Title: Trace metal incorporation in otoliths of pink snapper (Pagrus auratus Forster) as an environmental monitor

Authors:
Melinda Marie Ranaldi [1], Marthe Monique Gagnon [1, 2]

Address:
[1] Department of Environmental and Aquatic Sciences, Curtin University of Technology, GPO Box U1987, Perth, Western Australia 6845

[2] **Corresponding Author:**
Associate Professor Marthe Monique Gagnon
Department of Environmental and Aquatic Sciences,
Curtin University
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

Otolith metal concentrations may be related to the environmental exposure history of fish to contamination. Otoliths of pink snapper (Pagrus auratus) collected from the marine basin of Cockburn Sound and offshore near Rottnest Island were analysed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to measure the concentrations of 11 trace metals. The following metals were investigated using their respective isotopes: aluminum (^{27}Al), calcium (^{44}Ca), manganese (^{55}Mn), iron (^{57}Fe), copper (^{65}Cu), zinc (^{66}Zn), strontium (^{88}Sr), cadmium (^{111}Cd), barium (^{138}Ba), mercury (^{202}Hg) and lead (^{208}Pb). Significant differences in otolith metal concentrations were found between the sampling locations for Zn, Cd and Pb. These metals were significantly higher in the otolith edges of the pink snapper captured from the extensive industrial area bordering Cockburn Sound. Life history transects of Zn, Cd and Pb within otoliths of pink snapper sampled from Cockburn Sound typically showed temporal trends that may correspond to the movement of this fish species in and out of this contaminated area during the yearly spawning season.

Keywords: Cadmium, LA-ICP-MS, Lead, Otolith, Pink snapper, Zinc

1. Introduction

Cockburn Sound is an elongate, shallow, partially enclosed marine basin with an area of approximately 124 km^2, located immediately south of Fremantle, Western Australia. This relatively low energy coastal water body has contrasting shoreline environments with heavy industry along much of the eastern coast whereas the shoreline of Garden Island, located on the west side of the sound, is largely undeveloped. Extensive industrial development in Cockburn Sound began in 1954 with the construction of an oil refinery on its eastern shore. Subsequent development has been rapid and the area now includes iron, steel, alumina and nickel refineries, chemical and fertilizer plants, a wastewater treatment plant, electricity station and a bulk grain terminal (Department of Environment, 2005). Heavy metals are discharged into Cockburn Sound from a number of industries situated on the eastern boundary.

Pink snapper (Pagrus auratus Forster) is a commercially and recreationally important marine species throughout Southern Australia (Kailola et al., 1993). The semi-enclosed embayment that is Cockburn Sound is a significant spawning and nursery area for pink snapper where they aggregate and spawn for 3 to 5 months every year. There is limited knowledge of the historic and recent metal exposure to fish in this area.

Aquatic organisms accumulate trace metals from the environment (Phillips and Rainbow, 2008). Studies of metal contamination in fish have focused on metal accumulation in soft tissues, such as liver, kidney, gill, intestine and/or muscle. Liver, kidney and gill have efficient mechanisms for the elimination of potentially toxic metals (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. Therefore, metal analysis of soft tissue of fish requires continuous sampling to assess the ongoing status of an aquatic environment.

Otoliths are calcified structures located in the inner ears of teleost fish and may offer a permanent record of metal exposure. Otoliths grow continually throughout the lifespan of the fish and are formed by the deposition of calcium carbonate, primarily aragonite within a protein matrix. During formation, trace metals are incorporated into organic or inorganic portion of the otolith matrix (Campana 1999). The concentrations of these trace metals are thought to be influenced primarily by the environmental conditions. Bath et al. (2000) conducted experiments with the spot Leiostomus xanthurus that demonstrated a positive relationship between the Sr:Ca and Ba:Ca ratios in the otolith and the water. For other trace metals such as Zn, the primary mode of uptake and incorporation into otoliths appears to be via dietary exposure (Ranaldi and Gagnon, 2008a). Otoliths have the potential to act as a historic recorder of past exposure of fish to metal contamination. To date numerous studies have used chemical analyses of otoliths for stock discrimination (Edmonds et al. , 1991; Campana and Gagne, 1995; Milton et al., 2008), movement studies (Dove et al. , 1996; Gillanders and Kingsford, 1996; Hamer et al. , 2006, Milton et al., 2008) and to a lesser extent, as an indicator of environmental pollution (Ranaldi and Gagnon, 2008b; Saquet et al. , 2002).

The objective of this present study was to investigate whether fish collected from the industrial area of Cockburn Sound exhibited elevated otolith metal concentrations when compared to a reference population of the same species. The marginal region (edge) of the otolith was isolated for site comparison, permitting the examination of potential metal contamination exposure at the time of capture. In addition, temporal trends of metal incorporation across the otoliths (entire lifespan) were examined in an attempt to document the movement patterns of this fish species in and out of this industrial area as a monitor of exposure to past pollution.

