Chapter III Growth and Metal Accumulation within *Lates calcarifer* caged in Stanwell Corporation Limited Power Station Blow-down Wastewater Cooling Ponds

Abstract

Electrical power stations use water as a coolant to dissipate heat from turbines. Ponds are commonly used to dissipate heat from the resulting coolant wastewater for mechanical and environmental purposes. An experiment was set up to culture *Lates calcarifer* in a power station wastewater cooling pond. The objective of this experiment was to determine whether differences in fish fillet concentrations of arsenic, cadmium, copper, and mercury occurred between fish grown in the wastewater pond, and fish grown in a municipal tap water tank. Culture of barramundi was successful. Fish fillet from cultured fish met Australian distribution and consumption regulations and guidelines in regard to metal concentrations. However, one replicate fish cage in this experiment had significantly greater concentrations of all metals than fish cultured in other replicates. The elevated concentration of copper in those fish samples breached one of the ANZFSC 'general expected level' production guidelines. It is believed that subjecting the fish in that cage continuously to increased exposure to the pond substrate (bottom sediment re-suspension) during feeding was related to the relatively higher metal concentrations of fillet samples

1.0 Introduction

The Stanwell Corporation Limited (SCL) power station blow-down wastewater cooling ponds at the time of this experiment had not been dredged for 7-8 years, although dredging was scheduled for later that year. There was a question as to the ability of barramundi (*Lates calcarifer*) to survive on-site at the power station, in the blow-down wastewater, under the shortened HRTs and winter season operating conditions. An additional concern was whether accumulation of metals in fish tissues, rendering them unfit for distribution or consumption would occur if fish culture was successful. To address these issues SCL granted resources including access to the electrical plant cooling/settling ponds to conduct the research.

2.0 Experiment - Growth and Metal Accumulation within *Lates calcarifer* caged in Stanwell Corporation Limited Power Station Blow-down Wastewater Cooling Ponds

2.1. Experimental goals, objectives, and hypotheses

The goal of this experiment was to culture successfully *Lates calcarifer* in net cages located in blow-down wastewater treatment cooling pond # 3 at the Stanwell Corporation Limited Power Station (Gracemere, QLD) during the winter (dry) season. The objectives of this experiment were to determine if differences in fish fillet concentrations of arsenic (As), cadmium (Cd), copper (Cu) and mercury (Hg)

occurred over time, and between *Lates calcarifer* grown at SCL and at CQU in a freshwater tank. Completing the goals and objectives would provide information used (collectively with other experimental results) help determine whether or not developing an on-site, integrated wetland / fish culture system at the Stanwell Corporation Limited power station is an endeavor likely to be successful.

Null Hypothesis I

There will be no change in the concentration of arsenic, cadmium, copper, and mercury in fish fillet tissue of fish grown at Stanwell over the course of the experiment

Alternate Hypothesis I

Concentrations of arsenic, cadmium, copper, and mercury will increase in fish fillet tissue of fish grown at Stanwell over the course of the experiment.

Null Hypothesis II

There will be no difference in the concentrations of arsenic, cadmium, copper, and mercury between tissue samples of *Lates calcarifer* cultured in SCL wastewater ponds and the CQU tank (municipal tap water).

Alternate Hypothesis II

Stanwell Corporation Limited cage cultured *Lates calcarifer* will have greater concentration of arsenic, cadmium, copper, and mercury in tissue samples than *Lates calcarifer* cultured in municipal tap water at CQU.

2.2 Experimental and system design

Four individual cubic meter cages were constructed with 40 mm diameter PVC frames lined with 1.2 cm rigid-plastic mesh (Plate 2.1). Dual 10 cm diameter, 1.0 meter square, PVC floats (filled with foam) were lashed to the cages to provide floatation and stability. The floats supported a 115 cm^2 gray perspex lid which presented a 40 cm^2 opening, filled with PVC coated wire mesh (2 cm grid) to allow feeding and protect fish from pelicans, cormorants, turtles, and other predators.

