Chapter II Metal Accumulation within *Lates calcarifer, Cherax quadricarinatus*, and *Baumea articulata* cultured in Integrated Polyculture -Constructed Wetland Mesocosms Irrigated with Industrial Wastewater

Abstract

Electrical power stations use water as a coolant to dissipate heat from turbines. Dissipating heat from the resulting coolant wastewater for mechanical and environmental purposes is standard industry practice. The wastewaters produced and their thermal capacities are considered to be useable resources. An experiment was set up to culture *Lates calcarifer* (fish), *Cherax quadricarinatus* (crayfish), and *Baumea articulata* (wetland plant) using wastewater from a power station for irrigated wetland mesocosms. The objectives of this experiment were to determine the impact of the wastewater on animal and plant growth, and on tissue concentrations of selected metals (arsenic, cadmium, copper, mercury). Culture of experimental subjects was successful, and the wastewater did not negatively impact on the growth or metal concentration of the edible tissues of animal subjects. Edible tissue samples of *Lates calcarifer* and *Cherax quadricarinatus* met Australian distribution and consumption regulations and guidelines. *Baumea articulata* growth was not impacted by the wastewater, and showed the capacity to absorb copper into below-ground root biomass, a characteristic that could be useful in phytoremediation technology.

1.0 Introduction and literature review

At this point in the study the integration of polyculture and wetlands had been working well. However, the ability of the barramundi and red claw to survive in 100 % wastewater, and whether or not accumulation of metals would occur in animal tissues rendering them unfit for consumption (hence not useful in power station wastewater aquaculture) required investigation. Due to the loss of the Stanwell experimental site in 2000, it was impossible to access large enough volumes of SCL wastewater to run pilot scale experiments at CQU proper. Stanwell Corporation generously granted experimental funds to address selected concerns at CQU using mesocosm scale experiments.

1.1 Electrical power station wastewater and reuse

Coal burning electrical power stations generate and distribute electricity to consumers in most countries. At maximum conversion efficiency, coal combustion processes lose roughly 46 - 50 % of substrate potential energy to the environment as waste heat; partitioning 15% as atmospheric emission and 85% as cooling water dissipation (Parker and Krenkel, 1970, Buchwald, 1976). Parker estimated the amount of heat lost by American power stations at one time exceeded what would be required to heat every home in that country. Lake, river and estuary surface waters are well suited for use as power station cooling substrates because of their relative abundance, their cost, and their effective cooling properties. The quality of the recirculated water used in cooling purposes degrades as non-gaseous substances evapo-concentrate in the water. Periodic flushing out of solids from cooling water is

a necessary procedure resulting in 'blow down' wastewater (Keenan, 1989-90).

Engineered systems efficiently dissipate heat from coolant water for mechanical and environmental benefits. The wastewater produced and its thermal capacity are considered to be useable resources if harnessed with environmentally sound, profitable methods (Buchwald, 1976). Power stations produce approximately 75% of total industrial wastewaters (Reitemeier, 1973) and can be well suited for integration with primary production and methods of ecological management such as aquaculture, hydroponics, and constructed wetland systems. The thermal capacity of the wastewater can assist in maintaining efficient anabolism of cultured poikilotherms, as well as accelerating physical and biochemical processes that remediate many environmental pollutants in that system.

1.2 Aquaculture in power station wastewater

Filipiak (1997) conducted two experiments in which cultured European catfish (*Silurus glanis L.*) were cultured in 2 m^3 net cages in cooling water ponds of the Dolna Odra power plant (Nowe Czarnowo, Poland). The aim of the experiments was to optimize values for daily feed ration for two year old fish at different sizes in the summer season using fish assumed metabolic weight of the fish. Assumed metabolic weight of the fish was defined as 80% of the fresh weight of the fish.

Experiment one consisted of 7 feeding rate treatments (in triplicate) ranging from 0.5 - 3.0 % of the metabolic mean individual weight of fish (weight kg x 0.80). Sixty fish were stocked at weights ranging 554 - 585 g at stocking, and harvested at 814 – 1 649 g after 54 days. Mean growth rate ranged from 0.60 - 1.62 % bwd^{-1}; and

FCRs ranged 0.87 - 1.86 : 1.

Experiment two consisted of 7 feeding rate treatments ranging 0.5 - 3.0 % of the metabolic (weight kg x 0.80) mean individual weight of fish.. Sixty fish were stocked at weights ranging 1 318 – 1 638 g at stocking, and harvested at 1754 - 27419 g after 64 days. Mean growth rate ranged from 0.52 - 0.96 % bwd^{-1}; and FCRs ranged 0.86 - 1.55 : 1.

High water quality variability was experienced: temperature (19.2 - 31.2 ° C), DO (3.8 - 13.8 mg L^{-1}), and pH (8.3 - 9.2), chiefly due to environmental factors. The results indicated that in experiment one, 1.5 % metabolic weight (weight kg x 0.80) was the optimal feeding strategy, and in experiment two, 1.25 - 1.5 % metabolic weight d^{-1} was optimal. The authors suggested the results indicated that it was possible to grow European catfish in net cages in cooling ponds using trout feed pellets, and that the best feed ration was 1.0 - 1.5 % metabolic weight d^{-1}.

This research conducted by Filipiak (1997) was practical and well replicated within the constraints of a single wastewater source (that particular power plant). Successful growth trials of catfish cage culture in power station wastewater were followed with a recommendation on optimal feeding rate.

Heckman (1984) conducted experiments at power plants at Hunter and Huntington, Utah (USA). Water from evaporation ponds, cooling towers, and a freshwater spring were used in the study Three experiments were run: experiment 1 consisted of a short term (8 day) and a long term (60 day) toxicity bioassay, experiment 2 employed dual culture systems integrating plants, and experiment three utilized cage culture in a power station evaporation pond.

Experiment 1 - eight day bioassay. This assay used 1 gallon glass jars and 4 types of wastewater run with pre-aeration time intervals (72, 24, and 0 hrs pre-aeration) as treatments (Table 1.1). Five catfish (*Ictalurus punctatus*) were added to jars (the water was continually aerated in the jars over the assay). One-hundred percent survival was had in all but group 1 (72 hrs) and in group 2 (24 hrs); 80 % survival was measured in those jars.

	group 1 - 72 hrs pre aeration	group 2 - 24 hrs pre aeration	group 3 - no pre aeration
evaporation ponds	jar 1	jar 2	jar 3
cooling towers	jar 4	jar 5	jar 6
neutralizing basin	jar 7	jar 8	jar 9
freshwater spring	jar 10	jar 11	jar 12

Table 1.1. Experimental design Heckman (1984). Eight-day toxicity bioassay

Experiment 1 - 60 day bioassay. This assay utilized dual 10 gallon tanks plumbed together in tandem: one tank held fish and the other filtered fish tank water with oyster shell or plastic bio-ring filter media. The experimental design consisted of 4 types of wastewater supporting culture systems having either a) 15 catfish with a oyster shell filter, b) 15 tilapia (*Oreochromis aurea*) with a oyster shell filter, or c) 15 tilapia with a plastic bead filter (Table 1.2). Statistical analysis was not possible between treatments and neither growth nor feeding rates were reported.

	catfish and oyster filter	tilapia and oyster filter	tilapia and plastic filter
evaporation ponds	system 1	system 2	system 3
cooling towers	system 4	system 5	system 6
neutralizing basin	system 7	system 8	system 9
freshwater spring	system 10	system 11	system 12

Table 1.2. Experimental design Heckman (1984). Sixty-day toxicity bioassay

Experiment 2: Systems A and B in this experiment consisted of five tanks each (dimensions not reported) positioned in a series. Table 1.3 depicts the experimental design. Power station blow-down wastewater was circulated from the oyster shell filter to tank 1 (*Lemna minor* and *Macrobrachium rosenbergii*) → tank 2 (*20 Oreochromis aurea*) → tank 3 (*Lemna minor* and 2 *Oreochromis aurea*) → tank 4 (20 *Ictalurus punctatus*) → before being returned to the oyster shell filter (tank 5). The only difference between system A and system B is that the latter cultivated *Eleocharis dulcis* (water chestnut) in the oyster shell filter (Table 1.3). Tilapia and catfish were fed trout pellets at 2.5 % bwd^{-1} adjusted at 15 day intervals, and feed was not applied to other tanks.

system A		system B	
Lemna minor and *Macrobrachium rosenbergii*	tank 1	*Lemna minor* and *Macrobrachium rosenbergii*	tank 1
Ictalurus punctatus	tank 2	*Ictalurus punctatus*	tank 2
Lemna minor and 2 *Oreochromis aurea*	tank 3	*Lemna minor* and 2 *Oreochromis aurea*	tank 3
Oreochromis aurea	tank 4	*Oreochromis aurea*	tank 4
oyster shell filter	tank 5	oyster shell filter and *Eleocharis dulcis*	tank 5

Table 1.3. Experimental design for experiment 2 of Heckman (1984). Five tank design.

Fish fed on trout feed grew from 10-50 % every 15 days. When feeding strategies were changed to 15 % bwd^{-1} duckweed only (without trout pellets) over days 45 to 75 of fish culture period growth thereafter was marginal in tilapia, and catfish lost weight. Another change in feeding strategies (from day 76 to day 132) saw growth of up to 28 % every 15 days when duckweed was mixed with trout feed at ~ 0.2 - 15.0: 1 ratios; FCRs were lowest during the period when this feeding strategy was followed. An experimental mean FCR of 2.47 : 1 and 3.84 : 1 was reported for tilapia and catfish tanks over the experiment (inclusive of all feeding strategies). The water chestnut (*Eleocharis dulcis*) plant grown in system B oyster filter grew poorly under fluorescent lighting, but grew well under high intensity discharge (HID) lighting. Water quality of the systems ranged as follows: dissolved oxygen (1.6 - 7.2 mg L^{-1}), pH (7.4 - 8.5), conductivity (1.62 - 7.2 mohms) and temperature 23 - 31 °C.

Experiment 3: A 1.22 m^3 rubber coated mesh cage was floated in the evaporation pond at the Hunter Power Pant; 15 tilapia were cultured in the cage. The fish were fed 'some' of the mosses and insects that were found in the pond, and grew from a mean body weight of 46.5 g to 72.9 g in 26 days. Mayflies, dragonflies, damsel flies and numerous water beetles, and abundant vegetation were spotted in the cooling pond.

The authors suggest their tests showed that there was no apparent effect upon the organisms of using the wastewaters tested, and that the wastewater could be used without aeration pre-treatment

The experiments reported by Heckman (1984) in each case were un-replicated, a serious experimental design flaw. However, due to the repeatedly

successful fish growth Hackman achieved in independent systems at different time periods (when using commercial trout pellets), there is evidence that suggests fish (and possibly crayfish) can survive and grow well in Utah power plant cooling water. Also, the author noted abundant biodiversity in the cooling ponds that nourished the tilapia, showing the direct free-benefit afforded to the aquaculture activity by unintentionally integrated aquatic biodiversity.

In summary of section 1.2, it would appear that in the few cases reported, power stations (that use freshwater) have the capacity to grow fish and take advantage of the dissipating heat under certain conditions. There was no information concerning the impact of power station wastewater upon fish / crayfish growth rate, nor upon the nutritional or humans consumption regulation aspects of metals in the fish product.

This experiment aimed to culture barramundi and red claw in power station wastewater, and determine the impact on animal growth and nutritional status of the product in terms of concentration of metals in fish tissue. Additionally, the evaluation of emergent plant growth, and plant tissue metal concentrations will be completed as part of the integrated systems scope of the study.

1.3 Metals of Concern: Background levels and Standards of Seafood Products

Stanwell Corporation Limited power station cooling water sourced from the Fitzroy River (Queensland, Australia) holds vapor-concentrated naturally occurring ion and elements including metals after use by the power station. The accumulation of metals within species cultured in that water was of particular concern regarding

product safety regulations and consumption recommendations. Metal accumulation in seafood is an issue (e.g. disease in Japan) that has been given attention in recent global media. The potential for harm caused by consumption of metals ingested with food is generally dependent upon the specific element, concentration and form of that element in the consumed portions, and the amount consumed over time relative to gender, age, and physiological conditions such as pregnancy. The metals of regulatory concern in this study included arsenic, cadmium, copper, and mercury.

