CHEMICAL AND BIOLOGICAL MONITORING 1997 ANNUAL REPORT

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1.0 AIMS

To undertake biological and chemical monitoring of ANM's wastewater discharged to the River Murray in accordance with New South Wales Environment Protection Agency Licence No.01272; Sections W10 (Ecotoxicological and Biological Monitoring) and W11 (River Environment Monitoring Surveys) to April 1997; and, Sections W9 (Long term bioaccumulation monitoring using fish) and W10 (Microbial oxidation of manganese on artificial substrates) for May 1997 to April 1998. The null hypothesis tested in all cases "that there is no difference between control water and wastewater treatments." Some of the work is incomplete at this stage and will be finalised by June 30 1998, for inclusion in the 1998 Annual Report.

2.0 METHODS

2.1 Ecotoxicological Monitoring [W10]

2.1.1 Sample Preparation

All waters were collected as grab samples in 10L buckets on the morning of the test following ASTM (1990) guidelines. The dilution/control water was obtained from the Lake Hume Resort boat ramp at the Hume Dam on the River Murray upstream of ANM's discharge. The receiving water sample was taken from the River Murray approximately 2 km downstream of the wastewater discharge. The wastewater samples were collected on site at ANM from three locations; the final outfall, the 4-day holding pond and the inlet to the 4-day holding pond. All waters were sieved to 180 um to remove macro and micro fauna that could interfere with the tests, whilst still retaining the samples as close as possible to actual field conditions. The temperature, dissolved oxygen, conductivity, pH, hardness and alkalinity of all samples were measured prior to use (USEPA 1991 pp 44-46). The control and downstream river sample were tested undiluted (USEPA 1991 p47). The three on-site wastewater samples were routinely prepared at three concentrations 100%, 10% and 1%, diluted with control water and aliquots of these were distributed between replicates (ASTM 1990). The laboratory temperature was maintained at 20 ± 5 Celsius throughout the year.

Acute Toxicity Tests

Acute toxicity testing procedures were formulated from ASTM's "Standard Guide for Conducting Toxicity Tests on Aqueous Effluents with Fishes, Macroinvertebrates and Amphibians" (ASTM 1990) and USEPA's "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (USEPA 1991), but in some cases these have been modified slightly to accommodate the local organisms used in this monitoring program.

Acute toxicity tests were designed to obtain information on the immediate effects on test organisms following short term exposure to wastewaters under laboratory conditions. Results of these tests can be used to predict the likely effects of the wastewater on aquatic organisms in receiving waters. The use of locally occurring species ensures greater accuracy of these predictions. The organisms selected for this program occupy different functional groups:

Daphnia carinata is a pelagic microcrustacean and obligate filter feeder; *Chironomus tepperi* is a benthic midge larva which feeds on detritus. *D.carinata* and *C.tepperi* can be reliably cultured in the laboratory in sufficient numbers for the testing program.

Acute toxicity tests were conducted monthly. Test chambers used for both organisms were 60 mL clear round glass jars (resin acids, potential toxic components of paper mill effluent may be adsorbed onto plastic surfaces), approximately 40 mm high to ensure an adequate surface area to volume ratio for gas exchange. Test solutions were prepared in a single batch and apportioned between three replicates positioned randomly.

Daphnid tests were conducted using neonates (less than 24 hr old) from laboratory cultures. The neonates were collected by combining broodstock culture solutions in a 5L aquarium after the adults were transferred to fresh culture solutions. Neonates required for the test were carefully captured using a wide mouthed disposable pipette and released under the surface of the test solutions to minimise trauma due to handling. The neonates were distributed randomly between treatments and replicates so that there were 10 animals per jar. (ASTM 1990 pp 758-760, USEPA 1991 pp 49-51).

Chironomid tests were conducted using final instar larvae from laboratory cultures. Chironomid larvae were sieved from their culture solution and final instars carefully transferred to test solutions using flexible forceps to minimise trauma. The chironomids were distributed randomly between treatments so that there were ten animals per jar (ASTM 1990 pp 758-760). A small strip of facial tissue was added to each jar as a substrate to help prevent clumping of animals.

The organisms were not fed for the duration of the test as faecal matter and undigested food can reduce the dissolved oxygen level and reduce the biological activity of some test materials (ASTM p761). The numbers of dead animals were counted at 24 hours, and again at 48 hours, when the tests were terminated. Death of invertebrates is often difficult to determine, so immobilisation, lack of response to stimuli and opaque colouration or loss of colour were the symptoms interpreted as “effect” (ASTM p761). The results of these tests were reported as EC50 values (the concentration of effluent which results in the “effect” observed for 50% of the organisms), provided sufficient number of organisms were affected.

"Calculation of an EC50 is considered unacceptable if either or both of the following occurred: No treatment other than a control treatment killed or affected less than 37% of the test organisms exposed to it; No treatment killed or affected more than 63% of the organisms exposed to it." also if more than 10% of the controls exhibited signs of disease, stress or death (ASTM p762 , USEPA p55).