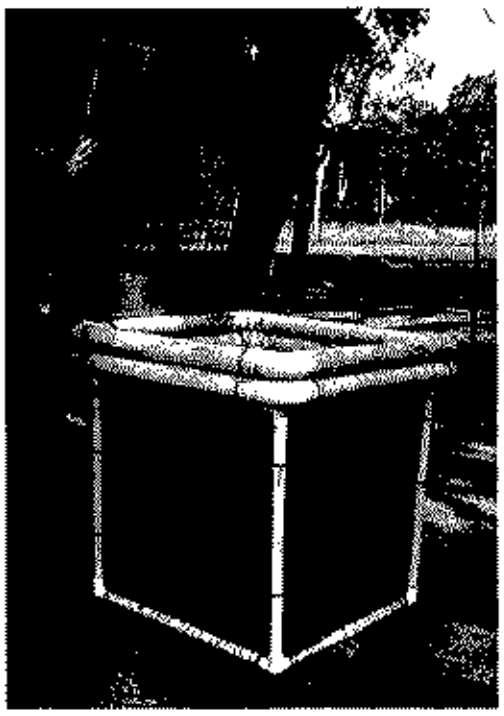

Plate 2.1. Net cage design.

A cement storm water spillway linking blow-down wastewater pond chosen for this experiment (pond # 3) with the neighboring up-stream pond #2 and #3 allowed vehicle access to the experimental site, and was the point of pond # 3 influent (via culvert) (Figure 2.1). The site provided the maximum heat available at a point down the wastewater treatment process, where the chances of intermittent chlorine pulses of fish-toxic concentrations of chlorine were thought to be low. Cages were lashed to a 200 m nylon rope located 20 meters off the storm water spillway in pond # 3, strung parallel to the western edge of the pond, and tied off to rebar anchors embedded in 75 kg circular cement footings positioned at the northern and southern ends.