Arsenic is a natural occurring element that exists in organic and inorganic forms. Anthropogenic sources of arsenic include agricultural applications, coal-fired power plant emissions, mine tailings, and product manufacturing sources (Edmonds and Francesconi, 1993). Freshwater ranges of arsenic are normally less than 10 μg L^{-1}(NRCC, 1978). The concentration of total arsenic in fish muscle sampled from numerous species taken from oceans, estuaries, rivers systems and lakes around the world is reported to range from 0.01 to 50.0 mg kg^{-1} wet weight with the vast majority of levels < 1.0 mg kg^{-1} (Chen, 2002, Eisler, 1988, Olsen, 1983, Swales et al., 1998). Dependent on species and growth stage, fish showed sub-lethal effects in growth and reproduction when subjected to waters containing 0.1 - 20.0 mg L^{-1} with lethal doses occurring at 15.0 - 250.0 mg L^{-1} (Eisler, 1988). Published concentrations of total arsenic in the tail meats of the Torres Straight crayfish, southern rock lobster, and Moreton Bay bugs range from 11.2 to 55.6 mg kg^{-1} wet weight (Olsen, 1983, Evans-Illidge, 1997).

Ingestion of food-bound arsenic is the primary route of exposure for humans (Munoz et al., 2000), and most foods be low levels of arsenic, although concentrations in seafood can be up to 10 times that of other foods (FSANZ, 2003). The arsenic found in seafood is primarily in the organic form which is less toxic than

the inorganic form. Due to the costs and complexity of arsenic speciation in fish muscle tissue analysis, it is common practice to calculate inorganic species concentration by multiplying total arsenic concentrations from 0.01 at lower element concentrations to 0.05 at higher element concentrations (i.e. 20 mg kg^{-1})(Olsen, 1983, Edmonds and Francesconi, 1993, Evans-Illidge, 1997). An ingestion limit of inorganic arsenic of 21 μg kg^{-1} / bw $week^{-1}$ is recommended (ANZFA, 1999).

Cadmium is found in low levels in the natural environment, and in higher concentrations in and around industrialized areas with one well recognized anthropogenic point source being the burning of fossil fuels (Edmonds and Francesconi, 1993). Freshwater ranges of cadmium levels are typically between 0.05 - 0.20 ppb diluting to open ocean concentrations of 0.01 - 1.0 ppb (Korte, 1983). The concentration of cadmium in fish muscle sampled from numerous species taken from oceans, estuaries, rivers systems and lakes around the world reportedly ranges from < 0.01 - 0.4 mg kg^{-1} wet weight (Eisler, 1985, Chen, 2002, Olsen, 1983). Eisler (1985) composed a cadmium synopsis, finding that, dependent on species and growth stage, fish show sub-lethal effects in growth and reproduction when subjected to waters containing 0.2 - 6.0 μg L^{-1} cadmium and mortality at 0.6 - 7.0 μg L^{-1}. Bioaccumulation of the element occurs in ambient concentrations was reported as low as 0.02 μg L^{-1}. Published concentrations of cadmium in the tail meats of marine crayfish, crab, and lobster sampled from Australia and the United States ranged 0.01 - 0.63 mg kg^{-1} wet weight (Olsen, 1983, Eisler, 1985, Evans-Illidge, 1997).

The ingestion of food-bound cadmium is the primary route of exposure for humans. An ingestion limit of 7 μg kg^{-1} / bw $week^{-1}$ total cadmium is recommended (WHO, 2001b).

Copper is found widely in the natural world and has many industrial and agribusiness uses. The concentration of copper in fish muscle sampled from numerous species taken from non-polluted oceans, estuaries, rivers systems and lakes around the world was reported to range 0.11 - 1.9 mg kg^{-1} wet weight (Chen, 2002, Swales et al., 1998, Olsen, 1983). Published concentrations of copper in the tail meats of the Torres Straight crayfish, southern rock lobster, and Moreton Bay bugs ranged from 0.69 to 11.0 mg kg^{-1} wet weight (Olsen, 1983, Evans-Illidge, 1997).

Ingestion of food-bound copper is the primary route of exposure for non-occupationally exposed humans (WHO 1996). An ingestion limit of 1.4 mg kg^{-1} / bw $week^{-1}$ total copper is recommended for adults (WHO, 1996). Copper is of particular concern in this study as SCL wastewater used in the fish culture experiments (Chapters 2 and 3) was unusually high in copper due to power station pipe leaching. Concentrations of the other metals investigated in this study (i.e. As, Cd, Cu, and Hg) were not of special concern with regard to wastewater concentrations, as their concentrations ranged within surface water values.

Naturally occurring mercury exists in organic, inorganic, and elemental forms, and major anthropogenic sources include fossil fuel combustion, mining, metal processing, pulp and paper milling, and production of paint, insecticide, herbicide, fertilizer, and disposable batteries (Edmonds and Francesconi, 1993). Unpolluted rivers, lakes and estuaries typically contain < 0.05 μg L^{-1} mercury diluting to open ocean concentrations of < 0.01 μg L^{-1} (Fitzgerald, 1979, Nishimura and Kumagai, 1983). The concentration of mercury in fish muscle sampled from numerous species taken from oceans, estuaries, rivers systems and lakes around the world reportedly ranges from < 0.01 - 14.0 mg kg^{-1} wet weight with the vast majority of levels reported < 0.5 mg kg^{-1} (Eisler, 1987, Chen, 2002, Olsen, 1983, Swales et

al., 1998). Eisler (1987) composed a mercury synopsis, and found that, dependent on species and growth stage, aquatic species show adverse effects in waters containing 0.03 - 0.1 μg L^{-1} mercury and fish mortality at 0.1 - 440 μg L^{-1}. Published concentrations of mercury in the tail meats of marine crayfish, crab, and lobster sampled from Australian, United States, and Canada ranged from 0.01 to 1.60 mg kg^{-1} wet weight (Olsen, 1983, Evans-Illidge, 1997, Eisler, 1987).

Ingestion of food-bound mercury is the primary route of exposure for humans, and seafood contains much higher concentrations of mercury than most other foods (FSANZ, 2003). More than 80 % of mercury found in fish is methylated, a form derived as an organic by-product of microbial chemotrophic activity, and toxic to humans (Huckabee et al., 1979, FSANZ, 2003). An ingestion limit of 5 μg kg^{-1} / bw $week^{-1}$ total mercury is recommended (WHO, 1989b).

Food Standards Australia New Zealand (2003) sampled the total dietary exposure of Australian individuals to As, Cd, Cu, and Hg for contrast to the established tolerable limits for metal ingestion. When dietary exposure data of Australian males (25-34 years) was expressed as a percentage of tolerable limits, mean intakes of that sample group were well below the tolerable limit in all cases (FSANZ, 2003).

With respect to regional enforceable guidelines, the Australia New Zealand Food Standards Code prescribes maximum levels (MLs) of specific metals in nominated foods. Maximum levels were set with respect to achievable production and natural resource management goals, and public health and safety, with consideration to Australia's international trade obligations under the Technical Barrier to Trade Agreement and the WTO Sanitary and Phytosanitary Agreements.

Maximum levels (MLs) have been established in foods that provide a significant contribution to the total dietary exposure of particular metal toxicity within the tissues that are ordinarily consumed. The MLs for fish and crustacean inorganic arsenic (2 000 $\mu g\ kg^{-1}$), fish mercury (1 000 $\mu g\ kg^{-1}$), crustacean mercury (500 $\mu g\ kg^{-1}$) were established. Cadmium MLs exist for foods ranging from wheat and rice (100 $\mu g\ kg^{-1}$) to sheep, cow and pig kidney meat (2 500 $\mu g\ kg^{-1}$), but not for fish or crustaceans. Although copper was not addressed in the ML sections of the code, it was addressed in the Generally Expected Levels (GELs) that complement MLs by providing food metal concentration assessment benchmarks (i.e. the levels of metals to be expected in the food) which help primary producers maintain product quality control. GELs are not enforceable. However, if production samples are higher than a listed GEL (at the 90th percentile) it is recommended to determine whether elevated concentrations are consistent, and if so, to identify the source and manage a solution. For the element copper, GELs exist for fish and crustaceans: at the 90th percentile, the limit is 2 000 $\mu g\ kg^{-1}$fresh weight for fish and 20 000 $\mu g\ kg^{-1}$fresh weight for crustaceans. GELs listed are limited to the availability of adequate data, and do not yet exist in fish and crustacean categories for arsenic, cadmium, or mercury.

2.0 Experiment - Metal Accumulation within *Lates calcarifer, Cherax quadricarinatus*, and *Baumea articulata* cultured in Integrated Polyculture - Constructed Wetland Mesocosms Irrigated with Industrial Wastewater

2.1 Experimental goals, objectives, and hypotheses

The goals of this experiment were to culture successfully and evaluate *Lates calcarifer* and *Cherax quadricarinatus* in mesocosm scale polyculture - wetland systems irrigated with Stanwell Corporation Limited (SCL) power station wastewater at 0 %, 50 %, and 100 % wastewater concentrations. Additionally this experiment was to provide a wastewater source for hydroponic rose cultivation trial (Chapter IV), and thus linking floral hydroponics to industrial wastewaters, wastewaters that in this experiment had been used to support integrated wetland polyculture of barramundi and red claw.

The objectives of this experiment were (a) to determine whether e differences occur between 0 %, 50 %, and 100 % wastewater culture treatments in terms of *Lates calcarifer, Cherax quadricarinatus*, and *Baumea articulata* growth; and (b) to quantify total arsenic (As), total cadmium (Cd), total copper (Cu) and total mercury (Hg) concentrations within the edible muscle tissue of *Lates calcarifer* and *Cherax quadricarinatus*, as well as in above ground (AG) and below ground (BG) *Baumea articulata* biomass.

It was hoped that the completion of the activities outlined above would offer practical knowledge in regard to culturing barramundi and red claw using power station wastewater, with emphasis on animal growth and animal / plant tissue metal

concentration (as outlined in hypothesis below), as well as offering a product consumption evaluation relative to Australian and multi-national nutritional and production recommendations and regulations.

Null Hypothesis I

There will be no difference in the fresh weight of *Lates calcarifer* and *Cherax quadricarinatus*, and the dry weights of *Baumea articulata* cultured in 0 %, 50 %, and 100 % SCL power station wastewater.

Alternate Hypothesis I

Lates calcarifer, *Cherax quadricarinatus*, and *Baumea articulata* cultured in 50 % and 100% power station wastewater will have lower fresh/dry weights than those cultured in the 0 % control.

Null Hypothesis II

There will be no difference in the concentration of arsenic, cadmium, copper, and mercury within the tissue samples of *Lates calcarifer, Cherax quadricarinatus* and *Baumea articulata* cultured in 0%, 50 %, and 100% SCL wastewater.

Alternate Hypothesis II

There will be greater concentrations of arsenic, cadmium, copper, and mercury within the tissue samples of *Lates calcarifer, Cherax quadricarinatus*, and *Baumea articulata* cultured in 50 % and 100% SCL wastewater than in those cultured in the 0 % control.

2.2 Experimental and system design

A control and two experimental concentrations (0 %, 50 %, and 100 %) of SCL wastewater were trialed in five replicates randomly positioned within the NW quadrant of the Plant Sciences Group (PSG) green house (Figure 2.1). The green-house was semi-enclosed, employing mesh walls and a clear poly top that reduced sun intensity by ~ 10 %. Each replicate consisted of an 80 L poly fish culture tank, a 50 L poly wetland tank, and a 25 L plastic crayfish culture bucket plumbed together, served to provide 5 polyculture-wetland mesocosm replicates for each of three experimental treatments.