2. Materials and Methods

2.1 Fish collection

A total of 33 adult pink snapper were examined for trace metals in their otoliths. Nineteen fish were captured in November 2002 by commercial fishermen from Cockburn Sound and 14 reference fish were collected offshore near Rottnest Island in Western Australia (Fig. 1).

2.2 Water quality sampling and analysis

Water samples were collected using a 1 L Niskin bottle approximately 2 m below the surface. A sub-sample was taken and subsequently filtered on site through a 0.45 µm filter and stored in a pre-acidified bottle containing concentrated HNO_3 to a pH of 2. This fraction was referred to as the dissolved metal concentration. Water samples were analysed for Al, Ca, Mn, Fe, Cu, Zn, Sr, Cd, Ba, Hg and Pb using inductively coupled plasma mass spectrometry (ICP-MS).

Temperature, dissolved oxygen, pH and salinity were measured using a YSI 6920 multi-parameter water quality probe (YSI, Yellow Springs, OH, USA).

2.3 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.

Otolith sections were handled only with nylon forceps to avoid potential metal contamination. All plastic, including forceps, and glassware used during the preparation of the otoliths, were acid-washed using 5% HNO_3 for 24hrs, rinsed once with deionised water then twice rinsed in Milli-Q water. Multiple otolith sections from different fish (5 per slide) randomly selected amongst geographic sites were mounted with super-glue (methyl methacrylate) onto labelled microscope slides. When glue was applied to the resin, extreme care was taken to avoid contact with the upper surface of the otolith. Mounted samples were then ultrasonicated in Milli-Q water, wiped with 5% HNO_3 and dried prior to analysis to minimise surface contamination.

2.4 Laser ablation ICP-MS analysis

Otolith sections were analysed using a CETAC LSX-200 plus Laser Ablation (LA) System coupled to a VG Elemental – Plasma Quad III inductively coupled plasma mass spectrometry (ICP-MS) with data acquisition by PQ Vision Version 4.3.

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 software 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 (Fig. 2). Transects were made along the same axis in all fish.

To assess the accuracy of the LA-ICP-MS instrument, calibration was achieved using NIST glass standard 610 (National Institute of Standards and Technology, Gaitherbury, 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 was pulsed at a repetition rate of 10 Hz and a laser energy of 0.65 to 0.85 mJ. These laser parameters resulted in a trench depth of ~15 µm. Transects of ablations were made across the otolith surface at a rate of 5 µm sec^{-1}. The ICP-MS continuously scanned for the selected isotopes during the course of each laser transect across the proximal margin. Otoliths were analysed for each of the following metals using their respective isotopes: ^{27}Al, ^{44}Ca, ^{55}Mn, ^{57}Fe, ^{65}Cu, ^{66}Zn, ^{88}Sr, ^{111}Cd, ^{138}Ba, ^{202}Hg and ^{208}Pb. Isotopic counts (counts per second) were converted into element concentrations through the use of an external standard (NIST 610) and an internal standard (^{44}Ca) measured and known both in the standard and the otoliths. The limit of detection (µg g^{-1}) achieved for each metal was based on three standard deviations of the blank gas and adjusted for ablation yield and are 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 0.01, ^{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.

2.5 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 elements 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.

2.6 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 pink snapper otoliths relative to the ablation transect. Fish aging techniques were used to determine the age at specific annuli (translucent 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 translucent 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 (Fig. 3a). For each otolith an annual mean was calculated from the series of metal readings that corresponded to each translucent zone (Fig. 3b). The outermost ~60 to 100 µm of otolith growth, representing approximately 3 to 4 months of the fish's life prior to capture, was used for site comparisons (REFERENCE).

2.7 Statistical Analysis

Analysis of the otolith edges allowed extraction of information on the recently inhabited environment. Therefore, comparison between sites was more reliable as pink snapper are mobile fish, 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), age, sex and reproductive stage on the metal concentrations in the otolith as these factors may confound the interpretation of the results.

For the comparison of temporal trends of metals across each otolith a mean value corresponding to each translucent 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 summer season during the fish's life. Given that pink snapper are known to aggregate and spawn during late spring/early summer in Cockburn Sound the translucent zone corresponding to this time was isolated for comparison of temporal metal trends.

Statistical analyses were performed using JMP® 5 statistical package (SAS Institute Inc. 2002). Significant differences between sites for dissolved metal concentrations were tested by t-test of the least square means. A multivariate analysis of variance (MANOVA) model was used to examine the relative differences in the otolith metal concentrations (otolith edge only), fish length, age, reproductive stage and sex. Where the residuals were found to be heteroscedastic, subsequent analyses were done on log-transformed data ($\log_{10}$ or $\log_{10}(1+x)$). Differences in otolith metal concentrations were evaluated by analysis of covariance (ANCOVA), using fish length as a covariate to control for differences in fish size between sites.

