Running head. Trace metal incorporation in otoliths.

Title. Trace metal incorporation in otoliths of black bream (*Acanthopagrus butcheri*, Munro), an indicator of exposure to metal contamination.

Author names and affiliations. Melinda M. Ranaldi, Marthe M. Gagnon

Department of Environmental Biology, Curtin University of Technology, GPO Box U1987, Perth, Western Australia 6845 Australia. m.gagnon@curtin.edu.au

Corresponding Author. Marthe Monique Gagnon

Department of Environmental Biology, Curtin University of Technology, GPO Box U1987, Perth, Western Australia 6845 Australia. Tel: +61 8 9266 3723, Fax: +61 8 9266 2495, Email: m.gagnon@curtin.edu.au

Abstract

Otoliths of black bream (*Acanthopagrus butcheri*) collected from the Swan River Estuary were analysed by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) to measure concentrations of 14 trace metals. Trace metal concentrations in the otoliths may be related to the environmental exposure history of fish to contamination. The following metal isotopes were investigated: aluminium (^{27}Al), calcium (^{44}Ca), manganese (^{55}Mn), iron (^{57}Fe), copper (^{65}Cu), zinc (^{66}Zn), strontium (^{88}Sr), cadmium (^{111}Cd), tin (^{120}Sn), barium (^{138}Ba), mercury (^{202}Hg), lead (^{208}Pb) and the metalloids arsenic (^{75}As, ^{77}As) and selenium (^{82}Se). Significant differences in otolith trace metal composition were found between sampling sites. Lead and ^{57}Fe were consistently lower in downstream fish relative to upstream fish, while ^{88}Sr varied with the salinity gradient in the urban estuary. Lead and ^{57}Fe followed similar patterns within the otoliths, and appeared to provide the best discriminatory power for relating otolith metal concentration to the environmental history of the fish.

Keywords: Contamination; Iron; LA-ICP-MS; Lead; Otolith; Strontium.

1. Introduction

The Swan River Estuary flows through the metropolitan area of Perth in Western Australia (31° 57’S 115° 51’E). The estuary, situated in a major urban area, is the recipient of pollutants, such as metals from anthropogenic sources (Eyre & McConchie, 1993; Gerritse et al., 1998). Urban runoff discharges directly into the river, or via stormwater drains, and is a major transport mechanism for metals and metalloids (Birch, 1996). There is limited knowledge of the historic and recent metal exposure to fish in this waterway.

Aquatic organisms accumulate trace metals from the environment (Rainbow, 1997b). The majority of metal contamination studies on fish focused on accumulation in soft tissues, such as liver, kidney, gill and/or muscle. Liver, kidney and gill have efficient mechanisms for the elimination of potentially toxic compounds (Olsson et al., 1998) and for the regeneration of damaged tissues (Tsonis, 2000). Although metals are probably retained in soft tissues for longer periods than they would be in water (Phillips, 1977), trace metals may be lost as the tissues regenerate. Thus, when metal analysis is performed on soft tissues of fish, continuous sampling is necessary to assess the ongoing status of an aquatic environment.

Alternatively, hard calcified tissues such as otoliths, which are located in the inner ears of teleost fish, may offer a permanent record of metal exposure. The alternating addition of calcium carbonate and protein layers to the otolith and the potential inclusion of trace metals, suggest otoliths may reflect a trace metal history of the water environment inhabited by the fish (Kalish, 1990; Edmonds et al., 1991; Campana, 1999). When coupled with fish age, the possibility for a detailed chronological record exists via annual growth increments visible on the otolith. Growth increments are routinely used to age the fish. This annular structure offers the possibility of examining metal incorporation over time, providing a historic record of past and recent exposure of fish to trace metals.

