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Otolith microchemistry of two amphidromous galaxiids across an experimental salinity gradient: A multi-element approach for tracking diadromous migrations

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ABSTRACT

An increasing number of studies are uncovering considerable flexibility in migration patterns of diadromous fishes. The development of otolith microchemical techniques has largely driven this research and led to an appreciation of the significance of facultative diadromy in the life history of numerous species. However, validation experiments need to be undertaken for each species and life stage of interest before diadromous migrations can be confidently reconstructed. These validation experiments are required to establish a salinity calibration series against which the otolith microchemistry of unknown individuals can be compared. To facilitate research on facultative amphidromy in galaxiids, we reared the larvae of two species, *Galaxias maculatus* and *G. argenteus*, in five different salinities (2, 5, 10, 20, 34). We tested whether trace element signatures of fish reflected their salinity treatment, and hence whether otolith microchemistry could reconstruct diadromous migrations. Distinguishing low salinity (2 and 5) from high salinity (20 and 34) treatments was straightforward using otolith Sr:Ca alone. The five salinity treatments resulted in five distinct multi-trace element signatures for both species (DFA classification success of 85% and 92% for *G. maculatus* and *G. argenteus*, respectively). Otolith lithium showed a similar trend to otolith Sr:Ca (ie. higher in saltwater), and otolith Rb:Ca showed a surprising negative trend with salinity despite higher ambient Rb concentrations in saltwater. Our results suggest otolith Li:Ca and Rb:Ca should be considered as part of a multi-trace element approach when investigating diadromous migrations, particularly when non-marine Sr levels may be high.

1. Introduction

Methodologies for characterising age-specific migration patterns are necessary to identify essential fish habitat at different life stages, as well as determining whether connectivity between different habitats is important for completion of typical life cycles (Elsdon et al., 2008). Being able to track individual migration patterns also allows an understanding of intergenerational exchange that can occur among spatially separated populations (as in a meta-population, Thorrold et al., 2007). Diadromous migrations are an extreme form of large scale movement and differential habitat utilisation, and diadromous species comprise important fisheries worldwide (McDowall, 1990). It has been difficult to track diadromous migrations due to the large distances these fishes can travel, the low recapture rates of tagged individuals, and the inability to physically tag small individuals with traditional methods, which limit the feasibility of mark/recapture studies (Secor et al., 1995). The development of otolith microchemical techniques, whereby chronologically deposited and metabolically inert geochemical information can be used to reconstruct the environmental history of a fish, has allowed diadromous migrations to be more efficiently reconstructed (Secor et al., 1995).

Otolith microchemistry has revealed a diverse range of life history strategies and habitat utilisation in a range of diadromous fish species (Secor et al., 1995). 'Facultative diadromy', whereby not all individuals in a presumed diadromous population actually make a migration between the river and the sea, has been identified in numerous species and populations (e.g.,: Herring, *Alosa aestivalis*, Limburg, 1998; Smelt, *Hypomesus nipponensis*, Katayama et al., 2000; Sturgeon, *Acipenser guldenstadti*, Arai and Miyazaki, 2001; Stickleback, *Gasterosteus aculeatus*, Arai et al., 2003; Striped bass, *Morone saxatilis* Zlokovitz et al., 2003; Shirauo, *Salangichthys microdon*, Yamaguchi et al., 2004; Smelt, *Retropinna semoni*, Crook et al., 2008). Facultative diadromy extends to all three forms of diadromy (as defined by McDowall, 1992). For example, facultative catadromy in *Anguillids* (Arai et al., 2004), whereby some eels complete their life entirely within the marine environment; facultative anadromy in brown trout (Arai et al., 2002), whereby some individuals are freshwater resident; and facultative amphidromy in bullies and galaxiids, whereby larvae develop in freshwater lake systems despite having open access to the ocean (Closs et al., 2003; David et al., 2004). In all cases, otolith

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microchemistry has been used to distinguish whether individuals have utilised marine and/or freshwater habitat during different stages of their lives.

Strontium, and to a lesser extent barium, are the two elements commonly used to distinguish residency in waters of different salinity (Elsdon et al., 2008). There is usually a much higher and lower abundance of strontium and barium, respectively, in saltwater compared with freshwater (Elsdon et al., 2008). Often, scientists are interested in distinguishing estuarine versus freshwater or marine habitat use (e.g., Arai et al., 2004), or even utilisation of different parts of an estuary (e.g., Kafemann et al., 2000). Distinguishing between freshwater and saltwater residency has usually been straightforward in previous validation experiments (e.g., Farrell and Campana, 1996; Tzeng, 1996; Kraus and Secor, 2003; Secor et al., 1995), but there has been mixed success in discriminating habitat utilisation over finer salinity scales. For example, Kraus and Secor (2003) suggested otolith Sr:Ca ratios could be used to determine residency in completely fresh, estuarine or open ocean water, but not over finer salinity scales. Importantly, the non-linear relationship between salinity and ambient element:Ca ratios would make it difficult to discriminate between medium to high salinity habitat utilisation (most variation occurs below 8 ppt — Kraus and Secor, 2003; Lowe et al., 2009), if ambient element:Ca ratio rather than ambient absolute concentrations dictate the rate of element uptake into otoliths.

The patterns of strontium and barium uptake can be both species and age dependent. Validation studies performed on one species will not necessarily extend to others (Zimmerman, 2005). Similarly, validation studies performed on adult fish will not necessarily extend to larvae. Hence, laboratory validation studies should be undertaken on the species and age group of interest before trying to infer patterns of diadromy or habitat utilisation, particularly if the goal is to reconstruct movements across finer salinity scales (Kraus and Secor, 2004; Elsdon et al., 2008).

