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# Changes in Selenium, Copper, Cadmium, and Zinc Concentrations in Mullet (*Mugil cephalus*) from the Southern Basin of Lake Macquarie, Australia, in Response to Alteration of Coal-Fired Power Station Fly Ash Handling Procedures

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**Abstract.** Selenium, copper, cadmium, and zinc concentrations were measured in mullet (*Mugil cephalus*) from the southern basin of Lake Macquarie, Australia, in 1997 to determine if improved ash-handling practices at an adjacent coal fired power station, implemented in 1995, had significantly lowered trace metal concentrations in mullet tissues. Mean muscle tissue concentrations of selenium (5.9 ± 0.7 μg/g dry mass), copper (3.6 ± 0.1 μg/g dry mass), and zinc (14 ± 1 μg/g dry mass) are lower than previously reported for mullet analyzed in 1993 (10 ± 2, 21 ± 3, 27 ± 3 μg/g dry mass, respectively). Cadmium concentrations in liver tissues increased from 2.3 ± 0.3 to 6 ± 2 μg/g dry mass. Significant intra-tissue correlations between metal concentrations were found for all tissues except muscle. Strong correlations of selenium, copper, and zinc concentrations were found in liver tissues, indicating a common primary source may exist for these metals, such as fly ash. All trace metals were found to have significant inter-tissue correlations, with strong correlations occurring for selenium between all tissues and for cadmium between all tissues except muscle. Regulation of copper, cadmium, and zinc appears to be occurring in muscle tissue. Selenium concentrations in mullet are still above levels considered to be of concern to human consumers. Trace metal concentrations are below that known to effect the health of fish. Mullet are directly exposed to trace metal concentrations as a result of feeding and the ingestion of contaminated sediment and detritus. Lower metal concentrations found in mullet tissues are attributed to the burial of highly contaminated sediment with material containing lower trace metal concentrations. Little of the variations in trace metal concentrations between mullet was explained by mass, gender, or age.

Approximately 280 species of fish are known to inhabit waters within and around Lake Macquarie, Australia (NSW Fisheries 1995) with the largest catches of finfish species in estuarine waters being the sea mullet (*Mugil cephalus*) (47%), luderick (*Girella tricuspidata*) (11%), bream (*Acanthopagrus australis*) (10%), and dusky flathead (*Platycephalus fuscus*) (4%) (Virgona *et al.* 1998). The commercial catch of sea mullet in 1996 was estimated to be valued at $11.4 million, with that caught in estuaries valued at $4.1 million (Virgona et al. 1998).

In a previous paper on trace metal concentrations in the tissues of mullet sampled from Lake Macquarie in 1993, selenium concentrations in muscle tissues were higher than that recommended as safe for human consumption of seafood and at levels likely to affect the health of mullet (Kirby *et al.* 2001). High selenium concentrations were attributed to power generation activities and the production of selenium-contaminated fly ash. Two coal-fired power stations are situated on the southern shores of Lake Macquarie at Vales Point and Eraring. Surface sediments collected from around the power station at Vales Point contain fly ash (Furner 1979) and have high concentrations of selenium and copper (Kirby *et al.* 2001; Peters *et al.* 1999; Batley 1987; Furner 1979). The surface sediments of the southern basin also contain elevated concentrations of cadmium and zinc compared to background concentrations (Kirby *et al.* 2001). The sources of these metals have not been established but are believed to be partly from the activities of the coal-fired power station and other urban and industrial inputs. Mullet are exposed to high trace metal concentrations as a result of feeding and the ingestion of contaminated sediment and detrital material. Human consumers and fish growth, reproduction, and survival are believed to be at risk from such high tissue concentrations of selenium.

Improved fly-ash handling procedures were implement by the Vales Point Power Station at the end of 1995 (Peters *et al.* 1999; Harston 1996). Before this time ash produced from power generation activities was mixed with lake water and pumped to a nearby ash dam, which drained directly into Wyee Bay via Mannering Bay. More than 80% of the water-soluble selenium originally present in the fly ash was found in the effluent from the ash dam corresponding to a flux of 1.53 ± 0.24 kg Se/day (Davies and Linkson 1991). Since this time water has been removed from the ash dam and recycled back to

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the power station, where it is mixed with cooling water before being discharged into the bay. These practices are expected to raise selenium concentrations within the ash dam but reduce the amount of suspended and dissolved trace metals reaching the lake (Peters *et al.* 1999). We have resampled mullet from the southern basin of Lake Macquarie to determine if these improved practices by the Vales Point Power Station have resulted in lower selenium, copper, cadmium, and zinc concentrations in mullet tissues.

In the previous study on mullet in 1993 (Kirby *et al.* 2001) we were unable to determine the influence of age and gender on trace metal concentrations in tissues. In this study we also report findings on the influence of age and gender on trace metal concentrations and verify the relative independence of trace metal concentrations with mass. We also report relationships between trace metal concentrations in mullet tissues to improve our understanding of accumulation and distribution of trace metals in different tissue types of this fish species.

## Materials and Methods

All equipment, vials, and microwave vessels used in collection, digestion, and analysis procedures were prewashed in 2% nitric acid (Aristar, BDH) and rinsed in deionized water.

### *Collection*

Sea mullet (*Mugil cephalus*) were collected from Wyee and Chain Valley Bays from 20 to 22 May 1997. The sampling sites and sampling procedures were the same as used to collect fish in 1993. A total of 36 mullet were collected using three gill nets (30 m × 3 m, with a mesh size of 37–50 mm) positioned perpendicular to the shore. Collection occurred each day approximately 2 h before dusk and continuing until noon the next day. Nets were checked every hour, with collected mullet being killed immediately by a blow to the head and snap-frozen on dry ice. Mullet were transported to the laboratory on dry ice and stored frozen (~−20°C) until dissection.

### *Tissue Removal*

Individual mullet were weighed for mass, measured for total length, and dissected to remove eight tissues (muscle, kidney, liver, stomach, intestine, heart, gonad, and gill) and otolith bone. Muscle tissue was removed from the left side of the fish between the pelvic and dorsal fins. Stomach and intestine tissues were dissected and rinsed with deionized water to remove food and sediment particles. Before freeze-drying, tissues were rinsed with deionized water and placed in acid-washed labeled vials and frozen (~−20°C).

### *Trace Metal Analysis*

Individual tissues were freeze dried for 72 h and then finely ground to a homogenous powder using a ZM 100 ultra centrifugal mill (Retsh, Germany). Tissues were digested using a low-volume microwave digestion procedure developed by Baldwin *et al.* (1994). Approximately 0.07g of freeze-dried tissue was weighed into a 7-ml Teflon polytetrafluroacetate closed digestion vessel and 1 ml of concentrated nitric acid added (Aristar, BDH). Each 7-ml vessel was then capped and tightened to 2.3 Nm, placed into larger 120-ml screw top vessels and tightened to 16.3 Nm. A model MDS-81 (CEM, Indian Trail, NC) microwave oven rated at 600 W was used for all digestions, with the microwave time procedure consisting of three stages: 2 min at 100% power, 2 min at 0% power, and 45 min at 75% power. After digestion the vessels were allowed to cool at room temperature for 20 min and then diluted in polyethylene vials to 10 ml with deionized water. Digests were stored in a cool room (~0–5°C) until analysis.