Otoliths have the potential to contain many elements that substitute for Ca in the aragonite crystalline matrix or through co-precipitation of another carbonate (e.g. $SrCO_3$) (Campana, 1999). A number of studies have shown that otoliths can integrate abundant elements such as Sr and Ba as well as trace elements such as Fe, Mn, Zn and Pb (Mugiya et al., 1991; Sie & Thresher, 1992; Campana & Gagne, 1995; Milton et al., 2000). In numerous studies otolith metal concentrations such as Sr, have been linked to water concentrations by comparison of trace metal profiles in the otoliths of fish sampled from different locations (Papadopoulou et al., 1978; Papadopoulou et al., 1980; Protasowicki & Kosior, 1988; Grady et al., 1989; Dove & Kingsford, 1998). Other studies of the composition of trace metals in the otoliths have shown that the otoliths and water concentrations are not always proportional, suggesting that fish physiology regulated the uptake and incorporation of some elements such as Cu and Zn (Thorrold et al., 1997; Hanson & Zdanowicz, 1999; Milton & Chenery, 2001). In addition to physiological factors, physiochemical parameters such as temperature, salinity and pH may also influence the bioavailability of trace metals to fish as reflected in their otoliths.

The Swan River Estuary is strongly affected by pollutants from different types of land-use in its watershed (Gerritse et al., 1998). Thus, the objective was to test the hypothesis that the trace metal concentrations in the otoliths collected from black bream (*Acanthopagrus butcheri*) are related to recent metal contamination exposure. Black bream were captured at different sites along the estuary however, it is recognised that this species is a relatively mobile fish. For this reason, the marginal region of the otoliths was isolated for between-site comparison, permitting the elucidation of metal pollution at each site prior to sampling. A second objective was to investigate the temporal trends of metal incorporation associated with the growth annuli over the lifespan of the fish. It is anticipated that the findings of this study will provide temporal information on metal exposure throughout the life history of the fish.

2. Materials and Methods

2.1. Fish and sample collection

Black bream were collected by a commercial fisherman during late September (spring) 2002 from four sites along the Swan River Estuary (Figure 1). Helena was chosen as the reference site as it has minimal impact from human activity. The fish were collected using a 120 m, 100 mm mesh monofilament haul net between 2200 and 2400 hr when the fish moved into shallow water for feeding. Water samples were collected at a depth of approximately 2 metres for physicochemical parameter measurements including salinity, temperature and pH. The fish were sacrificed after capture then weight (g), length (standard, fork and total (mm)), sex and reproductive stage were recorded. The otoliths were removed with nylon forceps and stored in polyethylene vials until further analysis.

2.2. Otolith preparation

Sagittal otoliths were cleaned of adhering tissues, rinsed with Millipore filtered (18.2 MΩ cm^{-1}) Milli-Q water and air dried. The otoliths were embedded in epoxy resin and thin-sectioned transversely through the nucleus (core), using a low-speed diamond-blade saw (Buehler®) lubricated with Milli-Q water, to approximately 250 µm thickness.

All plastic, including forceps and glassware used during the preparation of the otoliths, were acid-washed using 5% HNO_3 for 24 hr, rinsed once with deionised water then twice rinsed in Milli-Q water. Multiple otolith sections from different fish (5 per slide) were mounted with super-glue (methyl methacrylate) onto labeled microscope slides, all contact with otoliths was avoided when glue was applied to the resin. Mounted samples were then ultrasonicated in Milli-Q water and dried prior to analysis to minimise surface contamination.

2.3. Laser ablation analysis

Otoliths sections were analysed using a CETAC LSX-200 Plus Laser Ablation System coupled to a VG Elemental – Plasma Quad III ICP-MS with data acquisition by PQ Vision Version 4.3. Operating conditions of both the inductively coupled plasma mass spectrometer (ICP-MS) and laser ablation (LA) unit are given in Table 1.

The mounted otoliths were placed within a gas-tight ablation cell attached to the stage of a transmitted light microscope. The otolith sections were viewed on a computer screen using a CCD camera and high resolution monitor. The start and end positions of the laser transect were selected under soft control. Each transect was positioned to follow the growth annuli along the axis used for aging the fish, making it possible to match the metal profile with fish age. The laser transect entered the otolith from the distal edge, passed through the core and exited through the proximal edge of the otolith (Figure 2). Transects were made along the same axis in all fish.

