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# Response of otolith chemistry of the congollis Psuedaphritis urvilli (Teleostei: Psuedaphritigae) to element and salinity manipulations

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## Abstract

Otoliths are frequently used to determine the movement history of many species of fish, sometimes without validation of the behaviour of elements in response to periodic changes in their chemical environment. This study investigates whether the chemical composition of the otoliths from congolli, a small diadromous fish, reflect changes in the ambient elemental composition of strontium and barium or salinity. - methods. To determine the effects of water chemistry, juveniles were exposed to three levels of salinity (1, 17-19 and 35-38 $g.L^{-1}$) and three levels of elemental concentrations (ambient or x1, x2 Sr & x4 Ba and x4 Sr, x8 Ba) in a cross-factorial experiment. Otoliths were analysed using Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS). Otolith Sr:Ca and Ba:Ca ratios did not reflect differences among salinity treatments. Otolith and ambient Ba:Ca ratios were positively correlated. Otolith Ba:Ca ratios differed between the high-spiking and medium- and low-spiking treatments, but not between the medium and low treatments. No relationship was apparent between otolith and ambient Sr:Ca ratios. Sensitivity of otoliths to ambient Ba:Ca, but not to Sr:Ca, has not been previously reported. This finding may reflect the comparatively low metabolic rate of congolli and changing osmoregulatory mechanisms in diadromous fish. This study highlights the need for further research into factors affecting element uptake, particularly in diadromous fish.

## Introduction

Migrational excursions are characteristic of diadromous fish, which move between fresh- and salt-water (and/or *vice versa*) on a seasonal basis, usually for breeding (e.g. McDowall 2001). The nature and timing of migrations, are often obscure, particularly in non-commercial species, but previous work (e.g. Secor et al., 1995) has shown that the otoliths may contain a record of the environmental history of individuals. Chemical analyses of otoliths have been used to reconstruct migration histories for a range of fish species (e.g. Elfman et al., 2000, Kafemann et al., 2000, Secor et al., 1995, Elsdon and Gillanders, 2005a).

Calcium, the major constituent of otoliths, is readily substituted by barium and strontium, which have similar chemical affinities (Bath et al., 2000). Many investigations of the otoliths of diadromous fish have focused on the Sr:Ca ratios and assumed that these are related to the environment, without first determining the relationship between ambient and otolith elemental concentrations ( e.g. Arai et al., 2003, Kotake et al., 2003). Relatively high concentrations of strontium (~8ppm) occur in sea water, whereas concentrations in fresh water are typically about 4.8 times less (Campana, 1999). There is, however, considerable variability, with some estuaries having higher concentrations than sea water (Kraus and Secor, 2004, Rieman et al., 1994). Barium, on the other hand, is positively correlated to fresh water (Hanor and Chan, 1977, Coffey et al., 1997), and high Ba:Ca ratios in otoliths are considered to be indicative of freshwater residency (Bath et al., 2000, Milton and Chenery, 2001, Elsdon and Gillanders, 2005a).

The congolli (also 'sandy' or 'tupong'), Pseudaphritis urvilli (Valenciennes, in Cuvier and Valenciennes, 1832), is a small (total length (TL) <350 mm) diadromous fish endemic to southern Australia. Little is known of its life history (Hortle, 1978). Congolli inhabit estuaries and the slow-flowing lower reaches of rivers, and may travel up to 70 km from the sea (Allen, 1989). It is a benthic 'ambush' predator, feeding on invertebrates and smaller fish. Hortle (1978) suggested that congolli exhibit characteristics of a catadromous species, living in fresh water but spawning at sea or in estuaries. Downstream migrations of females occur through May to July, possibly associated with increased river flow (Koehn and O'Connor, 1990). Hortle (1978) reported that juveniles occur in estuaries, and older fish occur at progressively greater distances upstream, in fresh water. These characteristics make congolli of particular interest when considering the application of otolith chemistry to diadromous fish movements. The aim of this study was to determine the effects of salinity and water elemental concentrations on otolith elemental concentrations of congolli to determine if otolith chemistry can be used as an indicator of movement in this species.

## Methodology

Juvenile congolli were collected from an area surrounding the Goolwa Barrage in the Coorong, South Australia, during September and November 2004. Three single-wing fyke nets were placed perpendicular to the shore and left for 24 hours. Fish were then removed, sorted and congolli within the 30-80 mm TL size class were retained and returned to the laboratory for the salinity and elemental concentration experiment.