Results were reported quarterly to ANM and a summary of significant results is provided in this Annual Report.

Chronic Toxicity Tests

Chronic toxicity testing procedures for a local cladoceran, *Daphnia carinata* were adapted from ASTM's "Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*" (1991) and USEPA's "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (1989) which contain methods based on *Daphnia magna* and *Ceriodaphnia dubia*.

Chronic toxicity tests were designed to provide information to permit the prediction of the possible long term effects of wastewaters on the test organism in receiving waters. These tests were primarily concerned with sublethal effects which may not have been expressed in the short term tests. The cladoceran tests are life cycle tests, in which an animal is assessed for survival, growth and reproduction.

Chronic toxicity tests were conducted at two monthly intervals using *D.carinata*. Test chambers for these cladocerans were clear glass 60 mL jars. Test solutions were prepared in bulk (as for the acute tests) and apportioned between ten replicates. Daphnia neonates were obtained as for the acute tests. The neonates were distributed randomly between treatments and replicates so that there was one animal per jar. (ASTM 1990 p771, USEPA 1989 p106). The cladocerans were fed a daily dose of blended food solution made up of yeast and trout feed (ASTM 1990 p775) and transferred to fresh solutions three times per week (USEPA 1989 p110). Observations of survival, stress and reproduction/number of live young produced were noted at each transfer. The tests continued for a maximum of 21 days (*D.carinata*). The results were considered acceptable if survival of the controls was at least 80% and each surviving control animal had achieved at least three broods. Results rejected if

ephippia (desiccation resistant eggs produced in response to environmental stress) were produced in any of the controls. (USEPA 1989 p122, ASTM 1990 p 774).

Results were analysed in quarterly reports to ANM providing a summary of reproductive statistics for the duration of the tests. The mean number of young produced in each treatment were compared with the control using t-tests to determine their significance @ $p < 0.05$. A summary of the results for tests conducted this year is provided in this Annual Report.

2.2 Bioaccumulation Monitoring

Bioaccumulation trials were conducted to determine the levels of bioaccumulation of metals from ANM's final outfall wastewater using a crustacean (yabby, *Cherax destructor*) and a fish species (carp gudgeon *Hypseleotris spp.*).

2.2.1 Yabby *(Cherax destructor)*

Yabby trials were conducted on site at ANM using three preconditioned 8-9m^3 concrete flow-through tanks each containing ~90 pieces of PVC pipe as hides. Two control tanks were fed by sand filtered river water and the test tank was fed by 50% final outfall wastewater diluted with sand filtered river water. 300 male yabbies of approximately equal size (70 to 80 mm total length) were purchased from a commercial yabby farm. These yabbies were distributed randomly between the tanks to achieve a stocking density of 19/m^2. A subsample (5%) was measured and nine animals (three from each tank) were retained as initial control samples for metals analysis. Temperature, dissolved oxygen, conductivity, pH, hardness and alkalinity were measured fortnightly and 10-20 animals from each tank were measured (weight and length) monthly, and a smaller subsample (3) removed, frozen and freeze dried every three months and at the termination of the trial. One animal from each tank was separated into its three major components; shell + gastrolith; tail + claw muscle and viscera. These samples were then submitted to the MDFRC's chemistry laboratory for acid digestion and shipment to a contract lab for ICP metals analysis.

Mean growth data were analysed using t-tests to determine differences between the control and test treatments. These results were presented in quarterly reports to ANM. The results of

the metals assays were compared for each treatment using the means of the three subsamples. A summary of these results is provided in this Annual Report.

2.2.2 Carp Gudgeon (*Hypseleotris spp*).

Bioaccumulation studies using adult Carp Gudgeon spp. (primarily Western Carp Gudgeon (*Hypseleotris klunzingeri,* Ogilby) with some Lake's Carp Gudgeon (*Hypseleotris* sp.5, undescribed) and Midgley's Carp Gudgeon (*Hypseleotris* sp.4, undescribed)) were conducted in November 1996, using fish captured from a local billabong during the breeding season. The fish were contained in six 90L preconditioned polypropylene flow through tanks ('Nally' Tubs) containing filter boxes with aeration and artificial weed. Three tanks were randomly assigned to each treatment. The control tanks were fed by river water filtered to 1 micron, sterilised by Ultra Violet radiation and adjusted to 1000µS conductivity using stock feed grade salt, and the test tanks were fed by final outfall wastewater. Low stocking densities were used to reduce the impact of territoriality on the fish. Approximately 40 fish were added to each tank and maintained on a diet of frozen tubificid worms and trout pellets. Whole fish samples were collected at the termination of the trial for metals assays. Fish were anaesthetised using 1 mL/L of 'Benzocaine' stock solution (5g/100 mL alcohol) prior to being measured and were killed by overdose with 'Benzocaine' prior to being frozen and freeze dried in preparation for metals analysis. Samples were then submitted to the MDFRC's chemistry laboratory for acid digestion and shipment to a contract lab for ICP analysis. Temperature, dissolved oxygen, conductivity, pH, hardness and alkalinity were measured in each tank fortnightly.