To assess the performance of the LA-ICP-MS instrument, calibration was achieved using NIST glass standard 610 (National Institute of Standards and Technology, Gaithersbury, Maryland). The glass standard was analysed every hour in order to monitor the accuracy and precision of the instrument and minimise within-day and between-day differences in instrument operation. For all transects, a laser beam diameter of ~50 µm with a depth of ~15 µm was pulsed at a repetition rate of 10 Hz and a laser energy of <20 mJ. The ICP-MS continuously scanned for the selected isotopes during the course of each laser transect across the otolith. Otoliths were analysed for ^{27}Al, ^{44}Ca, ^{55}Mn, ^{57}Fe, ^{65}Cu, ^{66}Zn, ^{75}As, ^{77}As ^{82}Se, ^{88}Sr, ^{111}Cd, ^{120}Sn ^{138}Ba, ^{202}Hg and ^{208}Pb. Element counts were converted to concentration (ppm), using ^{44}Ca as an internal standard. The limit of detection (ppm) achieved for the metals found above background counts were as follows: ^{27}Al 0.5, ^{44}Ca 160, ^{55}Mn 1.8, ^{57}Fe 0.28, ^{66}Zn

0.44, ^{88}Sr 5.1, ^{111}Cd 0.1, ^{138}Ba 0.2, ^{208}Pb 0.01. LA-ICP-MS analysis of the epoxy resin confirmed that the levels of these metals were found to be similar to background values.

2.4. Standardisation

Data was checked for drift throughout the day of analysis and corrected for drift when necessary. Sample counts were corrected for blank values (background noise) with the mean blank value, for each element, subtracted from the sample count data set.

Calcium was used as an internal standard to compensate for signal variation caused by differences in mass of ablated material. Calcium concentration was assumed to be constant at 396000 µg g^{-1}. The concentrations of the isotopes were estimated against this using the relative response factor of the instrument to the known concentration in the glass standard (NIST 610) and that recorded for the samples.

2.5. Image analysis

Following laser ablation analysis, otolith sections were viewed using reflective light with the image captured and saved using a digital camera connected to a dissecting microscope. The images were used to locate the growth annuli of the black bream otoliths relative to the ablation transect. Fish aging techniques were used to determine the age at specific annuli (opaque zones) where the ablation occurred. A single growth annulus consists of a narrow opaque zone laid down during the winter (wet) period and a wide translucent zone deposited during the summer (dry) period. Using the software package Image J®, each opaque zone was counted and the width measured along the ablation transect. The metal concentration profiles were then divided into years according to the measured distances along this transect (Figure 3a). For each otolith an annual mean was calculated from the series of metal readings that corresponded to each opaque zone (Figure 3b). Only the results taken from the core to the last opaque zone were used in the statistical analyses. The outermost 40 µm of otolith growth, representing approximately 1.5 to 2 months of the fish's life prior to capture, was used for site comparisons.

2.6. Statistical Analysis

Analysis of the otolith edges allowed extraction of information on the recently inhabited environment. Therefore, comparison between sites was more reliable as black bream are possibly mobile within the estuary, potentially confounding the results if all growth annuli were included. Additionally, analysis of the outermost edge of the otoliths permitted testing of the effects of fish size (total length and weight), age, sex and reproductive stage on the metal concentrations in the otolith as these factors may confound the interpretation of the results.

For the analysis of spatial distribution of metals across each otolith a mean value corresponding to each opaque zone was calculated from a series of metal readings that occurred in a particular growth annulus. This provided a series of annual means relating to every winter season during the fish's life. The Swan River Estuary is highly seasonal, with freshwater flow during the winter (wet) season while saltwater intrudes up to 60 km upstream during the summer (dry) season when rain ceases for several months. Limiting the data to the use of the annual winter period restricted the inclusion of seasonal variability experienced by the fish. Opaque zones corresponding to the winter periods were selected for statistical comparisons as there is a higher potential for metal input into the river, via stormwater drains and surface runoff during periods of rainfall.