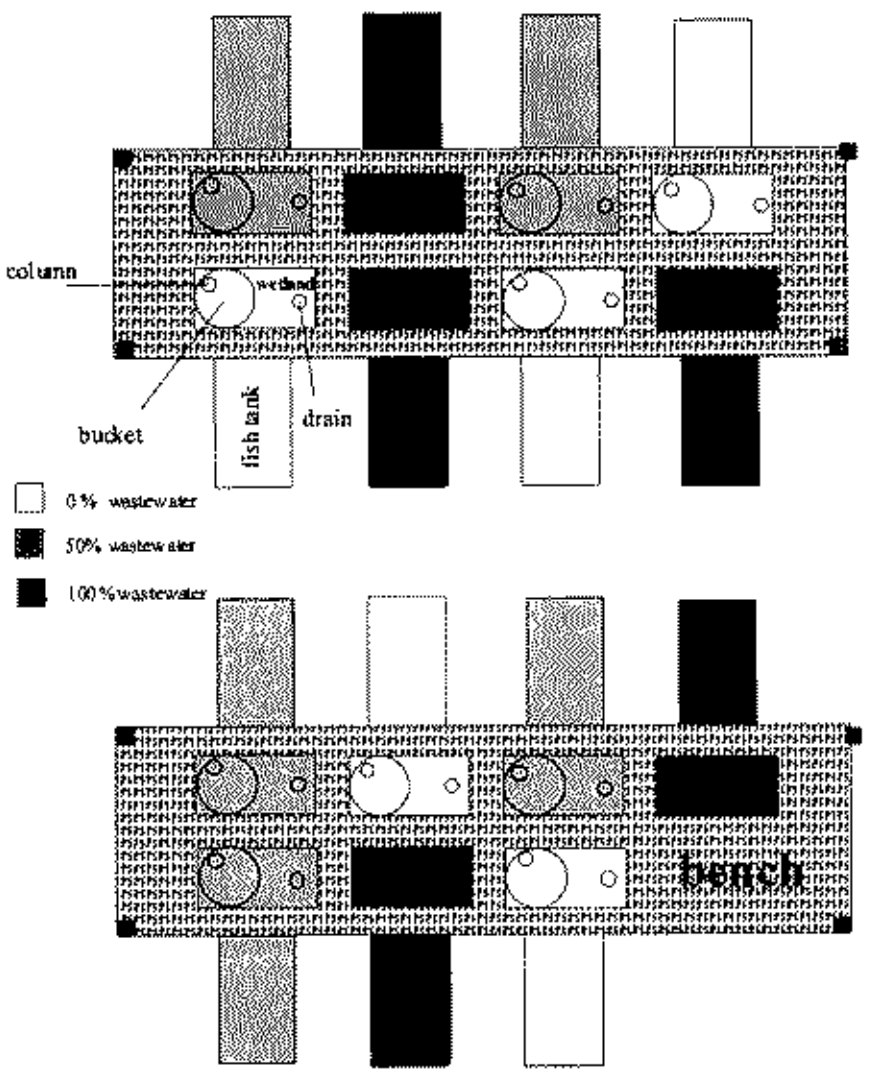

Figure 2.1. Mesocosms and experimental design.

The fish and wetland tanks (80 l and 50 L) were exteriorly painted with white UV resistant paint prior to use. A fifty centimeter long, 25 mm diameter PVC pipes perforated with fifteen 3 mm holes equally spaced down their lengths were laid on the bottoms of the fish tanks as culverts. One end of the culvert was fitted with a cap while the other was joined to a non-perforated 90 ° elbow. Each elbow was fitted with a 20 cm long, 25 mm PVC column that stood vertically at one end of each fish tank. Each column was fitted with aeration: a 2 mm diameter nylon air line entered the column 5 cm from its base, and was attached to 2 x 1 cm pumice air diffuser positioned inside the column and driven by a 250 W centrifugal pump to aerated and circulated the water. The fish tank culverts were covered by a 10 cm layer of quartz gravel (10 mm). Penplax 200 watt submersible heaters were laid horizontally on the gravel surface in the center of each fish tank, and provided heat for the system. Fish tanks had tight fitting lids to protect the fish against predators and from intense sun, and two 50 mm diameter holes drilled in each lid allowed adequate light to penetrate to keep fish healthy.

Lifetech AP 2000 power heads (placed in fish tanks on top of the gravel layer in the northwestern corner of each tank) pumped water from the fish tank up to its corresponding 50 L wetland tank positioned on a steel bench above (figure 2.1). Each wetland tank held a 25 L poly bucket within it, used to house crayfish. Each buckets was placed standing in one end of the wetland tank, was fitted with a 25 mm diameter PVC vertical column which directed incoming fish tank effluent to its base. The bucket was covered with rigid and filament protective mesh to exclude predators and prevent escape of crayfish. At the opposite end of the wetland tank, a vertical gravity drain was installed; a 25 mm bulk head fitted through the bottom of the wetland tank, with a 15 cm long piece of 18 mm diameter perforated poly tube

lodged in the opening to exclude gravel. The wetland tanks were filled to 10 cm with quartz (10 mm) substrate.

2.3 Methods

2.3.1 *Lates calcarifer, Cherax quadricarinatus,* and *Baumea articulata* culture

Each fish tank was stocked with two *Lates calcarifer*, individuals on March 31, 2003, that were harvested 85 days later on Jun 23, 2003. Individual fish fresh weights were measured at the time of stocking and harvest. Growth rate, feeding rate and feed conversion ratios were calculated using methods outlined previously in Chapter I, Section 2.37 and 2.4.1. Ridley Fish Culture pellets (10 % fat - 45 % protein) were used in this experiment.

Each crayfish culture bucket was stocked with one *Cherax quadricarinatus*, individual on March 28, 2003, that was harvested 88 days later on June 23, 2003. Individual crayfish fresh weights were measured each month, and growth rate, feeding rate and feed conversion ratios were calculated following methods outlined in sections observing methods outlined in Chapter I, Section 2.37 and 2.42. *Suprastock* red claw pellets (5 % fat - 20 % protein) were used in this experiment.

Each wetland tank was stocked with one *Baumea articulata* plant, on March 28, 2003, that was harvested 88 days later on June 23, 2003. Individual plant fresh and dry weights were measured at the time of stocking and harvest observing methods outlined in Chapter I, section 2.3.3. Plant growth rates (RGR) was

calculated according to methods outlined in Chapter I, section 2.4.6.

2.3.2 Hydrology and water quality

Stanwell Corporation Limited water was collected in a 6000 L water tanker from the Stanwell north pond 3 weir; the pond was part of a set of retention ponds that collected blow-down wastewater from the power generation plant allowing treatment prior to creek discharge via the pond # 3 north weir. Water was delivered to this experiment from a secured 7000 L poly tank that was fitted with 10 cm diameter vent covered with a micro-screen to prevent debris from entering it. After the system components were filled initially with their respective wastewater concentrations, periodic topping-up of water levels to compensate for evapotranspiration was necessary several times during the experiment. To maintain water quality, the replicates were drained and re-filled on one date (13/05/03) during the fish culture period.

When operating, water in the mesocosm was pumped from the fish tanks at ~ 4.0 L min^{-1} filling the crayfish bucket from the bottom, and then overflowing the bucket into the wetlands. After traversing the wetland, the water under gravity-drained back to the fish tank through the vertical bottom drain, thus completing a recirculation cycle every 22.5 minutes.

Residual wastewater remaining within the replicates at the conclusion of the experiment provided the water for the third trial of hydroponic roses (see Chapter IV), thus completing the linkage of industrial wastewater, barramundi culture, red claw culture, constructed wetlands, and rose hydroponics in this study.

Water collected from SCL was sub-sampled for metal concentration once at the time of collection (07/04/03), and again half way through the study (28/05/03). Samples were fixed with 2 ml nitric acid, put on ice, and then sent to SCL for analyses. Methodology followed by SCL to analyze metal elements is presented in plate 2.1.

METHOD DESCRIPTION SUMMARY PROFORMA

Analysis description: Trace metals in water
Matrix: seawater, fresh water, influents, effluents, waste water.
AGAL Method No: NT2.47
Reference Method(s): USEPA200.2 USEPA 6010 & 6020 , APHA 19th Edition, 3030b, c, d, e
LOR and units: 1-10 ppb
NATA Accredited: yes

Method Title
Determination of total and dissolved elements in water using inductively coupled plasma mass spectrometry and inductively coupled plasma atomic emission spectrometry

Preparation & Procedure:
1. Dissolved metals: water is filtered through 0.45 um filter, acidified and then analysed.
2. Total metals: water is digested in re-distilled nitric acid, filtered and then analysed .
3. The water sample is analysed for trace elements using ICPMS and ICPAES.

Comments, limitations or known interferences:
NT2.47 is a method for analysing a wide range of trace metals in water and other aqueous samples. For salt water HR-ICPMS is used for Cu, Zn, Ni, Cr, V, Mn, Co; CVAAS for Hg (2.44); hydride generation technique for As and Se (NT2.51). Quadrupole ICPMS and ICPAES are used for other elements including the non-metals, P, S, Si, B.
For Fresh water: Quadrupole ICPMS and ICPAES are used all other elements.

Equipment used:
ICPMS: Elan 6100DRC (Perkin Elmer)
ICPAES: Optima3000DV (Perkin Elmer)
Element: Finnigan High Resolution ICPMS

March 2004

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Plate 2.1. Proforma of methods used in SCL water analyses.

Fortnightly electrochemistry measurements (temperature, pH, DO, and EC) were taken using a HACH sension multi-parameter water quality meter. Each week at 10:00 am, one *in-situ* reading was taken from the front -center of each fish tank and

crayfish bucket at mid water depth.

2.3.3 Tissue metal analyses

Tissue metal concentration analyses were conducted on ten samples each of fish, crayfish, plant above ground (AG), and plant below ground (BG) biomass taken from stock sources at the beginning of the experiment. Analyses were also completed on fish, crayfish, plant AG, and plant BG samples taken from each replicate at the conclusion of the experiment.

Only edible muscle portions of fish and crayfish were selected for metal analyses, following methods outlined in regulatory references (Commonwealth, 2001) noted in Australian dietary intake studies (FSANZ, 2003). Fish samples consisted of 4 fillets per replicate (2 fillets per fish - skin intact). Fillets were cut with a small fillet knife and dried separately from the fish body. Crayfish tail meat was excised from pre-dried whole crayfish samples. Sample weighing and drying was completed following methods outlined in sections observing methods outlined in Chapter I, Section 2.4.1 and 2.42. Plant AG and BG sample weighing, drying and sub-sampling, was completed following methods outlined in Chapter I, Section 2.3.4, in addition, plant matter was chopped into a coarse mix after drying.

After drying all samples were individually sealed in plastic vials and stored in a desiccator until the third week of May (2003) when samples were sent to University of Queensland School of Land and Food Sciences - Analytical Services (FSAS) for arsenic (As), cadmium (Cd), copper (Cu), and mercury (Hg) analyses. At the FSAS laboratory, 500 mg of sample was digested following the EPA Method 3051 with 10 mL re-distilled nitric acid in a CEM MSP-1000 Microwave Digestion

Oven at 58% power for 10 min. The digests were diluted to 50 mL and further diluted 1+2 to give a final digest 7% HNO_3 + 10 ppb In as an internal standard. The analytes were measured on a Micromass Platform ICPMS using the isotopes 115In, 65Cu, 75As, 112Cd and 202Hg. Sample re-hydration calculations were used in making some comparisons to published regulations and research. The conversion factors used were derived from the inverse of the percentage dry-weight of samples.

2.4 Statistical analyses

A repeated measures ANOVA was used to determine whether differences in total weight of *Lates calcarifer, Cherax quadricarinatus*, and *Baumea articulata* occurred over time and between treatments, and to determine if differences in arsenic, cadmium, copper, and mercury accumulation occurred within *Lates calcarifer, Cherax quadricarinatus* and *Baumea articulata* over time and between treatments.

2.5 Results

2.5.1 *Lates calcarifer*

Culture of barramundi within mesocosms was successful. No statistical difference in individual fish fresh weights was evident between wastewater treatments at the time of stocking or harvest (Figure 2.2). The effect of time was significant across treatments ($p \leq .01$), and individual fish fresh weights increased

over the course of the experiment (Figure 2.2).