The results of the metals assays were compared for each treatment and the feed using the means of the three subsamples. A summary of these results is provided in this Annual Report.

2.2.3 Golden Perch from ANM's Holding Ponds

Following approval from NSW Fisheries and Charles Sturt University Animal Care and Ethics Committee, bioaccumulation monitoring for metals in fish will be conducted by surveying the golden perch population released into the 50ML (4-day holding pond) or Lake Ettamogah as fingerlings. Two surveys will be conducted in summer of 1997/98 using nets or electrofishing. The number of fish assessed will depend on the variability in size and representation of sex. The fish will be anaesthetised and killed using benzocaine™ and assessed for size, age (using otoliths), diet and gonad development. Flesh samples will be removed from behind the head, frozen, freezedried and homogenised then acid digested in preparation for assay of metals (Al, As, Cd, Cu, Fe, Mn, Pb, and Zn) by ICP at Australian Government Chemical Laboratories (AGAL).

2.2.4 Microbially Mediated Manganese Bioaccumulation

Artificial Substrates

Small pieces of inert plastic were used as artificial substrates in two flow through tanks, one river water (control) and one wastewater (treatment) in conjunction with a crustacean trial. 10 pieces of plastic 50mm x 80mm, bent in half to facilitate Mn extraction, were attached using fine cord to a piece of polythene pipe through a small hole so that both sides were available for biofilm deposition. The substrates were then lowered to a depth of 1.2m so that they were off the floor of the tank but as deep as possible to minimise fouling by filamentous green algae. A further six substrates were immersed in deionised water in glass conical flasks sealed with parafilm to prevent evaporation acting as blanks. Physico-chemical water quality data (temperature (minimum/maximum), dissolved oxygen conductivity, pH, hardness and alkalinity) was collected every fortnight. After six weeks exposure the substrates were carefully removed. Three substrates were randomly selected from each treatment and transferred to 50mL screw capped polyethylene centrifuge tubes. 30mL 1.0M HCl was added to each tube and the tubes were placed horizontally on a shaker table over night to facilitate Mn dissolution. Mn in the HCl extracts was determined by AAS and expressed per unit area of substrate. Triplicates of each treatment measured. These results are included in this Annual Report.

Isolation of Manganese Oxidising Bacteria

Samples of wastewater and small permanently inundated rocks from ANM's 4-day holding pond and irrigation dam (Lake Ettamogah) were collected for culture. Pedomicrobium (PC) agar (Tyler and Marshall 1967) plates containing agar, yeast extract and $MnSO_4$ were innoculated with either wastewater or rock biofilm. These were then cultured at 27°C and kept under observation for the presence of colonies which turned brownish/black as Mn oxide formed. These colonies were subcultured onto fresh media until a pure growth was obtained. These results are included in this Annual Report.

2.3 River Environment Monitoring Surveys

2.3.1 Water

Sample Collection and Handling.

Grab samples were taken at three locations on the river on a monthly basis. Site 1 samples were taken from Mungabarena Reserve (approximately 4 km upstream of the outfall). Site 2 samples were taken at a point approximately 200 m downstream of ANM's outfall (adjacent to the railway bridge). Site 3 samples were taken at a point approximately 1 km downstream of ANM's outfall (adjacent to Union Bridge).

5 samples were taken at each location (for analysis of physical parameters, phosphorus, forms of nitrogen, metals and mercury respectively). All samples were collected and preserved in accordance with Australian Standards AS2031.1 and AS2051 - all preservatives were "ANALAR" grade or better and, clean polyethylene gloves were worn at all times. Sampling blanks were handled and analysed in a similar manner to the samples.

Analysis of water samples.

All metal analyses were performed by NATA registered :-
EML (Chem) Pty Ltd
425 -427 Canterbury Road
Surrey Hills Vic 3127

Concentrations of recoverable aluminium, cadmium, cobalt, chromium, copper, iron, manganese, mercury, lead and zinc were determined.

Physical and nutrient analyses were performed at the Murray-Darling Freshwater Research Centre (MDFRC). Turbidity, colour, specific conductance, total filterable solids, ammonia, oxides of nitrogen (NO_x), organic nitrogen, and total phosphorus were determined according to the methods outlined in the MDFRC Chemistry laboratory's methods manual.

2.3.2 Sediment.

Sample Collection and Handling.

A series of forty sediment samples were taken in May 1997. Sediment samples were collected from three deposition zones on the River Murray. Deposition site A was located at Doctor's Point (about 2 km upstream of ANM's outfall). Deposition sites B and C were approximately equidistant (*ca* 500 m upstream and *ca* 500 m downstream respectively) of ANM's outfall. Samples were collected at 10 meter intervals along the 60 cm depth contour (approximately 2 meters from, and parallel to, the river bank). A total of 20 samples were taken from each deposition zone.