Statistical analyses were performed using JMP® 5 statistical package (SAS Institute Inc. 2002). Tree regression models were fitted for each metal concentration (response variable). Covariates used to build the tree were; site, fish weight (g), fish length (total) (mm), age (yr), sex, and reproductive stage. On completion of the tree regression modeling, the results were used to build a linear regression model for the data. Regression models, using metal concentrations as the response variable, were examined and the residuals plotted. Where the residuals were found to be heteroskedastic, subsequent analyses were done on log-transformed data ($\log_{10}$ or $\log_{10}(1+x)$). Both multivariate analysis of variance (MANOVA) and separate ANOVA for each metal were used with site as the categorical factor. These tests were followed by post hoc Tukey honest significant tests, applying Kramer's correction for unequal sample sizes (Ott, 1993). Figures associated with the Tukey-Kramer test were constructed using the standardised data (log-transformed) for the metals exhibiting heterogeneity (the actual mean concentrations (ppm) for these metals are shown in Table 3).

3. Results

3.1. Water parameters

The salinities ranged from 2.8 upstream to 21.6 ppt downstream during winter 2002 (Table 2). The temperature and pH measurements were similar between sites at the time of sampling (Table 2).

3.2. Location-specific differences (marginal region of otolith)

The age and total length of the fish ranged from 3 to 6 years and 247 mm to 320 mm respectively. Fish aging techniques revealed that majority of the fish were approximately 5 to 6 years old. There were no significant differences in fish length between sites (ANOVA; $F_{3,33} = 2.017$, $P = 0.131$).

Of the 14 trace metals analysed by LA-ICP-MS, ^{65}Cu, ^{75}As, ^{77}As ^{82}Se, ^{120}Sn and ^{202}Hg, were frequently found in background levels and subsequently excluded from further analysis. Fish length, weight, age, sex and reproductive stage were included in the regression model for each metal and none of these were a significant factor in explaining differences in metal concentrations ($P > 0.05$) and subsequently removed from the model. Metal concentration in the marginal (edge) regions of the otoliths showed significant differences between sites within the Swan River Estuary (MANOVA; Pillai's trace = 2.084, $F_{30,78} = 5.922$, $P < 0.0001$). A separate ANOVA for ^{27}Al, ^{55}Mn, ^{66}Zn, ^{111}Cd, and ^{138}Ba showed no significant site-specific differences in the marginal regions of the otoliths (ANOVA; $P > 0.05$ in all cases) (Figure 4).

Strontium concentrations had significant differences between sites (ANOVA; $F_{3,33} = 39.69$, $P < 0.0001$) (Figure 5a). Otolith ^{88}Sr concentrations were significantly higher in Freshwater Bay relative to the other sites (Figure 5a, Tukey-Kramer test). Strontium content in the otolith correlated significantly with salinity of the sites within the estuary during the winter period ($r^2 = 0.83$). The salinity measurements and otolith ^{88}Sr concentrations both increased at downstream sites where seawater intrudes into the estuary. (Table 2 and Figure 5a respectively). Otolith ^{208}Pb concentrations were significantly different between sites (ANOVA; $F_{3,33} = 40.17$, $P < 0.0001$). Ascot fish showed significantly greater ^{208}Pb content relative to other sites (Figure 5b, Tukey-Kramer test). Fish sampled from Barrack St and Freshwater Bay did not differ in otolith ^{208}Pb concentration although, both were significantly higher than Helena (reference site) (Figure 5b, Tukey-Kramer test). Iron concentrations also differed significantly between sites again decreasing downstream from Ascot to Freshwater Bay (ANOVA; $F_{3,33} = 88.14$, $P < 0.0001$) (Figure 5c, Tukey-Kramer test). The actual mean ^{208}Pb and ^{57}Fe concentrations (ppm) in fish otoliths for each site are shown in Table 3.

Trace metal analysis of sediment samples collected from these study sites in 2001 showed elevated ^{208}Pb concentrations at Ascot followed, in order of decreasing concentration, by Barrack St, Freshwater Bay then Helena (Table 3).

The mean otolith ^{208}Pb concentrations followed the same pattern as the ^{208}Pb concentrations found in the sediment at these study sites (Table 3).