Selenium and cadmium concentrations were determined using a Perkin Elmer 5100 PC atomic absorption spectrometer, equipped with Zeeman background correction, a HGA-600 graphite furnace and an AS-60 auto sampler. These metals were determined by atomization of 10 μl of sample from the surface of a pyrolytic graphite-coated tube inserted with a pyrolytic graphite L'vov platform. Palladium/magnesium nitrate and ammonium phosphate/magnesium nitrate modifiers were used for the determination of selenium and cadmium concentrations, respectively. Atomization programs are as described in Deaker and Maher (1995). Zinc and copper concentrations were determined using a Perkin Elmer 3100 Atomic Absorption Spectrometer equipped with deuterium background correction and an AS-90 auto-sampler.

Certified reference materials and blanks were routinely run with samples throughout the digestion and analysis procedure. Recoveries of trace metals from NIST RM50 Albacore tuna (measured: Se 3.7 ± 0.3, Cu 9 ± 1, Cd 0.16 ± 0.08, Zn 14 ± 1 μg/g; certified: Se 3.6 ± 0.4, Cu N/A, Cd N/A, Zn 14 ± 1 μg/g) and AGAL 2 shark tissue (measured: Se 1.5 ± 0.4, Cu 5.8 ± 0.6, Cd 0.09 ± 0.02, Zn 17 ± 1 μg/g; certified: Se 1.7 ± 0.3, Cu 5.7 ± 0.5, Cd 0.07 ± 0.02, Zn 16 ± 2 μg/g) were in close agreement with certified values.

### *Age Determination*

Two sagittal otoliths were removed from each mullet through a cranial incision. Individual otoliths were scrubbed with a synthetic bristle brush to remove excess tissue and allowed to dry at room temperature for 48 h. The right otolith from each mullet was set in resin and allowed to harden for 72 h. Several transverse sections of approximately 300 μm were taken using a Buehler Isomet low-speed diamond saw, including one section containing the center of the primordium. These sections were mounted on microscopic slides using epoxy adhesive and examined using a light microscope.

Otolith rings were counted by three readers, counts by one reader independent of the knowledge from previous readings. Age was accepted when all three readers agreed on the same number of rings. In situations of disagreement between readers a second count was performed. Age was accepted when counts by two of the three readers were agreed on. If all three readers disagreed on the second count the slide was given no age.

Mullet otolith sections in most cases were found to show clear ring patterns, consisting of light zones and dark rings. Narrower dark rings were counted for age estimation, with 95% of slides aged after the first reading. By the end of the second reading 100% of the slides were aged.

### *Data Analysis*

Data analysis was performed using the statistical package SAS (Statistical Analysis Systems, version 6.12, SAS Institute Inc., 1989–1996, Cary; NC), with data logarithmically transformed to meet requirements of normality and homogeneity of variances. Analysis of variance (ANOVA) was used to determine if significant differences existed between trace metal concentrations in tissues, mass, and years, followed by a multiple comparison Student Neuman Keus (SNK) test. Where mass was found to have a significant interaction with tissue

concentrations, analysis of covariance (ANCOVA) was used to remove this influence. Correlations and regressions were also used to determine relationships between metals, tissues, mass, and age.

## Results and Discussion

### *Metal Concentration Variation Between 1993 and 1997*

Selenium concentrations in muscle, stomach, and gonad tissues measured in this study were significantly less than in mullet analyzed in 1993 (Figure 1). Selenium concentrations in muscle tissues decreased from 10 ± 2 μg/g dry mass (mean ± SE) in 1993 to 5.9 ± 0.7 μg/g dry mass in 1997. Selenium concentrations were still greater than that reported for fish sampled from relatively uncontaminated environments for the same trace metal (Bebbington *et al.* 1977; Maher *et al.* 1992, 1997; Kirby *et al.* 2001). Mean selenium concentrations in muscle tissues appear to be declining, with mullet muscle tissue analyzed in 1998 by Barwick (1999) from the same location containing even less selenium (i.e., 1998, 4 ± 1; 1997, 5.9 ± 0.7; 1993, 10 ± 2 μg/g dry mass). Mean selenium concentrations in muscle tissues from this study were above that considered safe for human consumption (i.e., selenium 5 μg/g dry mass, assuming a wet/dry mass ratio of 5:1) (ANFA 1992). However, by 1998 selenium concentrations were below that considered to be of concern to human consumers.

Copper concentrations in muscle, stomach, heart, and gonad tissues have also significantly decreased from those measured in mullet in 1993 (Figure 1). Copper concentrations in muscle tissues decreased from 21 ± 3 μg/g dry mass in 1993 to 3.6 ± 0.1 μg/g dry mass in 1997. Similar to selenium, mean copper concentrations in muscle tissues appear to be declining, with muscle tissues analyzed in 1998 by Barwick (1999) having lower copper concentrations (i.e., 1998, 2.8 ± 0.4; 1997, 3.60 ± 0.02; 1993, 22 ± 3 μg/g dry mass).

Cadmium concentrations in liver tissues were significantly higher than in mullet analyzed in 1993 (Figure 1). Cadmium concentrations increased from 2.3 ± 0.3 μg/g dry mass in 1993 to 6 ± 2 μg/g dry mass in this study. Other tissues have similar cadmium concentrations to those measured in mullet collected in 1993. Cadmium concentrations measured in muscle tissues (0.05 ± 0.07 μg/g dry mass) are also similar to those measured by Barwick (1999) in 1998 (0.074 ± 0.055 μg/g dry mass).

Zinc concentrations in muscle and liver tissues have significantly decreased, and zinc concentrations in stomach, kidney, and heart tissues have significantly increased relative to mullet analyzed in 1993 (Figure 1). Zinc concentrations in muscle tissues decreased from 27 ± 3 μg/g dry mass in 1993 to 14 ± 1 μg/g dry mass in 1997. Zinc concentrations measured by Barwick (1999) in muscle tissues in 1998 are slightly higher (*i.e.*, 26 ± 3 μg/g dry mass). These concentrations are similar to those reported for mullet sampled from relatively uncontaminated environments (Plasket and Potter 1979; Bebbington *et al.* 1977; Eustace 1974; Denton and Burdon-Jones 1986; Brooks and Rumsey 1974).

Mean concentrations of copper and zinc measured in muscle tissues in 1993, 1997, and 1998 were below those considered safe for human consumption of seafood (*i.e.*, copper 20 μg/g dry mass; zinc 30 μg/g dry mass assuming a wet/dry mass ratio of 5:1) (ANFA 1992). No standard at present is set for cadmium concentrations in Australian seafood.

Selenium, copper, cadmium, and zinc concentrations measured in mullet tissues were below concentrations that have been shown to cause reduced growth and/or survival in mullet and other fish species (Coughlan and Velte 1989; Finley 1985; Hilton *et al.* 1980, 1982; Hilton and Hodson 1983; Murai *et al.* 1981; Lanno *et al.* 1985; Noel-Lambot and Bouguegnean 1977; Thomas *et al.* 1982; Westernhagen *et al.* 1980).