Approximately the top 5 - 10 cm of sediment was directly scooped into 500 mL wide mouthed polyethylene bottles which had previously been acid washed (5% HCl) and repeatedly rinsed with Milli-Q water. Sampling was such that every effort was made to completely fill the sampling bottle with sediment. The bottle was sealed while under water to minimise the loss of fine material.

The samples were immediately returned to the laboratory and air dried. The air dried samples were sieved (2 mm) - the fraction retained by the sieve was weighed and then discarded, the fraction passing through the sieve was weighed and then thoroughly mixed. All subsequent analysis were performed only on the sieved fraction (Grimshaw 1989).

Analysis of Acid Extractable Metals.

The fraction of acid extractable metals in the samples was determined by a modification of the method of Anon (1989). 5 g of sediment was accurately weighed into 50 mL polyethylene centrifuge tubes (which had previously been washed with 5% HCl and extensively rinsed with MILLI - Q water). 25 mL of 0.1 M "ARISTAR" grade HCl was subsequently added to the sediment. The tubes were then capped and placed on a "Ratek"

orbital shaking table for one hour. The samples were allowed to settle overnight and, subsequently filtered through acid washed Whatman GF/C filters. The filtrate was placed in 100 mL polyethylene bottles (which had previously been washed with 5% HNO_3 and repeatedly rinsed with MILLI-Q water) and dispatched to :-

Australian Government Analytical Laboratories (AGAL)
1 Suakin St
PYMBLE NSW 2073,

for analysis by Inductively Couple Plasma Atomic Emission Spectroscopy (ICP-AES). The elements assayed for were aluminium, arsenic, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lanthanum, lead, magnesium, manganese, molybdenum, nickel, silica, silver, strontium, tin, yttrium and zinc. An extraction blank and a standard reference material (Buffalo River sediment - SRM 2707) were processed in exactly the same manner as the samples.

***Analysis for Total Mercury*.**

Approximately 10 g of air dried sample was placed in clean polyethylene bags and dispatched to AGAL for digestion and subsequent analysis by Cold Vapour Generation Atomic Absorption Spectroscopy.

***Analysis for Total Nitrogen*.**

Total nitrogen was determined by a modification of the technique of Hosmoi and Sudo (1986). Approximately 0.25 g of sediment was accurately weighed into acid washed 50 mL centrifuge tubes. 10 mL of an alkaline persulfate digestion medium (0.9 % NaOH, 4.0 % $K_2S_2O_4$) and 20 mL of Milli-Q water was added to each tube. The tubes were sealed and subsequently heated in an autoclave for one hour. The solution was the analysed for nitrate by an automated version of the cadmium reduction method (Clesceri *et al* 1989). All analyses were done at least in duplicate.

***Analysis for Exchangeable Phosphorus*.**

Exchangeable phosphorus was determined by a modification of the method of Anon (1982). About 5 g of sediment was accurately weighed into 50 mL acid washed centrifuge tubes. The sediment was extracted into 25 mL of a 0.5 M sodium bicarbonate solution (pH adjusted to 8.5 with NaOH). The level of soluble reactive phosphate in the extractant was determined by an automated version of the ascorbic acid method (Clesceri *et al* 1989).

2.3.3 Macroinvertebrates

Monitoring of the macroinvertebrate fauna above and below the ANM wastewater discharge was performed using artificial substrate samplers as described in "Macroinvertebrates of the River Murray (Survey and Monitoring: 1980-1985)", (Bennison *et al* 1989). This standard sampling technique was used to obtain results that were directly comparable with respect to both temporal and spatial characteristics. The sampler, placed in an aquatic ecosystem acts as an artificial substrate so that colonisation by benthic organisms can be assessed.

Each sampler consists of a cylinder of black plastic "gutterguard" (mesh size ~ 10 mm^2) approximately 180 mm high x 180 mm diameter, the cylinder is closed on one end by a round piece of "gutterguard" and contains two knitted onion bags as complex substrate and a couple of small rocks as ballast. The top of the sampler is pinched and tied closed with a length of nylon cord which is attached to the limb of an overhanging tree. The sampler sits on the bed of the river for ~ 4 weeks before being retrieved using a 500 μm net.

Artificial substrate samplers were set at three paired sites - the 'controls' opposite Grey's farm approximately 500m above ANM's wastewater discharge; 'mixing zone' near the railway bridge 200m below the discharge; and 'downstream' at Union Bridge 2 km below the discharge. Ten samplers were set monthly at each of the three sites and after a minimum of four weeks, six of these were collected using a fine mesh net and all ten replaced with clean samplers. This allowed for the possible loss of four samplers each month due to disturbance ensuring that sufficient samples were collected. The samples were sieved to 500 μm to remove silt and the remaining portion retained and preserved in 70% alcohol. Samples were sorted using a stereo microscope and identified with reference to MDFRC's taxonomy collection.