3.3. Spatial variability of metals within otolith

The metals ^{208}Pb and ^{57}Fe, which showed significant differences between sites and possibly linked to contamination, were used for further investigation into the variation across the otolith to include the lifespan of each fish. LA-ICP-MS revealed significant spatial differences in metal concentrations within individual otoliths. Metal profiles for ^{208}Pb and ^{57}Fe were related to age for a total of 37 fish from the four sites (~9 per site). Profiles for two fish from each site demonstrating the typical variation in metal concentration with fish age are shown in Figure 6. Fish sampled from Helena, Ascot and Barrack St showed significant variability in ^{208}Pb and ^{57}Fe concentrations across each otolith ($p < 0.05$) (Figure 6a, b and c). There were no differences in ^{208}Pb and ^{57}Fe concentrations across the otoliths from Freshwater Bay fish ($P > 0.05$) (Figure 6d).

4. Discussion

Site-specific differences in the estuary were found for the trace metal concentrations in black bream otoliths from this study. Strontium concentrations in the marginal region of the otoliths were consistent with salinity measurements, with otolith ^{88}Sr concentrations increasing with higher salinity at downstream sites (Table 2 and Figure 5a). The estuary is highly seasonal with increased freshwater discharge during the winter months (Spencer & Imberger, 1956). The freshwater inflows (< 4 ppt) are relatively large compared to the tidal volume and as a consequence flush out most of the saline and brackish waters from the upper and middle estuary (Thomas et al., 2001). In contrast, the lower estuary is well mixed with intruding marine water driven by tides and barometric pressures (Twomey & John, 2001). Salinity was particularly high throughout the lower estuary, an indication that the lower rainfall, reported for the winter of 2002, was well below the average (BOM, 2002). Evidence of this was shown in the otoliths of fish collected from Freshwater Bay. The outer edges of the fish otoliths, representing approximately 2 to 3 months of life, had significantly higher ^{88}Sr concentrations relative to the upper estuary sites. Measuring Sr concentrations in otoliths as a proxy for salinity has been well documented (Mugiya & Tanaka, 1995; Secor et al., 1995). Although ^{88}Sr is not considered a trace metal used for the purpose of pollution studies, it does suggest that fish are resident at the site where they were collected, at least in the short-term (i.e. months).

The elevated levels of ^{208}Pb and ^{57}Fe found in the otoliths did not follow the pattern of ^{88}Sr, suggesting the variations were not influenced by salinity. However, the bioavailability of certain metals in solution decreases with increasing salinity (Rainbow, 1997a) and may have contributed to the lower concentrations of ^{208}Pb and ^{57}Fe in the fish collected at the most downstream site. The pH levels were similar between sites at the time of sampling, therefore it is unlikely any of the measured physicochemical factors influenced the uptake of these metals. Köck et al. (1996) also found that the Pb content of otoliths from arctic char (*Salvelinus alpinus*) did not correlate with pH and salinity and suggests otoliths may serve as sensitive indicators of Pb contamination in fish. Geffen et al. (1998) showed through laboratory experiments that the concentration of Pb in the otoliths of sandy goby (*Pomatoschistus minutus*) was related to that in the water.

Fish collected from the upstream reference site, Helena showed the lowest metal concentrations in their otoliths. This may be explained by the minimal human activity in the surrounding area, most of which is rural land. High ^{208}Pb concentrations in the otoliths of fish collected from Ascot is most likely associated with anthropogenic sources. The Bayswater Main Drain discharging at Ascot, is the largest urban catchment in the Perth metropolitan region with some reported Pb excesses (DOE, 2003). The Western Australian Department of Environment (2003) found Pb and Fe concentrations at several sites in the Bayswater drains exceeded acceptable environmental guideline levels. Rate et al. (2000) found high Pb concentrations in sediments sampled adjacent to stormwater drain outfalls, suggesting that Pb transported by stormwater was entering the estuary. This site is also in close proximity to a major industrial area. The sources of ^{208}Pb at Barrack Street and Freshwater Bay may include traffic emissions and subsequent runoff from roads. Particulate Pb-oxides from combusted fuel is likely to be a major source of Pb from road runoff (Gerritse et al., 1998). Additionally, the lower winter rainfall in 2002 and subsequent reduced river flow suggests that metal contaminants from runoff and stormwater drainage may have remained in the estuary for a longer period of time. Trace metal analysis of sediment samples collected from these study sites in 2001 found elevated Pb, in decreasing concentrations, Ascot (0.29 mg/kg dry), Barrack Street (0.17 mg/kg dry), Freshwater Bay (0.06 mg/kg dry) and Helena (0.04 mg/kg dry) (Webb, 2005). The mean otolith Pb concentrations in the fish collected at each of these sites show the same pattern of Pb distribution (Figure 4). This further suggests the fish reflected the trace metal history of their surrounding environment.