Decreases in selenium and copper concentrations in tissues may be attributed to improved practices implemented by Vales Point Power Station at the end of 1995 (Peters *et al.* 1999; Harston 1996). These practices were expected to reduce the concentrations of suspended and dissolved trace metals, especially selenium, entering the lake waters via ash dam overflows (Peters *et al.* 1999). Sedimentation rates from locations around Lake Macquarie are estimated to be 1.1–5.7mm per year (Peters *et al.* 1999; Batley 1987). Lower trace metal concentrations found in mullet tissues are attributed to the burial of highly contaminated sediments with material containing lower trace metal concentrations.

### *Tissue Metal Concentrations and Relationship to Mass*

Tissue concentrations for selenium ranged from 0.56 μg/g dry mass in intestine tissues to 53.1 μg/g dry mass in kidney tissues. Highest mean selenium concentrations were found in liver tissues (17.5 μg/g dry mass) and lowest in intestine tissues (4.2 μg/g dry mass) (Figure 2). Significant differences exist between selenium concentrations in various tissues ($p < 0.001$). Liver, kidney, and heart tissues were significantly higher than stomach, gonad, gill, muscle, and intestine tissues. Selenium concentrations within these two groups were not significantly different from each other. Significantly positive regressions were found between selenium concentrations in liver and intestine tissues and mass (Figure 3).

Tissue concentrations for copper ranged from 1.9 μg/g dry mass in intestine tissues to 692 μg/g dry mass in liver tissues. Highest mean copper concentrations were found in liver tissues (206 μg/g dry mass) and lowest in muscle tissues (3.6 μg/g dry mass) (Figure 2). Significant differences exist between copper concentrations in tissues ($p < 0.001$). Copper concentrations in liver tissues were significantly higher than all other tissues. Heart, kidney, stomach, and gonad copper concentrations were significantly higher than intestine and gill tissues, with copper concentrations in muscle tissues significantly less than all other tissues. Significant positive regressions were found between copper concentrations in liver, intestine and heart tissues and mass (Figure 3).

Tissue concentrations for cadmium ranged from 0.001 μg/g dry mass in muscle tissues to 37.9 μg/g dry mass in liver tissues. Highest mean cadmium concentrations were found in liver tissues (6.3 μg/g dry mass) and lowest in muscle tissues (0.05 μg/g dry mass) (Figure 2). Significant differences exist between cadmium concentrations in various tissues ($p < 0.001$). Cadmium concentrations decrease in the following order: liver > kidney > intestine > heart/gonad/stomach > gill > muscle. No significant regressions were found between cadmium concentrations in tissues and mass ($p > 0.05$).

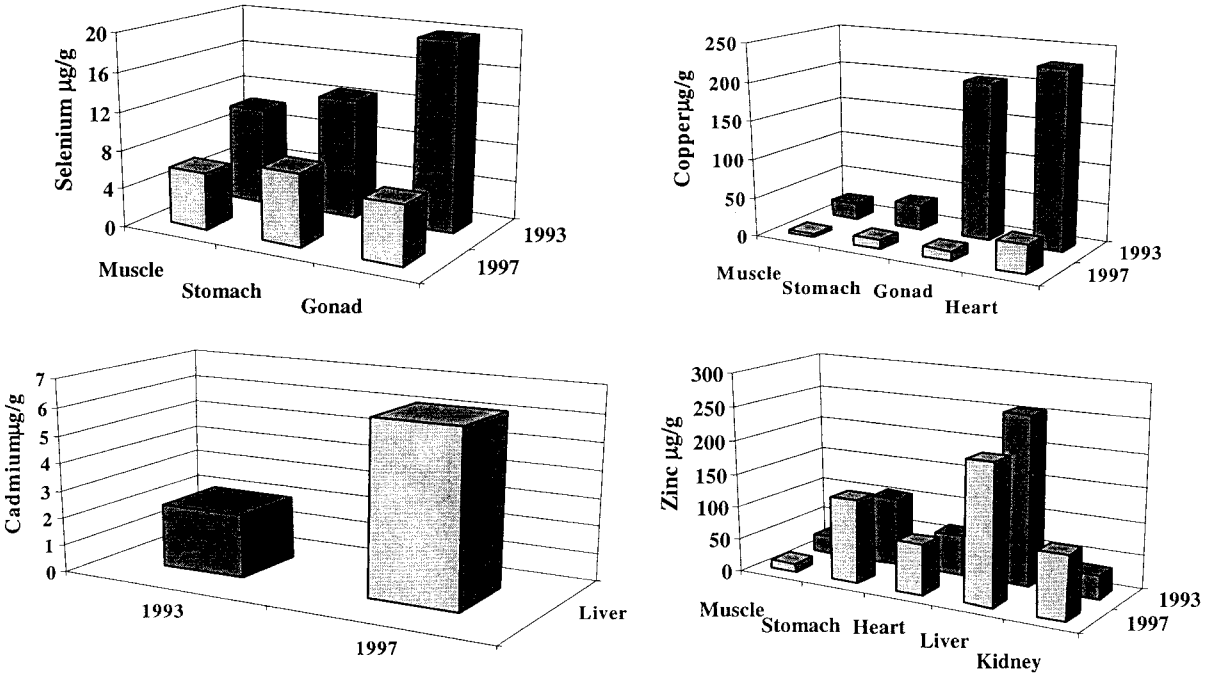


**Fig. 1.** Significant differences of tissue trace metal concentrations between *Mugil cephalus* sampled in 1993 and 1997

Tissue concentrations for zinc ranged from 2.9 µg/g dry mass in muscle tissues to 1996 µg/g dry mass in gonad tissues. Highest mean zinc concentrations were found in gonad tissues (580 µg/g dry mass) and lowest in muscle tissues (13.8 µg/g dry mass) (Figure 2). Significant differences exist between zinc concentrations in tissues ($p < 0.001$). Zinc concentrations decreasing in the following order: gonad/liver > stomach/intestine/gill/kidney/heart > muscle. Significant regressions were found between zinc concentrations in muscle, intestine, stomach, and kidney tissues and mass (Figure 3). Regressions were all negative, except for the intestine tissues.

The distributions of trace metal concentrations in tissues (highest to lowest) were similar to that previously reported for mullet analyzed in 1993. Liver tissues were again found to accumulate high concentrations of all trace metals, with gonad tissues accumulating elevated zinc concentrations. Liver tissues are believed to be the main site of trace metal detoxification within fish (Sorensen 1991). Significant differences in liver tissue concentrations of cadmium and zinc exist between mullet sampled in 1993 and 1997 (Figure 1). However, given the small differences in concentrations and the dynamic nature of trace metal concentrations in liver tissues these differences cannot be meaningfully interpreted because they reflect immediate past exposure, current metabolism, and excretion of trace metals.

Selenium and zinc concentrations in some mullet tissues were again found to have significant positive regressions with increasing mass (Figure 3). Similar significant positive regressions with mass were found for selenium concentrations in liver tissues and zinc concentrations in muscle tissues compared to mullet analyzed in 1993. Regressions for zinc concentrations and masses in stomach tissues were found to differ between years, with a significant positive regression found in this study and negative in mullet analyzed in 1993. Copper concentrations in this study were also found to have significantly positive regressions with increasing mass in liver, intestine, and heart tissues (Figure 3). In all cases the slopes of the regressions were mostly low ($r^2 = 0.1375–0.5636$) and explained little of the variation. The variation of trace metal concentrations in mullet tissues with mass may be masked to some degree by the combined influences of other factors such as gender, age, and feeding strategies (Dallinger *et al.* 1987).