Site data were analysed statistically to ascertain similarity/dissimilarity in community structure between site pairs using multivariate techniques developed at Plymouth Marine

Laboratories, England (Clarke 1993, Clarke and Warwick 1994). The Bray Curtis metric was used to compute similarity and construct a dendogram linking samples based on their similarity to each other. Hypothesis testing of predefined groups was performed using ANOSIM (analysis of similarity), which is analogous to the univariate ANOVA (analysis of variance). SIMPER (similarity percentages) were calculated to determine the proportional contribution of species to the dissimilarity between the predefined groups of samples.

Comparisons of the community structure data for a) location relative to ANM's wastewater discharge, and b) pre and post changes to the wastewater discharge, from 1 January 1997 were assessed with reference to flow and season. These resulted are presented in this Annual Report.

2.3.4 Fish

Following consultation with NSW Fisheries, and approval by ANM, NSW Environment Protection Authority and NSW Fisheries, fish surveys were no longer required as part of this monitoring program.

2.4 Reporting

Quarterly reports containing all test results and observations including physico-chemical data were submitted to ANM. This Annual Report containing a summary of results from the monitoring program was submitted to ANM for incorporation as an appendix to their annual report to fulfil their requirements for Condition W16 of Licence No.01272 issued by the NSW Environment Protection Authority.

3.0 RESULTS AND DISCUSSION

3.1 Ecotoxicological and Bioaccumulation Monitoring

3.1.1 Acute and Chronic Toxicity tests

Chironomid Acute Toxicity Tests

Three valid chironomid EC50 tests were conducted between January and July 1997, using three sources of ANM wastewater (final outfall, 4-day pond and pond inlet respectively). No significant mortalities (>20%) were recorded.

Daphnid Acute Toxicity Tests

Seven valid daphnid EC50 tests were conducted between January and July 1997. Figures 1a-c provide a summary of acute toxicity results for the daphnid tests conducted using three sources of ANM wastewater (final outfall, 4-day pond and pond inlet respectively) in 1997. Significant mortalities (>20%) were recorded for the final outfall sample in January (Figure 1a). No significant mortalities were recorded for the 4-day pond wastewater (Figure 1b) or the pond inlet wastewater (Figure 1c).

Daphnid ChronicToxicityTests

Four valid daphnia survival/maturation/reproduction tests were conducted between January and July 1997. A summary of these chronic toxicity test results using t-test values to compare the mean number of young produced in each treatment, compared with the control is provided in Figure 2. A 't value that exceeds +2.1 ($\alpha = 0.05$) denotes a significant reduction in the number of young produced, and conversely, a 't' value that exceeds -2.1 ($\alpha = 0.05$) denotes a significant increase in the number of young produced. A significant reduction in the number of young produced by daphnids exposed to ANM's treated wastewater occurred in the January test for the 4-day pond and pond inlet wastewater samples at all concentrations an in the May test for the pond 100% and pond inlet 100% concentrations. A significant increase in the number of young produced by daphnids exposed to ANM's treated wastewater occurred in the May test for 4-day pond and pond inlet wastewaters at 10% concentrations.

Given that the final concentration of ANM final outfall wastewater in the River Murray would not exceed 1% concentration, there is no evidence to suggest any acute or chronic

toxicity to riverine invertebrates from ANM's discharge, based on the sensitivity of these cladoceran crustacean tests.

3.1.2 Bioaccumulation Studies

Yabby

A four month *C.destructor* trial commenced in November 1996, terminating in March 1997. There was no significant difference in the length measurements between treatments, but the animals living in ANM's wastewater were significantly heavier than those living in river water (reported in the first quarter report to ANM, dated 5 April 1997).

The results (mean of three subsamples) for the eight metals assayed (aluminium, arsenic, cadmium, copper, iron, lead, manganese and zinc) are presented in Figures 3a-h. At each sampling date there are two control sample results and one test (wastewater) sample result. There was no consistent difference in the concentrations of aluminium (Figure 3a), arsenic (figure 3b), copper (Figure 3d), iron (Figure 3e), lead (Figure 4f), manganese (figure 3g), or zinc (Figure 3h) between treatments. Cadmium (Figure 3c) was higher in the wastewater treatment. Iron (figure 3e) was higher for control tank 1, possibly due to contamination from rusting overhead grating. The lead and cadmium concentrations were below 0.5 mg/kg. Arsenic levels (Figure 3b) remained below 1.2 mg/kg. Zinc concentration was variable ranging from 94 (initial) to 233 mg/kg (control 1 final). Aluminium levels ranged from 27 mg/kg (waste 3 final) to 247 mg/kg (initial). Copper levels ranged from 58 (control 1 final) to 92 mg/kg (initial). Iron levels ranged from 61 (waste 3 final) to 299 (Control 1 final). Manganese ranged from 84.2 (Control 1 final) to 394 mg/kg (initial).