Sediment type may have contributed to the reduced ^{208}Pb and ^{57}Fe content moving downstream from Ascot to Freshwater Bay. Moore et al. (1989) found that finer textured sediments have a tendency to retain higher metal content consequently increasing bioavailability. Similarly, the finer textured sediments with potentially higher bioavailable metals at the upstream sites of the Swan River Estuary corresponded to higher otolith metal content. In contrast, the lower otolith metal concentrations in the black bream may be related to the low metal retention of the coarse sandy sediment of the downstream sites.

The elevated ^{57}Fe concentrations in the otoliths of Ascot fish may reflect proximity to an industrial area. Iron is an essential metal for teleost fish, being an integral component of proteins involved in cellular respiration and oxygen transfer (Bury & Grosell, 2003). However, ^{57}Fe was found to be significantly higher in fish from Ascot relative to the other sites, suggesting the sources are likely to be anthropogenic rather than natural and suggests that the fish were unable to maintain Fe homeostasis. The temperature of aquatic environments has been shown to influence the accumulation rates of Fe and other metals into the otoliths (Gauldie et al., 1980; Gauldie et al., 1986; Townsend et al., 1992). A study by

Thomas et al. (2001) of water quality in the Swan-Canning system over a 4.5 year period found the pattern of seasonal variation in water temperature was similar for the lower, middle and upper estuary sites. In this study, water temperature was relatively similar between sites and therefore unlikely to have contributed to the high Fe levels found in the Ascot fish. It is well known that the availability of many metals increases as pH decreases (Heit et al., 1989; Spry & Wiener, 1991). The pH was fractionally lower at Ascot relative to the other sites and may have contributed to the increased bioavailability of Fe. The results further support the hypothesis that fish occupying different regions of the estuary contain otoliths with differing exposure histories possibly relating to metal contamination.

Variables such as age, size and weight may influence the trace metal content in fish otoliths. However, the influence of these factors was explored and found not to have contributed to the differences observed in otolith metal concentrations. Papadopoulou et al. (1980) found decreases in elemental content with increasing age suggesting it may be due to compositional changes in otoliths, decreased intake of metals in the diet of older fish, or dilution by growth. Increases may be the result of faster metabolism and higher growth rates in younger fish and thus an enhanced uptake of elements which can be incorporated into the otolith (Geffen et al., 2003).

The spatial distribution of ^{208}Pb and ^{57}Fe varied among growth annuli across otoliths. This variability may be related to the movement of fish in and out of areas contaminated by metals or to the changes in pollution to the local environment inhabited by the fish. Patterns in otolith metal concentrations among Helena, Ascot and Barrack St fish were not consistent between years. Conversely, the fish collected from Freshwater Bay showed a similar pattern with no significant difference in the distribution of ^{208}Pb and ^{57}Fe across the otoliths. Although the focus of this paper is not on the movement patterns of fish within the estuary, the results suggest the fish may have remained in the lower part of the estuary throughout their life and not moved into upstream regions where greater contamination exists. This supports the work by Sarre (1999), where black bream were found to have limited movement within the lower estuary. Of all the sites Freshwater Bay is closest to the mouth of the estuary which opens onto the Indian Ocean. Hence, tidal activity causing the ocean water to flow into the estuary may flush out the water-bound contaminates daily. The higher sand content of the sediment in the lower estuary may also contribute to low metal content retention in the substrate and consequently the low bioavailability of metals to fish is reflected in their otoliths. Given both metals often followed similar patterns within the otoliths, measured concentrations are more likely to be related to a fish’s environmental history rather than physiological processes. However, it cannot be completely ruled out that the physiological processes of uptake, accumulation and elimination (Rainbow, 1997b) may have contributed to the differences observed across each otolith over the fish’s lifetime. Additionally, the importance of physicochemical parameters in controlling the availability of the free metal ions, and hence the bioavailability of dissolved trace metals (Phillips & Rainbow, 1994) should not be ignored and may also be a contributing factor in the differences observed. This is especially relevant in Freshwater Bay where higher salinity was measured relative to the other sites, possibility contributing to a decrease in the bioavailability of metals to the fish (Rainbow 1997a).

