	0.02
	Encentrum	0.12	0.00	1.09	0.01	0.02
	Epiphanes	0.10	0.21	0.41	0.02	0.46
	Euchlanis	0.16	0.08	0.00	0.06	0.00
	Filinia	6.15	1.22	0.18	7.87	0.32
	Gastropus	0.35	0.15	0.18	0.69	0.00
	Hexarthra	0.00	0.03	0.00	0.00	0.00
	Keratella australis	0.68	2.42	1.50	0.06	0.02
	Keratella cochlearis	5.45	12.64	1.00	0.07	0.01
	Keratella procurva	0.84	3.60	3.08	49.59	27.77
	Keratella slacki	0.97	2.10	4.90	3.14	7.36
	Keratella tropica	0.02	0.00	0.00	0.00	0.00
	Itura	0.00	0.00	0.00	0.12	0.00
	Lecane	0.12	0.05	2.22	0.16	0.11
	Lepadella	0.12	0.35	0.00	0.03	0.02
	Lindia	0.00	0.18	0.68	0.00	0.05
	Lophocharis	0.02	0.00	0.00	0.01	0.00
	Monommata	0.04	0.36	0.00	0.00	0.00
	Monostyla	0.43	0.00	0.00	0.00	0.01
	Monostyla bulla	0.00	0.03	0.00	0.00	0.01
	Monostyla hamata	0.10	0.14	0.00	0.04	0.00
	Notholca squamata	0.00	0.06	0.00	0.00	0.00
	Polyarthra	15.00	4.03	2.77	2.64	0.33
	Pompholyx	1.98	1.05	1.09	0.00	0.02
	Platinonus patulus	0.00	0.00	0.00	0.00	0.04
	Synchaeta	10.08	3.29	2.45	0.09	0.20
	Taphrocampa cf selenura	0.00	0.06	0.00	0.00	0.00
	Testudinella patina	0.12	0.12	0.00	0.00	0.23
	Trichotria tetractis	0.06	0.56	0.00	0.01	0.16
	Trichocerca rattus	0.12	0.09	0.00	0.00	0.00
	Trichocerca tigris	0.00	0.00	0.00	0.00	0.02
	Trichocerca	0.04	0.00	0.00	0.00	0.00
	Trichocerca similis	0.54	0.58	1.13	3.87	9.02

Taxa		Site 1	Site 2	Site 3	Site 4	Site 5
	Australocyclops	0.23	0.58	0.00	0.00	0.00
Copepoda	*Boeckella*	0.02	0.00	0.00	0.00	0.00
	Boeckella fluvialis	1.48	1.63	0.36	0.01	0.15
		Site 1	Site 2	Site 3	Site 4	Site 5
	calanoid copepodite	0.97	0.85	2.59	0.07	0.09
	Calamoecia	0.04	0.12	0.23	0.00	0.00
	Calamoecia lucasi	0.60	0.42	0.00	0.00	0.00
	Cyclopoid	0.25	0.00	0.23	0.00	0.00
	cyclopoid copepodite	4.69	2.99	5.26	1.76	2.36
	Eucyclops	0.00	0.00	0.00	0.03	0.00
	Harpacticoid	0.02	0.03	0.00	0.00	0.00
	Macrocyclops	0.02	0.00	0.00	0.00	0.00
	Mixocyclops	0.00	0.00	0.00	0.08	0.00
	Mesocyclops	0.00	0.00	0.00	0.00	0.15
	nauplii	33.70	42.50	55.74	18.27	39.58
Ostracoda	Ostracod	2.18	0.22	1.68	0.64	4.38
Cladocera	*Alona*	0.00	0.08	0.82	0.02	1.74
	Bosmina meridionalis	0.19	0.20	0.54	0.00	0.01
	Ceriodaphnia	1.23	4.82	0.00	0.07	0.61
	Chydorid	0.00	0.00	0.00	0.00	0.02
	Chydorus	0.14	0.78	0.73	0.00	0.37
	Daphnia carinata	1.98	5.14	0.00	0.01	0.00
	Daphnia cephalata	0.04	0.00	0.00	0.00	0.00
	Daphnia lumholtzi	0.00	0.03	0.00	0.00	0.00
	Ilyocyrptus	0.00	0.18	0.00	0.00	0.00
	Macrothrix	0.02	0.00	0.00	0.00	0.00
	Moina	0.23	0.03	0.00	0.53	0.83
	Scapholeberis kingii	0.08	0.05	0.00	0.84	0.00
	Simocephalus	0.04	0.12	0.00	0.00	0.01
	Bosminopsis dietersi	0.00	0.00	0.00	0.00	0.00
Testate amoebae	*Arcella*	1.44	1.53	2.27	0.94	1.41
	Difflugia	2.78	1.27	0.45	1.92	0.01
	Difflugia corona	0.02	0.00	0.00	0.20	0.09
	Difflugia varians	0.10	0.06	0.00	0.80	0.15

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Apparent bioaccumulation of Mn derived from paper-mill effluent by the freshwater crayfish *Cherax destructor* — the role of Mn oxidising bacteria

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Abstract

Bioaccumulation studies of wastewater from a thermo-mechanical paper mill using the freshwater crayfish (*Cherax destructor*) consistently demonstrated elevated levels of manganese. Most of the Mn appeared to be associated with the carapace of the animals. It is suggested that the elevated Mn levels are the result of Mn-oxidising bacteria forming biofilms on the carapace of the crayfish followed by Mn oxide precipitation rather than active uptake of Mn by the crayfish.

Keywords: Heavy metal; Bioaccumulation; Mn oxides; Mn-oxidising bacteria

1. Introduction

Studies of metal-ion bioaccumulation by a target organism are frequently used to assess the potential impact of a waste-stream on the environment of the receiving water. The accumulation or concentration of metal ions by aquatic organisms is a complex process for which a variety of strategies are employed. Concentrations of intracellular trace metals may be regulated by facilitation of uptake, storage (hepatopancreas and kidney) and excretion (Phillips and Rainbow, 1989). The metal ions may be ingested in food or directly from the water column. In the latter case the metal ion may be accumulated either passively from the water column or, by an active uptake mechanism via ion channels (Phillips, 1992; Rainbow, 1992).

While Mn is not considered toxic at the levels often found in most environments (Johnson and Chiswell, 1996), nevertheless, because of its potential impact on water-reticulation infrastructure (Tyler and Marshall, 1967a,b; Tyler, 1970; Nealson, 1983; Ehrlich, 1990), monitoring of this metal ion in waste streams is often required by regulatory agencies. Such monitoring programmes may incorporate bioaccumulation studies. In addition to the mechanisms of metal bioaccumulation discussed above, an alternate mechanism potentially

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exists for the apparent bioaccumulation of Mn by some hard-body organisms (such as crustacea and molluscs). Specifically, Mn bioaccumulation may be the result of surficial deposition of poorly soluble Mn oxides facilitated by a biofilm containing Mn-oxidising bacteria on the organisms shell. Insoluble Mn(III) and Mn(IV) oxides are readily reduced to soluble Mn(II) in the anaerobic zone of sediments which in turn may result in a flux of Mn(II) ions to the water column. The rate of *abiotic* re-oxidation of Mn(II) to insoluble Mn(III, IV) oxides is very slow at pH levels less than 8.5 encountered in many aquatic systems (Chapnick et al., 1982; Ehrlich, 1990; Johnson et al., 1995). However, a number of bacteria have been isolated from various aquatic systems which have been shown to facilitate the oxidation of Mn(II). Indeed it has been suggested that microbial activity provides the primary catalysts for Mn(II) oxidation in some water bodies (Nealson, 1983, 1992; Johnson et al., 1995; Johnson and Chiswell, 1996). These Mn-oxidising bacteria are components of biofilms adhering to solid surfaces and have been implicated in the deposition of insoluble Mn oxides in water pipes (Tyler and Marshall, 1967a,b; Tyler, 1970). In the current study we examine the role of surficial biofilms on the bioaccumulation of Mn by the freshwater crayfish *Cherax destructor* exposed to treated wastewater from a thermo-mechanical newsprint mill. *C. destructor* is a large, omnivorous benthic-macroinvertebrate, ubiquitous in local aquatic ecosystems. This crayfish is a common component of the diets of many larger fish and avian predators.

2. Methods

2.1. Site description

A thermo-mechanical newsprint mill operated by Australian Newsprint Mills Ltd is located at Albury, New South Wales, Australia. The mill's water supply is pumped from the Murray River and treated (flocculation and sand-filtration) on-site prior to use. Wastewater from the pulping process is treated in aerobic activated sludge tanks, followed by secondary clarification and flocculation before discharge into a 40-Ml holding pond and finally, transfer to a 2100-Ml irrigation storage dam. During summer, water from the storage dam is pumped through a filtration plant before delivery to the drip-irrigated plantation.

2.2. Bioaccumulation of Mn in freshwater crayfish

Small streams of wastewater (after the holding pond) and treated river water were diverted to feed flow through tanks for bioaccumulation studies. Three hundred male crayfish (*Cherax destructor*) of approximately the same size (70–80 mm total length and 20–25 g) were obtained from a local commercial farm. One hundred crayfish were placed into each of three 9-m^3 flow-through tanks exposed to ambient conditions. The two control tanks were fed with the treated river water; the experimental tank was fed with a mixture of 50% wastewater and 50% sand-filtered river water. The crayfish were kept in the tanks for a period of approximately 8 months. At the end of the period three crayfish from each tank were removed, quickly frozen and then freeze-dried. The whole animal was homogenised and a smaller proportion digested in concentrated nitric acid under reflux. Total Mn concentrations in the digests were determined by ICP-AES. The bioaccumulation trials were repeated annually over a 5-year period.

In a separate experiment, 30 crayfish were placed in each of six 3-m^3 flow tanks, three fed with filtered river-water and three fed with 100% treated wastewater. After 3 months, four crayfish from each tank were removed, frozen and then separated into carapace, tail and claw muscle and viscera. Following digestion (as described above) Mn concentrations in the digests were determined by flame atomic absorption spectrometry using a GBC 903 spectrophotometer.

2.3. Mn oxide deposition on artificial substrates

Ten pieces of inert Mylar film (5.0×8.0 cm) were set at each of two depths (1.2 and 1.8 m) in each of two tanks (a control tank and a wastewater tank as described above). The strips were deployed for a period of 6 weeks, after which time three strips from each depth (a total of six strips

per tank) were selected randomly and removed. Six strips soaked in deionised water over the same period (experimental blanks) were also collected. Each strip was placed into a 50-ml polypropylene screw-top centrifuge tube. Thirty millilitres of 1.0 M HCl was added to each tube, the tubes were placed on an orbital shaking table (approx. 110 rev./min) overnight at room temperature. At the end of the extraction period, Mn concentrations in the acid extracts were determined by flame AAS. Results were expressed as amount of Mn per area of substrate.

2.4. Mn^{2+} oxidation studies on wastewater

Mn-oxidation in the wastewater was determined by measuring the rate of loss of added Mn^{2+} from both unfiltered wastewater and wastewater which had passed through a 'Spin-Klin™' filtration plant (nominal cut-off, 115 μm). Forty millilitres of filtered wastewater, unfiltered wastewater or Milli-Q water was placed into two series of 50-ml screw-cap polyethylene centrifuge tubes. Mn^{2+} [1000 $\mu g\ l^{-1}$ (as Mn_2SO_4)] was added to one series and none to the other. Microbial activity in half of the samples was inhibited by the addition of 0.25 ml of 1.75 M NaN_3. The tubes were placed on an orbital shaking table and maintained at 20 ± 1°C. Aliquots were periodically removed from the tubes and the concentration of Mn^{2+} was determined by the tetra(*p*-carboxyphenyl) porphyrin method of Ishii et al. (1982) as modified by Johnson et al. (1995).

2.5. Isolation and characterisation of Mn-oxidising bacteria

Mn-oxidising bacteria were obtained from wastewater by serial dilution and inoculation onto wastewater (WW) media, as well as from surficial biofilms by scraping artificial substrates and crayfish inoculated onto Pedomicrobium (PC) agar (Tyler and Marshall, 1967a). WW medium was prepared by supplementing wastewater (1 l) with NH_4Cl (0.1 g), KH_2PO_4 (0.2 g), $MnSO_4 4H_2O$ (0.02 g) and agar (15 g). All plates were incubated at 27°C and kept under observation for the presence of colonies which turned brownish/black as Mn oxide formed. Colonies testing positive for manganese oxide with Leucocrystal Violet (LV) indicator (ASTM, 1990; Spratt et al., 1994) were subcultured onto fresh media until a pure culture was obtained. Subsequent liquid culture was carried out as described by Green and Madgwick (1988). Fresh colonies were tested for catalase and oxidase activity with 3% pharmaceutical H_2O_2 and 'Pyo-test' strips (for detection of cytochrome oxidase), respectively. Standard Plate Count agar (BBL, Cockeysville, USA) was used to test growth in the absence of manganese.

3. Results

3.1. Bioaccumulation of Mn in freshwater crayfish

Annual crayfish trials (1993–1996) of 6–9 months duration have consistently exhibited an increase (60–274%) in total Mn for animals exposed to the newsprint mill wastewater compared with those exposed to river water (Table 1). (The wastewater typically had Mn concentrations of 0.4–0.5 mg Mn l^{-1} while river water typically had concentrations of 0.030–0.045 mg Mn l^{-1}). Prior to moult, the old carapaces of the animals exposed to wastewater were a dark brownish/black in colour, while those animals exposed to riverwater were much lighter. Mn concentrations in the

Table 1
Total manganese concentrations in *Cherax destructor* exposed to river water and newsprint mill wastewater for annual bioaccumulation trials and annual average Mn^{2+} concentrations in the waters

Year	Mn in river water crayfish (mg kg^{-1})	Mn in waste water crayfish (mg kg^{-1})	Difference (mg kg^{-1})	Increase (%)
1997	116	200	84	73
1996	142	329	187	132
1995	231	371	140	60
1994	169	457	288	170
1993	319	1193	874	274

former were observed to peak prior to the rapid growth period in summer and decrease following moults, before rising again (data not presented here). Thus, the Mn concentrations appeared to be linked to the period over which the carapace was exposed to wastewater following a moult.

Manganese concentrations in the whole animal (carapace + muscle + viscera) were 4.7 times greater for the animals grown in wastewater compared with those grown in river water controls (Table 2). The muscle samples contained the lowest proportion of the total Mn in both treatments (6–7%). The highest proportions of total Mn were associated with the viscera (58%) in the controls and the carapace (56%) in the wastewater treatment. Elevated levels of Mn in the viscera (300 compared with 97 mg kg^{-1}) are probably confounded by the gut, containing ingested biofilms or surface deposits from benthic grazing. There was a significant difference ($t = -6.7$, $P < 0.001$) between Mn concentrations in the carapace of animals exposed to wastewater (438 ± 106 mg Mn kg^{-1}) compared with animals from the freshwater control (59 ± 51 mg Mn kg^{-1}).

3.2. Mn-oxide deposition on artificial substrates

The Mylar substrates supported visible biofilms following 6 weeks exposure to treatments. These biofilms on the substrates in river water were predominantly green whereas those from the substrates in wastewater were predominantly brown. Examination using light microscopy found the former to contain abundant filamentous and single celled green algae, the latter was dominated by bacterial cells. Manganese was measured in the extractions from the substrates from both treatments and not detectable in the blanks. The mean/median concentrations of manganese for the aggregated control and wastewater treatments are depicted as box plots in Fig. 1. The control substrates contained 17–43 mg m^{-2} Mn, and the wastewater substrates 81–107 mg Mn m^{-2}. Students t-tests showed a significant difference ($t = -12$, $P < 0.001$) between the river water and wastewater treatments.

3.3. Mn^{2+} oxidation potential

Both native and added Mn^{2+} was oxidised in wastewater (Fig. 2). The shape of the oxidation curve is consistent with a biotically-mediated process rather than an abiotic process. A lag phase can be observed where little oxidation occurs in approximately the first 4 days of incubation. However, much of the Mn is oxidised in the next 4 days of incubation. The rate of oxidation was significantly reduced when microbial activity was inhibited by the addition of NaN_3 (Fig. 2). The rate of Mn^{2+} oxidation was also severely reduced in wastewater nominally filtered to 115 μm (to remove particulates) by the filtration plant (Fig. 3).

3.4. Isolation of Mn-oxidising bacteria

Colonies developed on wastewater agar within 3–4 days incubation, however, dark coloured colonies were only observed when plates were incubated for at least 4–5 weeks. The LV indicator test demonstrated that the dark coloured colonies contained manganese oxide. Pure cul-

Table 2
Total manganese concentrations in body parts of *Cherax destructor* exposed to river water and newsprint-mill wastewater

	Crayfish in river water		Crayfish in wastewater	
	(Mn mg kg^{-1}) (n) mean ± S.D.	% Mn Proportion of total	Mn mg kg^{-1} (n) mean ± S.D.	% Mn Proportion of total
Carapace	(4) 59 ± 51	35	(8) 438 ± 106	56
Muscle	(4) 11 ± 7	7	(8) 49 ± 30	6
Viscera	(4) 97 ± 48	58	(8) 300 ± 68	38
Total	167 ± 106		787 ± 204	

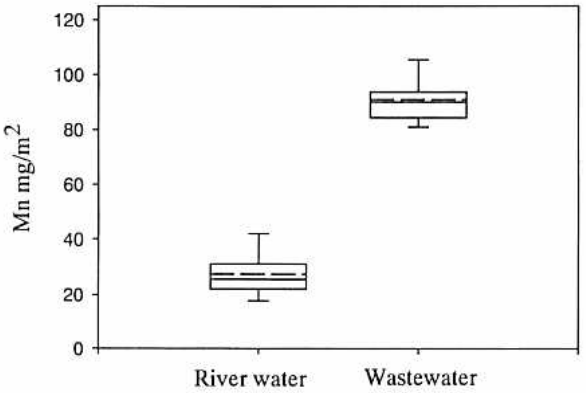

Fig. 1. Mn extracted from artificial substrate biofilms exposed to river water and wastewater ($n = 6$), where the solid line represents the median, the dashed line represents the mean, the box represents the interquartile range and whiskers indicate 90th percentiles ($t = -12$, $P < 0.001$, d.f. = 10).

tures were not obtained from the dilution series on the wastewater agar as the combined effect of slow growth of the Mn-oxidising bacteria and production of MnO_2 for confirmation of their identity meant the colonies were generally overgrown by organisms not oxidising manganese.

Surficial scrapings from the mylar sheets (see

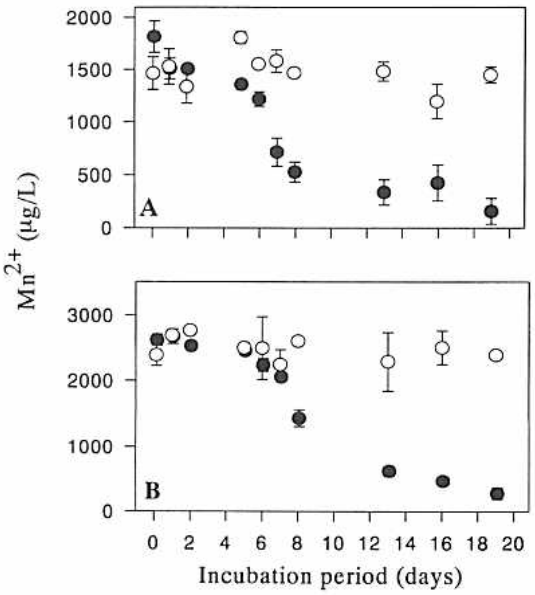

Fig. 2. Oxidation of Mn^{2+} in unsterilised (closed circles) and sterilised (open circles) storage dam wastewater, to which; (A) no Mn^{2+} and (B) 1000 μg Mn^{2+} l^{-1} was added.

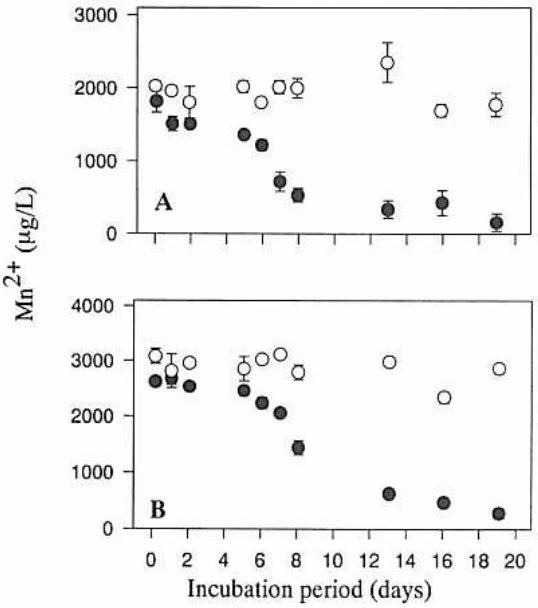

Fig. 3. Oxidation of Mn^{2+} in unfiltered (closed circles) and filtered (open circles) storage dam wastewater, to which; (A) no Mn^{2+} and (B) 1000 μg Mn^{2+} l^{-1} was added.

above) and crayfish produced dark coloured colonies that reacted with the LV indicator. Pure cultures were obtained with one isolate each from the artificial and crayfish surfaces selected for further study. Strain ANM1 was obtained from the artificial substrate and was a budding gram negative curved rod. After a 2-week incubation, colonies on PC medium were less than 1 mm, black and irregular, whereas the colonies on medium without manganese were 0.5–2.0 mm, yellow and circular with an entire margin. ANM1 was oxidase-positive, catalase-positive and did not grow fermentatively on glucose. Strain ANM2 isolated from the carapace of the crayfish was filamentous and slow growing. The colonies took upwards of 3–4 weeks before they were clearly visible on PC agar.