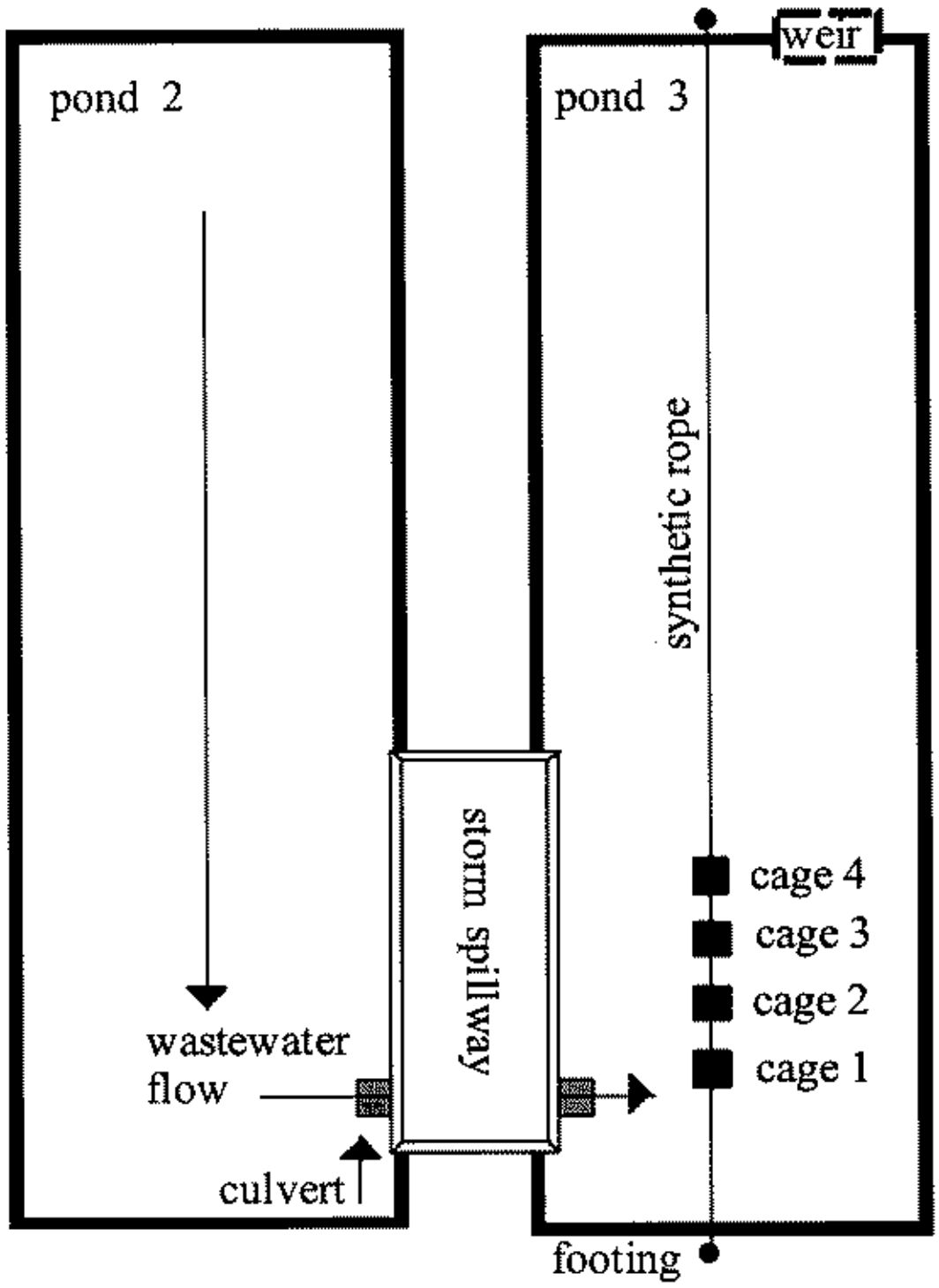

Figure 2.1. Location of barramundi culture cages in cooling pond # 3 of the Stanwell power station.

2.3 Methods

Power station operation dictated that the experiment take place prior to pond dredging operations scheduled for late 2003. The wastewater ponds had not been dredged for 8 years hence sediments were very thick, and represented the low end operational HRT in pond # 3 under normal electrical generation operations.

Consequently, once in place, access to the cages was difficult, requiring one person to pull the cages by their southern footing line toward the spillway to a second person waiting in the water. Due to thick sediments cage 1 ran aground *c.* 10 meters off the spillway; with the rope, the cages were pulled toward the spillway through the mud as far as possible before the waiting person waded out into the pond, and manually dragged the cages close enough to the spillway to work with.

A shipment of larger size fingerlings (*c.* 55 g body weight) were obtained from outside ponds at Kuranda Fish Farm on April 23, 2003 and were kept in the CQU quarantine tank as detailed in Chapter I, Section 2.3.7. Outdoor pond fingerlings were opted for stocking purposes given the harsh environmental conditions (e.g. cool air temperature, condensed wastewater due to no rain, avian and reptile predators) anticipated at SCL over the winter. Each cage was stocked with ten *Lates calcarifer* on May 6, 2003, and was harvested 84 days later on July 29, 2003. Fish used for stocking were weighed at CQU following methods outlined in Chapter I, Section 2.37, packed in plastic bags filled with source water and inflated with oxygen, and then placed in insulated cool boxes for travel to the SCL site. There, the fish were floated in bags for 10 minutes to equilibrate in the cages prior to release. At harvest, fish were netted from the cage and transferred immediately into ice-slurry filled insulated cool boxes for transport off site to CQU for fresh weight, dry weight, and metal analyses according to the methods outlined in Chapter I, Sections 2.4.1, and Chapter II, Section 2.3.3.

Cage fish feeding was completed daily by SCL staff. Prior to feeding, the cage line was unfastened from the southern, footing and cages were drawn in to *c.* 10 meters from the spillway, i.e. at the point at which the first cage became grounded.

Then feed was thrown manually into the cages through the mesh lid, and, after a five minute period, the cages were drawn back to their initial positions in the pond by the un-fastened line before it was refastened to the south footing. It was observed that more feed probably missed the cage openings and entered the surrounding water than entered the cages, and that fish were not breaching the water surface for feed.

Thirty of the remaining barramundi from the stock population were cultured in the 1.5 m^3 circular cement quarantine tank at CQU. The tank was aerated via a black poly hose tapped into the main airline of the CQU aquaculture unit. The hose end was pierced with 2 mm holes, then tied to a brick, and submerged in the tank to provide aeration. The influent rate of de-chlorinated tap water was 1.0 L min^{-1} (HRT = 1 day) and entered the tank via a black poly line tapped into the unit's main water line. The fish tank interior was coated in black isothallic resin, the water was not heated, and fish were fed by hand daily to satiation.

Lates calcarifer growth rates, feeding rates and feed conversion ratios were calculated using methods outlined in Chapter I, Section 2.4.1.

For this experiment, the study relied on weekly electrochemical water quality data collected for pond # 3 at the pond # 3 weir by SCL as part of their routine industry maintenance program. One sample was collected by SCL for metal analysis prior to the initiation of the experiment.