3. Results

3.1 Water quality

Physicochemical parameters were similar between sampling locations at the time of sampling (Table 1). The majority of dissolved metal concentrations were below the limit of detection (LOD). Those metals measured above the LOD were similar between the sampling sites apart from Zn and Cd. Mean dissolved Zn, Cd and Pb concentrations were significantly higher at the Cockburn Sound than the offshore location (t-test comparisons: Zn: $t = 5.376$, $P < 0.05$; Cd: $t = 5.157$, $P < 0.05$; Pb: $t = 5.842$, $P < 0.05$) (Table 1).

3.2 Site-specific differences (marginal region of otolith)

The age and total length of fish ranged from 3 to 8 years and 408 to 800 mm respectively. There was a significant difference in fish length between sampling sites (t-test comparisons: $t = 19.448$, $P < 0.0001$). The mean total lengths of fish collected from Cockburn Sound and offshore near Rottnest Island were 740.4 ± 9.02 and 470.9 ± 10.51 respectively. Fish aging techniques revealed that the majority of fish collected from Cockburn Sound were approximately 5 to 8 years old while the fish collected offshore were approximately 3 to 5 years old.

Fish length, sex, and reproduction stage were included in the MANOVA model for the 11 metals analysed (Al, Ca, Mn, Fe, Cu, Zn, Sr, Cd, Ba, Hg and Pb). Sex and reproductive stage were not significant factors ($P > 0.05$) in explaining differences in the metal concentrations and subsequently excluded from the model. Metal concentrations in the marginal (edge) regions of the otoliths were significantly different between the sampling locations (MANOVA; Pillai's trace = 2.682, approx. $F = 1.691$, $P < 0.01$). Analysis of covariance (ANCOVA) was used to test for site and fish size effects on otolith trace metal concentrations. After controlling for differences in fish length, the concentrations of ^{66}Zn, ^{111}Cd and ^{208}Pb in pink snapper otoliths differed between the two sampling sites (Table 2). There were significantly higher concentrations of ^{66}Zn, ^{111}Cd and ^{208}Pb in the marginal (edge) regions of the otoliths of fish from Cockburn Sound than those collected offshore (Fig. 4). The remaining 8 trace metals analysed showed no significant site-specific differences in the marginal regions of the otoliths (ANCOVA, $P > 0.05$).

3.3 Life history transect of metals within otoliths

The metals ^{66}Zn, ^{111}Cd and ^{208}Pb, which showed significant differences in concentrations between sites and may be 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 analyses showed temporal trends in the trace metal concentrations within the otoliths of the pink snapper collected from both Cockburn Sound and offshore. The typical temporal trend of the metal profiles for the fish collected from Cockburn Sound showed elevated metal concentrations in the core region of the otolith, and thereafter decreased gradually followed by a peak toward the edge of the otolith corresponding to the later years of life i.e. from 5 years old (Fig. 5) when the adult fish migrates to the Sound to reproduce. In contrast, the typical temporal trend in the offshore fish showed elevated metal concentrations in the core region of the otoliths only with a decrease toward the edge of the otolith (Fig. 5).

4. Discussion

Differences in the concentration of trace metals in the otoliths of pink snapper were found between the sampling locations of Cockburn Sound and offshore near Rottnest Island. Fish caught in Cockburn Sound showed higher concentrations of Zn, Cd and Pb in their otolith edges than the offshore fish. These results were consistent with the water quality measurements for Zn, Cd and Pb with higher dissolved concentrations in nearshore Cockburn Sound waters relative to the offshore waters. In Cockburn Sound, both Zn and Cd exceeded the ANZECC and ARMCANZ (2000) guideline trigger values of 0.015 mg L^{-1} and 0.0055 mg L^{-1} respectively for the 95% protection level. However, the dissolved Pb concentrations measured in Cockburn Sound did not exceed the trigger guideline value of 0.0044 mg L^{-1} for the 95% protection level. The pattern seen, from both the biological monitors and water samples may be explained by the industrial development in Cockburn Sound. Large quantities of metal contaminants have been discharged into Cockburn Sound from heavy industry on the eastern shoreline. These discharges impact the water quality and can settle out of the water column or bind with a fraction of surficial sediments. Tablot and Chegwidden (1983) examined the build up of several heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in sediments within Cockburn Sound. The contamination was found to be most likely related to industrial discharge from Woodman Point (metropolitan sewage discharge) and James Point (steel mill, oil refinery and superphosphate plant). Subsequent sediment studies in 1994 (Department of Environmental Protection, 1996) found that metal concentrations had decreased significantly however there was Pb contamination near areas of shipping and widespread

Zn contamination throughout the sound (DAL, 2001). Skene et al. (2005) found elevated sediment Zn concentrations at several sites within Cockburn Sound suggesting that fine contaminated sediment from industrial discharge has accumulated offshore. Cd contamination has been linked to a fertiliser plant in the past (Rosman et al., 1980) and may still be a source of contamination.