4. Discussion

Total manganese levels in crayfish living in the waste water were significantly higher than those living in river water. Furthermore, most of the manganese oxide was associated with the carapace of the crayfish. These observations suggest

either that the crayfish were taking up Mn from their environment and subsequently storing the accumulated Mn in the carapace or conversely, the Mn was deposited on the carapace by an external process (i.e. independent of the crayfish's physiology). To explore this latter hypothesis, inert substrates made from mylar film were suspended in both river water and wastewater to see if Mn would form surface deposits. The results clearly show that Mn was present on the surface of the inert substrates at the end of the experiment. Furthermore, significantly more Mn deposited on inert substrates which had been incubated in wastewater compared with inert substrates which had been suspended in river water. (Blank experiments where the inert substrates were suspended in sterile, de-ionised water showed that no Mn was actually associated with the mylar film). Therefore, it would appear that surficial deposition of Mn can be caused by some independent process.

Formation and deposition of solid Mn oxides requires the oxidation of dissolved Mn(II) to Mn(IV). The Mn oxidising potential of the wastewater was examined in order to get some understanding of the processes effecting Mn(II) oxidation. The observation that the potential for Mn oxidation in the wastewater was significantly reduced when a microbial inhibitor (NaN_3) was added to the wastewater, coupled with the observation that a significant lag phase was necessary before Mn oxidation occurred in non-sterile wastewater clearly indicates that Mn oxidation was a microbially-mediated process. This is consistent with other studies on Mn oxidation in aquatic systems (Chapnick et al., 1982; Nealson, 1992; Johnson et al., 1995). The observation that Mn oxidation was inhibited in filtered samples suggested that the Mn-oxidising bacteria were associated with the surface of particles rather than free floating.

The experiments carried out on wastewater demonstrated that the Mn oxidation occurring in the wastewater was biologically-mediated and furthermore, was associated with particulate material or surfaces. Chapnick et al. (1982) obtained similar results on enriched lake water samples filtered to 0.4 μm. Tyler and Marshall (1967a,b), Tyler (1970), Ehrlich (1990), and Nealson (1992) have documented the deposition of brown/black manganese oxides on pipes, rocks and sediments where the overlying waters were rich in manganese. These authors suggested that such manganese deposition occurred as a result of microbially-mediated oxidation of soluble Mn^{2+} where active Mn cycling was facilitated by oxic/anoxic interfaces. Direct microscopic examination of the surface Mn deposits in our study indicated the presence of bacteria. In addition, two different bacteria that could oxidise manganese were isolated from the surface oxide films. While no attempt is made to demonstrate whether these organisms are the dominant manganese-oxidising bacteria present, their presence serves to demonstrate that microbes were present that can carry out manganese oxidation.

The current study raises concerns about using hard-bodied species such as crayfish for bioaccumulation studies, and although it therefore highlights the importance of appropriate species selection for target chemicals; in this case the morphology of the animal provided an alternative pathway for bioaccumulation of the metal by surface deposition of the metal oxide. This study also suggests a pathway for potential bioaccumulation in higher order predators that ingest whole shelled animals as a component of their diets.

Acknowledgements

We wish to thank Dr T. Hillman (CRCFE) for first introducing us to Mn-oxidising bacteria. Dr Hillman as well as Dr J Whittington (CRCFE) and Dr D. Richardson (ANM) are thanked for commenting on earlier drafts of this manuscript. Mr S Dahl, Mr P Merrit, Mr J Lassman, Mr R. Coghill and Dr D Richardson (ANM) provided valuable assistance and information on the operations of ANM. Mn analyses by ICP-AES were performed by AGAL, Sydney; Mn analyses by atomic absorption spectroscopy were performed by J. Pengelly (CRCFE). This study was funded in part by Australian Newsprint Mills.

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4

The Fauna of ANM's Lake Ettamogah Forest Wastewater Re-use Scheme and Environs

1998

Handout for Students and Visitors

Helen King

AUSTRALIAN NEWSPRINT
MILLS LIMITED

THE FAUNA OF ANM'S LAKE ETTAMOGAH FOREST WASTEWATER RE-USE SCHEME AND ENVIRONS.

The wastewater re-use scheme operated by Australian Newsprint Mills Ltd at Ettamogah includes a large holding dam, grassland and a forest plantation containing both introduced and native tree species. The size of the operation and the inclusion of a variety of habitat types influences the diversity of fauna that use the area.

The fauna can be divided initially into two broad categories **vertebrates** (those with a spinal column) and **invertebrates** (those without a spinal column). These can be further divided into classes of animal. The number of species for each animal class (**Table 1 & 7**) indicates the diversity, eg. there are many more species of bird than mammal. Species lists for each of these classes (**Tables 2, 3, 4, 5, 6 & 8**) provide both scientific and common names where possible.

VERTEBRATES

TABLE 1: Vertebrate fauna recorded from ANM's Lake Ettamogah Forest Wastewater Re-use Scheme includes 163 species:

Number of species	Class of Animal
126	Birds
11	Mammals (including 4 Marsupials)
12	Reptiles
10	Amphibians
5	Fish

TABLE 2: BIRDS

The large number of **birds** can be grouped according to their dominant **habit** (way of life) which indicates which parts of the **habitat** (area they require to live, feed and breed) they use.

Habit	**Common Name**	***Species name***
Swimming Bird	Hoary-headed Grebe	*Poliocephalus poliocephalus*
Swimming Bird	Australasian Grebe	*Tachybabtus novaehollandiae*
Swimming Bird	Australian Pelican	*Pelecanus conspicillatus*
Swimming Bird	Darter	*Anhinga melanogaster*
Swimming Bird	Pied Cormorant	*Phalacrocorax varius*
Swimming Bird	Little Pied Cormorant	*Phalacrocorax melanoleucos*
Swimming Bird	Great (Black) Cormorant	*Phalacrocorax carbo*
Swimming Bird	Black Swan	*Cygnus atratus*
Swimming Bird	Australian Shelduck	*Tadorna tatorniodes*

Habit	Common Name	*Species name*
Swimming Bird	Pacific Black Duck	*Anas superciliosa*
Swimming Bird	Mallard	*Anas platyrhynchos*
Swimming Bird	Australian Grey Teal	*Anas gracilis*
Swimming Bird	Chestnut Teal	*Anas castanea*
Swimming Bird	Australasian Shoveller	*Anas rhynchotis*
Swimming Bird	Pink-eared Duck	*Malacorhynchus membranaceus*
Swimming Bird	Hardhead	*Aythya australis*
Swimming Bird	Maned Duck	*Chenonetta jubata*
Swimming Bird	Blue-billed Duck	*Oxyura australis*
Swimming Bird	Musk Duck	*Biziura lobata*
Wading Bird	Pacific Heron	*Ardea pacifica*
Wading Bird	White-faced Heron	*Ardea novaehollandiae*
Wading Bird	Great Heron	*Ardea alba*
Wading Bird	Intermediate Egret	*Ardea intermedia*
Wading Bird	Australian White Ibis	*Threskiornis aethiopica*
Wading Bird	Straw-necked Ibis	*Threskiornis spinicollis*
Wading Bird	Royal Spoonbill	*Platalea regia*
Wading Bird	Yellow-billed Spoonbill	*Platalea flavipes*
Wading Bird	Eurasian Coot	*Fulica atra*
Wading Bird	Dusky Moorhen	*Gallinula tenebrosa*
Wading Bird	Black-fronted Plover	*Elseyornis melanops*
Wading Bird	Black-winged Stilt	*Himantopus himantopus*
Wading Bird	Banded Stilt	*Cladorhynchus leucocephalus*
Wading Bird	Common Sandpiper	*Actitis hypoleucos*
Water Bird	Silver Gull	*Larus novaehollandiae*
Bird of Prey	Black-shouldered Kite	*Elanus notatus*
Bird of Prey	Brown Goshawk	*Accipiter fasciatus*
Bird of Prey	Whistling Kite	*Milvus spenurus*
Bird of Prey	Wedge-tailed Eagle	*Aquila audax*
Bird of Prey	Little Eagle	*Hieraaetus morphnoides*
Bird of Prey	Spotted Harrier	*Circus assimilis*
Bird of Prey	Peregrine Falcon	*Falco peregrinus*
Bird of Prey	Australian Hobby	*Falco longipennis*
Bird of Prey	Brown Falcon	*Falco berigora*
Bird of Prey	Australian Kestrel	*Falco cenchroides*
Night Bird	Barn Owl	*Tyto alba*
Night Bird	Southern-boobook Owl	*Ninox novaeseelandiae*
Night Bird	Tawny Frogmouth	*Podargus strigoides*
Aerial Bird	White-backed Swallow	*Cheramoeca leucosternum*
Aerial Bird	Welcome Swallow	*Hirundo neoxena*

Habit	Common Name	*Species name*
Aerial Bird	Fairy Martin	*Hirundo amel*
Aerial Bird	Rainbow Bee-eater	*Merops ornatus*
Tree Trunk Bird	White-throated Tree-creeper	*Cormobates leucophaea*
Tree Trunk Bird	Brown Tree-creeper	*Climacteris picumnus*
Bush Bird	Peaceful Dove	*Geopelia placida*
Bush Bird	Common Bronzewing	*Phaps chalcoptera*
Bush Bird	Crested Pigeon	*Geophaps lophotes*
Bush Bird	Galah	*Cacatua roseicapilla*
Bush Bird	Little Corella	*Cacatua pastinator*
Bush Bird	Sulphur-crested Cockatoo	*Cacatua galerita*
Bush Bird	Swift Parrot	*Lathamus discolor*
Bush Bird	Crimson Rosella	*Platycercus elegans*
Bush Bird	Eastern Rosella	*Platycercus eximus*
Bush Bird	Red-rumped Parrot	*Psephotus haematonotus*
Bush Bird	Pallid Cuckoo	*Cuculus pallidus*
Bush Bird	Fan-tailed Cuckoo	*Cuculus flabelliformis*
Bush Bird	Black-eared Cuckoo	*Chrysococcyx osculans*
Bush Bird	Horsefield's-bronze Cuckoo	*Chrysococcyx basalis*
Bush Bird	Laughing Kookaburra	*Dacelo novaguineae*
Bush Bird	Sacred Kingfisher	*Todirhampus sancta*
Bush Bird	Black-faced Cuckoo-shrike	*Corancina novaehollandiae*
Bush Bird	Jacky Winter	*Microeca leucophaea*
Bush Bird	Golden Whistler	*Pachycephala pectoralis*
Bush Bird	Rufus Whistler	*Pachycephala rufiventris*
Bush Bird	Crested Shrike-tit	*Falcunculus frontatus*
Bush Bird	Grey Shrike-thrush	*Collurincincla harmonica*
Bush Bird	Restless Flycatcher	*Myiagra inquieta*
Bush Bird	Grey Fantail	*Rhipidura fuliginosa*
Bush Bird	Willie Wagtail	*Rhipidura leucophrys*
Bush Bird	Superb Fairy-wren	*Malurus cyaneus*
Bush Bird	White-browed Scrub-wren	*Sericornis frontalis*
Bush Bird	White-throated Gerygone	*Gerygone olivcacea*
Bush Bird	Eastern Yellow Robin	*Eopsaltria australis*
Bush Bird	Brown Thornbill	*Acanthiza pusilla*
Bush Bird	Striated Thornbill	*Acanthiza lineata*
Bush Bird	Yellow-rumped Thornbill	*Acanthiza chrysorrhoa*
Bush Bird	Yellow Thornbill	*Acanthiza nana*
Bush Bird	Red Wattlebird	*Anthochaera carunculata*
Bush Bird	Noisy Friarbird	*Philemon corniculatus*
Bush Bird	Little Friarbird	*Philemon citreogularis*
Bush Bird	Noisy Miner	*Manorina melanocephala*
Bush Bird	White-plumed Honeyeater	*Lichenostomus pencillatus*
Bush Bird	Brown-headed Honeyeater	*Melithreptus brevirostris*

Habit	Common Name	*Species name*
Bush Bird	Black Honeyeater	*Certhionyx niger*
Bush Bird	Mistletoebird	*Dicaeum hirundinaceum*
Bush Bird	Spotted Pardalote	*Pardalotus punctatus*
Bush Bird	Striated Pardalote	*Pardalotus striatus*
Bush Bird	Silver-eye	*Zosterops lateralis*
Bush Bird	European Goldfinch	**Carduelis carduelis*
Bush Bird	Masked Woodswallow	*Artamus personatus*
Bush Bird	Dusky Woodswallow	*Artamus cuanopterus*
Bush Bird	Blackbird	**Turdus merula*
Bush Bird	Olive-backed Oriole	*Oriolus sagittatus*
Bush Bird	Pied Currawong	*Strepera graculina*
Bush Bird	Australian Raven	*Corvus coronoides*
Bush Bird	Little Raven	*Corvus mellori*
Grassland Bird	Masked Lapwing	*Vanellus miles*
Grassland Bird	Banded Lapwing	*Vanellus tricolour*
Grassland Bird	Golden-headed Cisticola	*Cisticola exilis*
Grassland Bird	Diamond Firetail	*Stagnopleura guttata*
Grassland Bird	Red-browed Firetail	*Neochemia temporalis*
Grassland Bird	Double-Barred Finch	*Taeniopygia bichenovii*
Grassland Bird	Flame Robin	*Petroica phoenica*
Grassland Bird	Red-capped Robin	*Petroica goodenovii*
Grassland Bird	Hooded Robin	*Melanodryas cucullata*
Grassland Bird	Brown Songlark	*Cincolorhamphus crucalis*
Grassland Bird	Rufous Songlark	*Cincolorhamphus mathewsi*
Grassland Bird	Singing Bushlark	*Mirafra javanica*
Grassland Bird	Stubble Quail	*Coturnix pectoralis*
Grassland Bird	Richards Pipit	*Anthus novaeseelandiae*
Grassland Bird	White-fronted Chat	*Ephthainura albifrons*
Grassland Bird	Chough, White-winged	*Corcorax melanorhamphos*
Grassland Bird	Australian Magpie-lark	*Grallina cyanoleuca*
Grassland Bird	Australian Magpie	*Gymnorhina tibicen*
Grassland Bird	Common Starling	**Sturnus vulgaris*
Grassland Bird	House Sparrow	**Passer domesticus*
Grassland Bird	Skylark	**Alauda arvenis*
		**introduced species*

Habit classification adapted from Slater *et. al* 1989 with assistance from Judy Frankenberg (MDFRC).

Reference: Slater, P., Slater, P. and Slater, R. (1989) "The Slater Field Guide to Australian Birds", Weldon Publishing, Sydney

TABLE 3: MAMMALS (including Marsupials)

Marsupials are a subclass of Mammals. Both have hair to help them maintain body temperature and give birth to live young which they feed with milk from mammary glands. These animals are most likely to be observed at night while they are feeding.

Common Name	*Species name*
Mammals	
Lesser Long-eared Bat	*Nyctophilus geoffroyi*
Chocolate Wattled Bat	*Chalinolobus morio*
Goulds Wattled Bat	*Chalinobus gouldii*
Large Forest Bat	*Vespardelus sagitula*
Southern Forest Bat	*Vespardelus regulus*
Small Forest Bat	*Vespardelus vulturus*
Southern Freetail Bat	*Mormopterus planiceps (lpf)*
Marsupials	
Short-beaked Echidna	*Tachyglossus aculeatus*
Common Ringtail Possum	*Pseudocheirus peregrinus*
Common Brushtail Possum	*Trichosurus vulpecula*
Eastern Grey Kangaroo	*Macropus giganteus*

TABLE 4: REPTILES

Reptiles cannot maintain their body temperature as well as mammals, but they can use some behaviours (eg. basking in the sun) to keep their temperature within an appropriate range. Their bodies are protected by tough skin or scales.

Common Name	*Species name*
Southern Rainbow Skink	*Carlia tetradactyla*
(no common name)	*Cryptoblepharus carnabyi*
Large Striped Skink	*Ctenotus robustus*
Tree Skink	*Egernia striolata*
Coventry's Skink	*Leiolopisma coventryi*
Grass Skink	*Leiolopisma delicata*
Bouganville's Skink	*Lerista bouganvillii*
(no common name)	*Morethia boulengeri*
Marbled Gecko	*Phyllodactylus mamoratus*
Lace Monitor	*Varinus varinus*
Brown Snake	*Pseudonaja texitilis*
Long-necked turtle	*Chelodina longicollis*

TABLE 5: AMPHIBIANS

Most amphibians require moist **habitats** so that they can regain the water they lose by evaporation through their moist skins during exercise.

Common Name	*Species name*
Banjo Frog (Eastern Pobblebonk)	*Limnodyastes dumerilli*
Giant Pobblebonk Frog	*Limnodyastes interioris*
Spotted Marsh Frog	*Limnodyastes tasmaniensis*
Plains Brown Tree Frog	*Litoria parewingi*
Southern Brown Tree Frog	*Litoria ewingi*
Common Froglet	*Crinia signifera*
Plains Froglet	*Crinia parinsignifera*
(no common name)	*Crinia sloanei*
Smooth Toadlet	*Uperoleia laevigata*
Wrinkled (Eastern) Burrowing Toadlet	*Uperoleia rugosa*

Data source for Tables 2-5: CSU Johnstone Centre Report No.93, June 1997
"ANM Ltd - Ettamogah Forest Fauna Study 1994-1996"

TABLE 6: FISH

Most of the fish were introduced by ANM as fingerlings purchased from a commercial hatchery. The introduced mosquito fish is common throughout Australia in most permanent fresh waters.

Common Name	*Species name*
Mosquito Fish*	*Gambusia holbrooki*
Golden Perch**	*Macquaria ambigua*
Silver Perch**	*Bidyanus bidyanus*
Murray Cod**	*Mccullochella peeli*
Catfish**	*Tandanus tandanus*

* introduced ** stocked

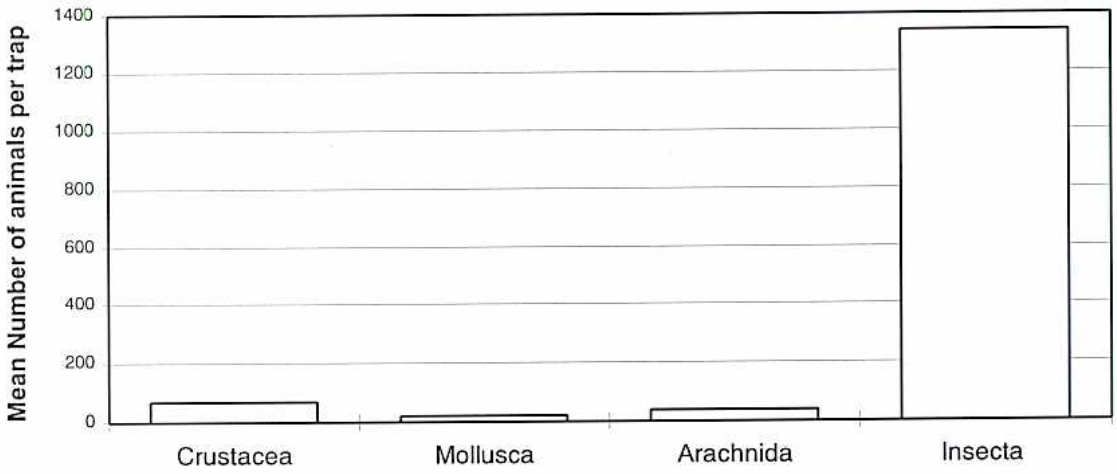
INVERTEBRATES

TABLE 7: Invertebrate fauna identified from the lake (aquatic) includes 24 species:

Number of species	Class of Animal
16	Insects
7	Crustaceans
1	Arachnid

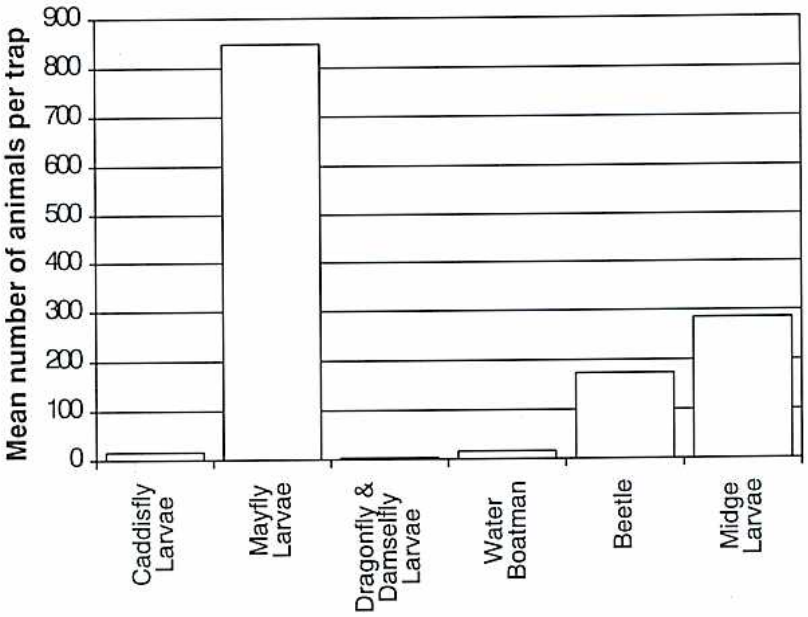
There are large numbers of these invertebrates. Some feed on algae (eg. molluscs and small crustaceans), some on decaying plant and animal material (eg. shrimp, yabbies and some insects) and many of the insects are predators feeding on other smaller invertebrates. As part of the many **food chains**, they in turn are food for some of the vertebrates. The aquatic invertebrates collected from the lake using artificial habitat traps can be divided into the four major groups shown in the following graph. **Insects** (invertebrates with 3 pairs of legs) are the most common group of these aquatic animals.