The concentrations of metals from the yabby samples at the termination of the trial, separated into three major body components are depicted in figures 4a-h. The greater proportion of the metals Al, As, Cd, Cu, Fe and Zn were found in the viscera; Pb in the tail + claw muscles; and Mn in the shell/carapace. The primary location of manganese in the shells is a further indication that the possible mode of manganese accumulation via bacterial biofilm adhering to the surface of the carapace which oxidise manganese in solution, (Ehrlich 1990, Tyler 1970, Tyler and Marshall 1967a & 1967b).

Carp Gudgeon

The 5 week Carp Gudgeon (*Hypseleotris spp*) trial was conducted in November-December 1996. No significant difference in growth between control and wastewater treatments was recorded and mortalities in the wastewater treatments were significantly lower than those in the controls (reported in the first quarter report to ANM, dated 20 January 1997).

The results (mean of three subsamples) for the eight metals assayed; aluminium, arsenic, cadmium, copper, iron, lead, manganese and zinc, are presented in Figures 5a-h. The data for one initial sample, three replicate control samples and three replicate test (wastewater) samples are plotted on the figure, and the final column depicts the commercial trout pellet feed used throughout the trial.

Aluminium (Figure 5a) and Manganese (Figure 5g) concentrations were slightly higher in wastewater treatments, although this is not considered significant when the variability between replicates is taken into account. Arsenic (Figure 5b) and Cadmiun (Figure 5c) concentrations were less than 1mg/kg, slightly higher in controls than the test replicates. Copper (Figure 5d), Iron (Figure 5e), Lead (Figure 5f) and Zinc (Figure 5h) and lead (Figure 5e) concentrations were consistently higher in controls compared with treatments.

Apart from inconsistent higher concentrations for Al and Mn, it appears that the wastewater treatment actually results in lower metals concentrations in these fish. The commercial pellets were higher in most metals (Al, As, Cd, Cu, Fe, Pb and Mn) assayed, compared with the fish samples but were much lower than the feed assayed in the previous years trial.

These bioaccumulation studies have demonstrated little effect from exposure to 50 - 100% ANM wastewater. The longer term yabby trial show increased growth and some bioaccumulation of manganese (the latter, probably due to the presence of a surficial bacterial biofilm on the the animals). The short term carp gudgeon trial showed that overall, metals concentrations in the fish living in ANM's wastewater were lower than those living in the river water controls.

Golden Perch from ANM's Holding Ponds

Sampling for this assessment is sheduled for December 1997 and January 1998 and will be reported in full in the 1998 Annual Report.

Microbial Oxidation of Manganese using Artificial Substrates

The concentrations of manganese for the two control treatments and two wastewater treatments with errors shown by standard deviation are depicted as box plots in Figure 6. The control substrates contained 20.5 to 34.5mg/m^2 Mn, and the wastewater substrates 90.0 to 91.9 mg/m^2 Mn. t-Tests showed no difference between the two wastewater treatments (t = 0.2, t crit = 2.8 $\alpha = 0.05$) permitting their aggregation. There were however, significant differences between the shallow and deep control samples (t =-0.3, t crit = 2.8 $\alpha = 0.05$); but more importantly between the shallow control and combined wastewater samples (t = -12.6, t crit = 2.4 $\alpha = 0.05$); and the deep control and combined wastewater samples(t = -9.1, t crit = 2.4 $\alpha = 0.05$).

Manganese Oxidising Bacteria

Isolation of manganese oxidising bateria from submerged rock biofilm was successful on agar in the laboratory. Work on these bacteria continues and may be published. Indications for ANM are that the holding ponds are active in reducing the concentration of manganese in wastewater and further supports the theory that Mn accumulation in yabbies is microbially mediated.

3.2 River Environment Monitoring Surveys

3.2.1 Water

A summary of the water quality data is presented in Figure 7. The figure shows the variation of metals (iron, manganese, aluminium, and zinc), nutrients (total phosphorus, organic nitrogen, ammonia and oxides of nitrogen), and, physical parameters (conductivity, turbidity, total filtrable solids and colour) between the three sites over time. (Site 1 samples are represented by circles, site 2 samples are represented by squares and site 3 samples are represented by triangles; lines are included only for clarity and no interpolation between data points is intended.) All the water-quality data accumulated since the commencement of the monitoring program (January 1992) has also been included for purpose of comparison. The figure does not include those analytes not detected in any of the samples or, those whose levels remained very close to their detection limit. Cadmium (0.001 mg/L), cobalt (0.006 mg/L), chromium (0.01 mg/L), lead (0.03 mg/L) and mercury (0.0005 mg/L) were not

detected in any of the samples (detection limits in brackets). Copper (detection limit of 0.004 mg/L) was detected on only two occasions - site 1, 2 and 3 on 9/12/96 (concentrations of 0.009, 0.007 and 0.006 mg Cu /L respectively) and at site 1 on 26/5/97 (0.005 mg Cu /L).