Amphidromy is a distinct form of diadromy with adults living in freshwater where most growth and reproduction takes place, but a short larval phase is expected to take place in the ocean (McDowall, 2007). Amphidromous galaxiids are a dominant freshwater fish fauna in New Zealand, Australia and South America (McDowall, 1990). Amphidromous galaxiid species typically spend a 3–6 month larval period in the ocean, before the transparent juveniles return to rivers where they are targeted as the basis of whitebait fisheries (McDowall, 1990). Genetic homogeneity at a continental scale suggests considerable larval dispersal occurs during the marine phase (Waters et al., 2001). Landlocked populations exist in large inland lake systems that have open access to the sea (McDowall, 1990), which suggests larvae are not actively dispersing (out of the lake system). There is also evidence for larval retention in galaxiids inhabiting coastal lake systems with open access to the sea (David et al., 2004). The presence of one and three endemic migratory galaxiid species in Australia and New Zealand and nearby islands, respectively, suggests colonisation abilities may differ among galaxiids despite all species having a pelagic larval period (McDowall, 1990). Being able to track a large number of individual larvae is thus necessary to determine the scale of movement of most individuals and effective population connectivity. This is difficult when trying to track movement through a comparatively homogeneous ocean, but determining retention in lakes or estuaries should be more straightforward. Determining the salinity history of larvae, and whether patterns of larval retention in lakes and estuaries are consistent among species, will thus help explore the magnitude of self-recruitment in galaxiid species.

Before relating otolith microchemistry of wild-caught fish to their salinity history, we needed to determine the degree to which movements across salinity gradients could be confidently inferred. We thus reared larvae of two migratory galaxiid species, *Galaxias maculatus* and *G. argenteus,* in salinities of 2, 5, 10, 20 and 34 to determine whether otolith trace element signatures could be related to a range of freshwater, estuarine and saltwater conditions. We primarily wanted to ensure we could identify individuals that had not spent time in a marine environment (i.e., were non-diadromous). We also wanted to explore whether there was potential to identify movements across finer salinity scales. To advance a more general understanding of factors regulating otolith microchemistry, we also explore whether otolith element:Ca is best predicted by the salinity, ambient element:Ca or ambient element concentrations in our experimental treatments.

2. Methods

Fish were reared in a constant temperature controlled room maintained at an average of 14.2 °C (range 13.5 °C–15.1 °C) and operating under a 12 h day/ 12 h night cycle. Eggs of both *Galaxias argenteus* and *G. maculatus* were obtained from a commercial breeding and culturing facility run by Charles Mitchell on the North Island of New Zealand. Eggs hatched between the 25/8/06–2/9/06 and larvae were transferred into several holding tanks of salinity approximately 17 and fed freshly hatched Artemia. A salinity of 17 was chosen for early development as a mid salinity range was associated with the highest larval survival rates (Charlie Mitchell *pers. comm.*). Mortality rates of newly hatched fish are typically high (>90%, Bath et al., 2000), so we kept larvae developing in holding tanks for two months until mortality rate had declined to less than a few individuals per day. This ensured there would be adequate survival of larvae during the experimental period (Bath et al., 2000). On the 4/11/06, surviving larvae were divided among identical 32 l tanks. This provided 2 replicate tanks per species for each of five salinity treatments, which were approximately 2.2, 5.4, 10.5, 20.5 and 34.2.

Salinities in tanks were gradually adjusted over a seven day period to the desired treatment levels by combining different volumes of Speight's spring water (a tapped, natural spring) with seawater from the Portobello marine station seawater filtration system. Galaxiid larvae appear to require live food to stimulate feeding behaviour (Mitchell, 1983), hence we constrained the salinity range to ensure consistency in the availability of live prey items across treatments. Our lowest salinity treatment was 2.2 as this was the lowest salinity that Artemia, our live prey, could tolerate.

Larvae were fed a surplus of freshly hatched Artemia each morning to provide a constant supply of small, actively swimming food items across the range of salinity treatments. We decided against filtration systems that would remove Artemia and potentially cause damage to fragile larvae. Instead we manually siphoned waste from the bottom of each tank, removed dead larvae and exchanged approximately 1/5 of the tank volume with water of matching temperature and salinity each day.

The experiment period lasted for 25 days. It takes time for otolith microchemistry to reach equilibrium with ambient element concentrations (Campana, 1999). This lag time was experimentally determined as two weeks for barramundi (Lates calcarifer, Milton and Chenery, 2001), 20 days for black bream (*Acanthopagrus butcheri*, Elsdon and Gillanders, 2005) and 21–22 days for largemouth bass (*Micropterus salmoides*, Lowe et al., 2009). These studies focused on the lag time for otolith Sr:Ca to reach equilibrium, and the relationship may differ among element:Ca ratios (Campana, 1999). Nevertheless, we assumed a 25 day experimental exposure would be sufficient for larval otoliths to reach equilibrium with ambient water chemistry.

Larvae were sacrificed and stored in ethanol. Handling and storing samples can affect otolith microchemistry (Milton and Chenery, 1998; Proctor and Thresher, 1998). However, Ca and Sr appear relatively robust to different handling and storage techniques (Proctor and Thresher, 1998) and the two most promising elements (Ba and Sr, Elsdon and Gillanders, 2005) appear to be unaffected by storage in ethanol (Hedges et al., 2004). As the bulk of wild samples we intended to analyse had been stored in ethanol, for consistency we felt it most