The experiment was conducted in a controlled environment room at 20°C, with a 12:12 h light:dark regime. All fish were fed daily, with brine shrimp (Artemia salina) ensuring that growth occurred throughout the experimental period, and the influence of diet on otolith elemental concentration was consistent. The experiment was designed to establish whether there was a predictive relationship between otolith chemistry and elemental composition and ambient salinity in the environment. A cross-factorial design with three levels of salinity and three levels of elemental concentrations (thus, nine treatments) was setup. To distinguish 'old' from 'new' growth in the otoliths, congolli were pre-treated by immersing them in fresh water containing 40 ppm alizarin complexone ($C_{19}H_{15}NO_8$). Fish were held in one 60-L tank for marking, and immersed for 24 h. Fish were then randomly assigned to treatments. Those destined for the more saline treatments were acclimatised from the freshwater holding tanks to one of the two higher salinities, over a two-day period. Salt water was added throughout this period until the desired salinity was reached. Fish were held in 12-L tanks (with a lid to prevent loss of Sr and Ba via evaporation) at densities of three fish per tank. PVC pipes were placed in the tanks to provide "hiding" areas. Experimental treatments were replicated twice to allow for the possibility of tank effects, giving a total of 18 tanks and 54 fish. The experiment was conducted for 27 days, and at the conclusion, all fish were placed in an ice slurry.

Three salinities (1, 17-18 and 35-38 $g.L^{-1}$) were chosen to be representative of the possible environments transgressed and inhabited by congolli throughout their life cycle. The intermediate salinity was controlled through the addition of bore water to seawater giving the 17-18 $g.L^{-1}$ treatments. To maintain water quality, 3 L of the water in each tank was changed every 3 days with accumulated detritus siphoned away, and replaced with freshly spiked water. Throughout the experimental period, salinity and temperature were randomly sampled, using a hand held meter ($n$=3).

Three elemental concentrations of barium (1x or ambient, 4x and 8x ambient levels) and strontium (1x or ambient, 2x and 4x ambient levels) were combined with the salinity treatments. Concentrations of the elements were controlled using standard solutions of $SrCl_2 \cdot 6H_2O$ and $BaCl_2 \cdot 2H_2O$ (Elsdon and Gillanders, 2003b). Replicate water samples ($n$=3) were taken from each tank on random days in acid-washed 50 mL polypropylene sample jars. Water samples were prepared and analysed following standard procedures (Elsdon and Gillanders, 2003b) to determine concentrations of strontium, barium and calcium.

### Analysis of Sr:Ca and Ba:Ca ratios

Concentrations of strontium, barium and calcium in otolith samples were determined using a Merchantek UP213 (New Wave Research) Nd: YAG deep UV laser microprobe, with a pulse rate of 5.00 Hz and an ablation spot size of 30 μm. The laser ablation station was connected to an Agilent 7500cs ICP-MS (Inductively Coupled Plasma – Mass Spectrometer). Ablations occurred within a sealed chamber with sample gases being extracted to the ICP-MS via the smoothing manifold in an argon and helium stream. The chamber was purged for 10 seconds after each ablation to remove any background gases from previous ablations. A reference standard (National Institute of Standards and Technology, NIST 612) was analysed after every 10-12 otolith ablations to correct for machine drift. The concentrations of strontium and barium within the otoliths were standardised to calcium using ratios.

The outside edges (within 30 μm) of otoliths from juvenile congolli were analysed using spot analyses to enable analysis of the material laid down during the experiment.