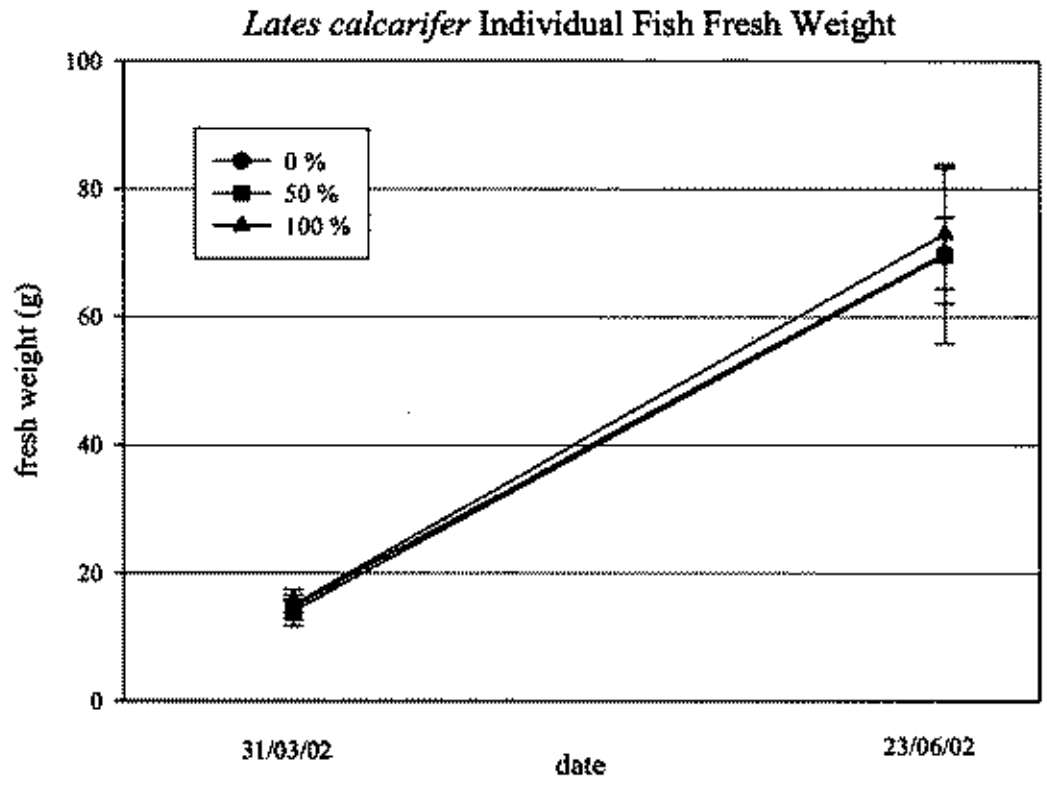

Figure 2.2. *Lates calcarifer* individual fresh weight and time.

In each wastewater treatment, mean fish feeding rates were above 2.0 % body weight d^{-1}, and ranging from 2.0 - 2.1 % body weight d^{-1}. Growth rates remained above 1.8 % body weight d^{-1}, ranging 1.8 - 1.9 % body weight d^{-1} (Table 2.1). Food conversion ranged 1.11 - 1.17 : 1 at culture densities that ranged from 2.3 to 2.4 g L^{-1}, and temperatures ranging from 27.5 to 27.8 ° C. Fish survival was ≥ 90 %.

	Feeding rate (% bw d^{-1})	SGR	FCR	culture density (g L^{-1})	Temp °C	Survival %
0%						
R1	2.22	1.75	1.23	2.1	26.2	100
R6	1.99	1.80	1.07	2.4	27.0	100
R9	1.94	1.91	1.99	2.6	27.0	50
R11	2.22	1.61	1.34	2.3	28.7	100
R12	2.14	1.71	1.22	2.3	28.7	100
mean	2.10	1.76	1.37	2.33	27.5	90.0
stdv	0.13	0.11	0.36	0.19	1.1	22.4
50%						
R3	2.17	2.15	0.99	2.6	26.2	100
R5	2.00	1.79	1.08	2.4	27.1	100
R7	2.18	1.89	1.22	2.1	27.4	100
R13	1.71	1.75	0.95	2.8	29.3	50
R15	2.65	1.44	1.76	1.6	29.2	100
mean	2.14	1.80	1.39	2.32	27.8	90.0
stdv	0.34	0.26	0.41	0.46	1.3	22.4
100%						
R2	1.80	1.73	1.01	2.8	26.6	100
R4	1.97	1.68	1.01	2.4	26.8	100
R8	2.05	1.82	1.10	2.3	27.2	100
R10	1.81	1.81	0.98	2.7	27.6	100
R14	2.54	1.69	1.47	1.9	29.9	100
mean	2.03	1.75	1.11	2.43	27.6	100.0
stdv	0.30	0.06	0.20	0.36	1.3	0.0

Table 2.1. *Lates calcarifer* culture efficiency values delineated by replicate: feeding rate, growth rate, food conversion ratio, culture density, temperature, and survival.

The manager of the UQ-FSAS analytical laboratories informed the author that the initial fish, crayfish, and plant samples sent to them for metal analyses were lost (presumably thrown out) in a move to new laboratory facilities. Therefore it was not possible to determine either the uniformity of initial tissue sample metal concentrations or the effect of time on tissue metal concentrations over the

experiment.

However, it was possible to determine whether differences existed between treatments at the conclusion of the experiment. There were no statistical differences in fish tissue concentration of arsenic, cadmium, copper, and mercury between wastewater treatments at harvest. In the 0 % treatment, the ranking of metals from greatest to least dry-weight concentration within fish fillets were copper (with a mean of 9.3 mg kg^{-1}), arsenic (485 µg kg^{-1}), cadmium (38 µg kg^{-1}), and mercury (32 µg kg^{-1}) (Table 2.2, Figure 2.3). In 50 % and 100 % treatments, ranking of metals from greatest to least concentration within fish fillets were copper (with a range of 4.9 - 6.0 mg kg^{-1}), arsenic (534 - 546 µg kg^{-1}), mercury (34 - 36 µg kg^{-1}), and cadmium (10 - 17 µg kg^{-1}).

	As µg kg^{-1}	Cd µg kg^{-1}	Cu mg kg^{-1}	Hg µg kg^{-1}		As µg kg^{-}	Cd µg kg^{-1}	Cu mg kg^{-1}	Hg µg kg^{-1}
fish 0 %					AG 0 %				
mean	485	38	9.3	32	mean	342	126	4.0	37
stdv	115	55	11.5	30	stdv	75	20	1.3	16
n = 5					n = 5				
fish 50 %					AG 50 %				
mean	534	17	6.0	34	mean	286	153	4.6	118
stdv	68	26	6.2	9	stdv	57	115	4.0	94
n = 5					n = 5				
fish 100 %					AG 100 %				
mean	546	10	4.9	36	mean	478	132	3.7	36
stdv	64	12	5.8	22	stdv	237	35	1.6	14
n = 5					n = 4				
crayfish 0 %					BG 0 %				
mean	221	45	31.9	79	mean	459	397	23.4	63
stdv	54	46	4.3	14	stdv	46	87	3.7	14
n = 4					n = 5				
crayfish 50 %					BG 50 %				
mean	238	47	24.8	74	mean	629	449	31.0	86
stdv	117	39	9.6	10	stdv	243	163	6.3	33
n = 4					n = 4				
crayfish 100 %					BG 100 %				
mean	102	27	25.6	90	mean	804	578	34.6	65
stdv	62	11	13.0	33	stdv	321	266	7.3	21
n = 5					n = 4				

Table 2.2. *Lates calcarifer, Cherax quadricarinatus*, and *Baumea articulata* sample concentrations of arsenic, cadmium, copper, and mercury grouped by 0 %, 50 %, and 100 % wastewater treatments. Table lists dry weight concentrations. AG = above ground plant matter, BG = below ground plant matter.

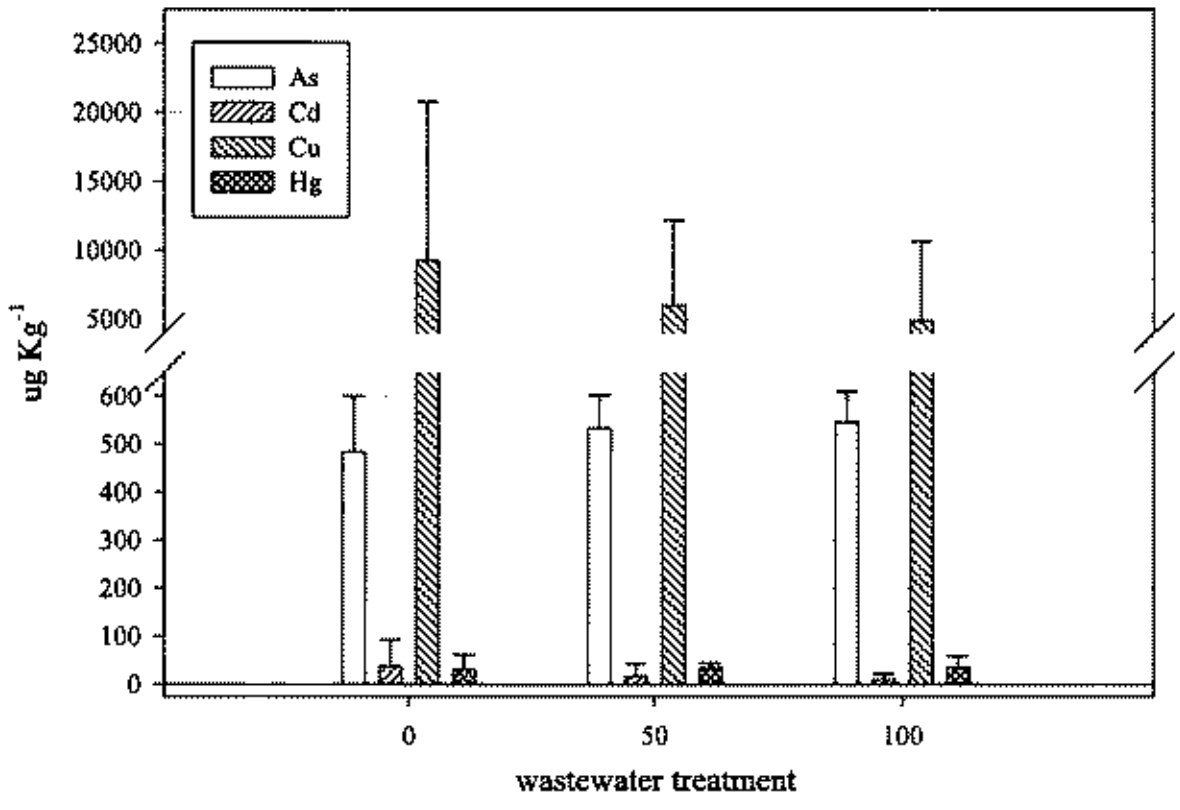

Figure 2.3. Metal concentrations in *Lates calcarifer* dry-weight tissue samples

2.5.2 *Cherax quadricārinatus*

Culture of red claw within mesocosms was successful and there were no statistical differences in individual crayfish fresh weight occurring between wastewater treatments at the time of stocking or harvest. The effect of time was significant across treatments ($p \leq .01$); individual crayfish fresh weights increased over the course of the experiment (Figure 2.4).