Graph 1: Summer Invertebrate Collections 1998



The insects can be futher separated into the six subgroups shown in the following figure. Mayflies were the most common insect present in these samples collected in summer. At other times of the year they may not be as common in comparison with the other groups as most of the insects have seasonal life cycles.

Graph 2: Summer Insect Collections 1998



Mayflies occur in large numbers in ANM's dam, living most of their life in the water as larvae, where they eat and grow. Their flying adult stage emerges on spring/summer evenings and lives for only a few days to mate before laying their eggs in the water.

Midge larvae are commonly known as bloodworms and live on the bottom sediments. Their adult stage looks like a mosquito but does not bite.

Aquatic **beetles** and **water boatmen** (bugs) are air-breathers like their terrestrial relations and many adults can live underwater by taking small bubbles of air down with them when they dive from the surface.

Caddisfly larvae often live on the bottom surfaces underwater in a case made of sticks, leaves or sand grains. They carry this case around with them with only their head and long legs sticking out. The flying adults look like delicate moths, not seen much during the day, but will swarm around lights at night.

Dragonfly and **damselfly larvae** are the larger of the insect predators and live underwater for many months. The adults are easily seen and may be brightly coloured. They are swift fliers often seen skimming along near the surface of the water feeding on adult midges or other small flying insects.

TABLE 8: INVERTEBRATES

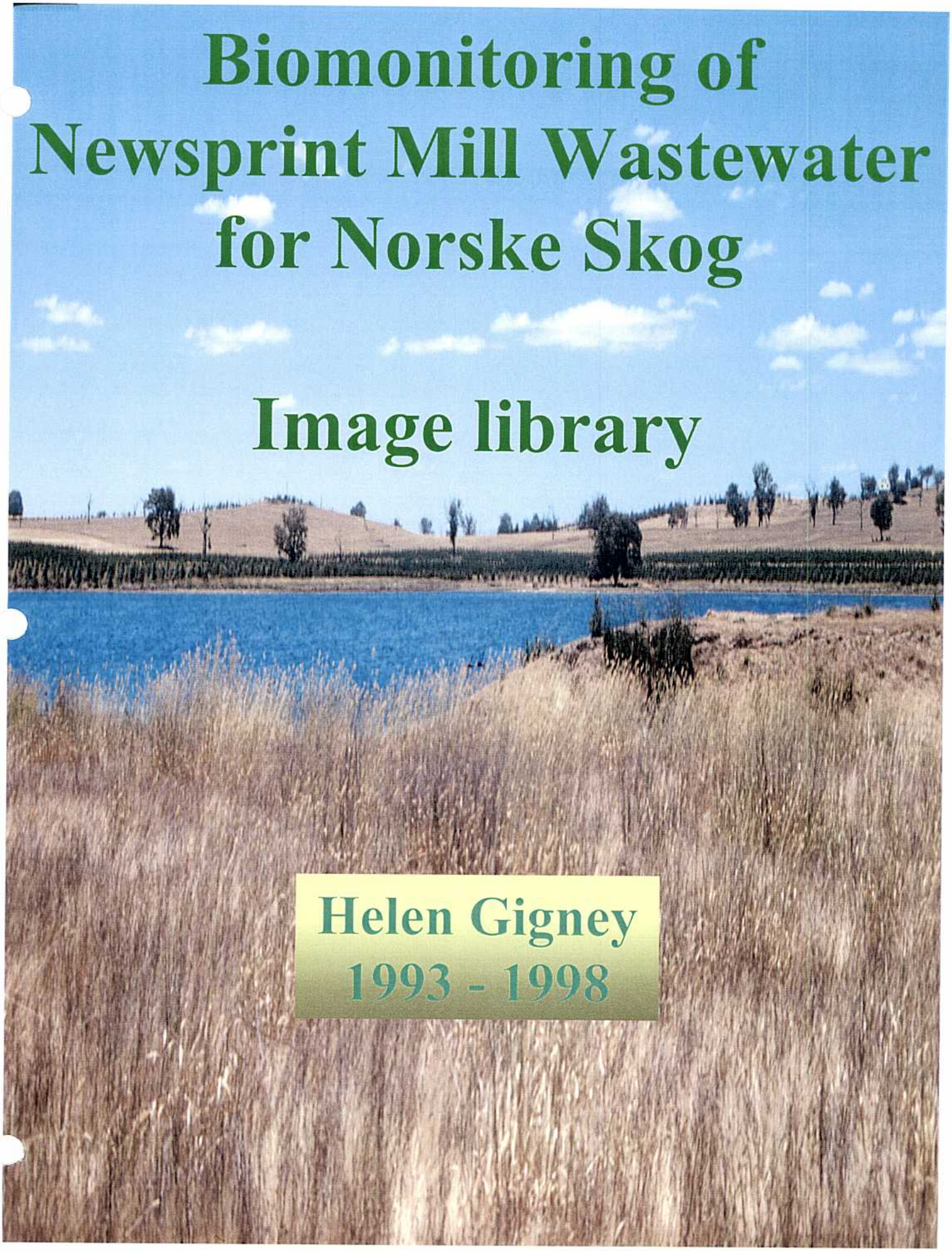
Common Name	*Species name*
Class: Insecta (Aquatic)	
Caddisfly Larvae	Oecetis sp.
Caddisfly Larvae	*Econmus continentalis*
Caddisfly Larvae	*Econmus pansus*
Caddisfly Larvae	*Ecnomus turgidus*
Caddisfly Larvae	Hydrobiosidae
Mayfly Larvae	Cloen sp.
Mayfly Larvae	Caenidae Genus B.
Dragonfly Larvae (mudeye)	*Hemicordulia tau*
Damselfly Larvae	*Austrolestes annulosus*
Damselfly Larvae	*Xanthagrion erythroneurum*
Damselfly Larvae	*Austroagrion watsoni*
Water Boatmen	*Micronecta annae*
Water Boatmen	*Micronecta robusta*
Backswimmer	Enithares sp.
Scavenger Water Beetle	Berosus sp.
Bloodworm (Non Biting Midge Larvae)	Chironomidae
Class: Crustacea	
Water Flea	Chydoridae
Water Flea	*Daphinia carinata*
Water Flea	Moinia sp.
Water Flea	Calanoida sp.
Seed Shrimp	Newnhamia sp
Freshwater Shrimp	*Parataya australiensis*
Yabby	*Cherax destructor*
Class: Arachnida	
Water mite	Acariformes

Invertebrate and small fish sampled using light traps, plankton trawls and artificial substrates in ANM's Lake Ettamogah January to April 1998

5

Biomonitoring of Newsprint Mill Wastewater for Norske Skog

Image library



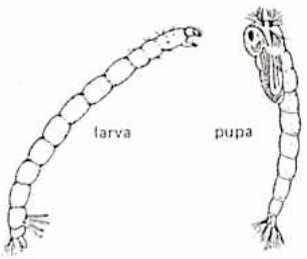
Helen Gigney

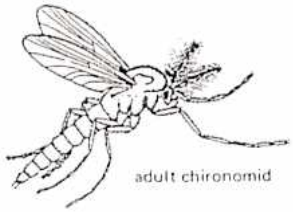
1993 - 1998

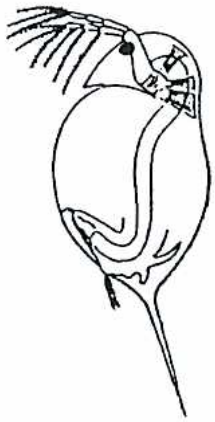
ANM's 4day holding pond and MDFRC Bioassay Lab 1993

Bioassay test animals

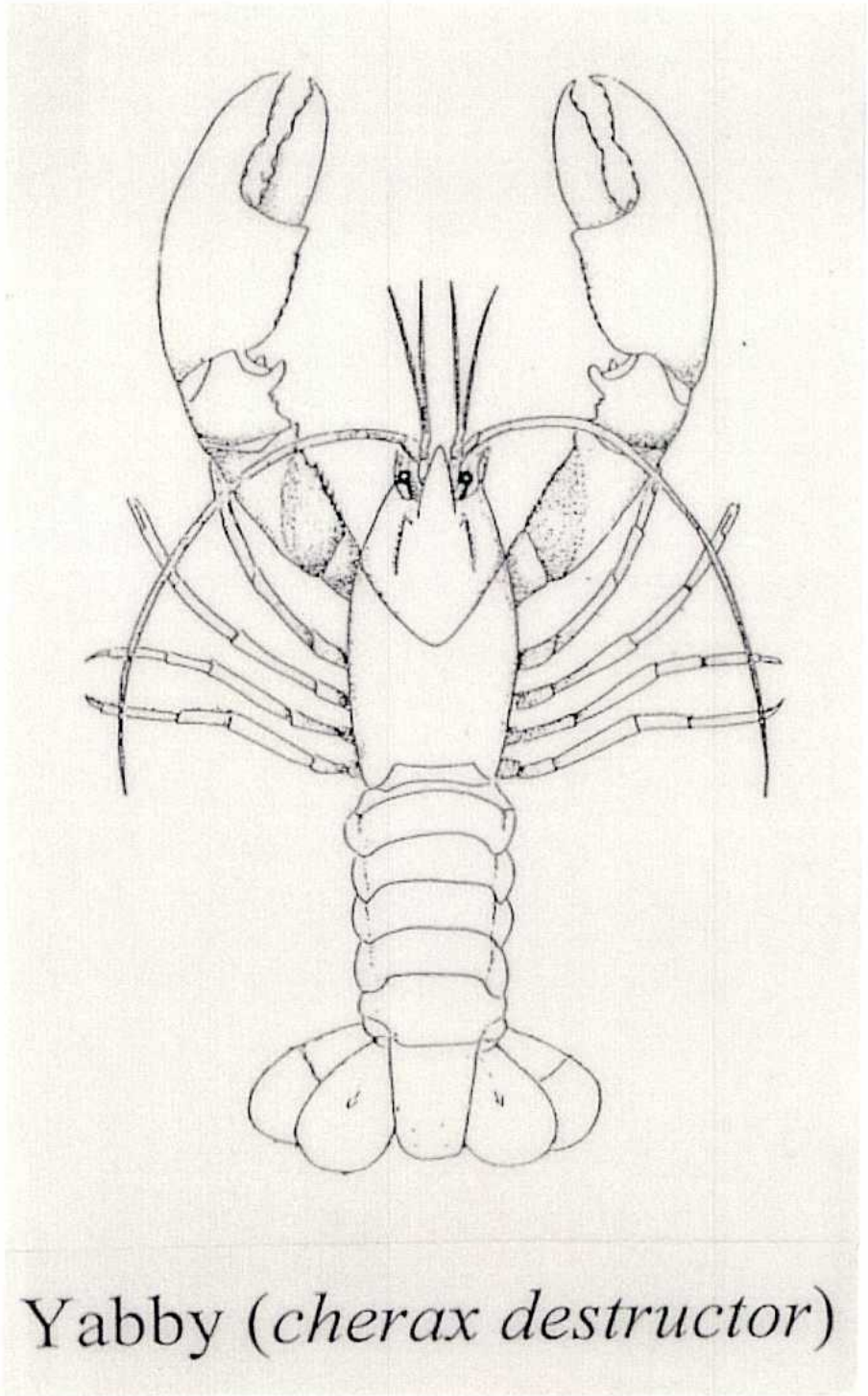
Fam. Chironomidae
(Gnats or midges).







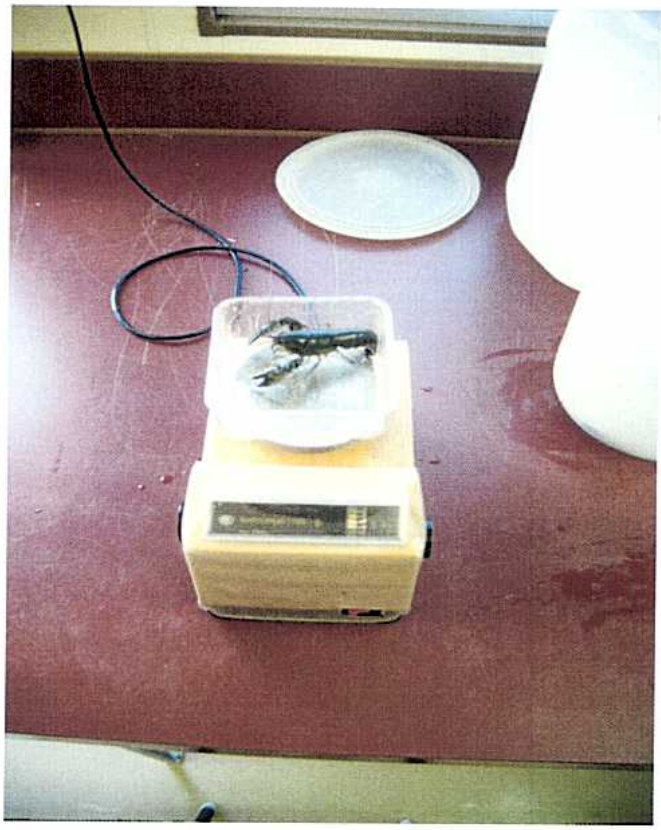
Daphnia (Daphniidae)



Yabby (*cherax destructor*)

Bioaccumulation Tank (with floating fish cage) at 4day Pond Final Filtration Unit 1993

Measuring yabbies from bioaccumulation trials 1993



Aerial View of Murray River @ Albury with Macroinvertebrate Monitoring Sites Indicated

Murray River Albury 1993

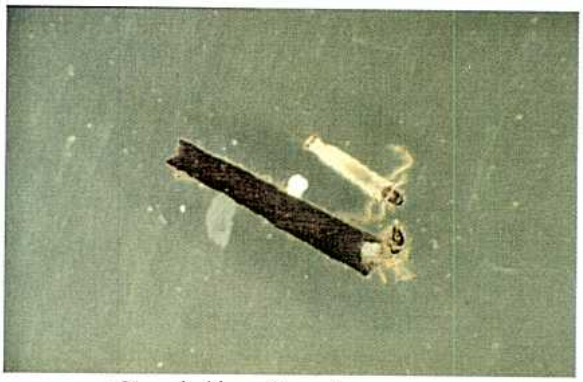
Wastewater Discharge Location

River Environment Monitoring Surveys. Monthly trip to collect artificial substrates 1993.

Collection of Macroinvertebrates in the River using Rock Baskets as Artificial Substrates 1993

Common Macroinvertebrate Taxa Collected from Artificial Substrates in the Murray River @ Albury

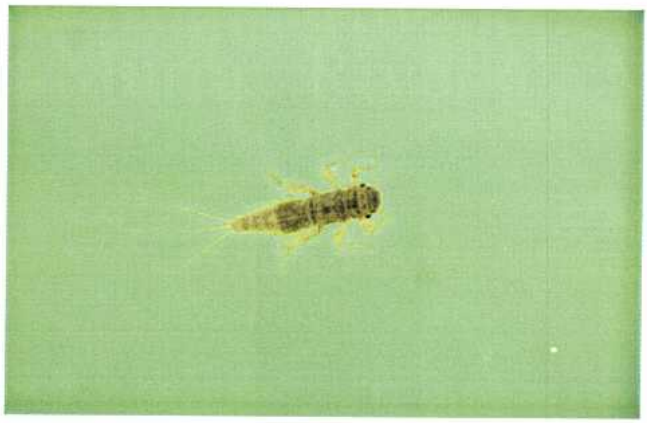
Caddis fly larva|
Chaematopsyche sp.



Caddis fly larva
Triaenodes sp.

Caddis Fly larva
Ecnomus pansus

Caddis Fly larva
Oecetis sp.



May Fly larva
Caenid Genus B

Lake Ettamogah 1997

From the dam wall looking West

From the track looking North

The filtration plant's backwash pond

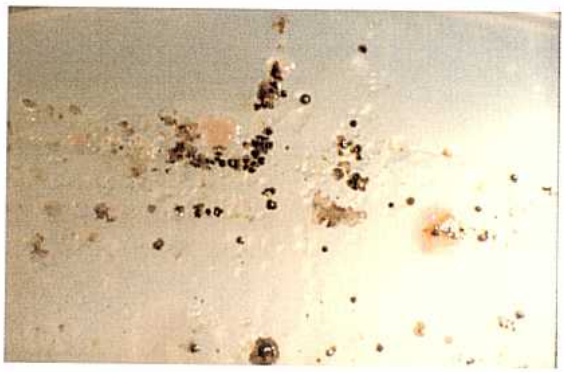
Lake Ettamogah and backwash pond 1998

Lake Ettamogah light trapping for fish larvae 1998

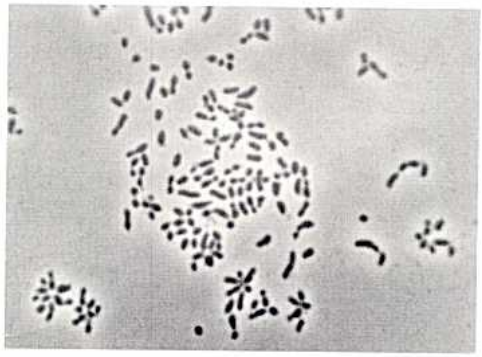
Light trapping yields large numbers of zooplankton bugs and dragonfly larvae but no fish larvae

Yabby bioaccumulation tanks at the backwash pond Lake Ettamogah 1997

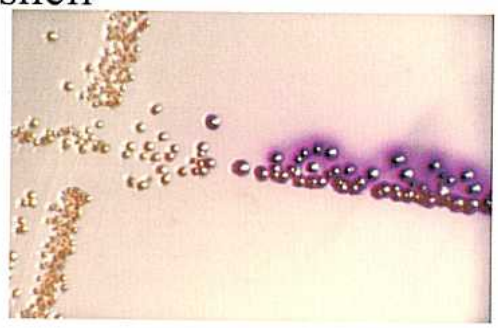
Mn-oxidising bacteria on Pedomicrobium agar 1998



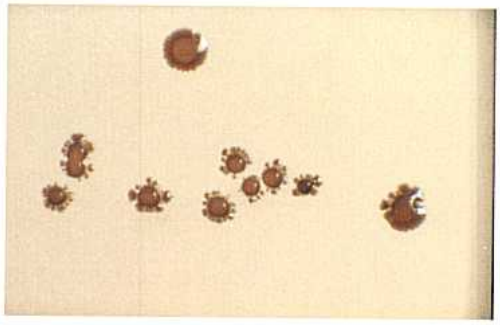
Mixed colonies from surface scraping of yabby shell



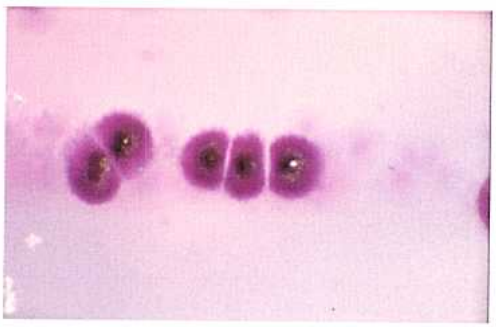
Sphingomonas sp. (ANM1)



Isolate ANM1 young colonies. Positive to LCV reagent for MnO



Isolate ANM1 older colonies with characteristic spreading pattern



Isolate ANM2 colonies. Positive to LCV reagent for MnO

Isolate ANM2 older colonies with characteristic crusty peaks

6

(Apparent) Bioaccumulation of Mn by the Freshwater Crayfish *Cherax destructor* - The Role of Mn Oxidising Bacteria.