### Statistical Analysis

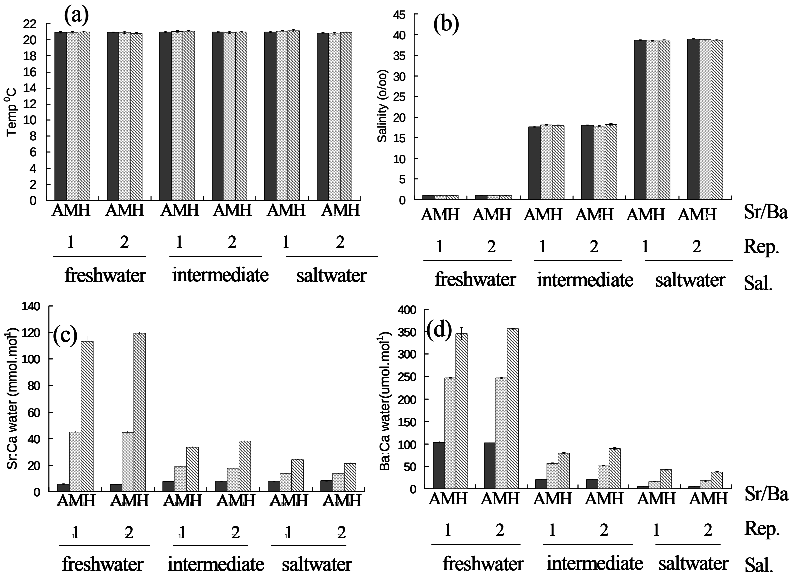
Permutational multivariate analysis of variance (PERMANOVA: Anderson 2001) was used to analyse the response of the otoliths to treatment groups, as the data did not satisfy assumptions of normality and homogeneity of variance. PERMANOVA allows the user to run both univariate and multivariate, multifactorial analyses of variance, using permutations in place of tabled *P*-values (Anderson, 2001). Univariate analyses were conducted to determine if elements in otoliths differed between treatment groups. A multivariate analysis was also conducted for the combined elemental signature of Sr:Ca and Ba:Ca. In both analyses salinity and element concentration were treated as fixed factors, with ‘tank’ as a random factor nested within these. Euclidean Distances were used and unrestricted permutations of raw data were performed, with 999 permutations done for the test, to detect differences at $\alpha=0.05$ (Anderson, 2001). If differences were detected, a stepwise regression was conducted.

### Results

Rearing Conditions

Temperature did not vary among experimental treatments (Fig. 1a). Salinity and element concentration differed among treatments (Fig 1. b-d), with both Ba:Ca and Sr:Ca being highest in the freshwater medium and high spiking treatments and decreasing with increased salinity (Fig. 1c-d, LSMeans contrast). Despite a significant difference in Sr:Ca among medium and high spiking treatments for differing salinities, ambient concentrations of Sr:Ca did not vary significantly with salinity. Elemental concentration was dependent on salinity, as indicated by a significant interaction (Table 1).

| **Source of variation** | **df** | **Salinity**<br>**MS** | **p** | **Sr:Ca**<br>**MS** | **p** | **Ba:Ca**<br>**MS** | **p** |
|---|---|---|---|---|---|---|---|
| Salinity | 2 | 6395.5 | <0.001 | 8681.4 | <0.0001 | 237396.2 | <0.0001 |
| Concentration | 2 | 0.014 | 0.85 | 12087.4 | <0.0001 | 602070 | <0.001 |
| Tank (S,C) | 9 | 0.15 | 0.11 | 12.00 | 0.0026 | 46 | 0.26 |



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Salinity x Concentration | 4 | 0.055 | 0.67 | 4198.3 | <0.0001 | 20263.71 | <0.0001 |
| Residual | 36 | 0.088 | | 3.31 | | 34.8 | |

Table 1: Results of analysis of variance (ANOVA) comparing the rearing conditions of salinity and element concentrations of strontium and calcium among treatment groups of salinity (S) and concentration (C).

Fig. 1. Mean concentrations (± s.e.) of (a) temperature, (b) salinity, (c) Sr:Ca and (d) Ba:Ca in rearing tanks of congolli in each experimental treatment (freshwater=1; intermediate= 17-19; saltwater=35-38) of salinity and element concentration (ambient or x1 =A; medium spiking x2 Sr, x4 Ba = M; high spiking x4 Sr, x8 Ba= H).

## Otolith Chemistry

The otoliths responded to the differences in concentrations of barium but not strontium in the rearing water. The varied element concentrations of the water produced differences in the otolith Ba:Ca ratios among treatments (Fig. 2b, Table 2). High ambient concentration produced otolith Ba:Ca ratios that differed significantly ($p < 0.05$) from the ambient and medium concentrations (Fig. 2b, stepwise regression). Otolith Sr:Ca ratios did not change with ambient Sr:Ca ratios among treatment groups (Fig. 2a, Table 2). Sr:Ca and Ba:Ca ratios within the otoliths were not affected by tank within treatments (Table 2). Analysis of the combined elemental signature using the PERMANOVA procedure for Sr:Ca and Ba:Ca within the otoliths suggested that element concentration had a significant effect, however, these values were similar to those obtained for a univariate ANOVA using Ba:Ca on its own.