2.4 Statistical Analyses

A repeated measures ANOVA was used to determine whether differences in total weight of *Lates calcarifer* occurred over time and between cages. The same test was used to determine whether differences in the concentration of fish fillet copper, cadmium, arsenic, and mercury occurred over time, between cages, and between SCL and CQU cultured fish.

2.5 Results

2.5.1 *Lates calcarifer*

Culture of barramundi was successful without statistical differences in individual fish fresh weights occurring between cages. The effect of time was significant across treatments ($p \leq .01$) as individual fish fresh weights increased over the course of the experiment (Figure 2.2).

Mean cage cultured fish feeding rates ranged 11.1 - 12.9 % body weight d^{-1}, while specific growth rates ranged 0.5 - 0.7 % body weight d^{-1}, and the food conversion ratio ranged 5.47 – 8.18 : 1.00 at culture densities ranging from 0.8 to 1.0 kg m^{-3} (Table 2.1, Figure 2.2). Average pond temperature was 21.2 ° C and fish survival was ≥ 90 % over the experiment. CQU cultured fish feeding rate was 0.5 % body weight d^{-1}. Specific growth rate was 0.2 % body weight d^{-1}, and the food conversion ratio was 2.92 : 1 at a culture density of 1.36 kg m^{-3} (Table 2.1, Figure

2.2). Mean ambient air temperature near the tank was 18.6 ° C and fish survival was 100 %.

	Cage 1	Cage 2	Cage 3	CQU
feeding rate (% body weight day^{-1})	12.85	12.51	11.05	0.5
SGR	0.53	0.51	0.65	0.16
FCR	8.18	7.43	5.47	2.92
cage culture density ($kg\ m^{-3}$)	0.81	0.83	0.99	1.36
individual weight at harvest (g)	90.4	82.5	99.2	68.2
average culture temperature °C	21.2			18.6*
survival %	90	100		
days of fish culture	84			

Table 2.1. Barramundi culture efficiency at SCL and CQU;
* mean ambient air temperature over trial

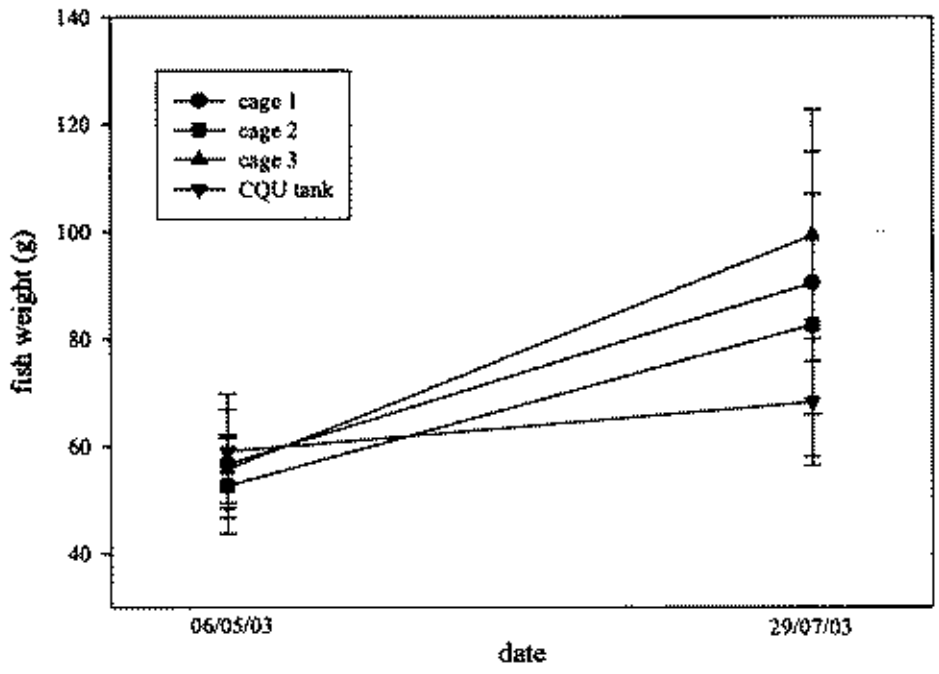

Figure 2.2. Barramundi growth at SCL and CQU.