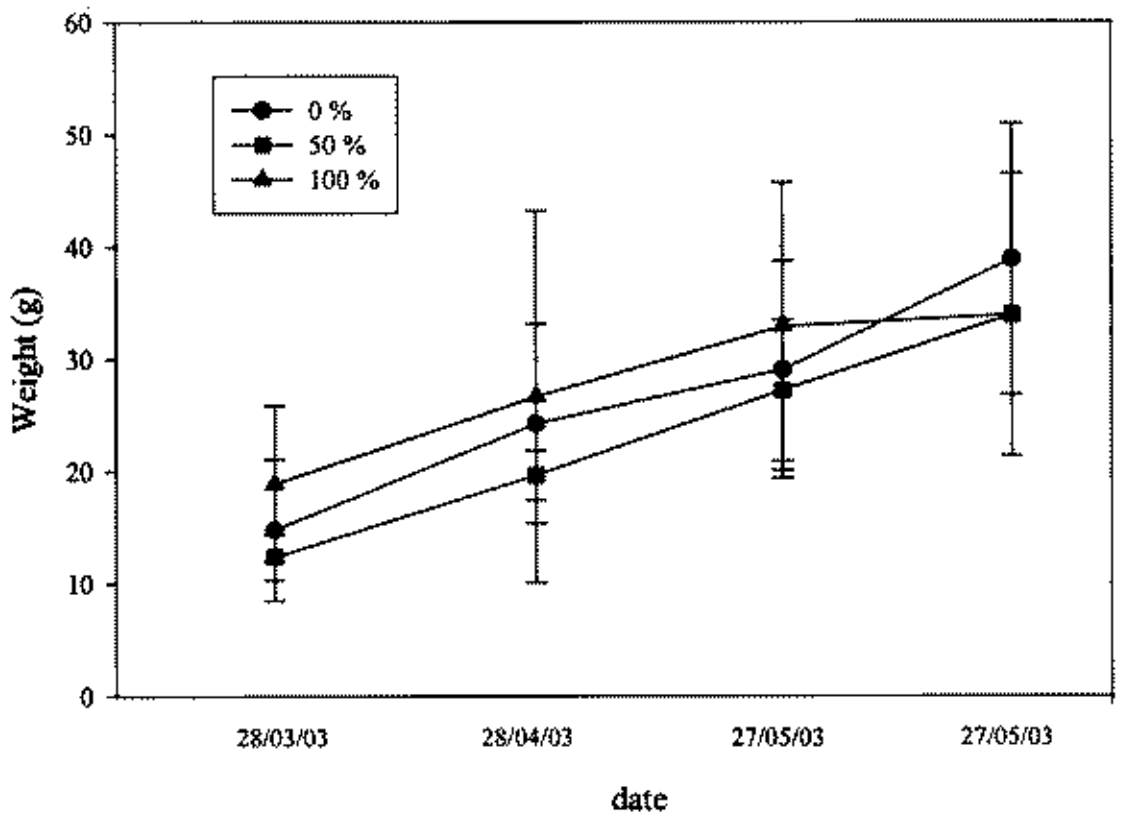

Figure 2.4. *Cherax quadricarinatus* individual fresh weight and time.

In each wastewater treatment, mean crayfish feeding rates were above 4.0 % body weight d^{-1}, ranging 4.8 - 6.0 % body weight d^{-1}. Growth rates remained above 0.5 % body weight d^{-1}, ranging 0.7 - 1.9 % body weight d^{-1} (Table 2.3). Food conversion ranged 7.37 - 8.10 : 1 at culture densities that ranged between 1.7 and 1.9 g L^{-1}. Culture temperatures ranged from 27.4 to 27.9 ° C, and survival was ≥ 80 %

(Table 2.3)

	Feeding rate (% bw d^{-1})	SGR	FCR	culture density (g L^{-1})	Temp °C	Survival %
0%						
R1	4.13	1.56	2.66	1.8	26.4	100
R6	6.40	0.58	11.05	1.9	27.1	100
R9	5.48	1.46	3.78	1.9	26.9	100
R11	3.36	0.63	5.38	1.6	28.8	100
R12						0
mean	4.84	1.06	7.37	1.81	27.30	80.0
stdv	1.36	0.52	3.78	0.13	1.05	44.7
50%						
R3	5.30	0.69	7.66	1.7	26.3	100
R5	4.15	1.81	2.31	2.0	27.1	100
R7						0
R13	6.10	1.04	5.91	1.8	29.3	100
R15	6.08	1.19	5.17	1.5	29.0	100
mean	6.09	1.18	5.26	1.65	27.91	80.0
stdv	0.01	0.47	2.23	0.25	1.48	44.7
100%						
R2	2.78	0.66	4.20	1.8	26.5	100
R4	4.97	1.06	4.71	1.6	27.0	100
R8	4.96	0.49	10.22	2.2	27.3	100
R10	4.71	0.52	9.07	1.8	27.5	100
R14	7.12	0.58	12.32	1.8	29.9	100
mean	4.91	0.66	8.10	1.85	27.64	100.0
stdv	1.54	0.23	3.53	0.21	1.32	0.00

Table 2.3. *Cherax quadricarinatus* culture efficiency values delineated by replicate: feeding rate, growth rate, food conversion ratio, culture density, culture temperature, and survival.

There were no statistical differences in crayfish tissue concentration of arsenic, cadmium, copper, and mercury between wastewater treatments at the time of harvest. The ranking of metals from greatest to least dry-weight concentration across treatments within crayfish tail meat were copper (with a range of 24.8 - 31.9 mg kg^{-1}), arsenic (102 - 238 μg kg^{-1}), cadmium (27 - 47 μg kg^{-1}), and mercury (74 - 90 μg

– kg^{-1}) (Table 2.2, Figure 2.5).

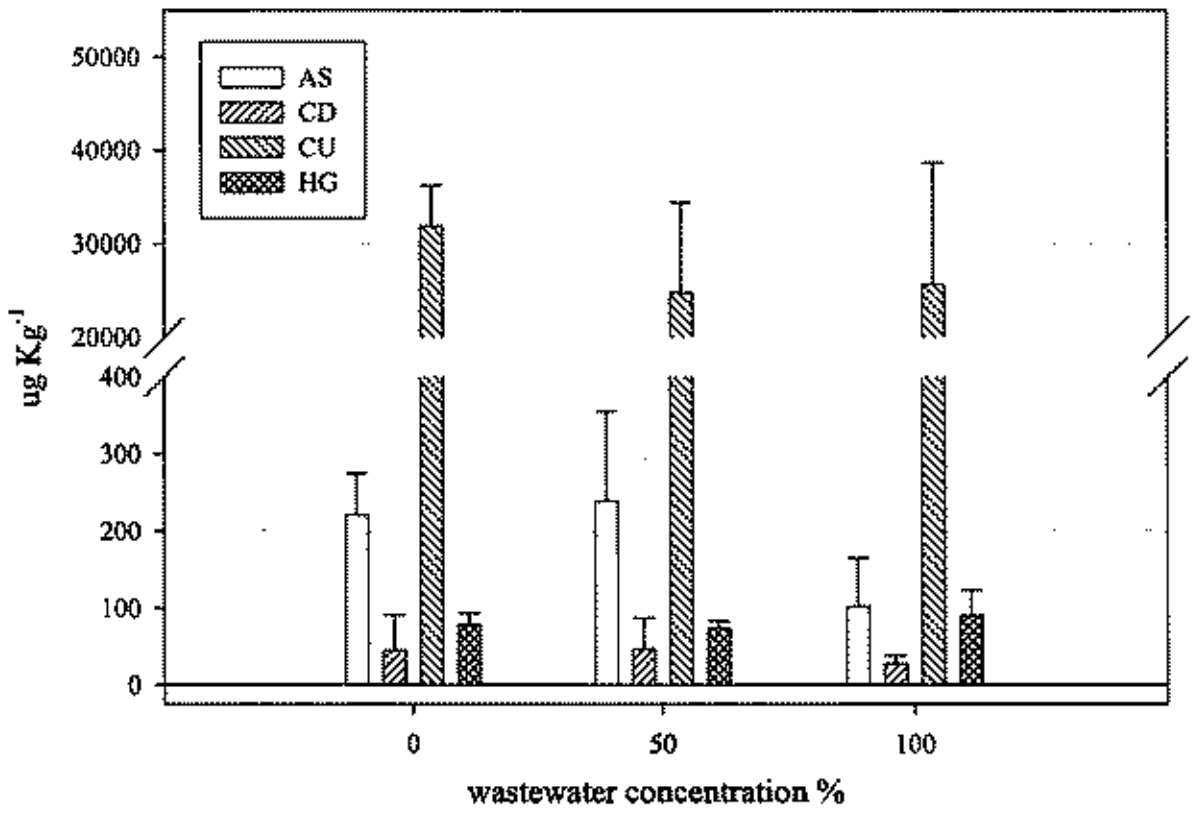

Figure 2.5. Metal concentrations in *Cherax quadricarinatus* dry-weight tissue samples (at harvest).

2.5.3 *Baumea articulata*

There were no significant differences in dry weight plant densities between treatments or over time (Table 2.4). *Baumea articulata* growth rates (RGR) were 0.004, 0.002, and 0.006 RGR in 0 %, 50 %, and 100 % wastewater treatments, respectively (Table 2.4). One plant within each of the 0 % and 50 % wastewater treatments, and two plants within the 100 % wastewater treatment decreased in biomass over the experiment. The aquatic plant *Lemna*, as well as algae, were recovered from the treatment replicates (Table 2.5). Dry weights of the *Lemna* and algae recovered were not significantly different between treatments.

	plant dw (g)	plant dw (g)	growth rate (RGR)	Survival %
	stocking	harvest		
0%				
T1	20.0	22.0	0.0011	100.0
T6	27.0	38.3	0.0040	100.0
T9	18.0	31.8	0.0064	100.0
T11	16.2	22.4	0.0037	100.0
T12	14.1	10.5	**	100.0
mean	19.05	24.99	0.0038	100.00
stdv	4.92	10.60	0.0022	0.00
50%				
T3	25.2	27.4	0.0010	100.0
T5	22.6	24.1	0.0007	100.0
T7	15.2	11.6	**	100.0
T13	13.5	22.3	0.0056	100.0
T15	15.8	17.6	0.0012	100.0
mean	18.45	20.58	0.0021	100.00
stdv	5.13	6.15	0.0023	0.00
100%				
T2 *	22.4	3.8	**	0.0
T4	16.6	18.9	0.0015	100.0
T8	18.0	14.9	**	100.0
T10	14.6	22.7	0.0050	100.0
T14	16.8	48.9	0.0121	100.0
mean	17.67	26.35	0.0062	80.00
stdv	2.93	15.34	0.0054	44.72

Table 2.4. *Baumea articulata* growth and survival in 0 %, 50 %, and 100 % treatments. * = dead plant; **g = loss of biomass

Plant below-ground (BG) biomass within the 100 % wastewater replicates had significantly greater concentrations of copper than plant below-ground (BG) in 0 % wastewater replicates ($p \leq 0.05$) (Table 2.2, Figure 2.6). Differences, however, between 0 % and 50 %, and 50 % to 100 % were not significant. Arsenic and cadmium concentrations within plant BG appeared to increase with greater wastewater concentrations, although measured differences were not significant. Plant above-ground (AG) metal concentrations did not differ significantly between treatments (Table 2.2 Figure 2.7).

	Lemna Dry weight (g)	Algae Dry weight (g)
0 %		
T1	9.94	0.61
T6	1.10	0.00
T9	0.46	0.10
T11	0.00	0.39
T12	5.50	0.00
mean	3.40	0.22
stdv	4.26	0.27
50 %		
T3	1.77	0.00
T5	2.56	0.00
T7	0.46	3.98
T13	18.60	0.00
T15	12.64	1.35
mean	7.21	1.07
stdv	8.00	1.73
100 %		
T2	1.02	0.42
T4	1.47	0.75
T8	1.29	1.33
T10	0.16	0.00
T14	10.80	0.31
mean	2.95	0.56
stdv	4.42	0.51

Table 2.5. *Lemna* and algal recove from 0 %, 50 %, and 100 % treatments.

The ranking of metals from greatest to least concentration across treatments within AG plant AG and BG matter were copper, arsenic, cadmium, and mercury. Above ground plant matter copper concentrations ranged 4.0 - 4.6 mg kg^{-1}, arsenic (286 - 478 µg kg^{-1}), cadmium (126 - 153 µg kg^{-1}), and mercury (37 - 118 µg kg^{-1}). Below ground plant matter copper concentrations ranged 23.4 - 34.6 mg kg^{-1}, arsenic (429 - 804 µg kg^{-1}), cadmium (397 - 578 µg kg^{-1}), and mercury (63 - 86 µg kg^{-1}). Metal assays were not conducted on recovered *Lemna* or algae.