### *Relationship of Metal Concentrations to Fish Age*

No relationships between trace metals concentrations in tissues and age were found ($p > 0.05$). Most mullet collected in this study were between 2 and 4 years old. Younger mullet reside in the Lake Macquarie estuary until approximately 3–4 years of age, when they leave to travel in a northern direction to spawn in open ocean waters (Harasti 1997). These nonsignificant results should be extended to other mullet populations with caution, as sufficient numbers were not obtained from all age groups. Other studies have reported both significant (positive and negative) and nonsignificant relationships of trace metal concentrations with age (Cronin *et al.* 1998; McFarlane and Franzin 1980). Differences in trace metal concentrations between fish of different ages have been proposed to occur because of variations in diet and better regulation of uptake and elimination of trace metals by older fish (Cronin *et al.* 1998; Vas *et al.* 1993). The clearest indication in fish of a true relationship between trace metal concentration and age or size exists only for mercury (Denton and Burdon-Jones 1986; Homung *et al.* 1993; Law and Singh 1991; Cross *et al.* 1973; Mackay *et al.* 1975; McFarlane and Franzin 1980). Mercury

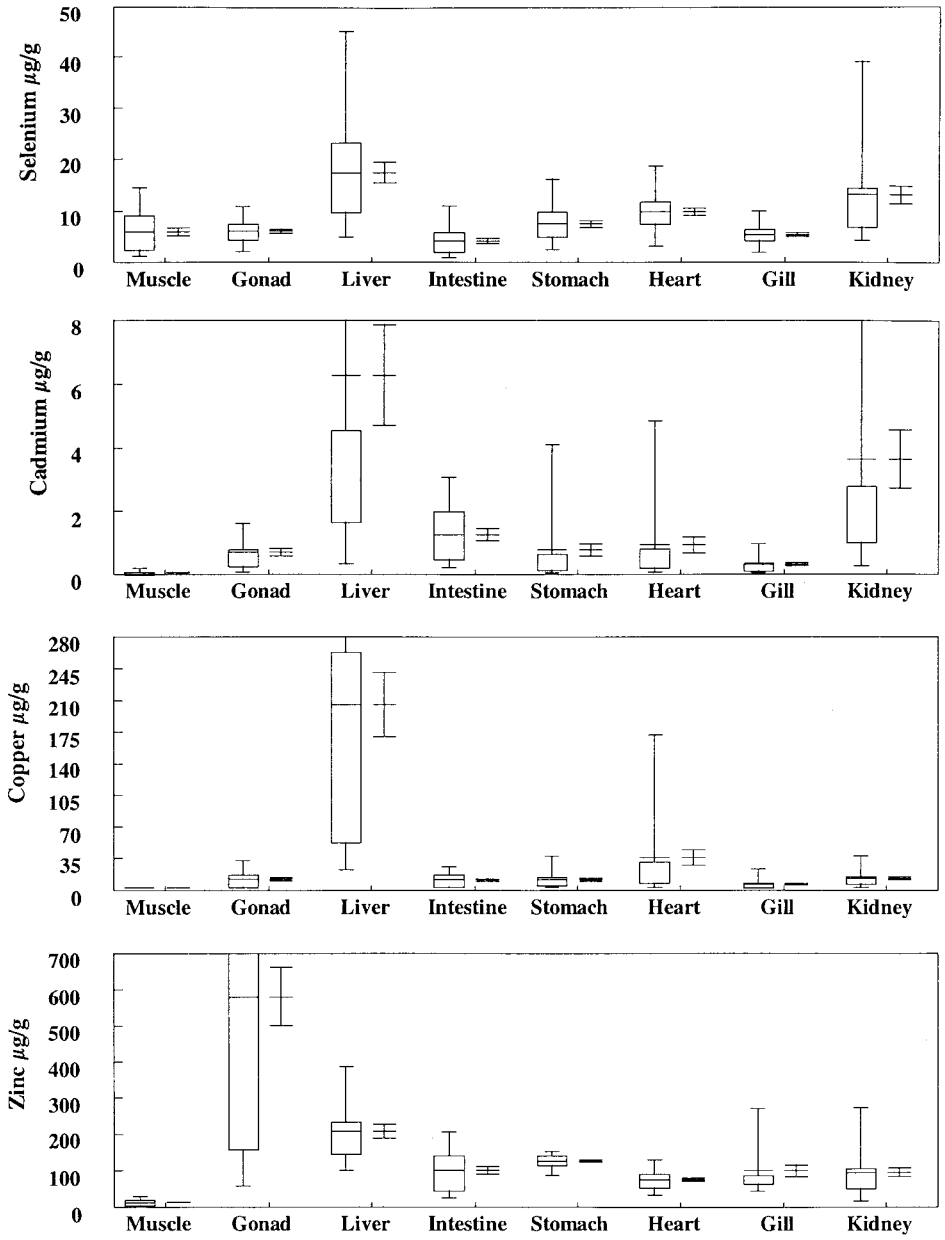


**Fig. 2.** Trace metal concentrations in *Mugil cephalus* tissues

concentrations probably increase with age due to the metal's long half-life, efficient uptake, and assimilation (especially of methylmercury), and low elimination rates that results in its almost complete retention (Bernhard and Andreae 1984; Cronin *et al.* 1998; Brooks and Rumsey 1974).

Mass and length for mullet, as reported for other fish species (Mackay *et al.* 1975; McFarlane and Franzin 1980; Cronin *et al.* 1998) were highly correlated (Figure 4). Size or dry mass of fish is often used in fish studies as an indicator of age (Hanna 1989; Aboul Naga 1996). Inherent variability in size at any age for many fish species often make precise estimates of age using these measurements difficult (Beckman *et al.* 1990; Zhao *et al.* 1997). In this study, mullet mass was found to be a poor indicator of age (Figure 4) with high variability found within age groups, best indicated by 3-year-old mullet having a mass range between 93 and 725 g.