Generally, most of the data show little (if any) variation between sites although, there may be significant variation over time (seasonal effects). The only observed difference of any significance was an elevated value of organic N at site 2 in the April 1997 sample relative to the other sites. The origin of this increased value are unknown but may simply reflect the semi-rural nature of the surrounding riparian zone. It is of note that the effects of the peak observed in June and July in many of the analytes particularly turbidity, Fe, Al, Organic N and total P (discussed in last years annual report) had dissipated by the time the August 1996 sample was taken.

3.2.2 Sediments

Mercury, tin, molybdenum, and silver (all with detection limits of 0.01 mg/kg) were not detected in any of the sediment samples.

The results for the sediment analyses for total persulfate nitrogen (N), exchangeable phosphorus (P) and acid extractable arsenic, aluminium, boron, barium, cadmium, chromium, cobalt, copper, iron, lanthanum, lead, manganese, nickel, strontium, vanadium and yttrium are summarised in Figure 8. For each analyte a box plot showing the analytes distribution for samples taken at Doctors Point (marked A on Fig 8), directly above the outfall (B) and below the outfall (c) are presented. The solid horizontal lines of the box plot represent the 10^{th}, 25^{th}, 50^{th}, 75^{th} and, 90^{th} percentiles of the data - the box itself represents the 25^{th} to 75^{th} percentile. All data outside the 10^{th} and 90^{th} percentiles are shown as open circles on the plots. The mean of the data is represented by a dotted line. From the figure it is clear that all of the samples from the 2 upstream deposition zones have a s greater range and mean concentration than those of the down stream sites. Indeed it can be seen that both the highest means and ranges for all analytes were from the deposition zone at Doctors Point.

In keeping with last years study, 2 upstream depositional sites were sampled - one, some distance upstream of the outfall (site A) and, a second site directly above the outfall (site B). As with last years samples, the sediments from site A contained the same or higher analyte

concentration ranges for all the elements examined compared with sediments from directly upstream (site B) or directly downstream (site C) of the outfall. Further, with the exception of Fe (which was higher at site B than C), the analyte concentrations for sediments from sites B and C tended to fall in the same range.

It is of note that the concentrations of analytes at the 3 sites in 1997 were substantially lower than in 1996. This may simply reflect a re-distribution of fine sediments following the floods towards the end of 1996.

3.2.3 Macroinvertebrates

The complete 1995 species list and abundance data for each of three replicate baskets at the 6 sites on the River Murray, presented by month is included in Table 1 (24 pages). The totals and % totals for each species on the last two of these pages show the mayfly (ephemeroptera) larva, Caenid Genus B as most common taxa, contributing 35.7% and the caddisfly (trichoptera) larva, *Ecnomus pansus* as next most common taxa, contributing 13.6% to the total macroinvertebrate abundance. The mean percentage abundance data for each site at each sampling time was analysed using a Bray-Curtis Similarity matrix (Clarke 1993, Clarke and Warwick 1994). These results are displayed in the cluster analysis dendogram (Figure 9). The dendogram groupings for the whole year show no biological differences between sites with limited grouping according to sampling period/season.

Location and Season

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 10) a high degree of overlap between the categories. The season MDS (figure 11) 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 $R = 0.741$ $\alpha = 0.001$.

One upstream control (Site 6) repeatedly grouped to one end of the ordinations, possibly the result of inappropriate site selection, as it tended to experience higher water velocities than

the other five sites, this interpretation reinforced by the presence of greater abundance and diversity of gripopterygid plecopterans (Williams 1980).

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. Despite the similarity of sites and stratified sampling design large variations in benthic macroinvertebrates between sites and seasons were obseved by Harris et al. (1992) in the La Trobe River. Barmuta (1989) was also concerned with patchyness, he 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.

Response to Alteration of Discharged Wastewater Quality

The quality of ANM's discharged wastewater is best described by its conductivity for the purposes of this study (figure 12) Conductivity measurements were reliable, reflect hardness and alkalinity, and indicate changes to wastewater processing. Throughout 1996 the conductivity of the water discharged to the Murray was generally between 1400µS and 2100µS (mean 1700µS). Following changes to wastewater processing from January 1997 until the end of the assessment in June, the conductivity of the discharge was 100µS to 650µS (mean 310µS). 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.

Mean abundance data for each site from January to May were compared for all sites in 1996 (pre change) and 1997 (post change) to limit seasonal influences that could confuse interpretation. The MDS ordination (figure 13) shows little separation, however some general trends are evident, even though there is considerable overlap between treatments. The upstream control samples 5&6 (pre change) and E&F (post change) lie towards positive on both axes, particularly 6 and F (the same site). The mixing zone samples 3&4 (pre change) and more particularly C&D (post change) lie towards negative on the y axis and around zero on the x axis. The downstream samples 1&2 (pre change) and A&B (post

change) lie scattered throughout the ordination within the boundaries of the other two treatment categories.