The initial fish fillet samples were sent to FSAS (with the initial samples collected in the experiment of Chapter II (as noted in Chapter II, section 2.5.1) and were lost in that organization's move to new laboratory facilities. Therefore it was not possible to determine the uniformity of initial tissue sample metal concentrations,

nor the effect of time. However, it was possible to determine whether differences in fillet metal concentration existed between cages, or between SCL cage cultured fish and CQU tank cultured fish grown over the same period.

Samples taken from fish cultured in cage 1 (Table 2.2) had significantly greater concentrations of all metals than fish cultured in cages 2 and 3, and fish grown at CQU ($p \leq 0.01$). Cage 1 fish samples had, on average, two to four times more arsenic, twice as much copper, and 1.7 times more cadmium and mercury than fillets taken from all other fish (Table 2.2, Figure 2.3). There were no statistical differences in fillet metal concentration between cage 2, cage 3, and CQU fish. The ranking of metals from greatest to least concentration within cage cultured fish fillets were copper with a mean of 9.9 mg kg^{-1}, cadmium (1.6 mg kg^{-1}), arsenic (904 µg kg^{-1}), and mercury (494 µg kg^{-1}) (Table 2.2, Figure 2.3).

	Cu mg kg^{-1}	As µg kg^{-1}	Cd µg kg^{-1}	Hg µg kg^{-1}
cage 1				
mean	16.8	1 924	3 322	532
stdv	4.4	1 068	726	100
cage 2				
mean	7.7	367	1 184	302
stdv	3.9	305	587	61
cage 3				
mean	5.3	422	1 922	259
stdv	1.8	265	939	42
CQU				
mean	6.5	883	1 888	245
stdv	2.8	517	726	32
n = 5				

Table 2.2. *Lates calcarifer* fillet sample concentrations of arsenic, cadmium, copper, and mercury (dry weight concentrations).

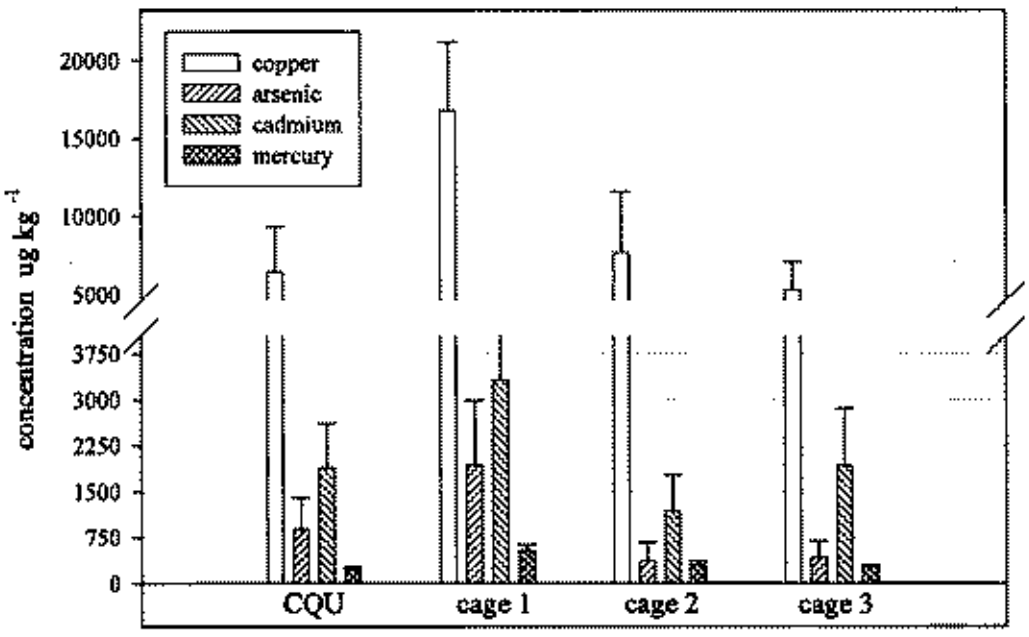

Figure 2.3. Metal concentrations in fillet samples ($\mu g\ kg^{-1}$ dry weight tissue).