Helen King[1]
Darren Baldwin[1]
Gavin Rees[1]
Suzanne McDonald[2]

Presented at the 1998 Congress of the Australian Society for Limnology, Brisbane, QLD

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Co-operative Research Centre for Freshwater Ecology
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ABSTRACT

[keywords: manganese, newsprint mill wastewater]

Studies of metal-ion bioaccumulation by a target organism are frequently used to asses the potential impact of a wastewater on the environment of the receiving water. Bioaccumulation studies using freshwater crayfish to monitor wastewater from a themo-mechanical newsprint mill have consistently shown elevated levels of manganese. The Mn appeared to be associated with the carapace of the animals. Raised manganese concentrations were also measured from surficial biofilms on artificial substrates.

Two strains of manganese oxidising bacteria were isolated. Mn^{2+} oxidation studies on the wastewater indicated a biotically mediated process probably associated with particulates. Although not toxic at levels often found in the environment, manganese has the potential to reduce the effectiveness of water reticulation infrastructure by deposition of metal oxide. The role of the holding dam and implication of wastewater management strategies on the Mn cycle and water quality is discussed.

INTRODUCTION
Why are we interested in Mn?

- Second most abundant heavy metal and essential trace element in living things.

- Deposition of oxides known to reduce efficiency of water delivery in pipes (Tyler and Marshall, Tasmanian Hydro work).

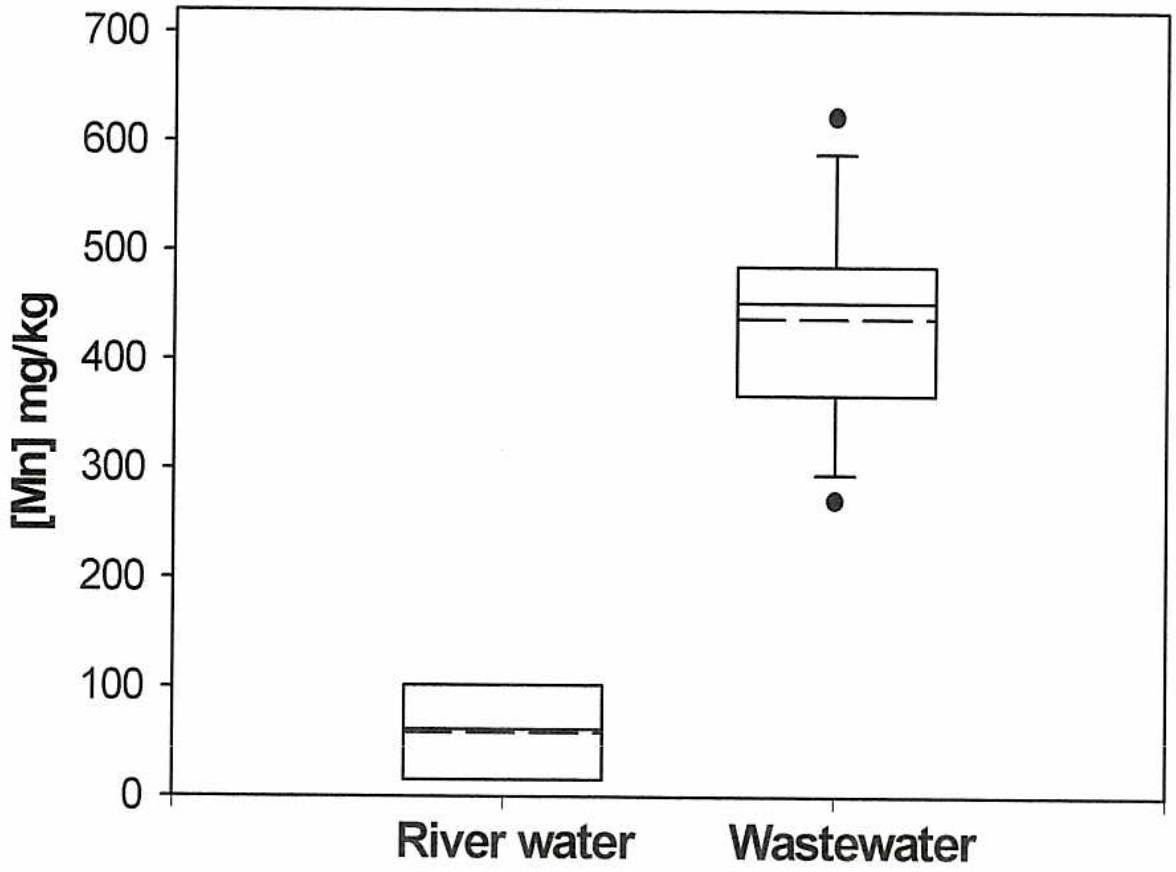
- Required by EPA as a component of metals testing for wastewater discharge

RESULTS

- Consecutive annual bioaccumulation trials of 7 to 9 months showed similar trends.

- The concentrations of manganese encountered in these trials were consistently greater in the animals from the wastewater treatments compared with those in the river water controls (Table 1).

Figure 1: Total Mn extracted from the carapaces of crayfish exposed to river water and wastewater where the solid line represents the median and the dashed line represents the mean (t = -6.665, p = 0.00006, df=10)



- Suspecting a role for biofilm in the observed carapace Mn concentrations, artificial substrates were used for biofilm collection

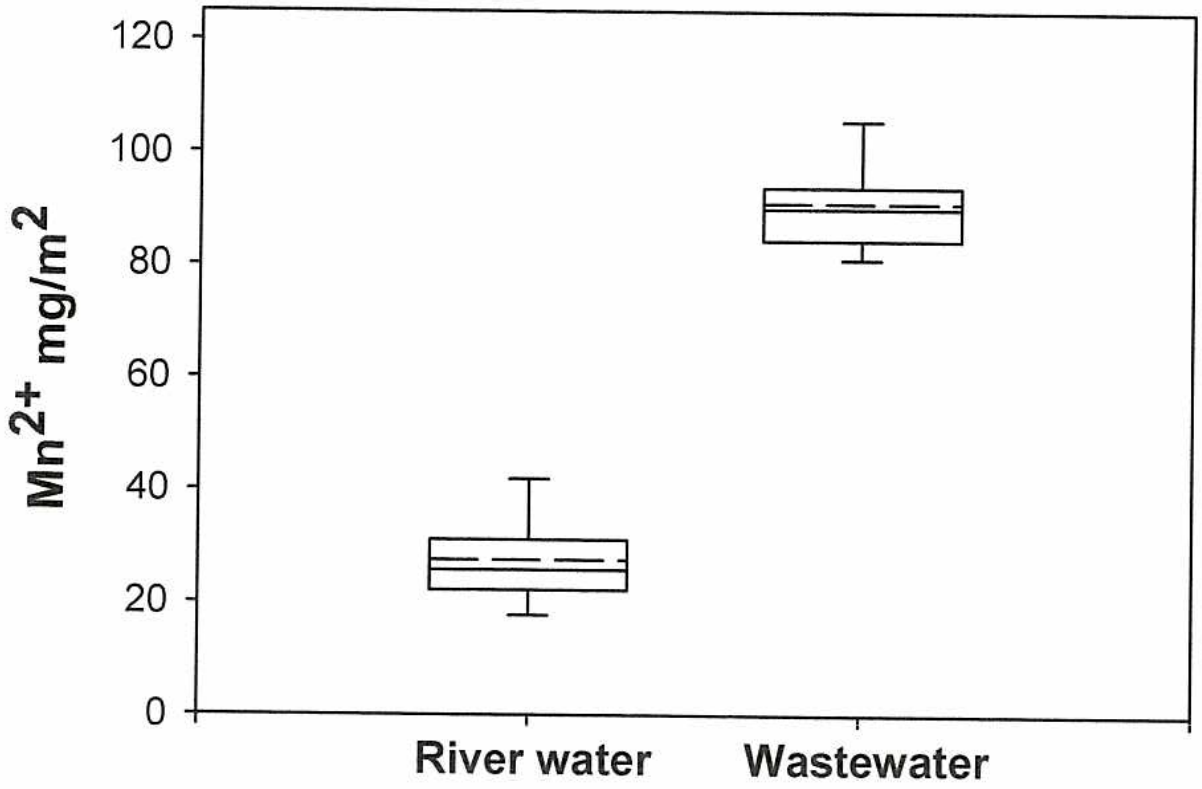
- Small racks of Mylar strips were added to each of two yabby bioaccumulation tanks (one river water control and one wastewater treatment).

- The racks were immersed at two depths (20cm and 2m (bottom)).

- Difference in colour was observed & Mn was extracted from the strips (Figure 2).

Figure 2: Mn^{2+} extracted from artificial substrate biofilms exposed to river water and wastewater (n = 6), where the solid line represents the median and the dashed line represents the mean (t = -11.965, p = 3x10-7, df = 10)

[manass.jnb]



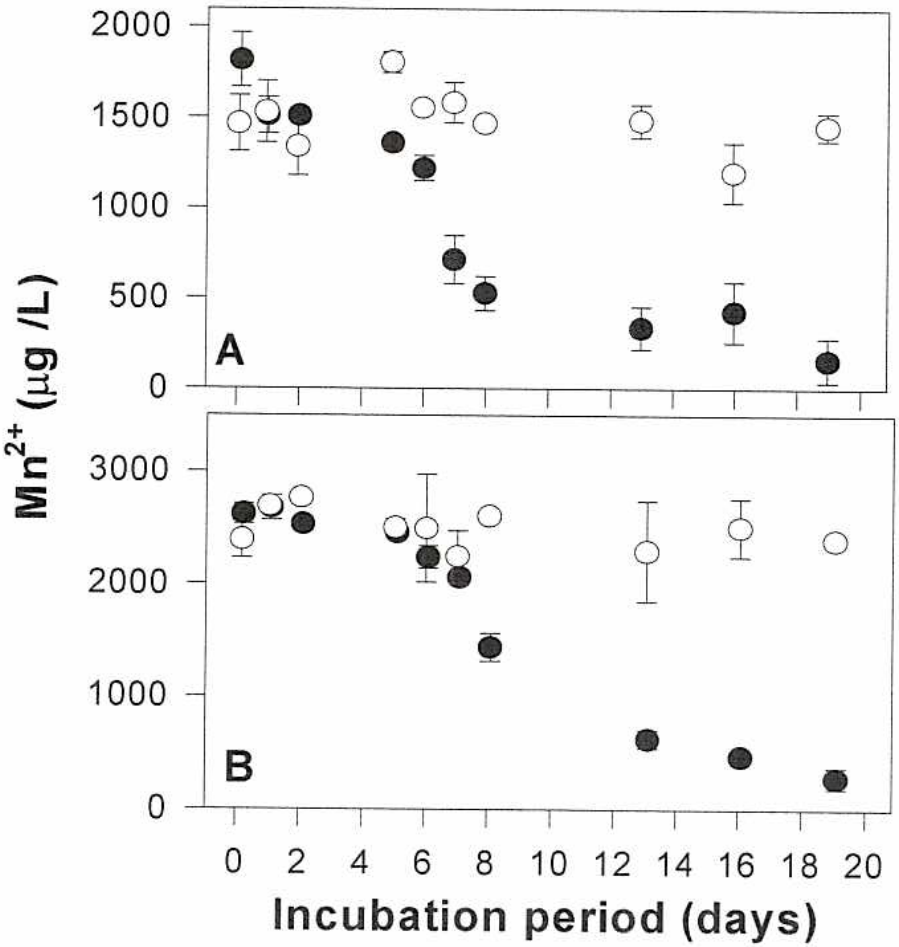
Mn(II) oxidation studies

- Abiotic oxidation of soluble Mn(II) to insoluble Mn (III) or (IV) oxides is very slow at pH < 8.5.

- So oxidation studies were conducted to determine the Mn oxidation potential in the wastewater from the irrigation dam.

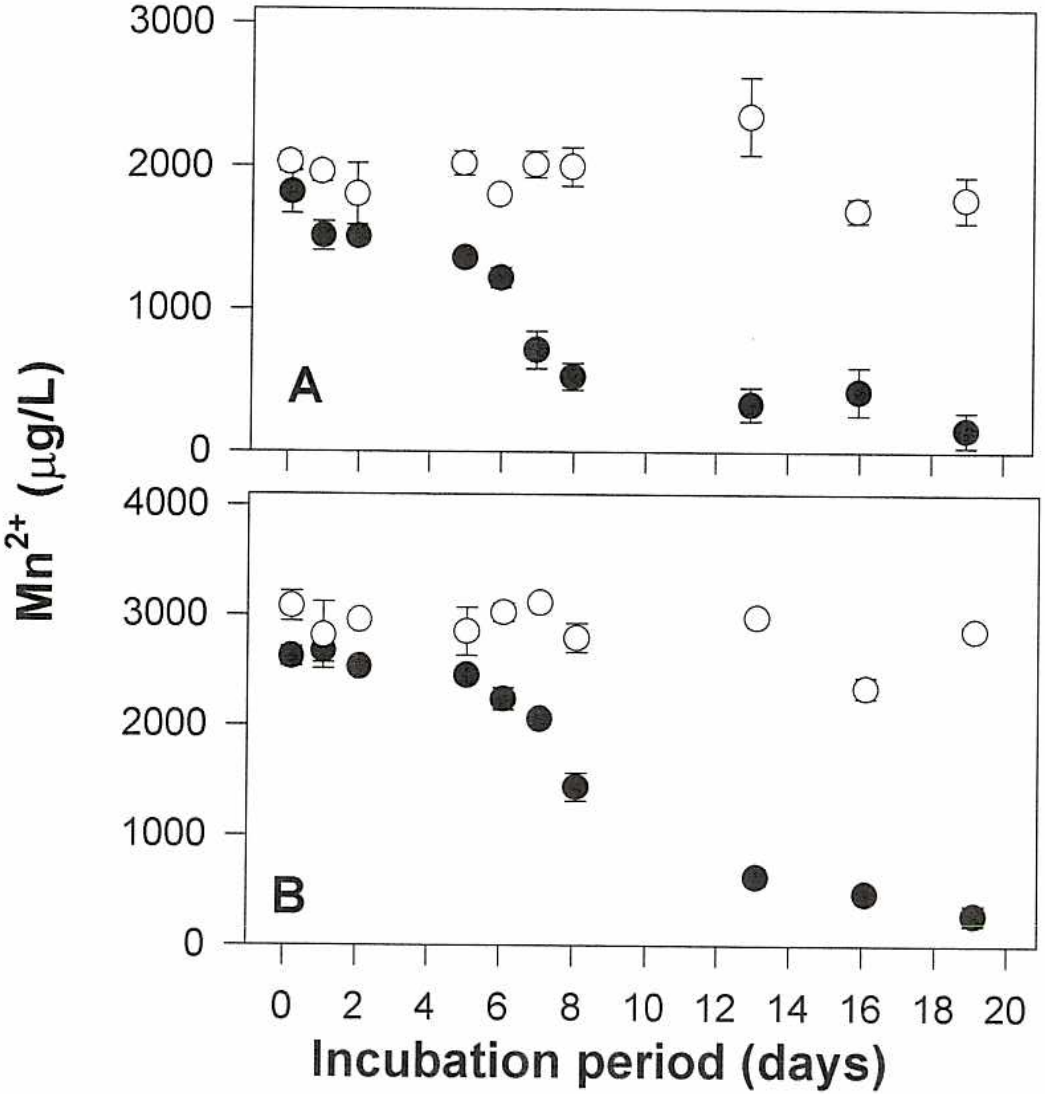
- In the first study one series was sterilised using azide and compared with un-sterilised wastewater (Figure 3).

Figure 3: Oxidation of Mn in unsterilised (closed circles) and sterilised (open circles) storage dam wastewater, to which no Mn^{2+} (A) and 1000μg/L Mn^{2+} (B) was added



- Both native and added Mn 2+ were oxidised in wastewater.
- The shape of the curve is consistent with a biologically mediated process. After an initial lag phase much of the Mn is oxidised.
- The rate of oxidation is significantly inhibited in the sterilised wastewater
- In the second oxidation study where wastewater was filtered to 115 μm by the onsite filtration plant and compared with un-filtered wastewater a similar reduction in oxidation rate was observed (Figure 4).
- This may indicate the presence of Mn oxidising bacteria in biofilms associated with particulates in the wastewater.

Figure 4: Oxidation of Mn^{2+} in unfiltered (closed circles) and filtered (open circles) storage dam wastewater, to which no Mn^{2+} (A) and 1000μg/L Mn^{2+} (B) was added

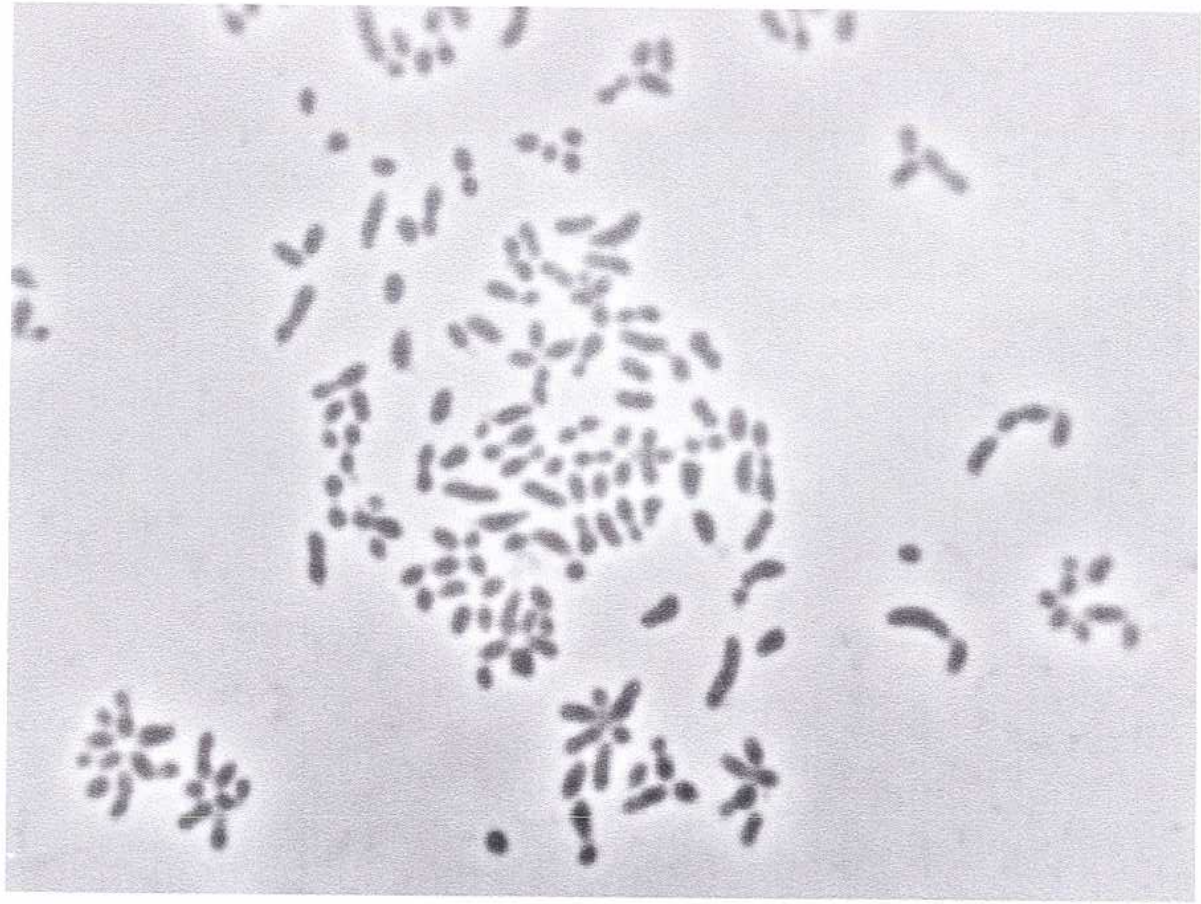


Isolation of Mn-oxidising Bacteria

- Artificial substrates tested Leucocrystal Violet positive for Mn oxide.

- Two strains of Mn oxidising bacteria were isolated by serial dilution of wastewater and scrapings from artificial substrates and yabby carapaces onto pedomicrobium agar.
- $\Rightarrow$ ANM1 from the artificial substrates (Figure 5)
- $\Rightarrow$ ANM2 Yabby carapace

Figure 5: Mn oxidising bacteria isolate ANM1
Sphingomonas sp



CONCLUSIONS

- A variety of microorganisms are known to oxidise Mn, we have only isolated two but there could be more.