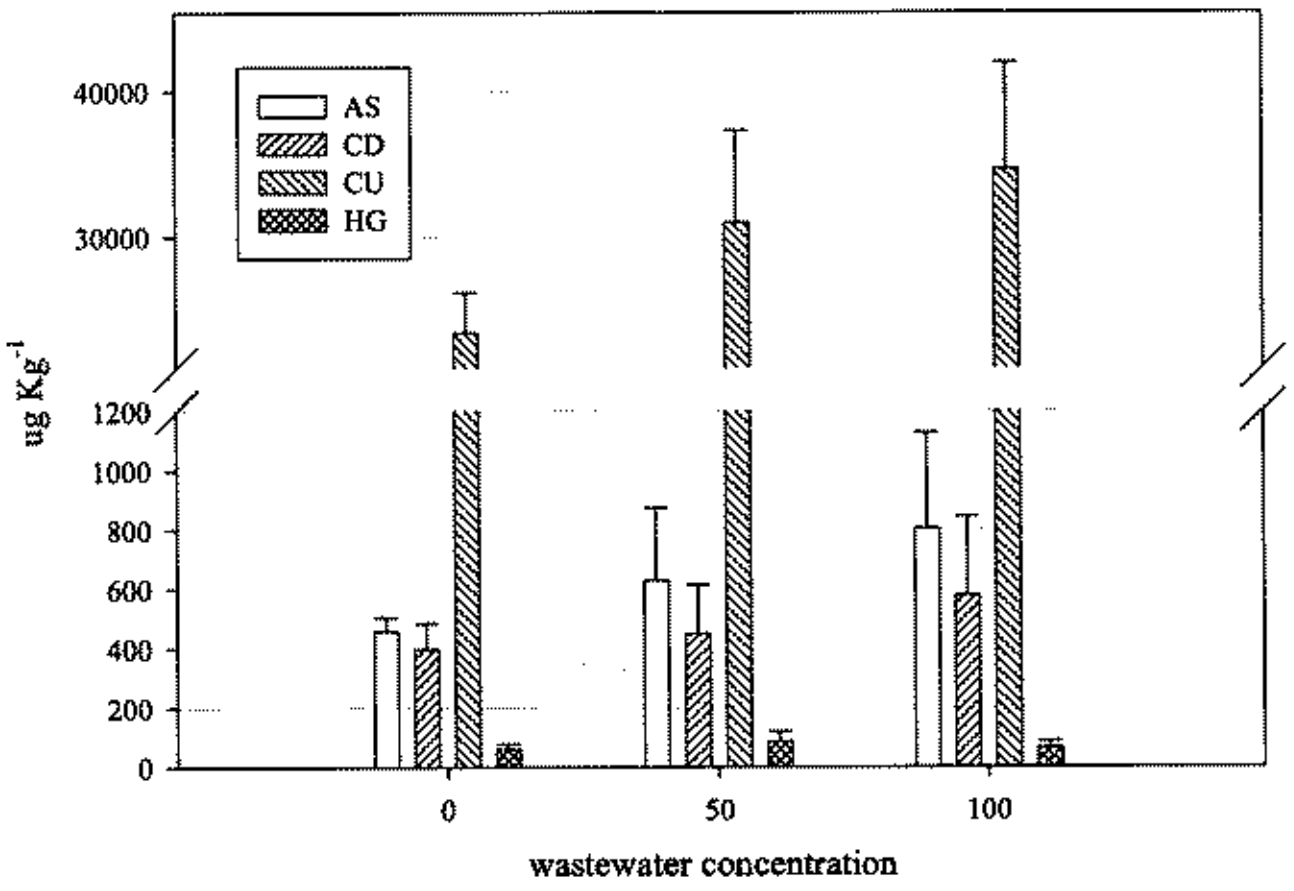

Figure 2.6. Metal concentrations in *Baumea articulata* dry-weight, below-ground matter samples.

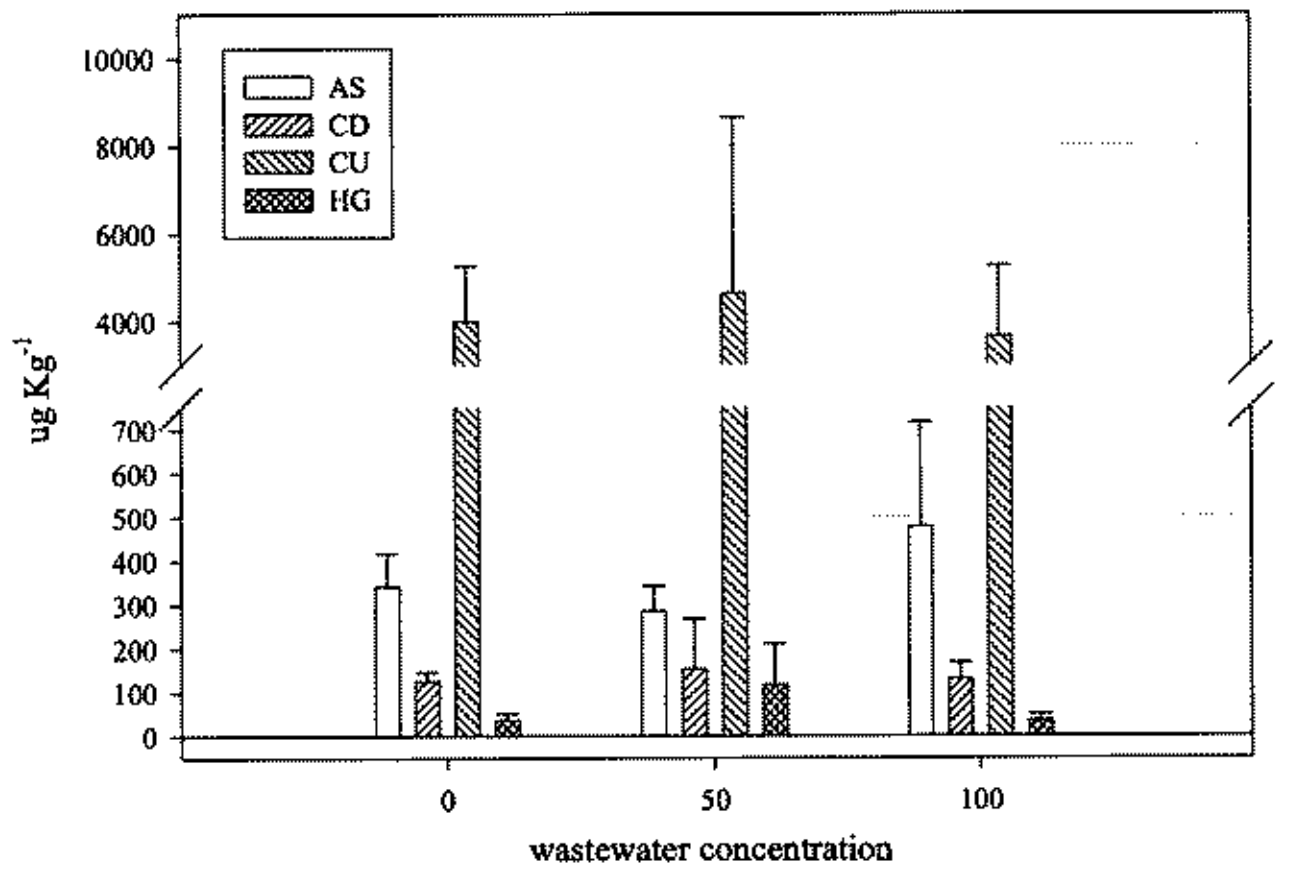

Figure 2.7. Metal concentrations in *Baumea articulata* dry-weight, above-ground matter samples.

2.5.4 Hydrology and water quality

There was no difference in water usage or evapotranspiration between wastewater treatments (Table 2.6). Replicates required between 311 - 317 L of wastewater over the experiment, of which 86 - 88 % was evapotranspired. The ratio of total water usage (L) to total fish production (g) was 5.4 - 5.8 : 1.0; when including red claw biomass produced, ratio decreased to 4.0 - 4.3 : 1.0 (Table 2.6)

	water usage (L)	evapotranspiration (L)	water use to fish production ratio ($L\ g^{-1}$)	water use to animal production ratio ($L\ g^{-1}$)
0%				
R1	322	279	6.5	3.7
R6	290	250	5.0	4.3
R9	327	288	5.1	3.6
R11	326	288	6.3	4.6
R12	319	283	6.1	*
mean	317	277.5	5.8	4.0
stdv	15.4	16.0	0.7	0.5
50%				
R3	336	293	5.0	4.2
R5	301	258	5.2	2.9
R7	313	269	6.1	*
R13	324	278	4.9	3.9
R15	306	266	8.7	5.6
mean	315.9	272.7	5.7	4.0
stdv	14.1	13.3	1.6	1.1
100%				
R2	312	270	4.7	3.5
R4	318	275	5.3	3.9
R8	285	248	5.2	4.4
R10	292	256	4.5	3.9
R14	349	302	7.9	6.6
mean	311	270.2	5.4	4.3
stdv	25.1	21.0	1.4	1.3

Table 2.6. Water use efficiency. Replicate water use and evapotranspiration including water use with respect to fresh barramundi production, and fresh barramundi plus red claw production. * red claw mortality

The 7000 L reservoir (used to irrigate the experiment) water held, on average, 6.9 μg L^{-1} arsenic, 0.1 μg L^{-1} cadmium, 14.6 μg L^{-1} copper, and 0.05 μg L^{-1} mercury (Table 2.7). Reservoir water DO and TDS increased, temperature decreased, and pH remained relatively stable over the experiment (Figures 2.8 - 2.11). Mean DO, TDS, pH, and temperature of reservoir water held over the experiment were 4.1 mg L^{-1}, 1 101.8 mg L^{-1}, 7.4, and 23.7 ° C, respectively (Table 2.8).

date	As μg L^{-1}	Cd μg L^{-1}	Cu μg L^{-1}	Hg μg L^{-1}
07/04/2003	6.1	0.05*	14	na
	6.2	0.06	16	na
	6.1	0.05	14	na
	6.2	0.05*	13	na
28/05/2003	11	0.05*	14	0.05*
	6.5	0.1	16	0.05*
	6.3	0.14	15	0.05*
	6.4	0.05*	15	0.05*
mean	6.85	0.07	14.6	0.05
stdv	1.68	0.03	1.1	0.00
* concentration ≤ 0.05 μg L^{-1}				

Table 2.7. Reservoir water concentration of arsenic, cadmium, copper, and mercury at two intervals over the experiment. na = not analyzed.

Total dissolved solids concentrations in each treatment were significantly different from one another ($p \leq 0.01$; $F_{2, 12}$), increasing from 0 % to 50 %, and 50 % to 100 %, treatments, as well as increasing over time ($p \geq 0.01$) (Table 2.8, Figure 2.8). *In-situ* DO, pH, and temperature measurements were not significantly different between treatments. Dissolved oxygen remained above 5.0 mg L^{-1}, and concentrations decreased over time (p 0.01). *In-situ* pH fluctuated significantly over time, including an unexplained pH drop in 0 % wastewater treatment replicates on 21/05/03. Temperature decreased over time ($p \leq 0.01$) in each treatment, and ranged 27 - 28 ° C on average.