2.5.2 Water quality

In pond # 3, DO, TDS, pH, and temperature averaged 7.0 mg L^{-1}, 1 256.9 mg L^{-1}, 8.2, and 21.2 ° C, respectively, over the experiment (Table 2.3). Pond # 3 water samples contained 7.2 $\mu g\ L^{-1}$ arsenic, $\leq 0.05\ \mu g\ L^{-1}$ cadmium, 24.0 $\mu g\ L^{-1}$ copper, and $\leq 0.05\ \mu g\ L^{-1}$ mercury at analysis.

	dissolved oxygen mg L^{-1}	temperature °C	pH	T.D.S mg L^{-1}	suspended solids mg L^{-1}
Date					
5/05/2003	6.2	22.6	8.2	1 198	7
12/05/2003	6.15	22.4	8	1 230	9
19/05/2003	6.95	25	8.3	1 219	8
26/05/2003	5.88	21.3	8.1	1 266	12
2/06/2003	6.84	23.7	8.2	1 277	12
9/06/2003	6.1	21.4	8.2	1 223	8
16/06/2003	7.43	19.4	7.9	1 242	12
23/06/2003	7.46	20	8.2	1 264	8
30/06/2003	7.21	21.3	8.2	1 253	13
7/07/2003	8.25	20	8.2	1 241	11
14/07/2003	7.57	20.5	8.5	1 300	7
21/07/2003	7.96	20.6	8.4	1 323	10
28/07/2003	no result	17.9	8.7	1 304	13
mean	7.0	21.2	8.2	1 256.9	10.0
stdv	0.8	1.9	0.2	36.8	2.3

Table 2.3. Electrochemical water quality at SCL pond # 3 (weir) over the course of the experiment.

2.6 Discussion

The goal of this study was met: *Lates calcarifer* were cultured successfully in net cages located in the Stanwell Corporation Limited power station cooling pond # 3 during the winter season. The objective of determining whether differences in fish fillet metal concentrations existed between cage cultured (SCL) fish and tank cultured (CQU) fish was met also. However, the loss of the initial samples rendered measuring changes over time impossible, forcing the abandonment of testing hypothesis I. Although null hypothesis II was rejected in the case of fish cultured in cage 1, culturing barramundi in the SCL wastewater ponds did not appear to impact fish fillet tissue concentrations of metals assayed. It is thought that subjecting the

fish in cage 1 daily to increased exposure to the pond substrate (through closer proximity and bottom sediment re-suspension when grounding the cage) during feeding was related to metal concentrations of fillet samples being elevated in comparison to samples taken from fish grown in cages 2 - 3 and tank cultured fish. Additionally, cage 1 floated directly in front of pond the # 3 influent culvert, the point at which if variances between ponds 2 - 3 existed (in suspended or soluble metals), the disparity would be at its greatest. It also was noted that during the time of the experiment, pond # 3 submerged vegetation proliferated, and the water was greener than in pond 2 which was depauperate in submerged vegetation with water browner in color. Stanwell technicians opined that the condition of pond 2 over the experiment was probably due to residual chorine. It is possible that the biotic conditions of pond # 3 sequestered soluble metals, thus a factor impacting variance in water quality between ponds 2 and 3.

Although barramundi survival was high, efficiency ratings of barramundi growth (feeding rate, growth rate, FCR) for the pond grown fish ranked at the inefficient end of values published in the literature (Chaitanawisuti et al., 2001, Awang, 1987, Lobegeiger et al., 2001, Rimmer, 1995, Williams and Barlow, 1996, Williams, 1998), probably due to the cool water temperatures, and stress induced by cage handling during feeding procedures.

In comparison to numerous world-wide samples of fish around the world, the concentrations of arsenic and mercury in the SCL fish from cages 2 - 3, and the CQU tank cultured fish were very low. In contrast, tissue concentrations of copper ranged at the high end, and cadmium concentrations were slightly higher than published values for global catches of various fish species (Eisler, 1988, Eisler, 1987, Eisler,

1985, Edmonds and Francesconi, 1993, Olsen, 1983, Swales et al., 1998, Chen, 2002). In further contrast, concentrations of copper and cadmium in the tissues of cage 1 fish were more than double the concentrations of published values from global catches of various fish species, while concentrations of arsenic and mercury in fish grown in cage 1 were low in comparison to the published data (Eisler, 1988, Eisler, 1987, Eisler, 1985, Edmonds and Francesconi, 1993, Olsen, 1983, Swales et al., 1998, Chen, 2002). With respect to the ANZFSC enforceable maximum levels (MLs) and suggested (GELs), fish samples taken from SCL cages 2 – 3 and the CQU tank were below ML and/or GEL limits, hence the products met Australian distribution and consumption regulations and guidelines (Table 2.4). Fish cultured in cage 1 were always below ML and/or GEL limits for all parameters except copper, which exceeded the GEL by twice the limit. There were no ANZFSC ML regulations or GEL guidelines for cadmium in fish. Cadmium levels in fish samples taken from cages 2 - 3 and the CQU tank were approximately 3 to 5 times greater than the ML for wheat and rice (100 $\mu g\ kg^{-1}$) and approximately $^1/_5$ of the ML for sheep, cow and pig kidney (2500 $\mu g\ kg^{-1}$) (Commonwealth, 2001). Cadmium levels in fish samples taken from cage 1 were approximately 9 times greater than that set for wheat and rice, and $^1/_3$ that of sheep, cow and pig kidney (Commonwealth, 2001).