### *Metal Concentrations and Gender*

Female and male mullet accumulated similar trace metal concentrations in tissues ($p > 0.05$) (Table 1). Differences would be

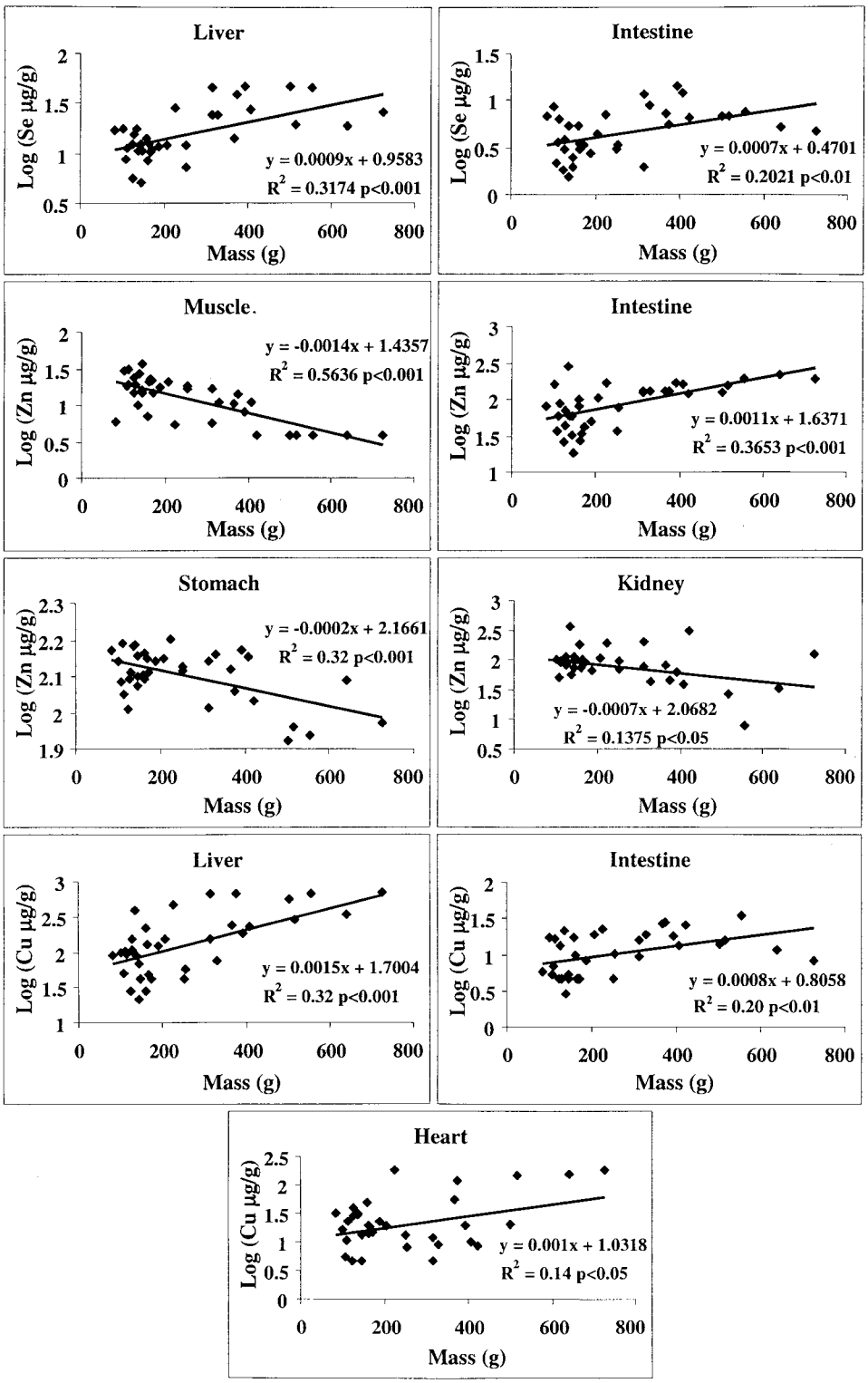


**Fig. 3.** Relationships for tissues with significant correlations of selenium, zinc, and copper concentrations with mass

expected to occur when one gender is less efficient in trace metal regulation as a result of more energy being used in egg or sperm production (Chernoff and Dooley 1979; Phillips 1969) and/or has to meet demands for sexual initiation, formation, or development. A number of other studies have also reported no differences in trace metal concentrations between female and male fish (Vas 1991; Chernoff and Dooley 1979; Glover 1979; Maher *et al.* 1997). When a significant difference has been found, higher trace metal concentrations are usually associated with female fish (Miller *et al.* 1992; Foerster *et al.* 1998).

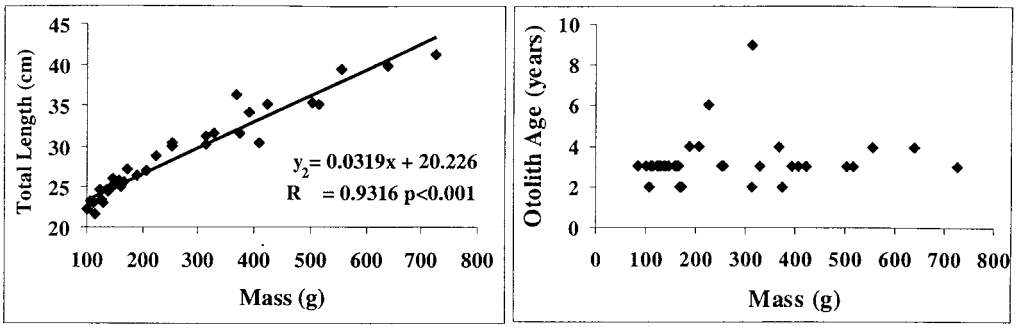


**Fig. 4.** Relationship between age, mass, and length for *Mugil cephalus*

**Table 1.** Trace metal concentrations in tissues of female and male *Mugil cephalus*

| Metal | Gender | Muscle | Liver | Kidney | Stomach | Intestine | Gill | Gonad | Heart |
|---|---|---|---|---|---|---|---|---|---|
| Selenium | Female | 5 ± 4 | 16 ± 11 | 16 ± 14 | 6 ± 3 | 4 ± 3 | 5 ± 2 | 6 ± 2 | 9 ± 5 |
| | Male | 7 ± 4 | 19 ± 14 | 12 ± 4 | 9 ± 5 | 5 ± 4 | 6 ± 2 | 7 ± 3 | 11 ± 4 |
| Copper | Female | 3.6 ± 0.2 | 203 ± 227 | 14 ± 9 | 11 ± 8 | 12 ± 7 | 6 ± 5 | 13 ± 12 | 42 ± 59 |
| | Male | 3.6 ± 0.1 | 209 ± 197 | 12 ± 11 | 13 ± 11 | 12 ± 9 | 8 ± 8 | 12 ± 11 | 31 ± 41 |
| Cadmium | Female | 0.055 ± 0.049 | 6 ± 9 | 4 ± 6 | 0.6 ± 0.8 | 1 ± 1 | 0.3 ± 0.3 | 0.8 ± 0.8 | 1 ± 1 |
| | Male | 0.044 ± 0.091 | 7 ± 10 | 4 ± 5 | 1 ± 1 | 1 ± 1 | 0.3 ± 0.3 | 0.6 ± 0.5 | 1 ± 1 |
| Zinc | Female | 13 ± 8 | 191 ± 75 | 100 ± 67 | 122 ± 18 | 96 ± 55 | 92 ± 61 | 625 ± 512 | 75 ± 27 |
| | Male | 15 ± 10 | 229 ± 146 | 91 ± 84 | 133 ± 21 | 109 ± 72 | 110 ± 131 | 528 ± 435 | 77 ± 32 |

Mean ± SD, n = 19 female, 17 male

### *Intra-tissue Correlations Between Metal Concentrations*

Strong significant correlations were found between essential trace metals selenium, copper, and zinc in liver tissues (Table 2). Significant correlations have also been reported in other studies between essential and nonessential trace metals in muscle and liver tissues (Galindo *et al.* 1986; Cronin *et al.* 1998; Ashraf and Jaffar 1991; Mackay *et al.* 1975; Brooks and Rumsey 1974; Lyle 1986; Chvojka *et al.* 1990; Glover 1979). Significant correlations of trace metal concentrations in tissues are proposed to occur due to similar pathways of accumulation (Brooks and Rumsey 1974) and may be associated with similar sources of exposure, methods of detoxification, sequestration, or elimination. New South Wales coal and Lake Macquarie fly ash are enriched in selenium, copper, and zinc (Swaine 1982, 1985; Davies and Linkson 1991) and would provide a common trace metal source of exposure to mullet.