| **Source of variation** | **df** | **Sr:Ca MS** | **p** | **Ba:Ca MS** | **p** | **Sr:Ca, Ba:Ca MS** | **p** |
|---|---|---|---|---|---|---|---|
| Salinity | 2 | 00.01 | 0.88 | 48.22 | 0.900 | 48.23 | 0.091 |
| Concentration | 2 | 0.08 | 0.44 | 97.47 | 0.023 | 97.55 | 0.023 |
| Tank (S x C) | 9 | 0.9 | 0.64 | 16.72 | 0.662 | 16.82 | 0.661 |
| Salinity x Concentration | 4 | 0.08 | 0.97 | 30.59 | 0.195 | 30.68 | 0.196 |

| | | | | |
|---|---|---|---|---|
| Residual | 33 | 0.1 | 22.39 | 22.49 |

Table 2: Results of permutational univariate analysis of variance (using PERMANOVA), comparing the Sr:Ca and Ba:Ca ratios in otoliths of congolli, among treatments of salinity (S) and element concentration (C), and results of permutational multivariate analysis of variance (PERMANOVA) comparing the elemental signatures of Sr:Ca and Ba:Ca in otoliths among treatments.

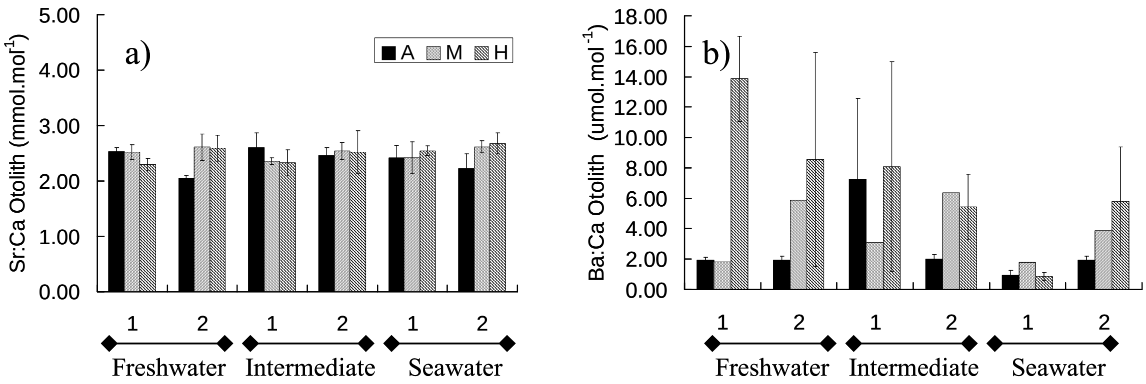


Fig. 2. Mean concentrations (± s.e.) of (a) Sr:Ca and (b) Ba:Ca of congolli otoliths in each experimental tank ( replicate 1 or 2) for salinity (freshwater = 1; intermediate = 17-19; saltwater = 35-38 g.L $^{-1}$) and element concentration (A= ambient or x1; M=x2 Sr, x4 Ba; H= x4 Sr, x8 Ba).

## Discussion

Analysis of the chemistry of otoliths may provide valuable information about the life history of fish. The processes involved in formation of growth increments in otoliths and the associated incorporation of elements are complex and governed by many interacting physiological and environmental factors (review: Elsdon and Gillanders, 2003a). Historical neglect of these interactions has compromised efforts to reconstruct the movements of diadromous fish from otolith chemistry. Results from this study suggest that the effect of metabolic rate in particular needs to be considered. Most prior work concerns demersal fish (e.g. Fowler et al., 1995, Bath et al., 2000, Milton and Chenery, 2001, Kraus and Secor, 2004, Elsdon and Gillanders, 2003b), and the present study is one of few to consider a less active, more sedentary species (but see Geffen et al., 1998, Swearer et al., 2003).

This study addressed the hypothesis that changing otolith chemistry in congolli is linked to changing salinity, and is a function of differences in ambient element concentrations between water bodies (fresh vs. saline). Otolith Sr:Ca and Ba:Ca ratios did not reflect differences among salinity treatments. However, a positive relationship was identified between otolith Ba:Ca and the Ba:Ca ratios in the rearing water of the high spiking treatments. Conversely, no relationship was detected between the Sr:Ca ratios in the rearing water and the otolith Sr:Ca ratios.