ANZFSC Regulations	As μg kg^{-1}	Cd μg kg^{-1}	Cu μg kg^{-1}	Hg μg kg^{-1}
fish ML	2000	-	-	1000
fish GEL	-	-	2000	-
SCL Cage Cultured Barramundi				
Cage 1:barramundi mean concentration	537	927	4682	149
Cage 2 - 3: barramundi mean concentration	110	433	1805	78
stdv	75.7	232.9	476	15.2
CQU - Tank Cultured Barramundi				
barramundi mean concentration	247	527	1801	68

Table 2.4. ANZFSC Australia and New Zealand Food Standards Codes maximum limits (ML) and general expected levels (GEL) for metals in fish tissues of fish and metal concentrations found in experimentally derived fish samples (fresh weight concentrations).

Dietary intake safety guidelines observed and recommended by FSANZ (2003) for dietary metal ingestion of cadmium, copper, arsenic, and mercury are calculated to be 1 575, 525, 105, and 375 μg $week^{-1}$ for a 75 kg man (FSANZ, 2003). Table 2.5 lists the amount of fresh weight 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. Cadmium was the element that limited fish fillet consumption to 0.6 kg $week^{-1}$ in cage 1, and from 1.0 - 1.6 kg $week^{-1}$ in cages 2 - 3 and the CQU tank. If a 75 kg male ate 1.6 kg of fresh weight fillet meat from cage 2, he would have reached 100 % of the total maximum dietary recommended intake of cadmium for one week (WHO, 1989b; (FSANZ, 2003).

Element	Ingestion limit 75 kg man ($\mu g\ week^{-1}$)	Kilograms of fresh weight barramundi fillets a 75 kg man could safely eat each week at WHO and ANZFA guidelines			
		cage 1	cage 2	cage 3	CQU
As	1575	2.9	15.4	13.3	6.4
Cd	525	0.6	1.6	1.0	1.0
Cu	105	22.3	50.0	70.0	58.3
Hg	375	2.5	4.5	5.2	5.5

Table 2.5. 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 eat each week.

2.7 Conclusion

This experiment illustrated that suggested that cage culture systems could be used to culture nutritionally safe (with respected to metals assayed) barramundi, possibly to market size, even under sub-optimal temperatures, and handling procedures, with low hydraulic residence times in Stanwell Corporation Limited power station cooling wastewater treatment ponds during the dry winter season.

There was no evidence to suggest that the SCL water used in the experiments caused accumulation of metals in barramundi fillet tissues. In comparison to global data, the edible tissues of the experimentally produced fish in regard to elemental concentrations were normal (with the exception of one cage in which cultured fish were exposed to chronic sediment re-suspension). Furthermore, it is suggested that re-suspension of sediments into culture water, or fish cultured in water with suspended solids values >10.0 mg L^{-1} over prolonged periods, may result in increased concentrations of metals such as arsenic, cadmium, copper, and mercury, within barramundi fillets; and that copper in particular may accumulate to levels that

breach suggested GELs. Additionally, if and when cadmium regulations become drafted, further investigations including food source manipulation may be required to address the issue of that element accumulating in the fish tissues.

Roughly 53 % of barramundi fillets sampled in this experiment exceeded the copper GEL, although the amount one could eat is not restricted in realistic terms of consumption safety guidelines; the same paradox that resulted in the barramundi grown in the experiment of Chapter II. It is hoped that the information gained in both experiments regarding this paradox is reviewed by regulating bodies, possibly resulting in resolution of the discrepancy between their guidelines.

Thus, this experiment offered practical information regarding the efficacy and nutritional aspects of culturing barramundi in 100 % (SCL) power station blow down wastewater.