- It is apparent that these organisms are active in reducing the amount of soluble Mn in the wastewater and cycling it into the sediments.

- This investigation of an interesting phenomenon utilising a multidisciplinary team has given us information to assist ANM with their mgt of water quality

IMPLICATIONS FOR MANAGEMENT

- **Continued removal of Mn^{2+}** in holding ponds by bacterial biofilms.
- **Process dependent on maintenance of oxic conditions.** If the anoxic conditions do occur at the water sediment interface there would be great potential for release of Mn back into the water column
- Evidence of the **importance of a multi-disciplinary team** approach - 2 biologists, 1 research chemist, 1 microbiologist and 1 student analytical chemist.
- **The microbial factor ! A significant part of the picture**

7

The Murray-Darling Freshwater Research Centre

Seasonality of Benthic Macroinvertebrates in the River Murray at Albury and Impact of Newsprint Mill Wastewater.

Helen King

Murray Darling Freshwater Research Centre

Poster Presentation

Australian Society for Limnology

37th Congress 1997 Albury/Wodonga

AUSTRALIAN NEWSPRINT MILLS LIMITED

ABSTRACT

A newsprint mill operating at Albury improved the quality and reduced volume of it's wastewater discharge to the River Murray. In this study, benthic macroinvertebrate community abundance data were compared using artificial substrate samples from six sites on the River Murray, upstream and downstream of the discharge, over a period of 18 months (including 6 months following the change in the discharged wastewater quality). Approximately 100 taxa from 16 orders were recorded. The fauna was dominated by an ephemeropteran (Caenidae Genus B) and a trichopteran (*Ecnomus pansus*) which together constituted 93% of the total abundance. Abundance data were compared using Bray-Curtis Similarity Matrices. ANOSIM results and MDS ordinations indicated that the flow regime imposed by river regulation combined with season were the dominating influences on macroinvertebrate assemblages. There was no detectable influence on these river benthos attributable to the wastewater discharge of the Newsprint Mill.

INTRODUCTION

- The Murray River at Albury is strongly influenced by regulation from the Hume Dam. Apart from some spring floods, maximum flows (10 000 to 22 000 ML/day) occur in summer when irrigation demands are greatest, and the river is maintained at a minimum of 1 200 ML/day below the confluence of the Kiewa River through winter (Ward 1990).

- Australian Newsprint Mills Ltd (ANM) operates a thermomechanical paper mill at Albury. ANM has discharged its treated wastewater to the River at Albury since July 1981, but from January 1997 changes to their wastewater processing resulted in a dramatic reduction in the volume and an improvement of the quality of this discharged water.

- Benthic macroinvertebrates have been collected from six sites in the Murray as part of an extensive wastewater monitoring program since 1991.

SITE DESCRIPTION & METHODS

Monitoring of the macroinvertebrate fauna of the River Murray at Albury, above and below the ANM wastewater discharge was performed using artificial substrate (Bennison *et al.* 1989). Landuse at the sampling locations is primarily cattle grazing, with some irrigated pasture. The river's banks are fringed by river red gums (*E.camaldulensis*) and willows (*Salix babylonica* and *Salix x rubens*) with little understorey (Figure 1b).

The Artificial substrate samplers were set at six sites in three paired locations in a 2 km stretch of the Murray River

Sites 5 and 6 - the "**upstream**" (controls) near "Grey's farm" approximately 500 m upstream of ANM's discharge;

Sites 3 and 4 - "mixing zone" near the railway bridge 100 to 200 m downstream of the discharge;

Sites 1 and 2 - "downstream" at Union Bridge 2 km downstream of the discharge.

Sampling

Five samplers were set monthly at each of the six sites and after a minimum of four weeks, three of these were randomly collected using a 500 μm mesh net and all five replaced with clean samplers. The samples were sieved to 500 μm to remove silt and the remaining portion retained and preserved in 70% alcohol. The samples were sorted using a stereo microscope and identified to the lowest practicable taxonomic level with reference to MDFRC's taxonomy collection.

Data Analysis

Taxa abundance data were interrogated using "Primer version 3.1b". Ordinations were performed on Bray-Curtis Similarity Matrices from 4th root transformed data. These ordinations were then plotted in "Sigmaplot". Hypothesis testing was conducted on the same similarity matrices in using "ANOSIM" (analysis of similarity) and identification of the species contributing to the differences between groups was performed using "SIMPER" (similarity percentages).

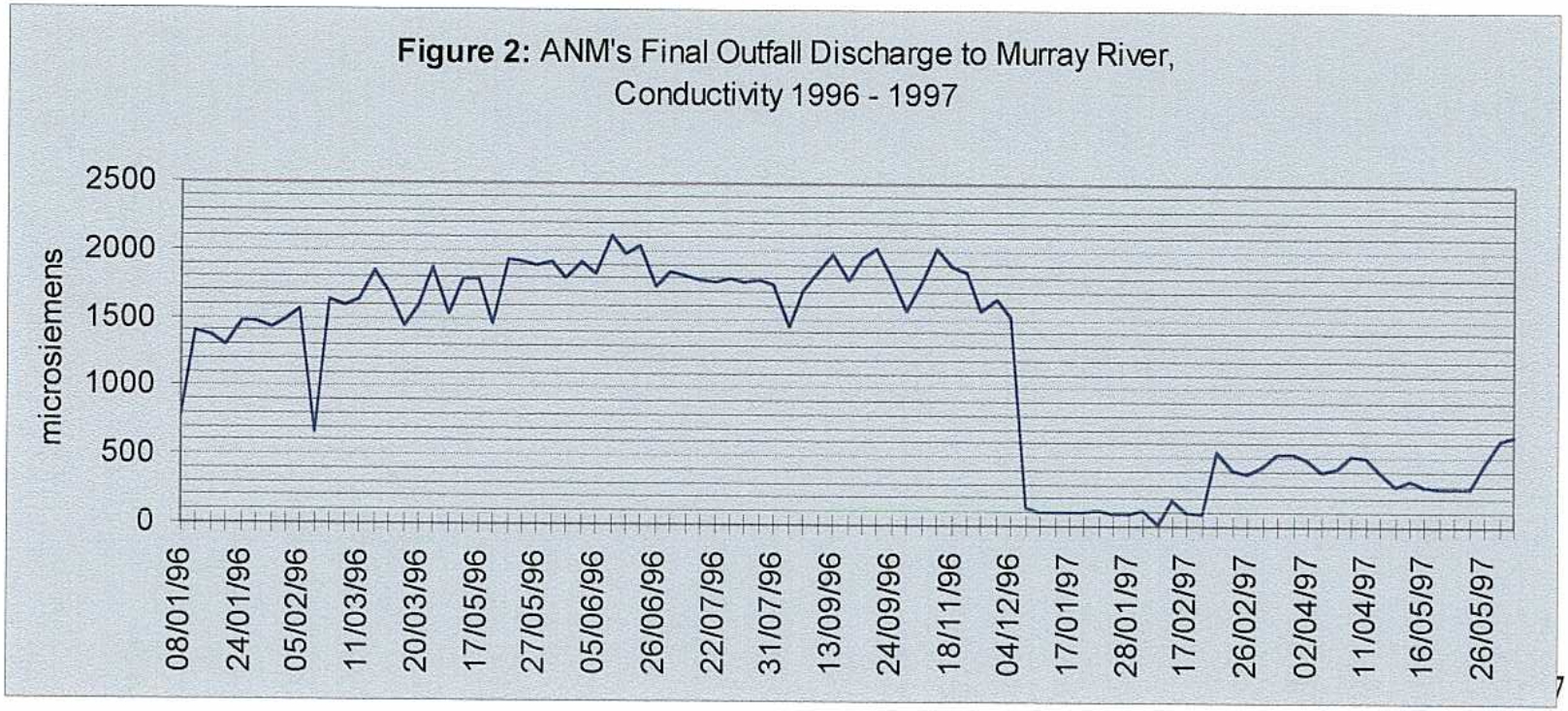
Physico-chemical Parameters

Aqueous physico-chemical parameters were measured using a "TPS Lab Analyser". Flow data for the River were obtained from Murray Darling Basin Commission's fortnightly reports for Doctors Point, Albury (below the confluence of the Kiewa river and above the sampling sites).

RESULTS

Changes To Wastewater Quality

The conductivity (Figure 2) of ANM's wastewater discharged to the Murray changed from a mean of 1700µS in 1996 to 310µS in 1997. The mean conductivity of the River over both periods was stable at 60µS, the influence of the discharge on the physico-chemical parameters undetectable even at low flow periods

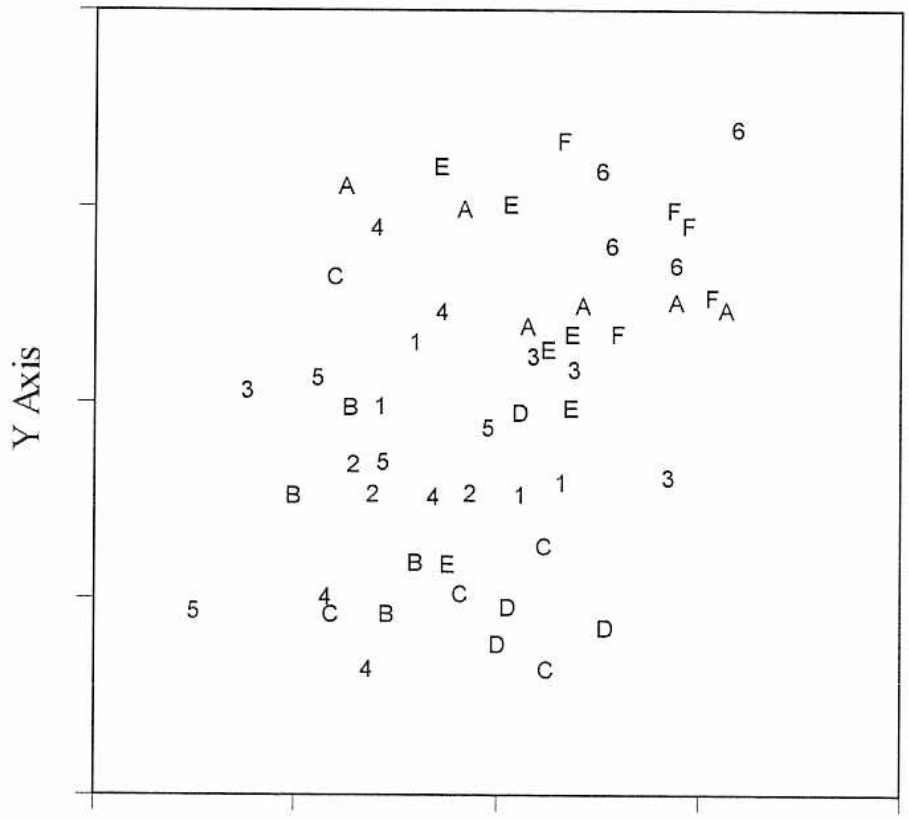


RESULTS

Mean macroinvertebrate abundance data for each site from January to May were compared for all sites in 1996 (pre change) and 1997 (post change). The MDS ordination (figure 3) shows little separation, and considerable overlap between treatments. ANOSIM results (one-way) testing for homogeneity of samples pre and post discharge changes, indicated that the mixing zone samples were different ($R = 0.350$ $\alpha = 0.004$) however the upstream controls were also different ($R = 0.381$ $\alpha = 0.040$) but the downstream samples were not different ($R = 0.019$ $\alpha = 0.33$).

Macroinvertebrate Community Comparisons using MDS

Figure 3: Changes to ANM's Wastewater Quality.
1-6 = downstream to upstream sites pre change
A-F = downstream to upstream sites post change
Stress = 0.16



RESULTS

Season & Location

Mean abundance data for each site in 1996/97 were compared to elucidate differences in macroinvertebrate community data between locations (relative to ANM's discharge) and seasons. The location MDS ordination (figure 4) a high degree of overlap between the categories. The season MDS (figure 5) showed greater aggregation of categories but again, no real separation and major overlap. ANOSIM (two way nested) results indicated some difference between site groups $R = 0.174$ $\alpha = 0.001$ and a greater difference between season groups $R = 0.741$ $\alpha = 0.001$.

Figures 4 & 5: Macroinvertebrate Community Comparisons using MDS

Figure 4: Location Relative to ANM's Wastewater Discharge
Upstream, Mixing zone and Downstream
Stress =0 .19

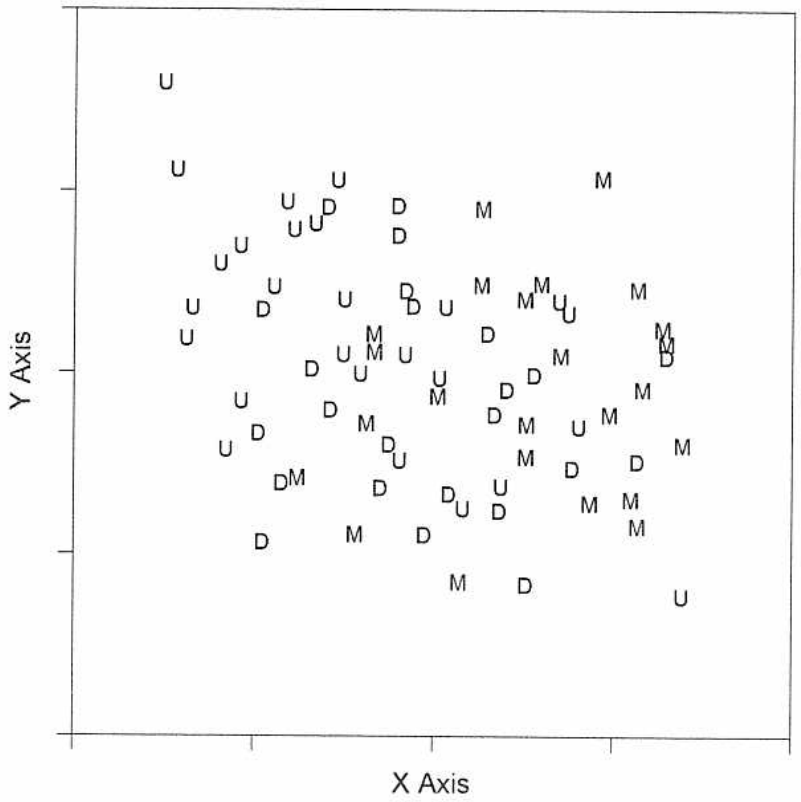
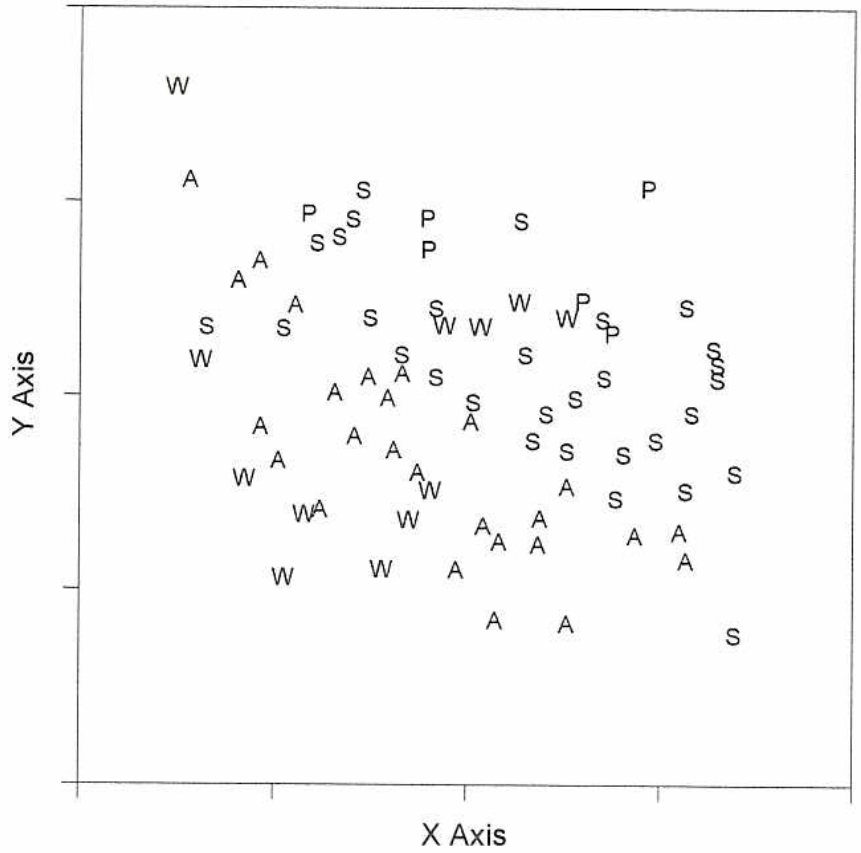


Figure 5: Season of collection:
Summer, Autumn, Winter and Spring
Stress = 0 .19



RESULTS

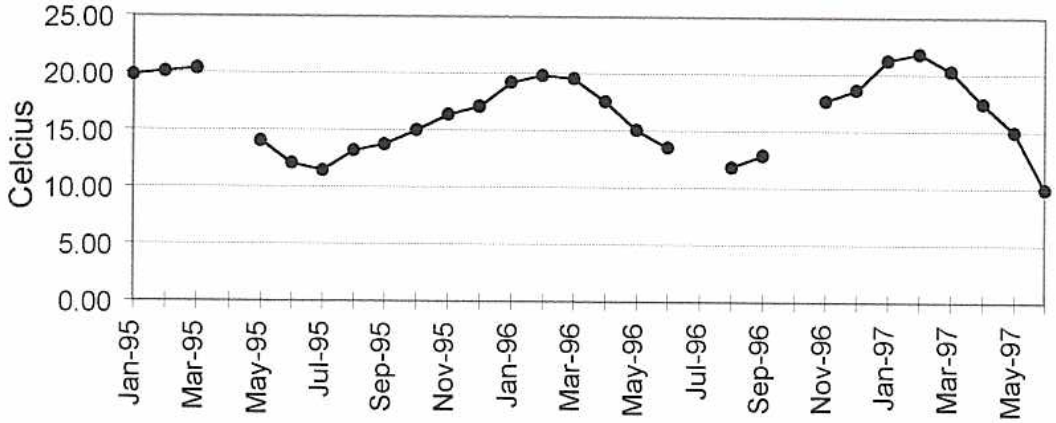
Flow & Season

The flow of the Murray (figure 6) tends to be high (~20 000 ML) in summer, early autumn and mid to late spring, and low (2 000 ML - 10 000 ML) in late autumn and winter.

Figure 6: Mean daily flow each week for the River Murray at Doctors Point, Albury, 1992-1997

Mean monthly water temperatures for 1995-1997 (figure 7) show summer temperatures ~20°C, autumn falling to ~15°C, winter falling to a low in July of ~10°C and spring increasing to ~17°C.