No significant correlations were found between trace metals in muscle tissues (Table 2). This is in contrast to other studies that have reported significant correlations between Cu-Zn, Cu-Cd, and Cd-Zn in fish muscle tissues (Brooks and Rumsey 1974; Glover 1979; Hornung and Ramelow 1987; Cronin *et al.* 1998). The lack of coaccumulation in muscle tissues suggests some ability to regulate these metals in muscle tissues as proposed by several authors (Cross *et al.* 1973; Wiener and Giesy 1979; Bernhard and Andreae 1984).

### *Inter-tissue Correlations Between Metal Concentrations*

Numerous significant inter-tissue correlations were found for all trace metals (Table 3). Correlations were positive, except for zinc in muscle and intestine tissues. Selenium concentrations in muscle tissues are highly correlated ($R^2 > 0.5$) (Figure 5) with stomach and gill tissue concentration indicating that selenium concentrations in muscle tissues may increase or decrease depending on exposure to trace metal contaminated food or water. This is in contrast to Mackay *et al.* (1975) who reported that black marlin (*Makaira indica*) had no significant correlations of selenium concentration in muscle and liver tissues. Strong correlations were also found between cadmium concentrations in stomach, kidney, and liver tissues (Figure 5). Cadmium is a nonessential metal to fish and has no known biological function (Sorensen 1991). Cadmium correlations between detoxification and elimination tissues indicate that a mechanism may be in place to remove this metal and prevent cellular damage. Copper, cadmium, and zinc correlations in muscle and other tissues were low indicating little coaccumulation. This possibly is indicating that tissues such as muscle can regulate their metal content to some extent as proposed by other researchers (Cross *et al.* 1973; Wiener and Giesy 1979; Bernhard and Andreae 1984). In situations of high metal exposure, such as sediments containing selenium from fly ash, this regulatory mechanism may be overcome.

## Summary

Selenium and copper concentrations in muscle and other tissues are less than those previously reported for mullet analyzed in 1993. This decrease may be attributed to improved practices implemented by the Vales Point Power Station at the end of 1995. Mullet are exposed to these lower trace metal concentrations as a consequence of feeding and the ingestion of less

**Table 2.** Intratissue correlations between trace metal concentrations

| | Muscle | | | | Liver | | | Stomach | | | | Kidney | | | | Gonad[a] | | | | Intestine | | | | Gill | | | | Heart | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cd | Se | Zn | Cu | Cd | Se | Zn | Cd | Se | Zn | Cu | Cd | Se | Zn | Cu | Cd | Se | Zn | Cu | Cd | Se | Zn | Cu | Cd | Se | Zn | Cu | Cd | Se | Zn | Cu |
| Selenium | n/s | — | | | 0.128* | — | | | | | | 0.350*** | — | | | n/s | — | | | n/s | — | | | n/s | — | | | | | | |
| Copper | n/s | n/s | n/s | — | 0.196** | 0.728*** | 0.604*** | — | 0.375*** | n/s | 0.159* | — | n/s | n/s | 0.429*** | — | n/s | n/s | n/s | — | 0.407*** | 0.243*** | 0.653*** | — | 0.126* | n/s | 0.121* | — | 0.308*** | 0.149* | 0.474*** | — |
| Cadmium | — | | | | — | | | — | | | | — | | | | — | | | | — | | | | — | | | | — | | | |
| Zinc | n/s | n/s | — | | 0.286*** | 0.497*** | — | | n/s | n/s | — | | 0.129* | 0.139* | — | | n/s | 0.128* | — | | 0.256** | 0.344*** | — | | 0.178* | n/s | — | | 0.143* | n/s | — | |

[a] n = 35, all other tissues n = 36.
Significance *p < 0.05, **p < 0.01, ***p < 0.001.
n/s = nonsignificant.

**Table 3.** Significant correlations between trace metals concentrations in different tissues

| Interactions | | $R^2$ |
|---|---|---|
| Selenium | | |
| Muscle | Stomach | 0.84*** |
| Muscle | Gill | 0.50*** |
| Stomach | Gill | 0.49*** |
| Liver | Heart | 0.47*** |
| Heart | Gill | 0.43*** |
| Stomach | Heart | 0.34*** |
| Liver | Intestine | 0.29*** |
| Intestine | Heart | 0.27** |
| Muscle | Heart | 0.24** |
| Liver | Gill | 0.15* |
| Copper | | |
| Stomach | Gill | 0.47*** |
| Stomach | Heart | 0.34*** |
| Heart | Gill | 0.31*** |
| Liver | Heart | 0.30*** |
| Liver | Intestine | 0.29*** |
| Gonad | Kidney | 0.19** |
| Liver | Gill | 0.18* |
| Liver | Stomach | 0.16* |
| Zinc | | |
| Liver | Intestine | 0.33*** |
| Liver | Gill | 0.25** |
| Gill | Kidney | 0.22** |
| Muscle | Stomach | 0.19** |
| Stomach | Kidney | 0.16* |
| Heart | Kidney | 0.16* |
| Muscle | Intestine | −0.34*** |
| Cadmium | | |
| Liver | Kidney | 0.86*** |
| Liver | Stomach | 0.70*** |
| Stomach | Kidney | 0.64*** |
| Stomach | Heart | 0.56*** |
| Liver | Intestine | 0.49*** |
| Gonad | Heart | 0.44*** |
| Intestine | Kidney | 0.43*** |
| Heart | Gill | 0.37*** |
| Intestine | Stomach | 0.36*** |
| Liver | Heart | 0.36*** |
| Intestine | Heart | 0.35*** |
| Stomach | Gill | 0.35*** |
| Muscle | Stomach | 0.33*** |
| Heart | Kidney | 0.31*** |
| Gill | Kidney | 0.30*** |
| Liver | Gill | 0.30*** |
| Muscle | Gill | 0.29*** |
| Gonad | Stomach | 0.29*** |
| Muscle | Heart | 0.26** |
| Gonad | Liver | 0.22** |
| Muscle | Intestine | 0.22** |
| Muscle | Gonad | 0.21** |
| Gonad | Kidney | 0.18* |
| Muscle | Liver | 0.17* |
| Muscle | Kidney | 0.13* |
| Intestine | Gill | 0.13* |

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

contaminated sediment and detritus. Selenium concentrations in mullet muscle tissues in 1997 are still above that considered to be of concern to human consumers. Muscle tissue concen-