The accumulation of trace metals in fish otoliths depends on a number of factors, including the trace metal concentration in the water environment, bioavailability, the physiological state of the individual fish, and the affinity of the calcium carbonate otolith for different metals. The behaviour of different metals is complex and the mechanisms for uptake and incorporation differ substantially between metals. The elevated Zn concentrations in the otoliths of pink snapper collected from Cockburn Sound although correlated to the dissolved Zn concentrations may in fact be related to dietary Zn exposure. A recent laboratory experiment conducted by Ranaldi and Gagnon (2008a) demonstrated that juvenile pink snapper accumulate Zn in their otoliths predominantly via dietary exposure rather than through waterborne exposure. Therefore, the measured otolith Zn concentrations in the Cockburn Sound pink snapper may be indicative of the dietary Zn bioaccumulation in this environment. Prey species such as benthic invertebrates, an important food source for this fish species, may strengthen the food-chain effect (Dallinger et al., 1997) contributing to the higher Zn concentrations found in the edges of the otoliths from the Cockburn Sound pink snapper.

In contrast, the elevated Cd concentrations found in the edges of the Cockburn Sound pink snapper otoliths are likely to be related to both waterborne and dietary Cd exposure. Ranaldi and Gagnon (2009) demonstrated that both dietary and waterborne Cd are important uptake routes for Cd incorporation in the otoliths of this marine fish species.

The higher Pb concentrations in the otoliths of the Cockburn Sound fish relative to the offshore fish may reflect proximity to the industrial area. 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. In addition, field studies by both Ranaldi and Gagnon (2008b) and Köck et al. (1996) found elevated otolith Pb concentrations in black bream (Acanthopagrus butcheri) and arctic char (Salvelinus alpinus) respectively. Therefore, Pb may serve as a sensitive monitor of environmental contamination in fish otoliths.

It would appear that non-essential metals, such as Cd and Pb represent good markers of dietary/waterborne exposure while essential metals, such as Zn, may be predominantly related to the dietary uptake route. Therefore, the exposure history should be taken into consideration when interpreting trace metal incorporation in the otolith of wild fish and their subsequent susceptibility to metal pollution.

The life history transects of Cd, Zn and Pb showed a typical temporal trend across the otoliths of pink snapper captured from Cockburn Sound. The fluctuation patterns in the otolith metal concentrations along the life history transect appear to be consistent with what is known about the spawning behaviour of the pink snapper and the distribution of contaminants in the Sound. Cockburn Sound is the site of the largest known aggregations of pink snapper in the West Coast Bioregion and is thought to be critical to the maintenance of adequate breeding stocks of this long-lived and slow growing fish (Department of Fisheries, 2009). Spawning in Cockburn Sound can extend over a 3 to 5 month period during late spring to early summer (October to February) (Department of Fisheries, 2009). In adult fish, this reproductive period appears to be represented by the elevated metal concentrations in the translucent regions of the growth annuli corresponding to the summer period of growth which coincide with the spawning period of this species in Cockburn Sound. The elevated metal concentrations in the core region of the Cockburn Sound pink snapper may represent the time spent in the sound by the juvenile fish as this area is an important nursery ground for this fish species. The gradual decrease in the otolith metal concentrations during the early years of life may represent the movement of the fish out of the contaminated area to pristine offshore waters. The elevated metal concentrations toward the edge of the otoliths during the later part of life may represent the movement of the fish into the sound during the spawning months.

The life history transects of Cd, Zn and Pb among the growth annuli across the otoliths of the fish collected offshore near Rottnest Island did not exhibit the same pattern of temporal variability. Typically only the core region of the otoliths showed elevated concentrations of these metals. This may be explained by the age difference between the groups of fish captured. The offshore fish were significantly smaller than those sampled in Cockburn Sound. Pink snapper take four to five years to reach maturity and in the lower west coast region the fish are likely to be 50 to 70 cm in length. By the time the fish reach about six to seven years old, over 90% will have spawned at least once (Department of Fisheries, 2009). Therefore, these offshore fish may not have reached full maturity and therefore unlikely to have migrated back to Cockburn Sound to spawn during their life span.

The elevated otolith metal concentrations for Zn, Cd and Pb typically followed similar temporal patterns across the otolith transect suggesting that the differences measured are more likely related to the fish’s environmental history rather than physiological processes. However, it can not be ruled out that the physiological changes during development may have contributed to the changes in the concentrations of these metals across the otoliths of the individual fish.