Figure 7: Mean Monthly Water Temperatures Murray River, Albury, 1995 - 1997

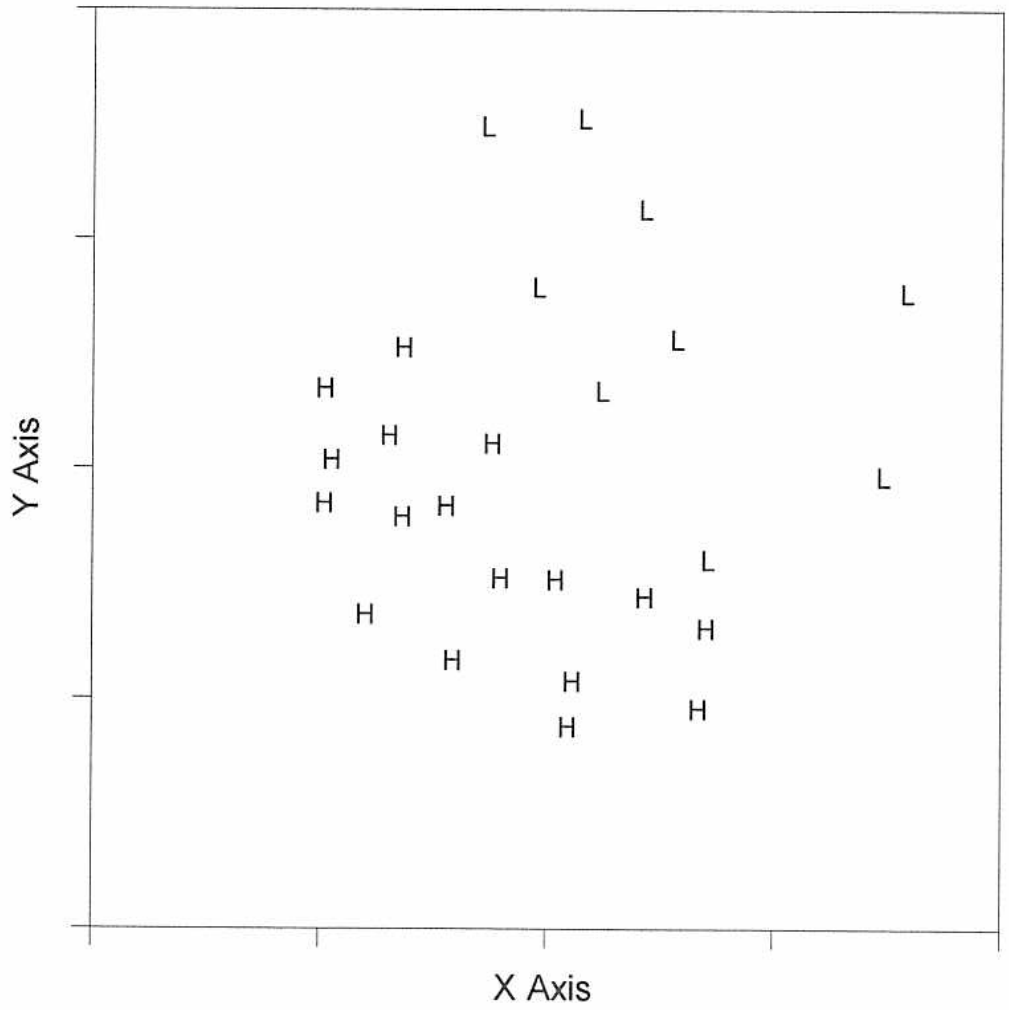


RESULTS

Flow & Season

Percentage composition data from each site were used to calculate monthly means for 30 months from January 1995 to June 1997. These mean values were then compared for flow and season differences. The MDS ordination for flow (figure 8) showed diagonal separation of Low flow (<2m at Union Bridge gauge and < 10 000ML at Doctors Point) and high flow (>2m at Union Bridge Gauge and > 10 000 ML at Doctors Point) samples. The ANOSIM result, $R = 0.568$ $\alpha = 0.000$ confirmed that samples from high flow months were significantly different to samples from low flow months.

Figure 8: Flow Regime - Macroinvertebrate MDS 1995-97
4th Root Transformed Bray-Curtis Matrix
Stress = .18
where L=<10000 >=H (ML/day)



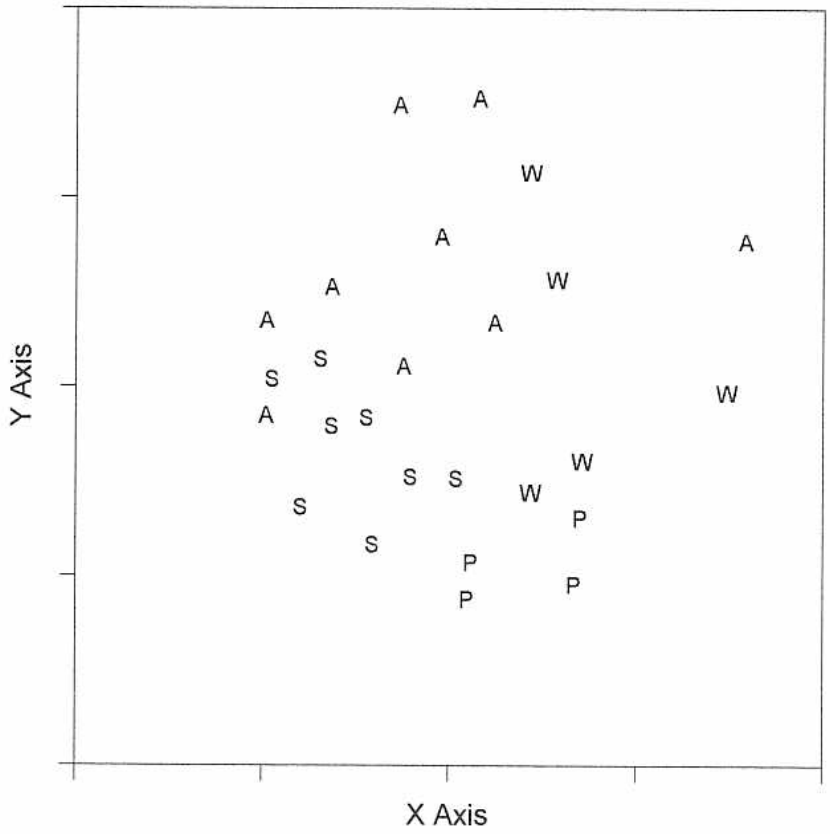
RESULTS

Flow & Season

SIMPER analysis of the dissimilarity between flow groups identified nine low flow taxa (*Triaenodes* spp., *Chaematopsyche* spp., *Micronecta annae, Nososticta solida, Ischneura heterosticta, Hypogasturidae* spp., *Caenidae Genus C, Pisidium* spp. *and Leptoperla* spp.) and six high flow taxa (*Parachironomus* spp., *Rietha* spp., *Paracladapelma* spp., Simulidae spp., *Ecnomus pansus* and Nematode spp.) the abundance of which, contributed to 25% of this dissimilarity.

The same ordination was allocated season symbols (figure 9) and some grouping of seasons was evident. The ANOSIM result for the four season groups ($R = 0.444$ $\alpha = 0.000$) indicates significant difference between these groups.

Figure 9: Season - Macroinvertebrate MDS 1995-97
4th Root Transformed Bray-Curtis Matrix
Stress = .18
where
S = summer (Dec,Jan,Feb)
A = autumn (Mar,Apr,May)
W = winter (Jun,Jul,Aug)
P = spring (Sep,Oct,Nov)



RESULTS

Superimposing the flow ordination on the season ordination, the high flow group includes the spring summer and early autumn group and the low flow groups includes the two late autumn winter group. The two late winter (August) samples although similar to the spring sample are positioned in accordance with their flow character (one high flow and one low flow).

DISCUSSION

Changes To Wastewater Quality

ANM's discharge of tertiary treated wastewater to the Murray River was not detectable from physicochemical data. Multivariate analyses of macroinvertebrate community structure have been successful in detecting the impact of pulp mill discharges (eg. Thomas and Munteanu 1997) and sewage effluent (eg. Cao *et al.* 1996) in freshwater systems. In this study, no differences were detected in the colonising benthic macroinvertebrates of the Murray River prior to and following the improvement of wastewater quality entering the river, but given the overall quality of this tertiary treated wastewater and its dilution in the River this is not surprising. Similarly, Harris *et al.* 1992 were also unable to detect any difference in macroinvertebrates of the LaTrobe River in response to tertiary treated pulp and paper effluent.

DISCUSSION

Season & Location

The greater value of the statistical difference between the season groups compared with the site groups and the position of the samples in the ordinations indicate a gradual change between the seasons, but the high degree of overlap implies either another factor is involved or that habitat patchyness has confounded the results and a greater number of replicates may have improved the resolution. Barmuta (1989) found pronounced seasonal changes confined to erosional habitats, but, overall, temporal and spatial continuity of community structure that did not correspond with easilly identifiable habitats in an upland stream.

DISCUSSION

Flow & Season

Pooling the data for an average from all sites for each month helped to address the problem of habitat/community patchyness and provided a clearer picture of the influence of season and flow on the composition of these benthic macroinvertebrate communities. The most distinctive influence on these data was flow, but this of course, may intrinsically include temperature and other seasonal differences. Lowest flows correspond with lowest temperatures in winter and similarly highest flows with highest temperatures in summer, even though the range of both is severely restricted due to river regulation for summer irrigation.

DISCUSSION

The dominance of the mayfly genus Caenidae and the trichopteran genera Ecnomidae and Hydropsychidae are indicative of lotic depositional and erosional habitats (Merritt and Cummins 1984) and disturbed sites (Marchant *et al.* 1984).

Insect taxa with aquatic larvae are the major contributors to taxonomic diversity in this study and many of these (eg. Trichoptera, Ephemeroptera, Chironomidae) have quite distict seasonal life histories (Marchant *et a*l. 1984 and Lake *et a*l. 1985), which helps to explain the seasonal differences detected here. Examination of the time series data for some of these taxa may yield lifecycle information to permit a greater understanding of this seasonality.

Although it was not possible to separate season from flow and temperature, it is clear that these three factors are major influences on the benthic macroinvertebrate assemblages of the mid Murray River.

CONCLUSIONS

- An appropriate number of sample replicates is required to address habitat patchiness, even when a uniform substrate is used, so that patterns/trends are not lost in noisy data.

- Benthic macroinvertebrates downstream from the newsprint mill's discharge were not different to those upstream, and no changes were observed in this fauna for the six months following the change in discharged water quality.

- The flow regime imposed by river regulation appeared to explain differences in these macroinvertebrates over time although a seasonal continuum was also evident and further analysis of individual taxa may provide a clearer understanding of these macroinvertebrate communities in the Murray River.

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8

BIOLOGICAL MONITORING OF PAPER MILL WASTEWATER

H.M. King

The Murray-Darling Freshwater Research Centre

Poster presented at the 2nd Annual Conference of the Australasian Society for Ecotoxicology: Ecotoxicology and Environmental Management – Towards and Integrated Approach. Sydney, NSW June 1995

ABSTRACT

Australian Newsprint Mills Ltd (ANM) has operated a thermo-mechanical pulp mill in Albury since 1981 and discharges treated wastewater to the Murray River. The Murray-Darling Freshwater Research Centre was contracted to undertake environmental, chemical and biological monitoring of ANM's wastewater, and assess its influence on the River Murray at Albury. The monitoring was conducted in accordance with specifications included in a wastewater discharge licence issued to ANM by the NSW Environment Protection Authority.

The ecotoxicological and biological monitoring program consists of toxicity bioassays using microcrustacea and dipteran larvae, as well as bioaccumulation studies for metals using macrocrustacea and fish, supported by measurements of basic physico-chemical water quality parameters.

Although there was some evidence of bioaccumulation of manganese in the *Cherax destructor* trial, monitoring in 1994 indicated that the potential impact of ANM's wastewater discharge on the fauna of the Murray River would be negligible.

METHODS - GENERAL

The biological monitoring program is conducted independently of any mill operations and testing schedules vary from month to month to eliminate any bias in sampling. Bioassays are conducted in a small on site laboratory at ANM where fresh wastewater samples are readily available.

METHODS - GENERAL

The ecotoxicological and biological monitoring program consists of laboratory bioassays and bioaccumulation studies supported by measurements of temperature, dissolved oxygen, conductivity, pH, hardness and alkalinity.

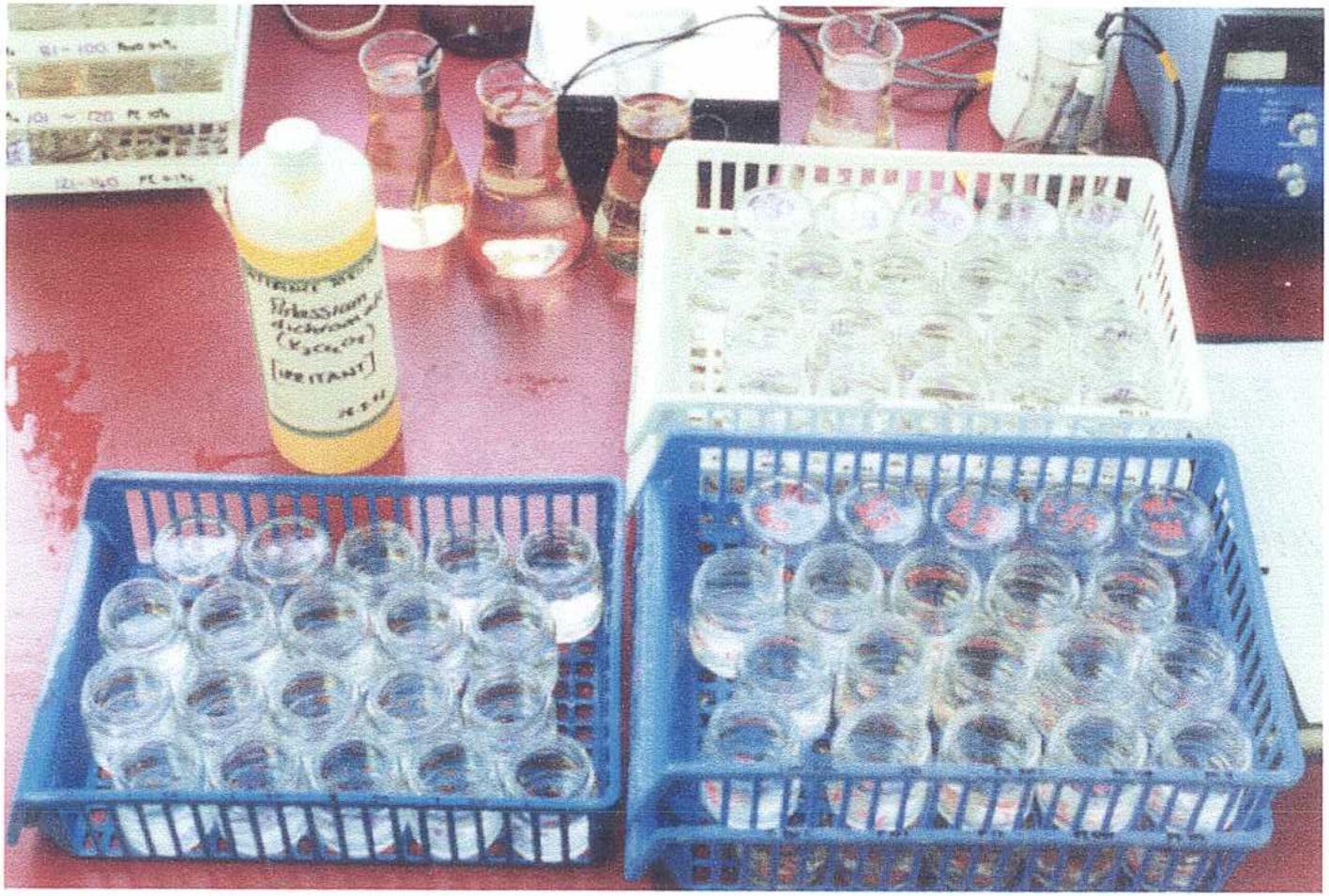
Following range finding tests, all wastewaters are tested at 100%, 10% and 1% concentration. The lowest concentration only being included as a worst case for the receiving waters, given that the wastewater concentration in the river under winter low flow conditions should be less than 0.1%.

Acute Toxicity Tests

Acute toxicity tests using *Daphnia carinata* and *Chironomus tepperi* are conducted using procedures formulated from ASTM's "Standard Guide for Conducting Toxicity Tests on Aqueous Wastewaters with Fishes, Macroinvertebrates and Amphibians" (1990) and USEPA's "Methods for Measuring Acute toxicity of Wastewaters and Receiving Waters to Freshwater and Marine Organisms" (1991).

Tests are performed monthly using 3 replicates of 10 animals in a 60mL vial for each test concentration. Observations of mortality are recorded every 24 hours for 96 hours. Results are considered significant when mortalities exceed 20% and an EC50 value calculated when more than 50% mortality occurs in any of the concentrations. The mean values for each concentration are tabulated for the quarterly reports.

Daphnia carinata – Acute Toxicity Bioassay



Chronic Toxicity Tests

Chronic toxicity tests using *Daphnia carinata* are based on ASTM's "Standard Guide for Conducting Renewal Life-cycle Toxicity Tests with *Daphnia magna* and USEPA's "Short-term Methods for Estimating the Chronic Toxicity of Wastewaters and Receiving Waters to Freshwater Organisms" (1989) which contains methods for *D.magna* and *Ceriodaphnia dubia*.

Chronic toxicity tests are performed on alternate months, using 10 replicates per treatment. A summary of reproductive statistics is provided for each test and total young are compared between treatments using *t*-tests.

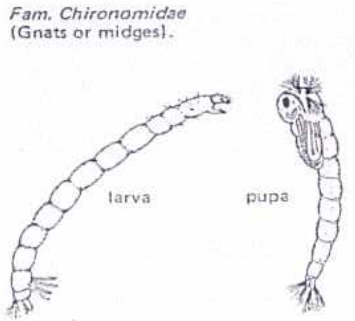
Bioaccumulation

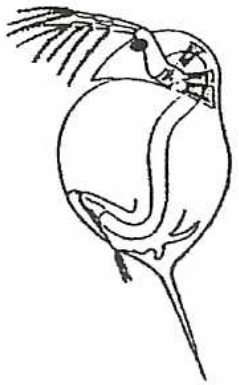
Yabby (*cherax destructor*) bioaccumulation trials were conducted using three 8m3 concrete tanks housing 100 animals each on site at ANM. The control is fed by river water and the test tanks are fed by a mixture of 50% ANM wastewater and 50% river water.

Silver perch (*Bidyanus bidyanus*) trials are conducted in six 60L tanks each housing 150 fingerlings in the lab which is maintained at 20 Celcius. Three tanks are controls fed by river water and three are test tanks fed by 100% pond outlet wastewater.

A subsample of 20 of each is assessed each four weeks (length and weight measurements) and a smaller subsample of these is removed every 3 months and whole animal freeze dried and analysed for; Co, Al, Cr, Ni, Mn, P, Mg, As, Mo, Ag, Y, Ba, Fe, Zn and Cu.

RESULTS & DISCUSSION





Daphnia (Daphniidae)

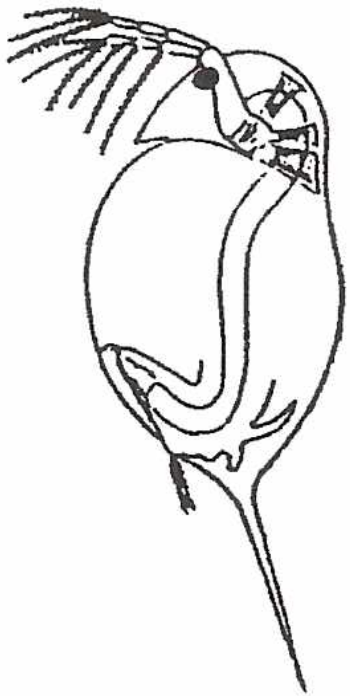
Acute Toxicity Tests

Five out of twelve Daphnia acute toxicity tests conducted in 1994 showed mortalities greater than 20% (Table 1). The test solutions in which this occurred were almost exclusively 100% concentrations of wastewater. There were two tests where the Murray River water downstream of the wastewater discharge resulted in mortalities greater than 20% and these seemed to coincide with highly turbid waters following heavy local rainfall. No EC50 values were achieved. No significant mortalities were recorded for any of the chironomid tests.

Table 1: Summary of 1994 Acute Toxicity Tests
(mortalities exceeding 20%)

Test	Water	Test Period (hr)	Month
D.carinata 96hr	Pond Inlet 100%	48	March
D.carinata 96hr	Pond Inlet 100%	72	April
D.carinata 96hr	Murray below outfall	24	May
D.carinata 96hr	Pond Outlet 100% Pond Inlet 100% Pond 10% & 100%	48 72 96	July
D.carinata 96hr	Pond Inlet 100% Pond 100% Murray below outfall Pond Outlet 100%	72 72 72 96	August

Chronic Toxicity Tests



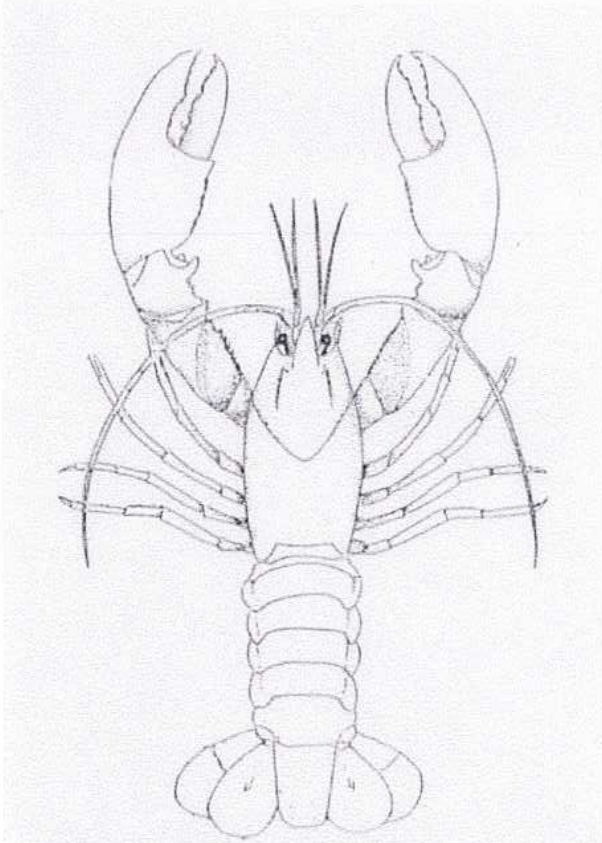
Daphnia (Daphniidae)

All chronic toxicity tests resulted in some statistically significant variations in numbers of young compared with the controls (Table 2). Inhibitory effects on reproductive potential resulted from exposure to 100% wastewater in January, March and August. The most common significant effect was an increase in the mean number of young produced over the 21 day period. This may have been due to supplementary food available in the wastewater samples, as the samples are only filtered to 90um to remove microcrustacea and macro fauna.