## Environmental influences

There was no apparent relationship between salinity of the rearing waters and either the Sr:Ca or the Ba:Ca ratios in the otoliths of congolli. The use of these ratios to track movements between environments of differing salinities assumes that changes in otolith Sr:Ca ratios and otolith Ba:Ca ratios, correlate with salinity. Salinity will indirectly affect the ambient elemental concentration, but not always in a consistent manner (Kraus and Secor, 2004, Rieman et al., 1994), and this is likely to account for the differing relationships reported.

For congolli there was an anomalous response in that no relationship was found for the otolith Sr:Ca ratio, while a positive relationship existed for Ba:Ca. This type of divided response has not been previously reported. The low concentrations of the elements used to spike the treatments may explain why otolith Sr:Ca did not respond to water Sr:Ca. However, low spiking concentrations (1.2-1.8 times ambient strontium) have been used, with detectable responses in otoliths of other species (Elsdon and Gillanders, 2003b, Bath et al., 2000).

In summary there was no obvious explanation for the anomalous response of otolith Ba:Ca when compared with the Sr:Ca response relative to ambient concentrations. It seems unlikely that environmental factors are causing this response. Rather, it may be that biological processes in congolli differ from other species.

## Biological processes

Biological processes, such as the metabolic rate and changing osmoregulatory mechanisms, are often neglected when studies on the otolith chemistry of diadromous fish are conducted. These may have attributed to the novel response of otolith Ba:Ca but not Sr:Ca to ambient concentrations for congolli. While limited information is available on the relationship between metabolic rates and activity of fish, there is reasonable evidence to suggest that aerobic capacity is linked with metabolic rates and this in turn should differ between fish with differing activity rates. Metabolic rate of different fish species may influence the rate of element uptake by affecting the rates of otolith growth (Wright, 1991, Yamamoto et al., 1998), and water flow through the gills (Swearer et al., 2003, Geffen et al., 1998). Diadromous fish will change the pathway of element uptake from the gills when in freshwater, to the intestines when in saltwater (Campana, 1999). The impact of this change on the rate of element uptake remains undetermined.

Rate of element uptake influences element concentrations of otoliths. Rate of uptake is controlled by the physiological barriers the elements must first pass, resulting in a time lag between exposure and incorporation into the otolith matrix (Elsdon and Gillanders, 2005b). The length of time fish are exposed and the concentration of rearing waters will interact, so higher concentrations are likely to be reflected more quickly in the otoliths (Pollard et al., 1999). In the current study otolith Ba:Ca ratios differed between the high-spiking treatments and the low- and the medium-spiking treatments, but these did not differ significantly from each other. Thus, these results may be a function of the high Ba:Ca spiking, producing a more pronounced response. In a separate study the minimum experimental period identified for black bream before Sr:Ca in the otoliths saturated binding sites (and thus was representative of ambient concentrations) was 20 days (Elsdon and Gillanders, 2005b). The duration of the current experiment (27 days) was determined based upon those results.

Congolli are sedentary fish (Allen, 1989) and would be expected to have lower metabolic rates. This is supported by the relatively small otolith increment width (1 $\mu m.d^{-1}$), (KC, BMG, KFW and QY Unpublished data). A combination of the low concentrations of strontium used to spike the treatments, relatively short experimental period and a low metabolic rate, is likely to account for the otolith Sr:Ca ratios not responding to water Sr:Ca ratios. This may account for the longer time period required for element uptake in congolli, when compared with faster moving, demersal species, such as black bream.

Less active fish with reduced metabolic rate, such as congolli, are likely to use less oxygen than more active fish. The amount of water passing through the gills (and possibly gill surface area) may influence the rate of element uptake into the otolith. Fish such as congolli may have a lower volume of water passing through the gills, and thus a reduced quantity of elements passing through the gill per unit time.

## Conclusions

The positive relationship between otolith Ba:Ca and ambient Ba:Ca ratios identified for congolli is not simply a function of salinity, these patterns could be used to track movement given prior knowledge of the Ba:Ca ratios in the environment. In essence, without water chemistry data it is difficult to show that the fish are actually moving between environments. However, further work is required into the effects of metabolic and activity rates on element uptake into the otoliths before it can be determined whether or not otolith chemistry can identify movements in congolli.

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