5. Conclusions

The use of LA-ICP-MS provides precise positional information with the potential to aid in the accurate reconstruction of the environment inhabited by the fish. Otoliths hold the potential to monitor past contamination exposure particularly for non-essential metals such as Cd and Pb which for pink snapper are incorporated into the otolith through both waterborne and dietary exposure. Monitoring changes in otolith metal concentrations through the life history of the fish may not only provide information on contamination exposure but may also provide important information on movement patterns of recreationally and commercially significant fish species.

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Table 1. Summary of water quality results for Cockburn Sound and offshore near Rottnest Island. [a]

Parameter	Cockburn Sound (n = 6)	Offshore (n = 6)
Temperature (°C)	22.2 ± 0.13	22.0 ± 0.08
Dissolved oxygen (mg/L)	8.9 ± 0.8	9.0 ± 0.6
pH	7.5 ± 0.1	7.8 ± 0.3
Salinity	35.6 ± 0.02	35.6 ± 0.08
Al (mg $L^{-1)}$	0.02 ± 0.003	0.02 ± 0.006
Ca	438.3 ± 14.47	440 ± 9.3
Mn	<0.001	<0.001
Fe	<0.005	<0.005
Cu	<0.005	<0.005
Zn	0.02 ± 0.002	<0.005
Sr	8.46 ± 0.07	8.52 ± 0.15
Cd	0.01 ± 0.002	<0.002
Ba	0.01 ± 0.0003	0.01 ± 0.001
Hg	<0.0001	<0.0001
Pb	0.002 ± 0.0001	<0.001

[a] Data are reported as mean and standard error.

Table 2. Comparison of metal concentrations in the otolith edges of pink snapper collected in Cockburn Sound and offshore near Rottnest Island.[a]

Trace Metal	Site effect	Length effect	Length-Site interaction
Zn	$F_{1,33} = 30.05$, $p < 0.0001$	$F_{1,33} = 2.90$, $p = 0.09$	$F_{1,33} = 0.116$, $p = 0.736$
Cd	$F_{1,33} = 278.25$, $p < 0.0001$	$F_{1,33} = 0.364$, $p = 0.551$	$F_{1,33} = 2.412$, $p = 0.131$
Pb	$F_{1,33} = 8.96$, $p < 0.05$	$F_{1,33} = 2.95$, $p = 0.096$	$F_{1,33} = 0.358$, $p = 0.554$

[a] F values are given for ANCOVA tests of site effects, length effects and length and site interaction effects.

Figure Captions

Fig. 1. Map of Cockburn Sound and offshore sampling location near Rottnest Island.

Fig. 2. Transverse section of pink snapper otolith showing growth annuli and laser transect.

Fig. 3. An example of data handling to generate age-related mean values for each metal, based on output data from LA-ICP-MS analysis of the transverse sections of pink snapper otoliths. a) Pb concentrations across the otolith of an individual fish collected from Cockburn Sound, approximate position of growth annuli shown; b) mean Pb values (± SE) calculated for each translucent growth zone.

Fig. 4. Mean Zn, Cd and Pb otolith concentrations (µg g^{-1}) (± SE) in the marginal region (edge) of the pink snapper otoliths for fish collected from Cockburn Sound and offshore.

Fig. 5. Mean metal profiles (Zn, Cd and Pb (µg g^{-1})) (± SE) for three fish from each sampling location (Cockburn Sound and offshore) demonstrating the typically observed temporal trend in metal concentration across the life history transect.