Table 2: Summary of 1994 *D.carinata* 21 day Reproduction - Chronic Toxicity Tests

Water	Result	Month
Pond Inlet 100% **Pond 100%** Pond 10%	**Reduced** **Reduced** Enhanced	January
Pond Outlet 100% Pond Outlet 1% Pond 10% Pond Inlet 0.1% and 1%	**Reduced** Enhanced Enhanced Enhanced	March / April
Murray below outfall Pond 100% & 10% Pond Outlet 10%	Enhanced Enhanced Enhanced	May
Pond 100% & 10% Pond Outlet 100%	Enhanced Enhanced	July
Pond Inlet 100% **Pond Outlet 100%**	**Reduced** **Reduced**	August / September

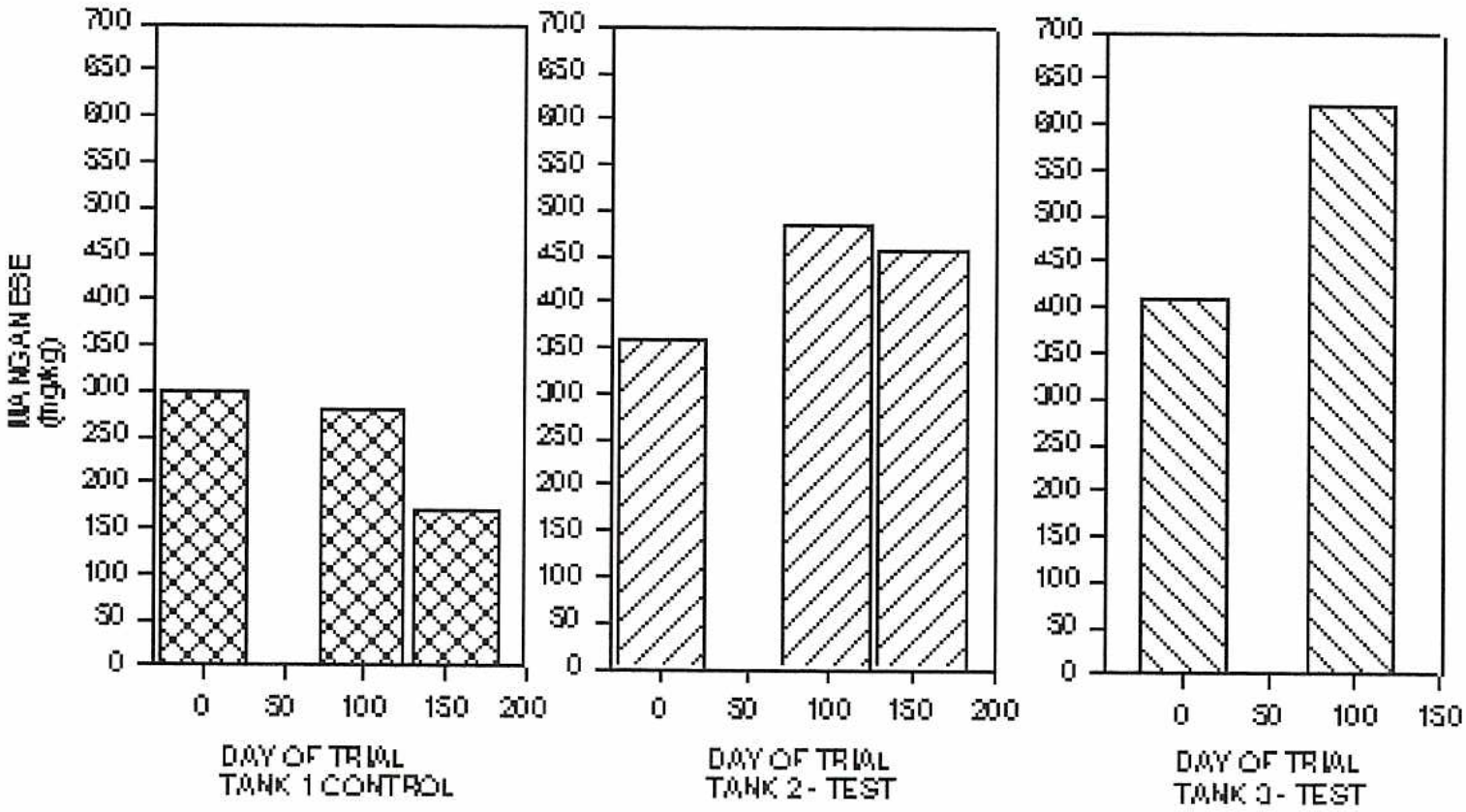
Bioaccumulation Trials



Yabby (*cherax destructor*)

Successive trials to date have shown no difference between the growth and health of animals in the two treatments and the only consistent bioaccumulant of all the elements tested was manganese in *C.destructor* (Figure 1). The manganese is suspected to occur in the form of Mn reducing bacteria which appear as a black discolouration on the shells of the animals in wastewater. The original source of manganese according to ANM is the *Pinus radiata* wood its self and the quantity varies depending on the origin of the logs. The National Water Quality Management Strategy's Australian water Quality Guidelines for Fresh and Marine Waters sets no limits for Manganese.

Figure 1: ANM Bioaccumulation Trial 1994
Cherax destructor - manganese



ANM's Point of Discharge to the Murray River at Albury

CONCLUSIONS

- ✓ Wastewater enters the Murray River at less than 0.1% concentration even during low winter flows.

- ✓ The ecotoxicological and biological monitoring of ANM's wastewater has provided data on acute toxicity, chronic toxicity and bioaccumulation using a variety of organisms.

- ✓ The data for 1994 shows only sporadic low levels of toxicity even at 100% concentration and some bioaccumulation of manganese in *C.destructor*.

- ✓ Considering this data the potential impact of ANM's wastewater discharge on the fauna of the Murray River is negligible.

Acknowledgement

This work was performed as part of a wastewater monitoring consultancy for Australian Newsprint Mills Ltd Albury and the data is presented with their consent.

Many thanks to Ralph Coghill for his support.

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Biomonitoring of Paper Mill Effluent using Fish Ventilatory Signals

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Abstract

Many industries discharge waste waters into our river systems. It is important that these discharges be monitored on a regular basis to ensure that there is no disruption to the overall health of the system. In conjunction with Australian Newsprint Mills Ltd (ANM) at Albury, NSW, the Murray-Darling Freshwater Research Centre (MDFRC) has designed, built and installed a prototype fish ventilatory biomonitoring system into ANM's wastewater discharge, similar to commercial systems produced in the United States and Europe. Eight eastern rainbow fish (*Melanotaenia duboulayi*) are housed in separate one litre flow-through chambers. Each fish is constantly monitored by computer for changes in frequency and strength of opercular movement. Due to the sensitivity of the system, changes in effluent quality are detectable at a sublethal level of 5% concentration of raw effluent, allowing ANM to rectify the problem before chronic levels are reached, minimising the effect on river biota.

Key Words: fish, ventilation, sub-lethal, biomonitor, effluent.

Introduction

The health and well being of the Murray River is important to the health of Australia. A large proportion of Australia's population and industry rely on the Murray as their source of water as well as for disposal of aqueous wastes. Many of these industries discharge effluent directly into the river. This effluent is generally only monitored for physical/chemical changes and rarely assessed for its biological effects directly. Where biological monitoring does occur it is generally in the form of standard toxicity tests on invertebrates (e.g. *Daphnia* 96h EC50 tests) (APHA, 1989). Current legislation in the state of NSW prohibits the use of vertebrates for routine lethal concentration testing, except with the conditional permission of the Minister, given on the recommendation of the Animal Research Review Panel. The drawback to lethal concentration tests is that they use periodic discrete sampling and may completely miss an event or only detect the event after damage to the environment has been done (Gruber *et al.*, 1991; Cairns and Garton, 1982; Morgan and Kuhn, 1984).

Automated biomonitoring systems that continually monitor an effluent stream overcome the problem of missed events. Systems that measure changes in respiration patterns of fish have the ability to detect changes caused by a toxicant at a sublethal level (Diamond *et al.*, 1990; Gruber *et al.*, 1991). The prototype system developed by the Murray Darling Freshwater Research Centre (MDFRC) in conjunction with Australian Newsprint Mills Ltd (ANM) has the ability to monitor continuously the strength of opercular movement and, if required, the frequency of opercular movement. The system has been developed to detect small changes in wastewater quality, specifically as an early warning monitoring device.

Materials and Methods

The fish ventilation monitor at the MDFRC's bioassay laboratory has been purpose-built to monitor ANM's wastewater quality. The wastewater stream is composed of treated process water (derived from activated sludge treatment) plus cooling water and is generally non-toxic. Failure of the tertiary treatment facility or contamination of the wastewater stream could result in the discharge of toxicants. The monitor

is installed in line with the wastewater stream and is designed to detect fish responses to changes in wastewater quality.

Eight eastern rainbow fish (*Melanotaenia duboulayi*, Castelnau) (Allen, 1989), 40mm to 50mm in length are housed in individual one litre flow-through chambers, receiving mill wastewater (Figure 1). The flow-through chambers are constructed in banks of four and enclosed in a darkened box to minimise disturbance to the fish. Individuals are exposed to the wastewater stream for a maximum of two weeks by changing a bank of chambers on a weekly rotation. New batches of fish are allowed 48 hours to acclimatise before monitoring commences. Fish are not fed for the two week period, however some food is available in the effluent stream. Each box is shielded via an earthed Faraday cage to minimise external electrical interference. In each chamber, three electrodes surround the fish. The central electrode is an earth. The other two electrodes are connected to one of eight paired amplifier/filters, designed and built by Rossoft, Albury. The primary amplifier is a high gain, low impedance amplifier based on an "Analog Devices"®AD621 chip. This is coupled to a 5th order, active low-pass filter with the cut-off frequency set to 10Hz (Maxim MAX-580® chip). The raw data is collected at a speed of 50Hz. Signal processing is performed using a 486DX2 66MHz personal computer combined with "Labtech Notebook"® software which execute the calculation and triggering functions.

An estimation of the strength of opercular movement (distance from peak to trough) is determined in Labtech, by calculating the absolute value of the signal and multiplying by two. A half-second (25 point) moving average is used to remove excessive peaks. One point every ten seconds is then plotted, and displayed on computer screen.

Under normal conditions the strength of opercular movement for each individual fish will lie within a range specific to that fish. If a fifty percent reduction in strength occurs, an ON/OFF switch is triggered, sending a digital output to a voting box, which records a "vote" for that fish. If the signal returns to normal the "vote" is removed.

If four or more fish are found to vote at the same time, three auxiliary triggers are set off. The first locks the voting box, which can only be reset manually. The second switches the inflow to fresh water, removing stress before causing harm to the fish. The third enables collection of ten minutes of raw data signal for all fish for later analysis.

The system is checked bimonthly by dosing the wastewater flow in the laboratory with untreated process water at concentrations based on results of EC50 tests conducted on *Daphnia carinata* neonates (APHA, 1989) and third instar *Chironomus tepperi* larvae (ASTM, 1990). These EC50 values are generally between 5% and 10% for the untreated process water.

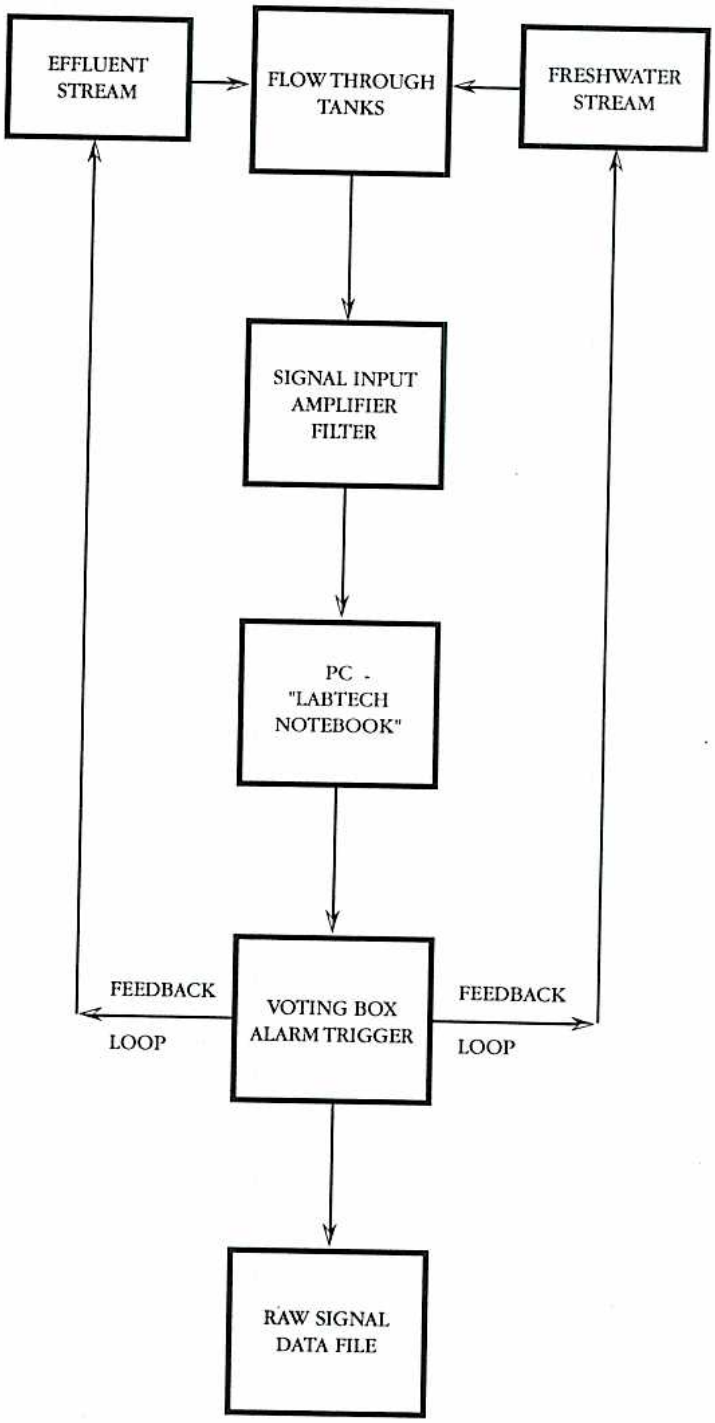

Figure 1: *Flow diagram of configuration of fish ventilation monitor.*

Results and Discussion

A five second plot of the raw signal generated by the opercular movement of one fish under normal conditions and after exposure to 5% primary wastewater is shown in Figure 2.

Ventilation signal strengths (amplitude), collected simultaneously from all eight fish under normal conditions are illustrated in Figure 3. When these fish were then exposed to wastewater contaminated with primary effluent a significant reduction (Table 1) in the strength of opercular movement occurred (Figure 4).

The variation in the degree of change in response to the contaminated wastewater is due to the innate variation in ventilation patterns between individual fish. This makes it important to determine the normal ranges for individual fish during the initial acclimation period (Gehrke, 1988; Diamond *et al.*, 1990; Gruber *et al.*, 1991) . Once established, these ranges determine the trigger value assigned to each individual.

The response time for this trial was 15 minutes, following the introduction of a stock solution of 5% primary effluent. In that time 20L of stock solution was used. Due to the dilution of effluent in the flow through system the final concentration in the exposure tanks was less than half the stock concentration. Comparison with the 96h EC50 value of 5% to 10% primary effluent for *Daphnia carinata* and *Chironomus tepperi* suggests the greater sensitivity of the ventilation monitor. Similar studies with juvenile blue gill exposed to dieldrin demonstrated a significant response at 25% to 30% of the LC50 concentration (Diamond *et al.*, 1990). Gruber *et al.* (1989) reported that a system installed to monitor effluent from an ammunition factory was able to detect 10% of the 96hr LC50 concentration in 24hrs.

Early ventilation monitors analysed the frequency of ventilatory movements. Gruber *et al.* (1989) suggested that ventilatory rate alone was sometimes insufficient to determine acute toxicity. In more recent work Gruber *et al.* (1991) indicated that ventilatory response may be toxicant specific. Diamond *et al.* (1990) showed a depression in amplitude of the signal for blue gill exposed to heavy metals and an increase in the ventilation rate for the same fish exposed to hydrocarbons.

Trials at MDFRC have indicated that a variation in signal amplitude is the more effective parameter for determining stress, caused by exposure of eastern rainbow fish to untreated paper mill effluent.

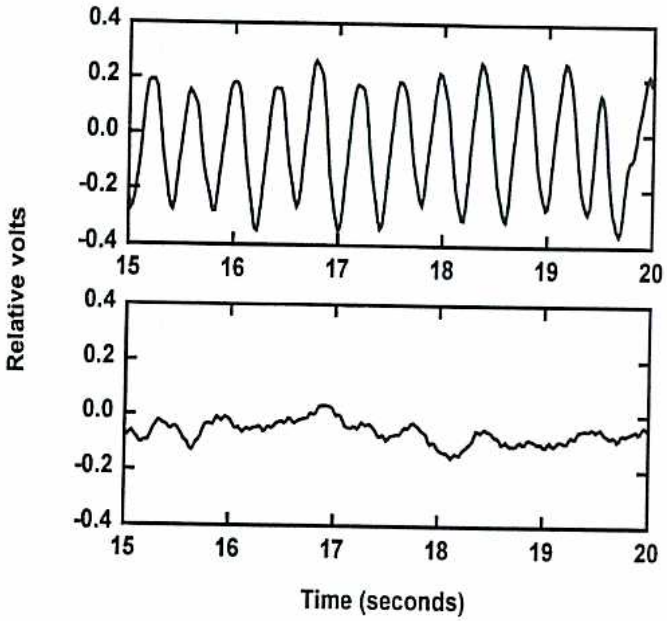

Figure 2: *Ventilatory patterns of M. duboulayi under control (top) and test conditions (bottom).*

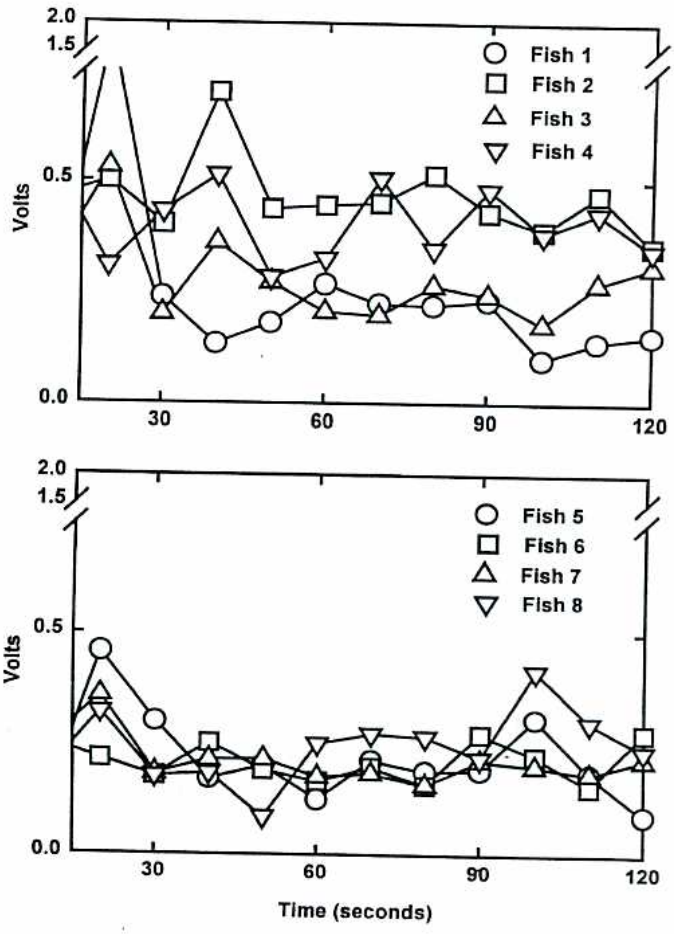

Figure 3: *Ventilation signal strengths of eight M. duboulayi under control conditions.*

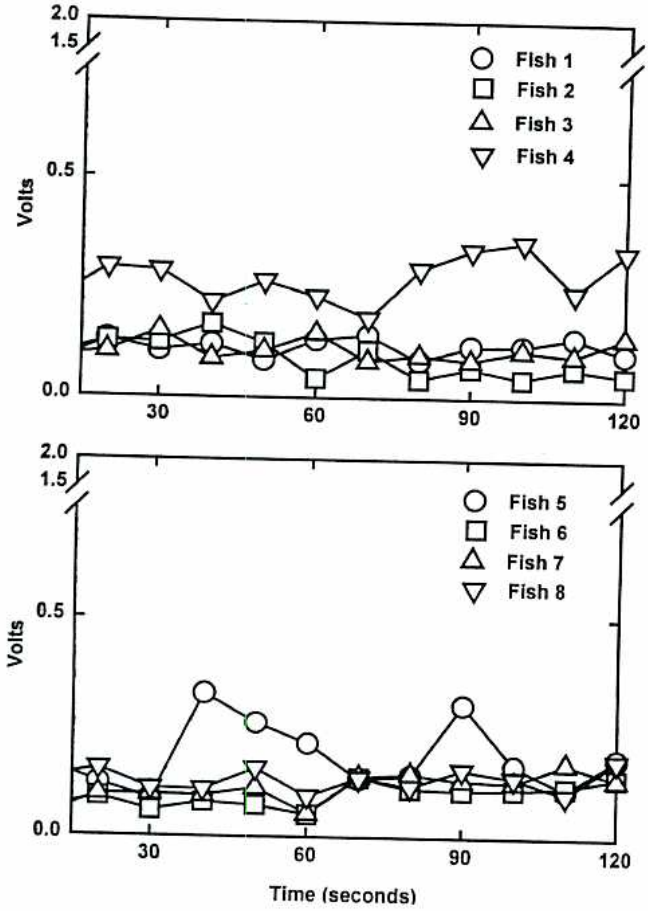

Figure 4: *Ventilation signal strengths of eight M. duboulayi following exposure to 5% primary effluent.*

Conclusion

The fish ventilation monitor installed at Australian Newsprint Mills Ltd, Albury is able to detect contamination of discharged wastewater at a sublethal level. In the event of such contamination, the warning system enables steps to be taken to avoid impact on the receiving waters of the River Murray.