5. Conclusions

The use of LA-ICP-MS provides precise positional information with the potential for chronological studies of otoliths and the history of the environment that the fish have lived in. Otoliths coupled with this analytical approach appear to be useful indicators of exposure to metals and in obtaining a coarse historic picture of past exposure of fish to contamination. The findings also suggest that ^{208}Pb and ^{57}Fe can be used to provide information on the bioavailability of these metals throughout the life of the fish. The use of a sedentary fish species would serve better to represent a historical record of pollution changes to the local habitat.

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FIGURES LEGENDS

Figure 1 Location of fish collection sites in the Swan River Estuary (adapted from Swan River Trust, 1998).

Figure 2 Transverse section of black bream otolith showing growth annuli and laser transect.

Figure 3 An example of the data handling to generate age-related mean values for each metal, based on data from LA-ICP-MS analysis of transverse sections of black bream otoliths. (**a**) ^{208}Pb concentration in transect across otolith of individual fish from Ascot site, approximate position of growth annuli shown (**b**) mean ^{208}Pb values calculated for each opaque growth zone.

Figure 4 Concentration (ppm relative to Ca) (mean ±SE) of the metals measured in the marginal regions of black bream otoliths which did not differ significantly between sites. Middle horizontal line and vertical span of each diamond represents sample mean and 95% confidence interval respectively. Overlap marks are drawn above and below the group mean.

Figure 5 Mean metal values (±SE) in the marginal regions of black bream otoliths, sampled at four sites along the Swan River Estuary. Middle horizontal line and vertical span of each diamond represents sample mean and 95% confidence interval respectively. Overlap marks are drawn above and below the group mean. Significant differences are between non-intersecting circles (Tukey-Kramer test, $P < 0.05$). (**a**) ^{88}Sr (**b**) ^{208}Pb (**c**) ^{57}Fe.

Figure 6 ^{208}Pb and ^{57}Fe concentration (ppm relative to Ca) profiles for two fish from each site within the Swan River Estuary, demonstrating the typically observed variation in metal concentration with fish age.

Table 1 Operating parameters of the LA-ICP-MS used in this study.

ICP- MS	VG Elemental PQ III
Acquisition mode	Time resolved
Coolant gas flow rate	14 L.min^{-1}
Auxiliary gas flow rate	0.95 L.min^{-1}
Nebuliser gas flow rate (Ar)	1 L.min^{-1}
RF power	1150 W
Dwell time	20 milli-sec
Laser	CETAC LSX-200 Plus Laser Ablation
Laser type	Nd:YAG laser
Wavelength	266 nm
Energy level	65%
Repetition rate	10 Hz
Spot size	50µm

Table 2 Water parameters measured at the time of fish collection. [a]

Site	Temperature (°C)	Salinity (ppt)	pH
Helena	16	2.8	8.0
Ascot	18	3.9	7.7
Barrack St	18	9.1	7.9
Freshwater Bay	17	21.6	8.1

[a] Water collected at a depth ~2 m in September 2002

Table 3 Mean ^{208}Pb and ^{57}Fe concentrations (ppm) measured in the marginal (edge) regions of black bream otoliths, sampled at four sites along the Swan River Estuary [a].

Site	n	^{138}Pb (ppm) (Mean ± SE)	^{57}Fe (ppm) (Mean ± SE)	Pb (mg/kg dry) in sediment [a]
Helena	10	0.04 ± 0.01	11.26 ± 2.29	0.04
Ascot	8	0.38 ± 0.03	102.02 ± 7.65	0.29
Barrack Street	9	0.21 ± 0.03	40.49 ± 3.72	0.17
Freshwater Bay	10	0.14 ± 0.02	21.06 ± 2.52	0.06

[a] Lead concentrations in sediment collected at the study sites in 2001. Sediment results extracted from Webb (2005).