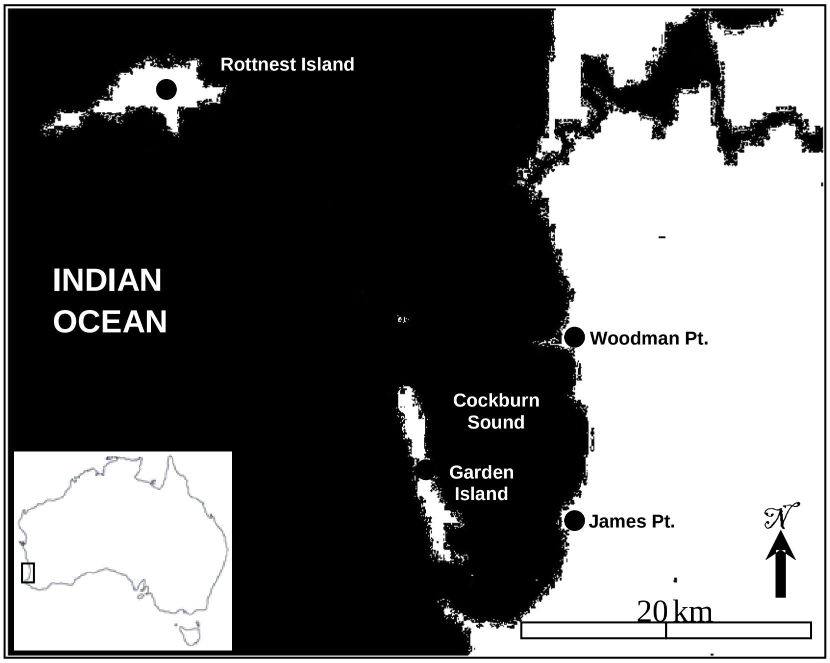

Rottnest Island
INDIAN OCEAN
Woodman Pt.
Cockburn Sound
Garden Island
James Pt.
𝒩
20 km

Fig. 1.

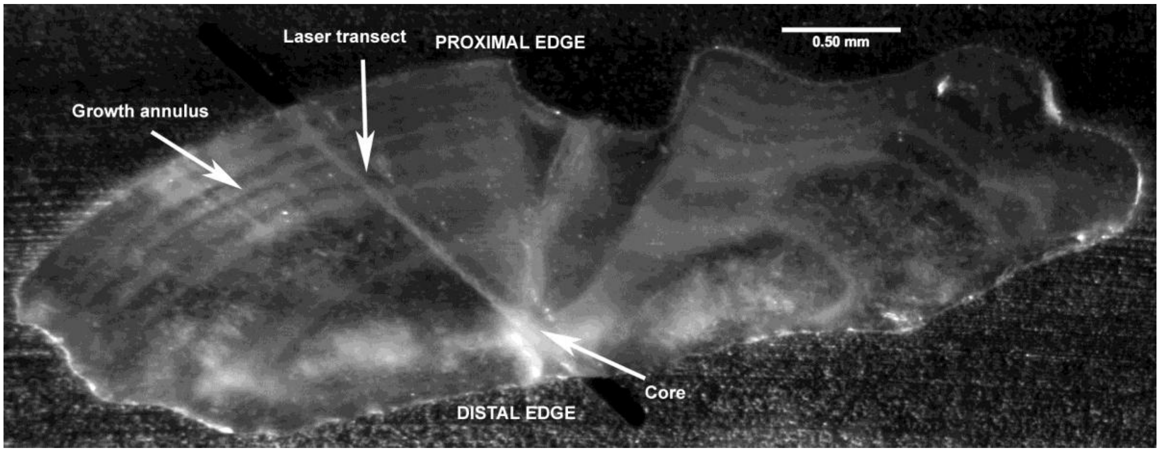

Laser transect
PROXIMAL EDGE
0.50 mm
Growth annulus
Core
DISTAL EDGE

Fig. 2.