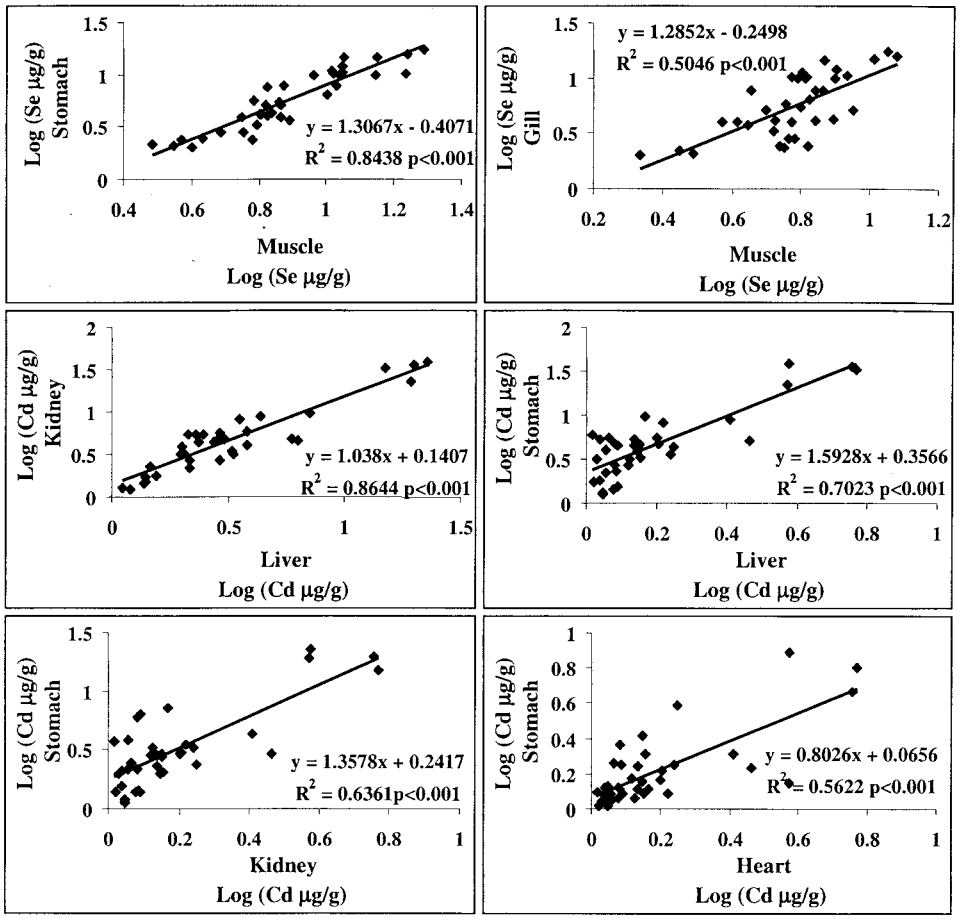


**Fig. 5.** Intertissue trace metal concentrations for *Mugil cephalus*

trations of cadmium, copper, and zinc are below that recommended for safe human consumption of seafood. All trace metal concentrations are below those likely to affect the health of mullet.

Some of the tissue trace metal concentrations were significantly correlated to dry mass. However, regressions explain little of the variation, and relationships may be masked to some degree by the combined influence of other factors.

Female and male mullet tissues contained similar trace metal concentrations. Trace metal concentrations in mullet tissues were not correlated with age. This nonsignificant result for age should be extended to other mullet populations with caution, as sufficient numbers were not obtained from all age groups for a robust analysis to be undertaken.

Significant intra-tissue correlations between metal concentrations were found for all tissues, except muscle tissues. Strong correlations of selenium, copper, and zinc concentrations were found in liver tissues, indicating a common primary source may exist for these metals such as fly ash. All trace metals were found to have significant inter-tissue correlations, with strong correlations occurring for selenium between all tissues and for cadmium between all tissues except muscle. Regulation of copper, cadmium, and zinc appears to be occurring in muscle tissue.

## References

Aboul Naga W (1996) Comparative study of trace metals accumulated in the muscle tissues of the most common and marketable seafood in Alexandria waters. Int J Environ Health Res 6:289–300

ANFA (1992) Australian food standards code. Australian New Zealand Food Authority, ACT Australia

Ashraf M, Jaffar M (1991) Relationships between heavy metal content and body weight of fish from the Kelang Estuary, Malaysia. Mar Poll Bull 22:86–89

Baldwin S, Deaker M, Maher W (1994) Low-volume microwave digestion of marine biological tissues for the measurement of trace elements. Analyst 119:1701–1704

Barwick M (1999) Assessment of copper, cadmium, zinc, arsenic, lead and selenium biomagnification within a temperate eastern Australian seagrass food web. B. App. Sci. Honours Thesis (unpublished), University of Canberra, Australian Capital Territory, Australia

Batley G (1987) Heavy metal speciation in waters, sediments and biota from Lake Macquarie, New South Wales. Aust J Mar Freshw Res 38:591–606

Bebbington G, Mackay N, Chvojka R, Williams R, Dunn A, Auty E (1977) Heavy metals, selenium and arsenic in nine species of Australian commercial fish. Aust J Mar Freshw Res 28:277–286

Beckman D, Louise Stanley A, Render J, Wilson C (1990) Age and

growth of black drum in Louisiana waters of the Gulf of Mexico. Trans Am Fish Soc 119:537–544

Bernhard M, Andreae M (1984) Transport of trace metals in marine food chains. In: Nriagu J (ed) Changing metal cycles and human health. Springer-Verlag. Berlin, pp 143–147

Brooks R, Rumsey D (1974) Heavy metals in some New Zealand commercial sea fishes. NZ J Mar Freshw Res 8:155–166

Chernoff B, Dooley J (1979) Heavy metals in relation to the biology of the mummichog, *Fundulus heteroclitus*. J Fish Biol 14:309–328

Chvojka R, Williams R, Fredrickson (1990) Methyl mercury, total mercury and selenium in snapper from two areas of the New South Wales coast, Australia. Mar Poll Bull 21:570–573

Coughlan D, Velte J (1989) Dietary toxicity of selenium-contaminated red shiners to striped bass. Trans Am Fish Soc 118:400–408

Cronin M, Davies I, Newton A, Pirie J, Topping G, Swan S (1998) Trace metal concentrations in deep sea fish from the North Atlantic. Mar Environ Res 45:225–238

Cross F, Hardy L, Jones N, Barber R (1973) Relation between total body weight and concentrations of manganese, iron, copper, zinc, and mercury in white muscle of bluefish (*Pomatomus saltatrix*) and a bathyl-demersal fish (*Antimora rostrata*). J Fish Res Bd Can 30:1287–1291

Dallinger R, Prosi F, Segner H, Back H (1987) Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. Oecologia (Berlin) 73:91–98

Davies W, Linkson P (1991) Selenium discharges from power station ash dams: Eraring, Vales Point, quantity, speciation and strategies for control. Report from the Department of Chemical Engineering, University of Sydney to Electricity Commission of NSW

Deaker M, Maher W (1995) Determination of selenium in seleno compounds and marine biological tissues using electrothermal atomization atomic absorption spectrometry. J Anal At Spec 10: 423–431