Acknowledgements

The authors wish to thank Ross Wheeler (Rossoft) for his ability and expertise in computing and electronics, Chris Knight (MDFRC) for his help in building the electronic components and Rod Hoffman (ANM) for supplying infra structure support. Special thanks to Terry Hillman (MDFRC) and Ralph Coghill (ANM) for supporting the project and for comments on the manuscript.

Table 1: *Statistical analysis of opercular ventilation amplitude measurements of M. duboulayi following exposure to 5% primary effluent (Cooling pond "Cp 5%").*

FISH	TREATMENT	MEAN	t-VALUE	SIGNIFICANCE p<0.01
1	Control	0.2144	85.879	**
	Cp 5%	0.1206		
2	Control	0.4672	187.293	**
	Cp 5%	0.1627		
3	Control	0.2511	144.460	**
	Cp 5%	0.1232		
4	Control	0.4130	114.888	**
	Cp 5%	0.2711		
5	Control	0.2335	40.496	**
	Cp 5%	0.1656		
6	Control	0.1995	137.917	**
	Cp 5%	0.0931		
7	Control	0.2237	150.026	**
	Cp 5%	0.1078		
8	Control	0.3198	75.073	**
	Cp 5%	0.1179		

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10

The Murray-Darling Freshwater Research Centre

Sublethal biomonitoring of papermill effluent using fish ventilatory signals

D L Nielsen & H M King

Poster presented at the inaugural conference of the Australian Society for Ecotoxicolgy, Sydney 1994

Abstract

Many industries discharge wastewaters into our river systems. It is important that these discharges be monitored on a regular basis to ensure that there is no disruption to the overall health of the system. In conjunction with Australian Newsprint Mills Ltd (ANM) at Albury, NSW, the Murray-Darling Freshwater Research Centre (MDFRC) has designed, built and installed a prototype fish ventilatory biomonitoring system into ANM's wastewater discharge, similar to commercial systems produced in the United States and Europe. Eight eastern rainbow fish (Melanotaenia duboulayi) are housed in separate one litre flow through chambers. Each fish is constantly monitored for changes in frequency and strength of opercular movement. Due to the sensitivity of the system, changes in effluent quality, are detectable at a sublethal level allowing ANM to rectify the problem before chronic levels are reached, minimising the effect on river biota.

Key Words

Fish, ventilation, sub-lethal, biomonitor, effluent.

Introduction

The health and well being of the Murray River is important to the health of Australia. A large proportion of Australia's population and industry rely on the Murray as their source of water as well as for disposal of aqueous wastes. Most of these industries discharge effluent directly into the river. This effluent is generally only monitored for physical/chemical changes and rarely assessed for its biological effects. Where biological monitoring does occur it is generally in the form of standard toxicity tests on invertebrates (i.e. Daphnia 96hr LC50 tests). Current legislation in the state of NSW inhibits the use of vertebrates for lethal concentration testing. The draw back to lethal concentration tests is that they use periodic discrete sampling and may completely miss an event or only detect the event after damage to the environment has been done (Gruber et al 1991, Cairns and Garton 1982, Morgan and Kuhn 1984).

Automated biomonitoring systems that continually monitor an effluent stream overcome the problem of missed events. Systems that measure changes in respiration patterns of fish have the ability to detect changes caused by a toxicant at a sub lethal level (Diamond et. al. 1990; Gruber et. al. 1991).

The prototype system developed by the Murray Darling Freshwater Research Centre (MDFRC) in conjunction with Australian Newsprint Mills Ltd (ANM) has the ability to continuously monitor the strength of opercular movement and, if required, the frequency of opercular movement. The system has been developed to detect small changes in wastewater quality, specifically as an early warning device.

Methods

The fish ventilation monitor at the MDFRC's bioassay laboratory has been purpose built to monitor ANM's wastewater quality. The wastewater stream is composed of treated process water (derived from activated sludge treatment) plus cooling water and is generally non-toxic. Failure of the tertiary treatment facility or contamination of the wastewater stream could result in the discharge of toxicants. The monitor is installed in line with the wastewater stream and is designed to detect fish responses to changes in wastewater quality.

Eight eastern rainbow fish (Melanotaenia duboulayi) are housed in individual one litre flow-through chambers, fed by the Mill wastewater (Figure 1.). The flow-through chambers are constructed in banks of four and enclosed in a darkened box to minimise disturbance to the fish. Individuals are exposed to the wastewater stream for a maximum of two weeks by changing a bank of chambers on a weekly rotation. Each box is shielded via an earthed faraday cage to minimise external electrical interference. In each chamber, three electrodes surround the fish. The central electrode is an earth. The other two electrodes are connected to one of eight paired amplifier/filters, designed and built by Rossoft, Albury. The primary amplifier is a high gain, low impedance amplifier based on an "Analogue Devices" AD621 chip. This is coupled to a 5th order, active low-pass filter with the cut-off frequency set to 10Hz (Maxim MAX-580 chip).

Data is collected as analogue input at 50HZ using a 486DX2 66MHz personal computer combined with "Labtech Notebook" software that performs calculations and triggering functions.

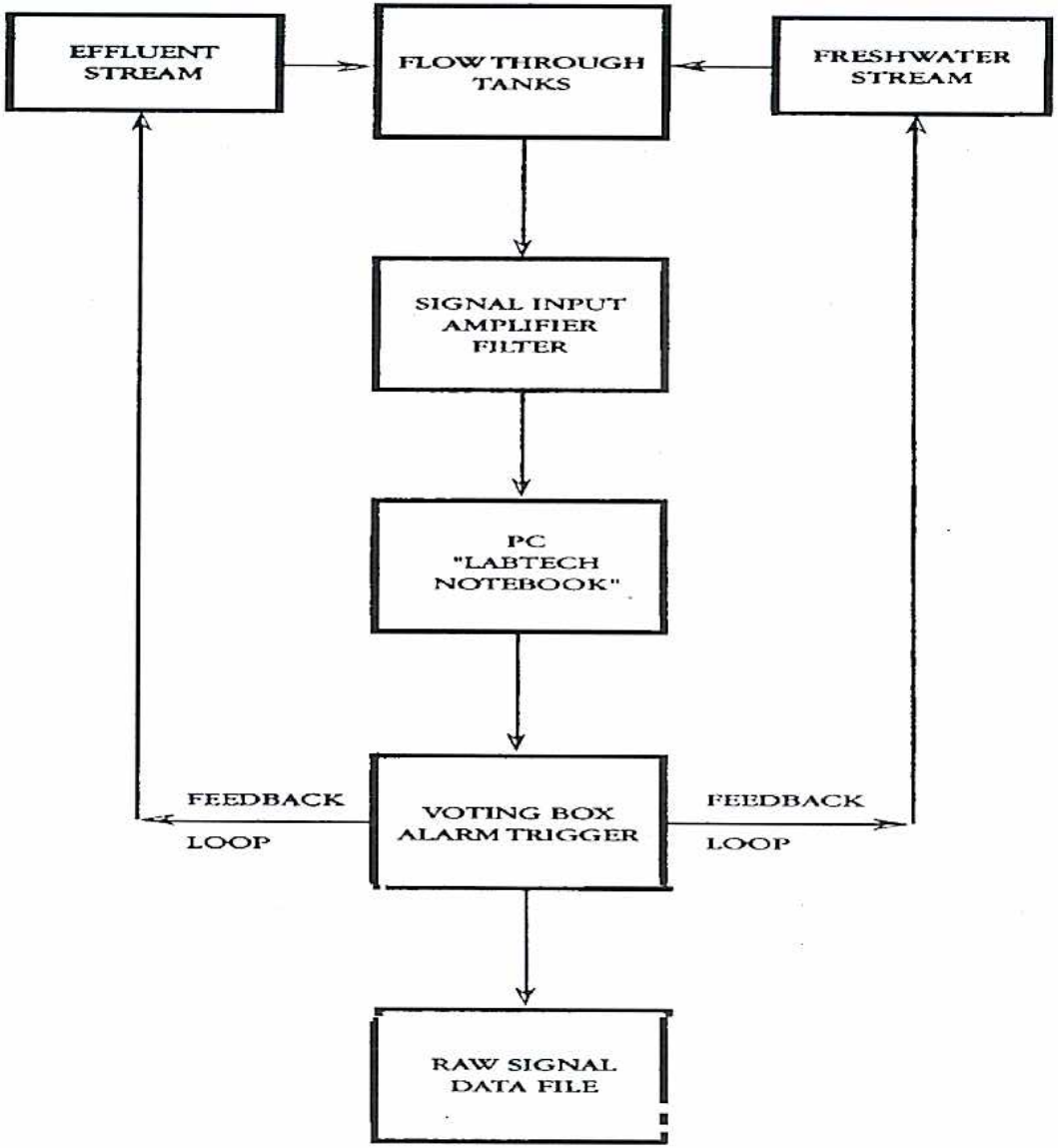
An estimation of the strength of opercular movement (distance from peak to trough) is determined in Labtech, by calculating the absolute value of the signal and multiplying by two. A half-second (25 point) moving average is used to remove excessive peaks. One point every ten seconds is then plotted, and displayed on computer screen.

Under normal conditions the strength of opercular movement for each individual fish will lie within a given range specific to that fish. If a fifty percent reduction in strength occurs, an on/off switch is triggered, sending a digital output to a voting box, which records a "vote" for that fish. If the signal returns to normal the "vote" is removed.

If four or more fish are found to vote at the same time, three auxiliary triggers are set off. The first locks the voting box, which can only be manually reset. The second switches the fish to fresh incoming water, removing stress before causing harm. The third enables collection of ten minutes of raw data signal for all fish for later analysis.

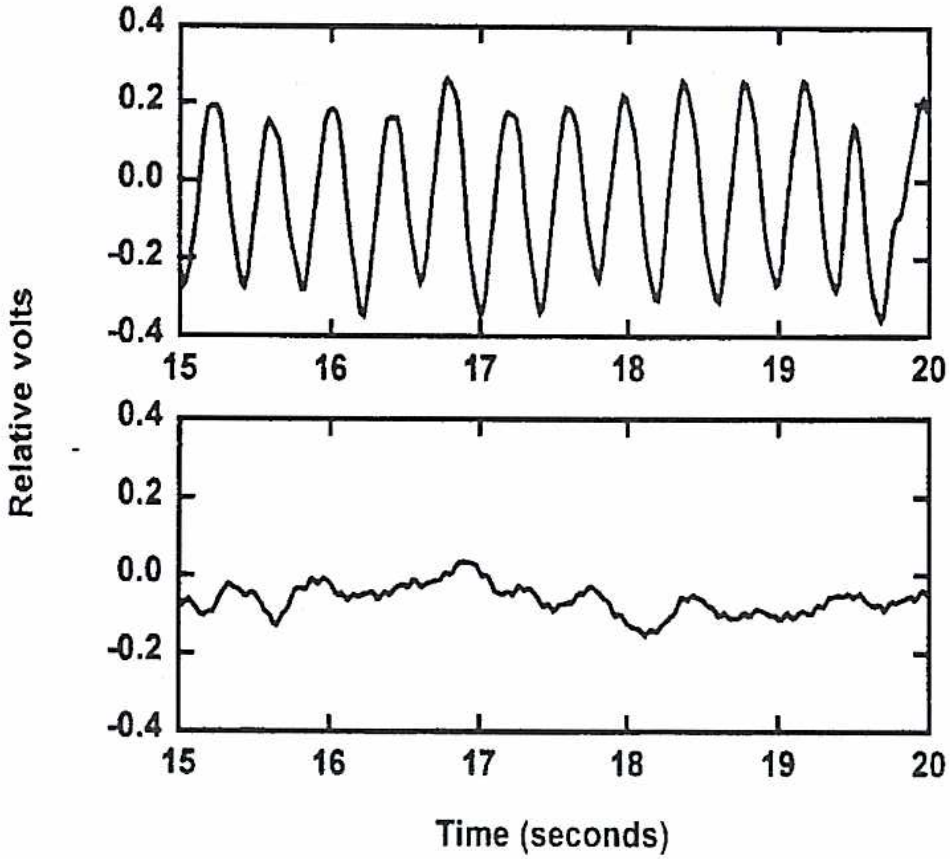
The system is checked bimonthly by dosing the wastewater flow in the laboratory with untreated process water at concentrations based on results of EC50 tests using Daphnia carinata and Chironomus tepperi.

Figure 1: Flow diagram of configuration of fish ventilation monitor



Results

A five second plot of the raw signal generated by the opercular movement of one fish under normal conditions and after exposure to 5% primary wastewater is shown in figure 2.



Ventilation signal strengths (amplitude), collected simultaneously from all eight fish under normal conditions are illustrated in figure 3. When these fish were then exposed to wastewater contaminated with primary effluent a marked reduction in the strength of opercular movement occurred (figure 4).

Figure 3: Control condition signals

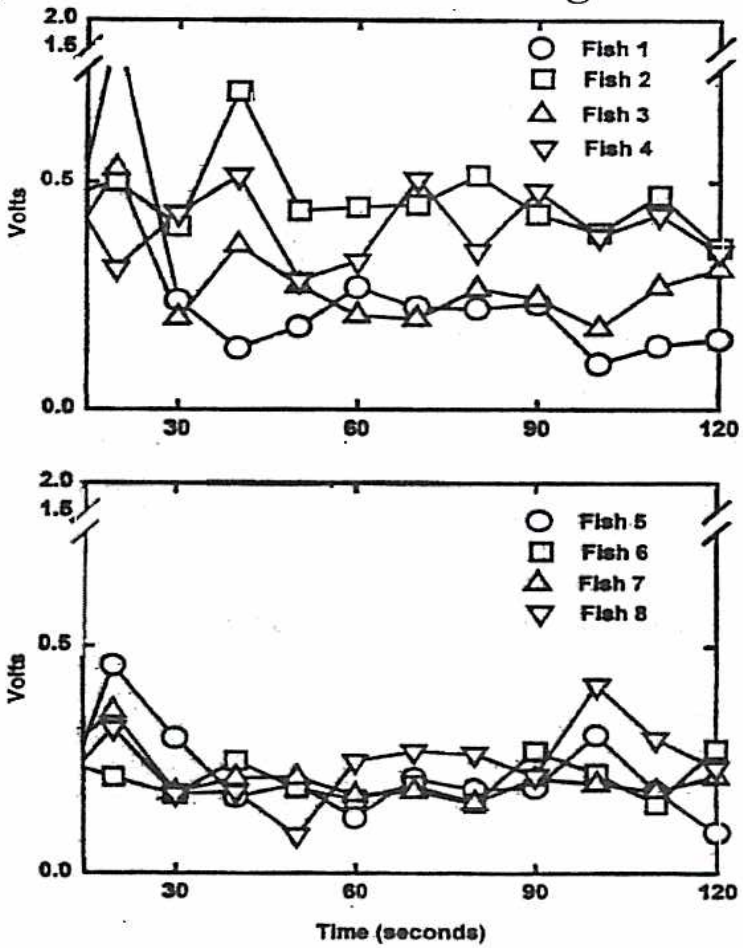
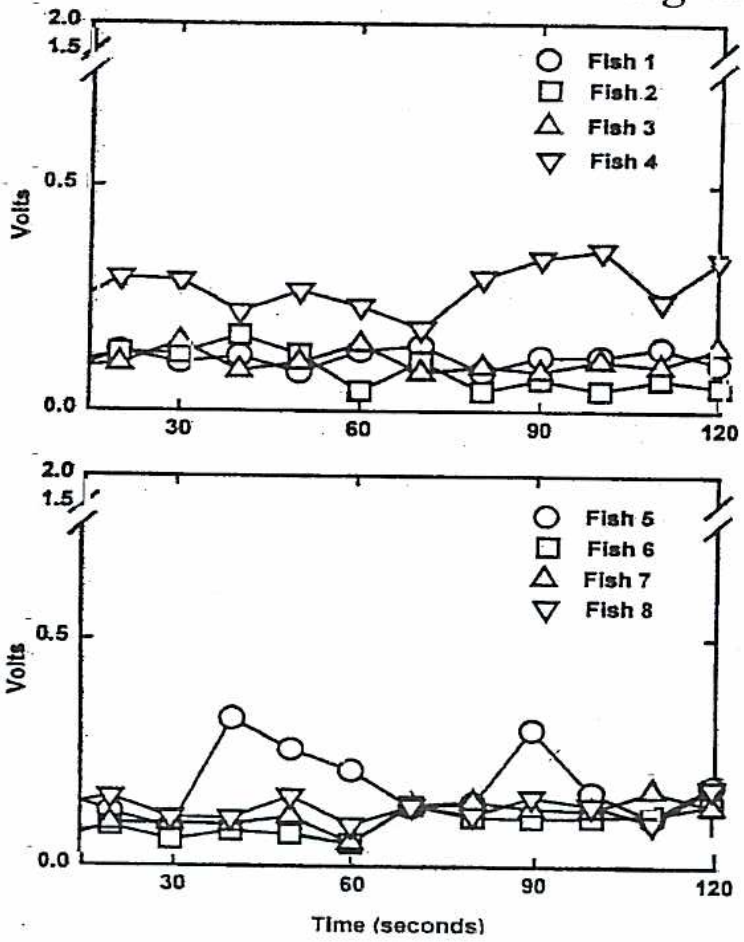


Figure 4: Contaminated condition signals



Discussion

The variation in the degree of change in response to the contaminated wastewater was probably due to innate variation between fish. Gruber et. al. (1991) and Diamond et. al. (1990) noted the importance of determining the normal ranges for each animal during the acclimation period. Once established, these ranges determine the trigger value assigned to each fish.

The response time for this trial was 15 minutes, following the introduction of a stock solution of 5% primary effluent. In that time 20L of stock solution was used. Due to the dilution of effluent in the flow through system the final concentration in the exposure tanks was less than half the stock concentration. Comparison with the 96hr EC50 value for Daphnia carinata suggests the greater sensitivity of ventilation monitor. Similar studies with juvenile blue gill exposed to dieldrin demonstrated a significant response at 25% to 30% of the LC50 concentration (Diamond et. al. 1990). Gruber et. al. (1989) reported that a system installed to monitor effluent from an ammunition factory was able to detect 10% of the 96hr LC50 concentration in 24hrs.

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Trials at MDFRC have indicated that a variation in signal amplitude is the most effective parameter for determining stress, caused by exposure of eastern rainbow fish to untreated paper mill effluent.

Conclusion

The fish ventilation monitor installed at Australian Newsprint Mills Ltd Albury, is able to detect contamination of discharged wastewater at a sub-lethal level.

In the event of such contamination, the warning system enables steps to be taken to avoid impact on the receiving waters of the River Murray.

Acknowledgements

Ralph Coghill and Rod Hoffman, Australian Newsprint Mills Ltd.

Ross Wheeler, Rossoft.

Chris Knight, Murray-Darling Freshwater Research Centre.

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