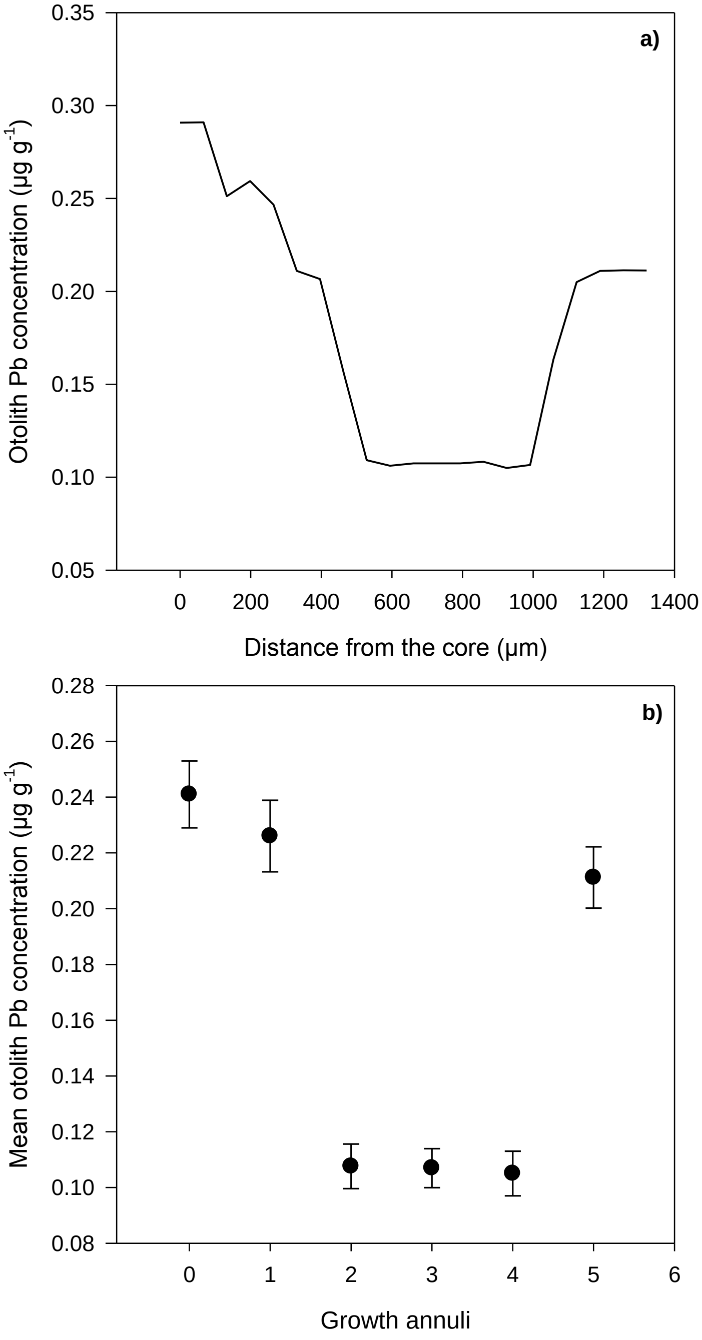

a)
Otolith Pb concentration (μg g^{-1})
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
200
400
600
800
1000
1200
1400
Distance from the core (μm)
b)
Mean otolith Pb concentration (μg g^{-1})
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0
1
2
3
4
5
6
Growth annuli

Fig. 3.