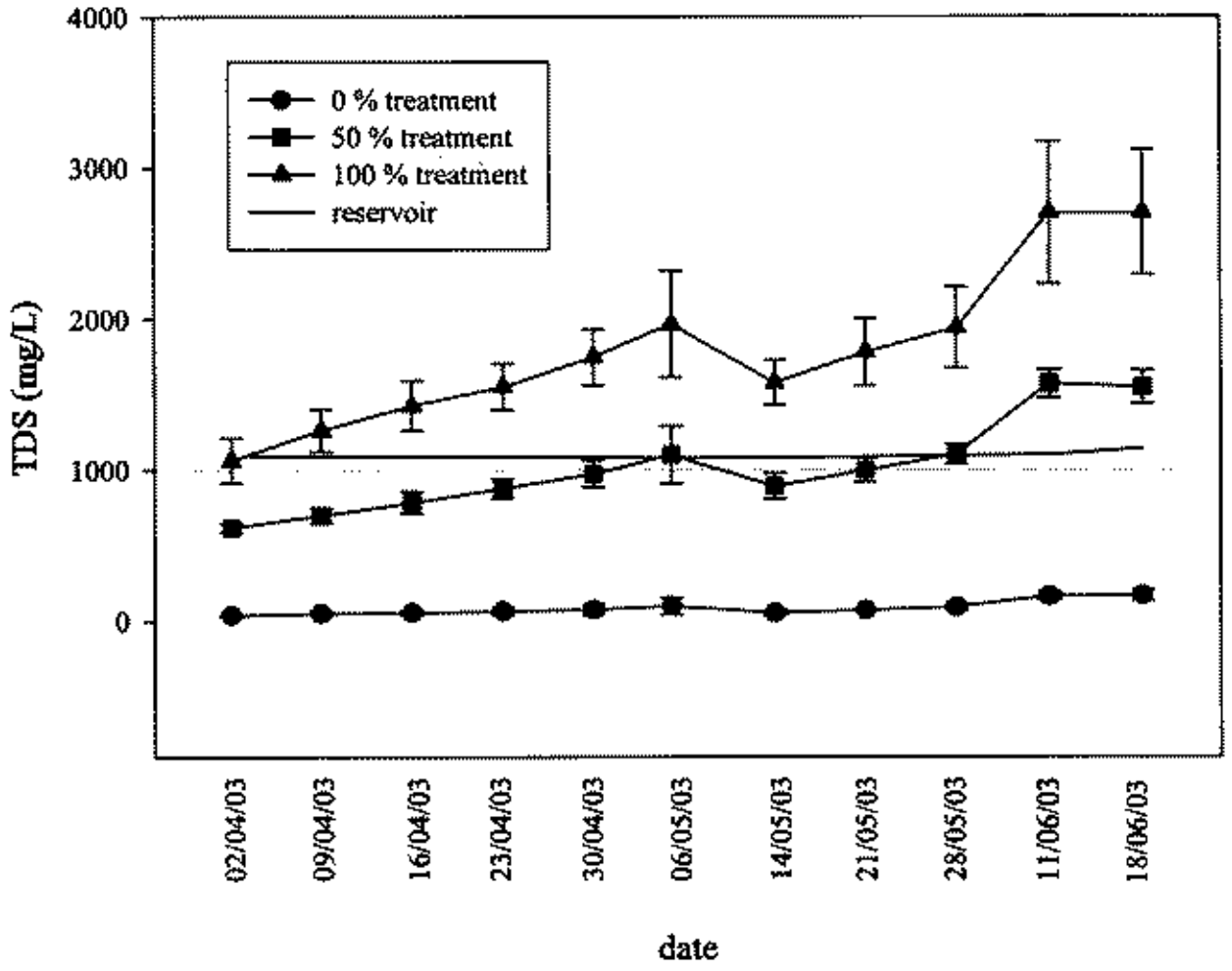

Figure 2.8. Mesocosm and reservoir *in-situ* total dissolved solids concentrations and time.

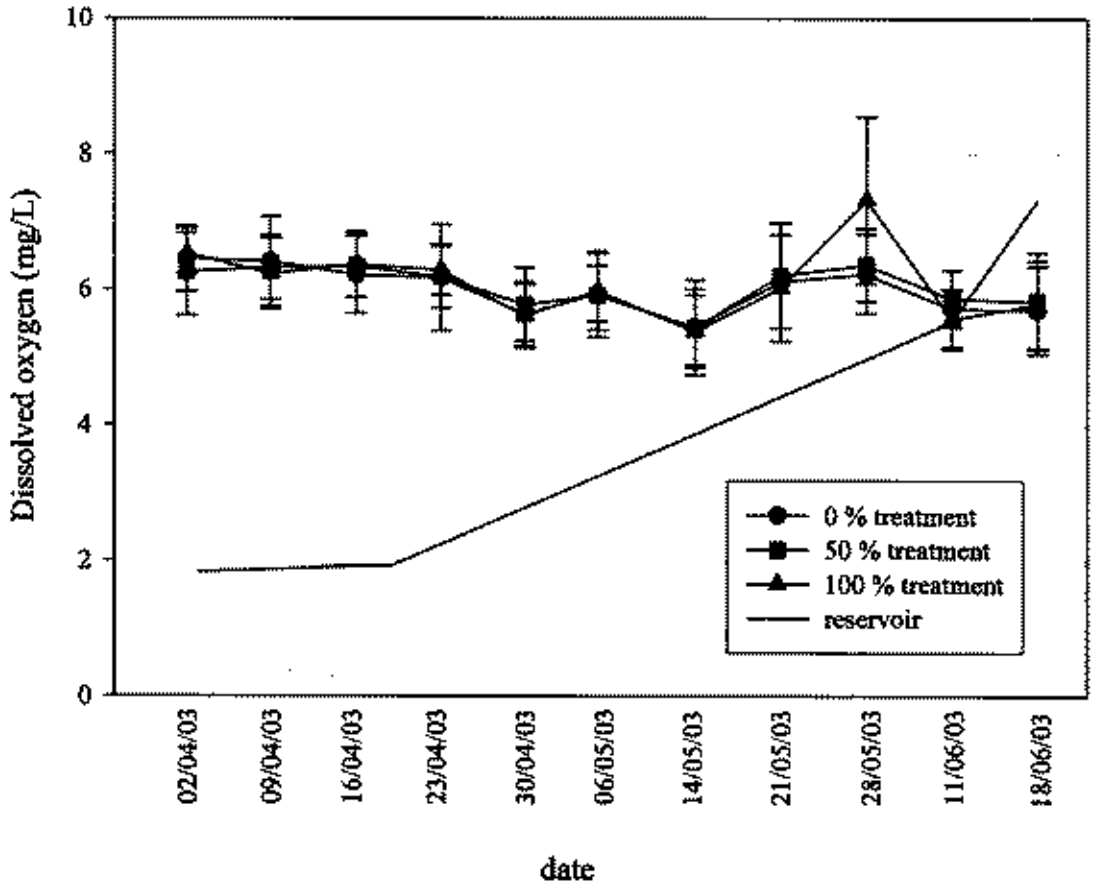

Figure 2.9. Mesocosm and reservoir *in-situ* dissolved oxygen concentrations and time.

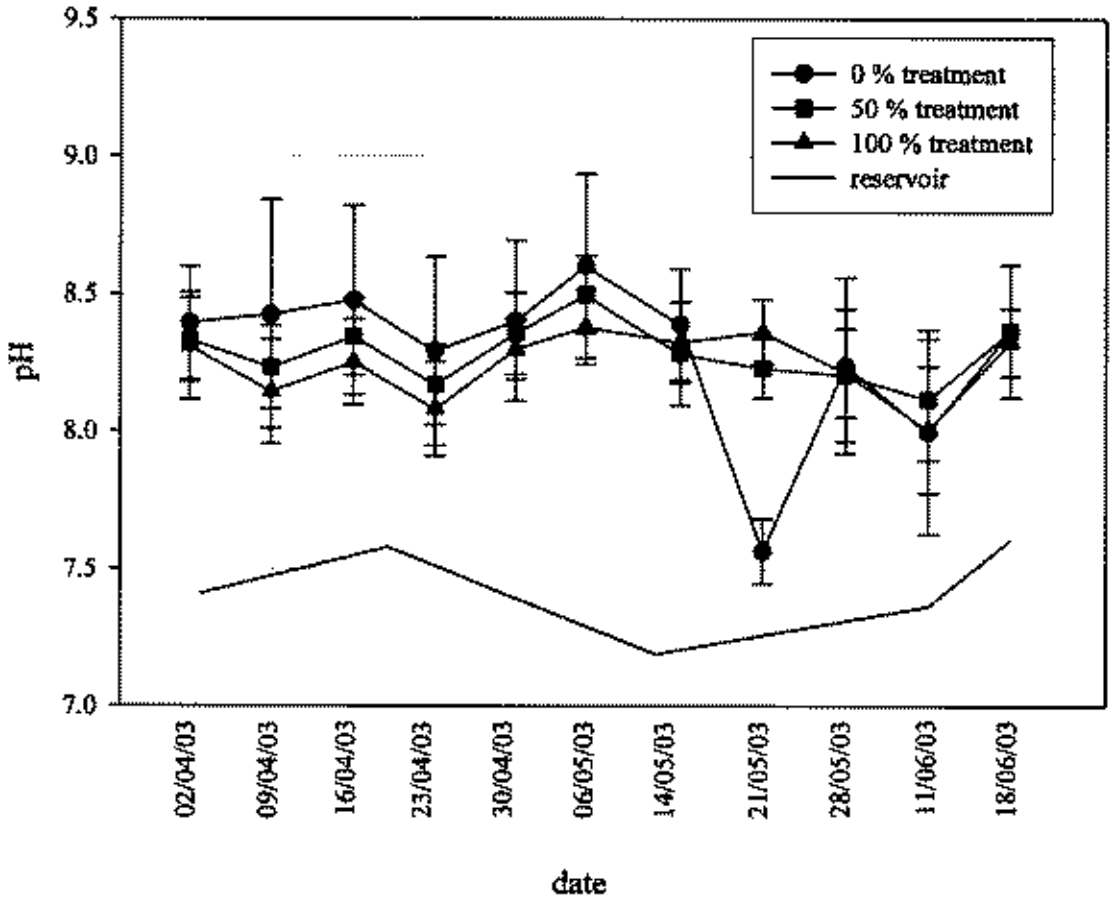

Figure 2.10. Mesocosm and reservoir *in-situ* pH and time.

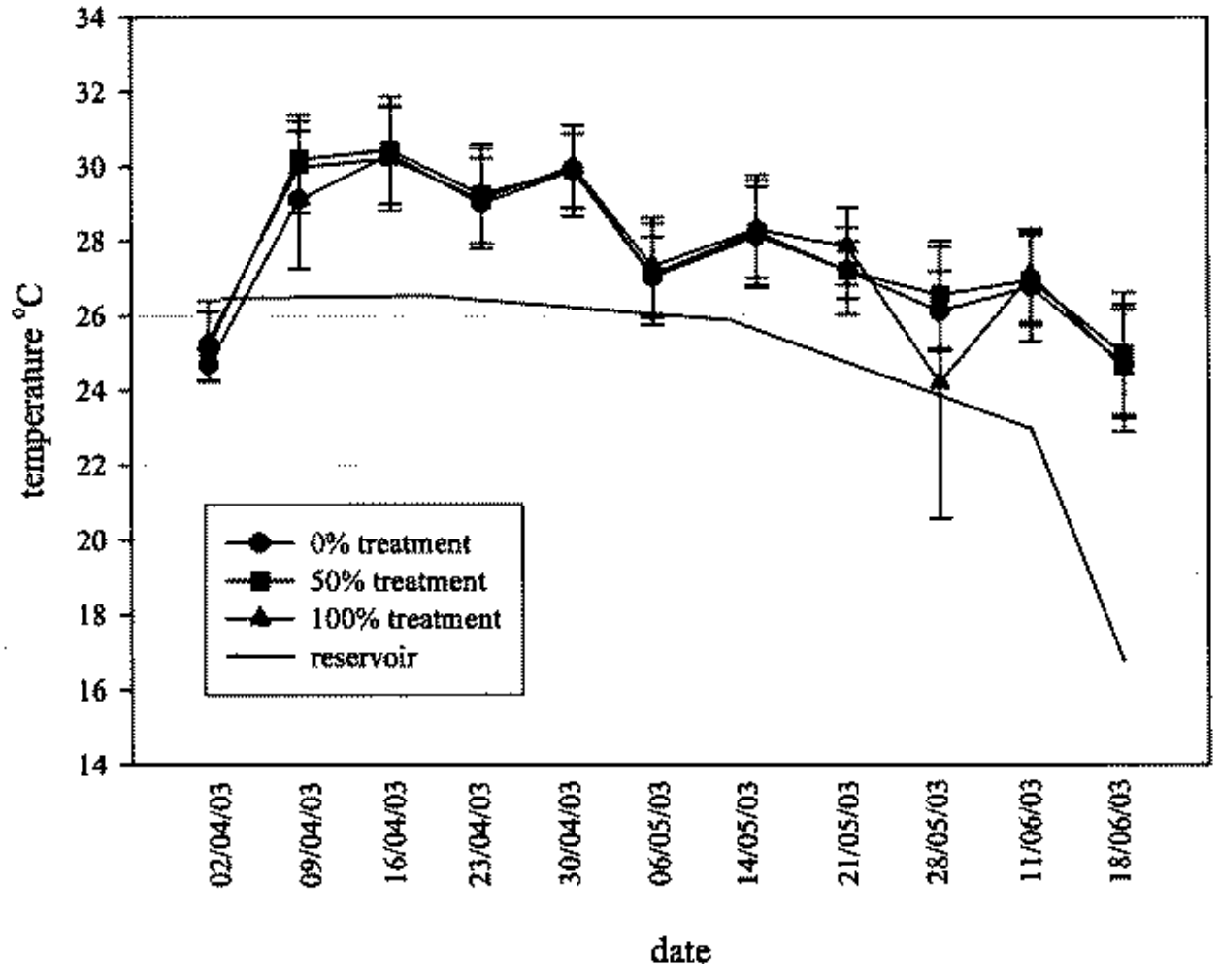

Figure 2.11. Mesocosm and reservoir *in-situ* temperatures and time.

replicate		DO (mg L^{-1})	TDS (mg L^{-1})	pH	Temp (°C)
R1	0 % Treatment	6.41	67.7	7.92	26.3
R6		6.13	73.1	8.49	27.1
R9		6.19	102.1	8.55	27.0
R11		5.57	101.3	8.36	28.7
R12		5.70	83.5	8.11	28.7
mean		6.00	85.5	8.29	27.5
stdv		0.35	15.8	0.26	1.1
R3	50 % Treatment	6.47	1042.1	8.38	26.3
R5		6.24	893.1	8.41	27.1
R7		6.31	993.4	8.22	27.4
R13		5.48	1045.6	8.07	29.3
R15		5.70	1104.1	8.37	29.1
mean		6.04	1015.7	8.29	27.8
stdv		0.43	79.0	0.14	1.3
R1	100 % Treatment	6.48	1557.1	8.13	26.5
R6		6.29	1822.1	8.33	26.9
R9		6.17	1799.4	8.37	27.3
R11		6.09	1643.5	8.18	27.6
R12		5.44	2143.9	8.22	29.9
mean		6.09	1793.2	8.24	27.6
stdv		0.39	224.7	0.10	1.3
reservoir		4.06	1100.8	7.43	23.7

Table 2.8. Wetland replicate (treatment) and reservoir *in-situ* electrochemical water quality: dissolved oxygen, total dissolved solids, pH and temperature.