ANOSIM results (one-way) testing for homogeneity of samples (excluding site 6 and F due to its consistent difference from all other sites) 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 ($R = 0.019$ $\alpha = 0.33$).

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. 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 despite a 20-25% increase in TDS and conductivity in the river attributable to the Maryvale Mill.

Overall, there was no difference in the macroinvertebrate community structure between river sites for each sampling period, or between first quarter samples for 1996 (whole wastewater - prior to the change in discharged wastewater) and 1997 (cooling water only - following the change in discharged wastewater). Hence, no detected effect of ANM's wastewater discharge on colonising macroinvertebrate communities in the receiving waters of the Murray River.

3.2.4 Fish

Fish surveys were not required following a decision reached at the annual meeting (2 November 1995) and ratified by Allan Lugg from NSW Fisheries (29 January 1996).

4.0 COMMUNICATION

The revised monitoring program entitled “Biological Monitoring Program Proposal for Australian Newsprint Mills Ltd Albury Commencing on 1 January 1997” incorporating modifications to the program following the cessation of wasterwater discharge to the River Murray, submitted to NSW Fisheries and EPA NSW in April 1997, was approved and adopted in June 1997.

A poster titled “Seasonality of Benthic Macroinvertebrates in the River Murray at Albury, and Impact of Newsprint Mill Wastewater” was presented at the Australian Society of Limnology (ASL) Congress in Albury, 26 to 28 September 1997.

A talk titled “Bioaccumulation of Mn in Yabbies - Is It Microbially Mediated?” was presented at the RACI Riverina Branch Meeting, in Albury, 31 October 1997.

The annual review meeting was held on 25 November 1997, with participants from Department of Land and Water Conservation, Environment Protection Authority, Australian Newsprint Mills and The Murray-Darling Freshwater Research Centre.

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APPENDIX 1 (Figures)

Figure 1a-c: Acute toxicity results for eight Daphnia tests exposed to three concentrations of three types of ANM wastewater during 1996. Where a percentage mortality greater than 20% is considered a significant result.

Figure 2: Chronic toxicity results for four Daphnia tests exposed to three concentrations of three types of ANM wastewater during 1996. Where the mean number of young produced by the animals in each treatment is compared, using "t-tests" with the control. A "t" value greater than ±2.1 is considered a significant difference, the +ve exceedences indicate a reduction and the -ve exceedence indicates an increase in the abundance of young produced.

Figure 7: A summary of the water quality data for 1992 to 1996. The figure shows the variation of metals (iron, manganese, aluminium, and zinc), nutrients (total phosphorus, organic nitrogen, ammonia and oxides of nitrogen), and, physical parameters (conductivity, turbidity, total filtrable solids and colour) between the three sites over time. (Site 1 samples are represented by circles, site 2 samples are represented by squares and site 3 samples are represented by triangles; lines are included only for clarity and no interpolation between data points is intended.)

Figure 8: Box plots for the distribution of elements from deposition sites A (Doctor's Point), B (directly above the outfall) and C (directly beneath the outfall).

Figure 9: Dissimilarity classification dendogram of ANM river monitoring sites using macroinvertebrate species abundance for 1995. Where:

Sites 1 and 2 = ‘downstream’ - 2km below the discharge

Sites 3 and 4 = ‘mixing zone’ - immediately downstream of discharge

Sites 5 and 6 = ‘control’ - upstream of the discharge.

APPENDIX 2 (Tables)

Table 1: 1996/7 macroinvertebrate species list and abundance data for three replicate samplers at the six paired ANM river monitoring sites:

Sites 1 and 2 = ‘downstream’ - 2km below the discharge

Sites 3 and 4 = ‘mixing zone’ - immediately downstream of discharge

Sites 5 and 6 = ‘control’ - upstream of the discharge.

5 December, 1997

Our Ref: YH/6/21/1 and YH/6/21/3

Mr Jeff Lassman
Australian Newsprint Mills Limited
Private Bag
LAVINGTON NSW 2641

Dear Jeff,

1997 ANNUAL REPORT - BIOLOGICAL AND CHEMICAL MONITORING

Please find enclosed an unbound copy of the 1996 Annual Report of Chemical and Biological Monitoring for Australian Newsprint Mills Limited, undertaken by The Murray-Darling Freshwater Research Centre. This Annual Report complies with Licence Condition W16 on the ecotoxicological and bioaccumulation monitoring and the river environment monitoring surveys.

Please do not hesitate to contact me on 582355 for any additional information.

Wishing you and your staff a Merry Christmas and a Happy New Year.

Yours sincerely

Helen King

Scientific Officer

Enc. 1997 Annual Report.