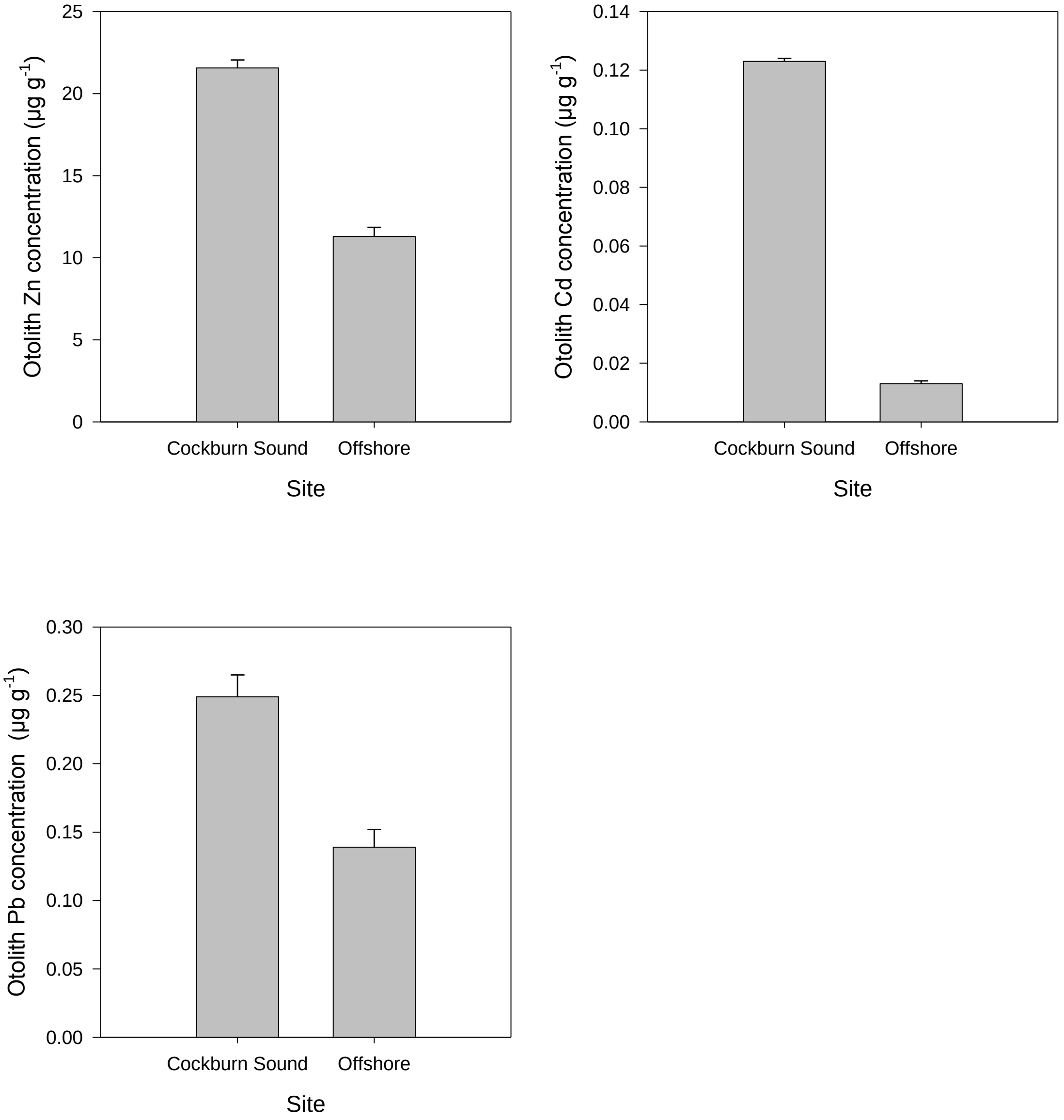
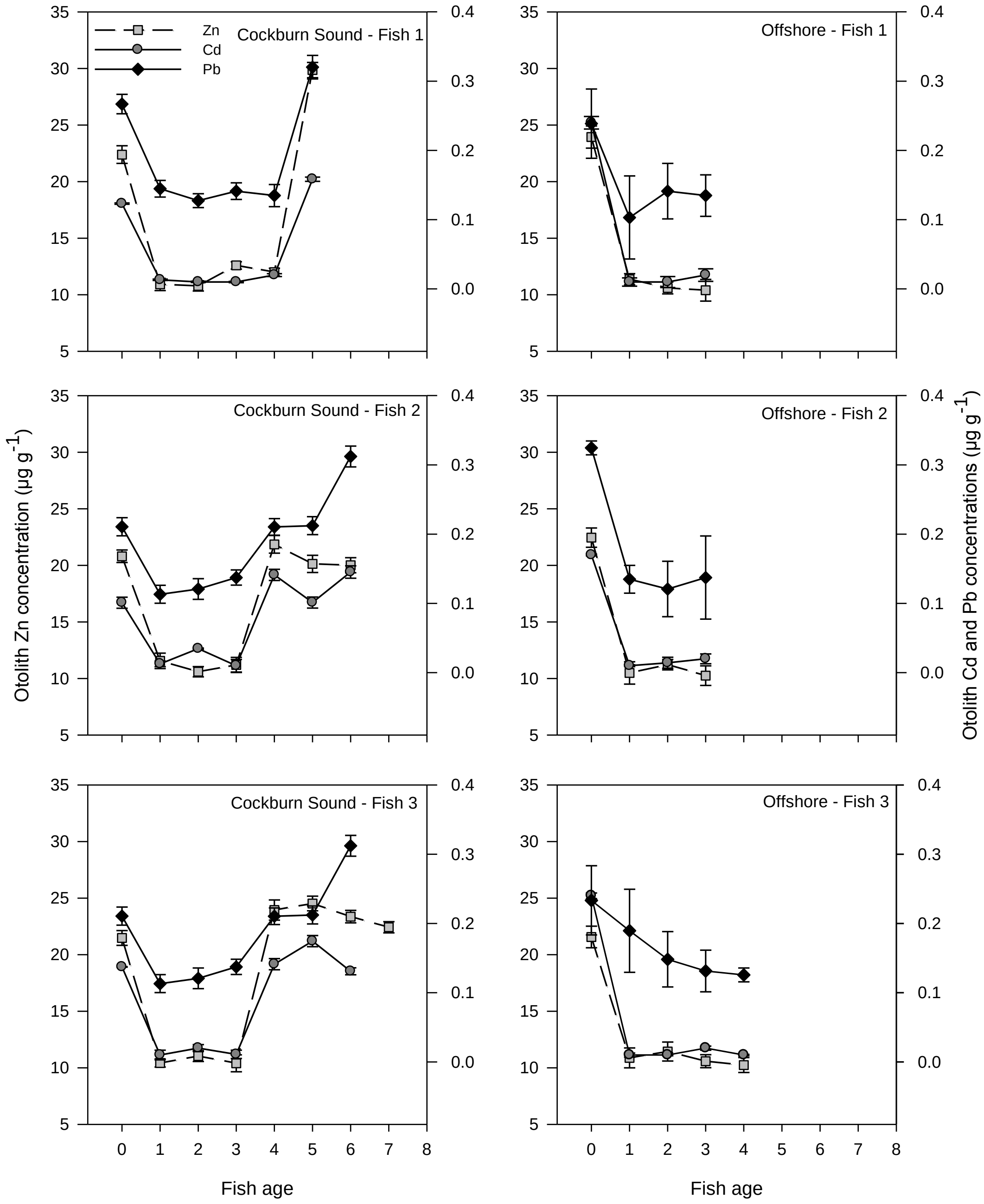

25
20
15
10
5
0
Otolith Zn concentration (µg g-1)
Cockburn Sound
Offshore
Site
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Otolith Cd concentration (µg g-1)
Cockburn Sound
Offshore
Site
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Otolith Pb concentration (µg g-1)
Cockburn Sound
Offshore
Site

Fig. 4.

581



582

583 Fig. 5.