2.6. Discussion

The goals of this study were met: *Lates calcarifer* and *Cherax quadricarinatus* were cultured successfully in mesocosm scale integrated wetland systems irrigated with power station wastewater at 0 %, 50 %, and 100 % concentrations, and with resulting wastewater re-used for hydroponic rose cultivation (see Chapter IV).

The objective of determining whether differences occurred between wastewater treatments in terms of animal and plant growth, and animal and plant tissue sample metal concentrations was met also, however the loss of the initial samples rendered the proposed measurement of changes in the latter over time impossible.

It is reasonable to assume that stratification of metal concentrations in subjects at the initiation of the experiment would be reflective of a normally stratified population in respect to the sample size taken, and, it is unlikely the metal concentrations would be more variable at the initiation of the experiment, becoming less variable between wastewater treatments over the course of the experiment. This lends confidence to the result of hypothesis 2 (see section 2.1).

Null hypotheses 1 and 2 were accepted for both barramundi and red claw: that wastewater did not impact on the growth of either organism, nor did the level of metal concentrations within tissues samples.

The overall efficiency of barramundi growth (feeding rate, growth rate, FCR, and survival) ranked with more efficient published values in the literature (Chaitanawisuti et al., 2001, Awang, 1987, Lobegeiger et al., 2001, Rimmer, 1995,

Williams and Barlow, 1996, Williams, 1998).

When compared with numerous samples of fish from unpolluted ecosystems around the world, concentrations of arsenic, cadmium, and mercury of fish grown in the experiment were very low, although copper ranged at the higher end (Eisler, 1988, Eisler, 1987, Eisler, 1985, Edmonds and Francesconi, 1993, Olsen, 1983, Swales et al., 1998, Chen, 2002). In respect to the ANZFSC enforceable maximum levels (MLs) and generally expected levels (GELs), concentrations of each metal element in all fish samples met Australian distribution and consumption regulations and guidelines.

Barring copper concentration, mean concentrations of metals within all fish samples were well below MLs and GELs (Table 2.9). The mean copper concentration of experimental fillets was 1800 $\mu g\ kg^{-1}$, only 200 μg below the 2000 $\mu g\ kg^{-1}$ GEL guideline.

	inorganic	total	total	total
ANZFSC Regulations	As $\mu g\ kg^{-1}$	Cd $\mu g\ kg^{-1}$	Cu $\mu g\ kg^{-1}$	Hg $\mu g\ kg^{-1}$
fish ML	2 000	-	-	1 000
fish GEL	-	-	2 000	-
Crustacean ML	2 000	-	-	500
Crustacean GEL	-	-	20 000	-
Experimental Animals				
barramundi mean concentration	139	6	1 800	9
stdv	26	9	2 100	5
red claw mean concentration	48	10	7 200	22
stdv	26	8	2 600	6

Table 2.9. ANZFSC Australia and New Zealand Food Standards Codes maximum limit (ML) and general expected level (GEL) of fish and crustaceans with respect to the elemental concentrations found in experimentally derived fish and crayfish samples. note: table lists fresh weight values.

Dietary intake safety guidelines observed and recommended by FSANZ regarding total dietary metal intake of cadmium, copper, arsenic (inorganic), and mercury are calculated to be 1 575, 525, 105, and 375 μg week^{-1} for a 75 kg man (FSANZ, 2003). Table 2.10 lists the amount of fresh weight fish fillets a 75 kg man could safely eat per week, in the absence of other sources of heavy metals, in respect to metal assayed and experimental treatment.

Element	Ingestion limit 75 Kg man (μg week^{-1})	Kilograms of fresh weight barramundi fillets a 75 kg man could safely eat each week at WHO and ANZFA guidelines		
		0 % treatment	50 % treatment	100 % treatment
As - inorganic	1575	12.2	11.1	10.9
Cd - total	525	52.5	105.0	175.0
Cu - total	105	42.0	65.6	80.8
Hg - total	375	46.9	41.7	37.5

Table 2.10. Food Standards Australia New Zealand recommended total dietary consumption limits of arsenic, cadmium, copper and mercury in respect to the amount of experimentally derived fresh-weight barramundi fillets a 75 kg man could safely ea each week.

Arsenic was the element that limited fish fillet consumption to 10.9 kg week^{-1} in the 100 % wastewater trial. Arsenic concentration analysis in this study combined both inorganic and organic forms of the element (the latter form predominates in fish tissues), thus over estimating the significance of arsenic concentrations when comparing them to FSANZ guidelines which consider only the inorganic form.

When considering the guidelines apply to inorganic arsenic only, the amount of experimentally derived fillets that could be eaten observing FSANZ guidelines is largely under-estimated. This is because the analysis performed in this study included both inorganic and organic forms of arsenic, the latter predominates in fish tissues.

Although the experimental mean for fish fillet copper was only 200 μg kg^{-1} under the ANZFSC GEL, and in one treatment breached that guideline, realistic consumption of fillets by a 75 kg male would not be limited due to copper concentration under FSANZ / WHO guidelines (Table 2.10).

Crayfish growth in the study was slightly lower than comparable published values (Pinto and Rouse, 1992, Austin, 1992), possibly due to the restricted growing space and lack of natural forage. An improved mesocosm design that afforded more surface area for forage food growth, and forging activity, would help ensure no scale-effect. Red claw feeding efficiency was comparable to less efficient than published values (Rouse and Kahn, 1998), possibly due to over-feeding. Crayfish survival was comparable to published values (Pinto and Rouse, 1996, Rouse and Kahn, 1998, Jones, 1990, Meade and Watts, 1995).

The elemental concentrations within the tail meat derived from this experiment were within published values of numerous samples of crayfish and other crustaceans from Australia, Canada, and the USA: cadmium concentrations ranked at the lower end, and arsenic and mercury concentrations ranked well below published values (Olsen, 1983, Evans-Illidge, 1997, Eisler, 1985, Eisler, 1987),

Concentrations of all metals within samples were well below ANZFSC enforceable maximum levels (MLs) and suggested (GELs), meeting Australian distribution and consumption regulations and guidelines. Table 2.11 lists the amount of fresh weight red claw tail meat a 75 kg man could safely eat per week, in the absence of other sources of heavy metals, in respect to metal assayed and experimental treatment.

Element	Ingestion limit 75 K man (μg week^{-1})	Kilograms of fresh weight red claw tails a 75 kg man could safely eat each week at WHO and ANZFA guidelines		
		0 % treatment	50 % treatment	100 % treatment
As - inorganic	1575	27.2	25.0	58.3
Cd - total	525	43.8	40.4	75.0
Cu - total	105	13.1	15.0	15.0
Hg - total	375	1.7	1.8	1.5

Table 2.11. Food Standards Australia New Zealand recommended total dietary consumption limits of inorganic arsenic, cadmium, copper and mercury in respect to the amount of experimentally derived fresh-weight red claw tail meat a 75 kg man could safely eat each week.

Mercury is the element that limited tail meat consumption to between 1.5 - 1.8 kg week^{-1}. If a 75 kg male ate 1.5 kg of fresh weight tail meat (not taking into consideration other dietary sources) he would have reached 100 % of the total maximum dietary recommended intake of mercury for one week (WHO, 1989, FSANZ, 2003).

Barring total copper concentrations in BG biomass (addressed further in this section), null hypotheses 1 and 2 were accepted for *Baumea articulata*: that wastewater did not measurably impact on the growth of plants, or the level of metal element concentrations within plant AG and BG samples.

On average, *Baumea articulata* plants grew well in the replicates over the autumn season although individual results were highly variable since one-third of the plants distributed among treatments grew at a rate of < 0.10 RGR, or lost biomass over the experiment. The variability was thought to be due to the strategy of transplanting physically small plants into the replicates during the senescent (colder) season, and into and environment in which *Lemna*, algae, and other organisms already established competed for the nutrients that took time to concentrate in the

low animal density recirculating systems. Thus, some plants were unable to establish and degenerated over the 88 day trial, or died, as in one case.

Under these less than optimal abiotic conditions exacerbated by plant / microbial competition, *Baumea articulata* concentrated copper in BG biomass in 100 % wastewater treatments, and in doing so, highlighted further potential use of the plant. Considering the water generated from SCL power stations is rich in copper, *Baumea articulata* may show promise in copper phyto-stabilization applications on soils of moderately hydric to permanently flooded condition, and possibly phyto-extraction of copper from water using modified growing techniques linked with integrated production systems using such wastewater.

As expected, water use in relation to animal production was inefficient at low volumetric animal culture densities. Water loss due to evapotranspiration was high due to fish culture tank aeration, evaporation from the crayfish bucket 'water falls', and plant transpiration vented water from the system. Water quality remained of a standard requirement for barramundi and red claw culture; and, although TDS concentrations increased over treatments at statistically different concentrations, the rise did not measurably affect animal production efficiency or plant growth at 100 % strength wastewater. Review of Stanwell data collected from the point at which water was drawn for this experiment showed that metal and electrochemical concentrations of water used in the experiment were representative of normal operating cooling pond conditions for 2003. At the conclusion of the experiment, the use of residual wastewater remaining within the replicates for the third trial of hydroponic roses (see Chapter IV - Rose Hydroponics Supported by Industrial and Aquaculture Wastewater Re-use) completed the linkage of rose hydroponics to

industrial wastewaters that had been initially used to support integrated wetland polyculture of barramundi and red claw.

2.7 Conclusion

Through this experiment valuable information regarding the impact of power station wastewater on animals and plant in performance and nutritional safety has been gained through the integration of power station wastewater into fish, crayfish, and plant based primary production. In comparison with global fish catch data, edible tissue elemental concentrations were normal. The evidence suggests that polyculture of barramundi and red claw to market size in 100 % SCL waste water would be successful in recirculating wetland systems. However, a precautionary note concerns metal accumulation with regard to GEL regulations (specifically barramundi and copper) and suggested consumption values (specifically red claw and mercury) that would require further investigation (including food sources of those elements). Paradoxically, approximately 25 % of barramundi fillets sampled in this experiment exceeded the copper GEL, although the amount one could eat is not restricted in terms of consumption safety guidelines; and conversely, red claw tail meat mercury concentrations were well below ML regulations, although the amount one could eat is restricted in terms of consumption safety guidelines.

This research showed that SCL power station cooling waste had no significant effect on *Baumea articulata* growth. Furthermore a serendipitous outcome of this facet of the project was that *Baumea articulata* had the capacity to absorb copper into below ground biomass in 100 % SCL power station wastewater treatments; a characteristic that could be for phytoremediation, and which should be the subject of further research.