Denton G, Burdon-Jones C (1986) Trace metals in fish from the Great Barrier Reef. Mar Poll Bull 17:201–209

Eustace I (1974) Zinc, cadmium, copper and manganese in species of finfish and shellfish caught in the Derwent Estuary, Tasmania. Aust J Mar Freshw Res 25:209–220

Finley K (1985) Observations of bluegills fed selenium-contaminated *Hexagenia* nymphs collected from Belews Lake, North Carolina. Bull Environ Contam Toxicol 35:816–825

Foerster J, Smart S, Correll F, Edsall D (1998) Sentinel species: biologically active trace metals in the livers of the livers of the oyster toadfish (*Opsanus tau*). Second Annual Marine and Estuarine Shallow Water Science and Management Conference, NJ, 3–7 April 1995

Furner I (1979) Lead, cadmium, zinc and copper in organisms of Lake Macquarie. B. App. Sci. Honors Thesis (unpublished), University of Newcastle, New South Wales, Australia

Galindo L, Hardisson A, Garcia Montelongo F (1986) Correlations between lead, cadmium, copper, zinc, and iron concentrations in frozen tuna fish. Bull Environ Contam Toxicol 36:595–599

Glover J (1979) Concentrations of arsenic, selenium and ten heavy metals in school shar, *Galeorhinus australis* (Macleay), and gummy shark, *Mustelus antarcticus* Gunther, from south-eastern Australian waters. Aust J Mar Freashw Res 30:505–510

Hanna R (1989) Levels of heavy metals in some Red Sea fish before hot brine pools mining. Mar Poll Bull 20:631–635

Harasti D (1997) The accumulation and distribution of selenium in the sea mullet *Mugil cephalus* from Lake Macquarie, NSW, Australia. BSc thesis, University of Canberra, Australian Capital Territory, Australia

Harston E (1996) Evaluation of selenium and heavy metal contamination in Wyee Creek, NSW. Thesis (unpublished), University of Sydney, New South Wales, Australia

Hilton J, Hodson P (1983) Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). J Nutr 113:1241–1248

Hilton J, Hodson P, Slinger S (1980) The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). J Nutr 110:2527–2535

Hilton J, Hodson P, Slinger S (1982) Absorption, distribution, half-life and possible routes of elimination of dietary selenium in juvenile rainbow trout (*Salmo gairdneri*). Comp Biochem Physiol 71C:49–55

Hornung H, Ramelow G (1987) Distribution of Cd, Cr, Cu and Zn in eastern Mediterranean fishes. Mar Poll Bull 18:45–49

Hornung H, Krom M, Cohen Y, Bernhard M (1993) Trace metal content in deep-water sharks from the eastern Mediterranean Sea. Mar Biol 115:331–338

Kirby J, Maher W, Krikowa F (2001) Selenium, cadmium, copper, and zinc concentrations in sediments and mullet *(Mugil cephalus*) from the Southern Basin of Lake Macquarie, NSW, Australia. Arch Environ Contam Toxicol 40:246–256

Lanno R, Slinger S, Hilton J (1985) Maximum tolerable and toxicity levels of dietary copper in rainbow trout (*Salmo gairdneri* Richardson). Aquaculture 49:257–268

Law A, Singh A (1991) Relationships between heavy metal content and body weight of fish from the Kelang estuary, Malaysia. Mar Poll Bull 22:86–89

Lyle J (1986) Mercury and selenium concentrations in sharks from northern Australian waters. Aust J Mar Freshw Res 37:309–321

Mackay N, Kazacos M, Williams R, Leedow M (1975) Selenium and heavy metals in black marlin. Mar Poll Bull 6:57–61

Maher W, Baldwin S, Deaker M, Irving M (1992) Characteristics of selenium in Australian marine biota. Appl Organom Chem 6:103–112

Maher W, Deaker M, Jolley D, Krikowa F, Roberts B (1997) Selenium occurrence, distribution and speciation in the cockle *Anadara trapezia* and the mullet *Mugil cephalus*. App Organom Chem 11:313–326

McFarlane G, Franzin W (1980) An examination of Cd, Cu, and Hg concentrations in livers of northern pike, *Esox lucius*, and white sucker, *Catostomus commersoni*, from five lakes near a base metal smelter at Flin Flon, Manitoba, Can J Fish Aquat Sci 37:1573–1578

Miller P, Munkittrick K, Dixon D (1992) Relationship between concentrations of copper and zinc in water, sediment, benthic invertebrates, and tissues of white sucker (*Catostomus commersoni*) at metal-contaminated sites. Can J Fish Aquat Sci 49:978–984

Murai T, Andrews J, Smith R Jr (1981) Effects of dietary coper on channel catfish. Aquaculture 22:353–357

Noel-Lambot F, Bouquegneau J (1977) Comparitive study of toxicity, uptake and distribution of cadmium and mercury in the seawater adapted eel *Anguilla anguilla*. Bull Environ Contam Toxicol 18:418–424

NSW Fisheries (1995) A review of the information on the factors affecting the fisheries of Lake Macquarie. NSW Fisheries Research Institute, Sydney, Australia

Peters G, Maher W, Jolley D, Carroll B, Gomes V, Jenkinson A, McOrist G (1999) Selenium contamination, redistribution and remobilisation in sediments of Lake Macquarie, NSW. Org Geochem 30:1287–1300

Phillips A (1969) Fish physiology. Academic Press, New York

Plaskett D, Potter I (1979) Heavy metal concentrations in the muscle tissue of 12 species of teleost from Cockburn Sound, Western Australia. Aust J Mar Freshw Res 30:607–616

Sorensen E (1991) Metal poisoning in fish. CRC Press, Boca Raton, FL

Swaine D (1982) The importance of trace elements in Australian coals. Energy Res Technol 4:18–22

Swaine D (1985) Trace inorganic constituents in fly ash. Symposium on Coal Combustion, University of Newcastle, NSW

Thomas P, Bally M, Neff J (1982) Ascorbic acid status of mullet, *Mugil cephalus* Linn., exposed to cadmium. J Fish Biol 20:183–196

Vas P (1991) Trace metal levels in sharks from British and Atlantic waters. Mar Poll Bull 22:67–72

Vas P, Gordon J, Fielden P, Overnell J (1993) The trace metal ecology of ichthyofauna in the Rockall Trough, north-eastern Atlantic. Mar Poll Bull 26:607–612

Virgona J, Deguara K, Sullings D, Halliday I, Kelly K (1998) Assessment of the stocks of sea mullet in New South Wales and Queensland waters. New South Wales Fisheries Final Report Series No. 2, ISSN 1440-3544

Westerhagen H V, Dethlefsen V, Rosenthal H (1980) Correlation between cadmium concentration in the water and tissue residue levels in dab, *Limanda limanda* L., and plaice, *Pleuronectes platessa* L. J Mar Biol Assoc UK 60:45–58

Wiener J, Giesy J Jr (1979) Concentrations of Cd, Cu, Mn, Pb and Zn in fishes in a highly organic softwater pond. J Fish Res Bd Can 36:270–279

Zhao B, McGovern J, Harris P (1997) Age, growth and temporal changes in size at age of the vermilion snapper from the southern Atlantic bight. Trans Am Fish Soc 126:181–193