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CHRONOSEQUENCES OF STRONTIUM IN THE OTOLITHS OF TWO NEW ZEALAND MIGRATORY FRESHWATER FISH, INANGA (*GALAXIAS MACULATUS*) AND KOARO (*G. BREVIPINNIS*)

B. J. HICKS[1], D. W. WEST
Centre for Biodiversity and Ecology Research, Department of Biological Sciences, School of Science and Engineering, The University of Waikato, Private Bag 3105, Hamilton, New Zealand

B. J. BARRY, A. MARKWITZ
Rafter Research Centre, Institute of Geological and Nuclear Sciences Ltd, P.O. Box 31-312, Lower Hutt, New Zealand

C. F. BAKER
National Institute of Water & Atmospheric Research Ltd, P.O. Box 11-115, Hamilton, New Zealand

C. P. MITCHELL
Charles Mitchell and Associates, Ohautira Road, RD 1, Raglan, New Zealand

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Half of the freshwater fish fauna in New Zealand is diadromous (i.e., migrates between the sea and freshwater); unimpeded access to and from the sea is important for the conservation of diadromous fish. The genus *Galaxias* (Galaxiidae: Osmeriformes) contains five diadromous species that spawn in freshwater, migrate to sea as larvae, and then migrate back into freshwater as 50-55-mm juveniles. Microchemistry of the otolith, a calcified structure in the fish's head, allows an independent test of assumptions about these migrations. Concentric layers of $CaCO_3$ with some Sr as $SrCO_3$ comprise the otolith, creating a chronosequence that reflects a fish's migratory history. More Sr accumulates in the otolith when a fish is in seawater than when it is in freshwater. High-resolution nuclear microscopy was used to measure the molar ratios of Sr/Ca in two galaxiid species. Otoliths of inanga (*G. maculatus*) caught in freshwater all showed a central zone of 100-200 μm in radius with Sr/Ca of 0.008-0.012, indicating early rearing in the sea. Sr/Ca values decreased to 0.001-0.002 as the fish moved into freshwater. Of six adult koaro (*G. brevipinnis*) caught in a river with sea access and no lakes, five had migrated to sea but one had not, raising questions about the generalized assumptions of migration.

Keywords: otolith microchemistry, migratory history, freshwater fish, calcium, strontium

1. Introduction

Calcified structures in the fish's body absorb mostly Ca but also some Sr directly from the water surrounding the fish.[1] The otoliths in the fish's head are composed of concentric layers of $CaCO_3$ with some Sr as $SrCO_3$. More Sr accumulates in otoliths when fish are in seawater than when they are in freshwater, creating a chronosequence that reflects a fish's migratory history, and shows if a fish has had a marine phase.[2] Half of the freshwater fish fauna in New Zealand is diadromous, i.e., migrates between the sea and freshwater.[3] Two genera with a number of diadromous representatives are *Galaxias* (Osmeriformes: Galaxiidae) and

[1] Correspondence to: Dr Brendan Hicks, email: b.hicks@waikato.ac.nz

Gobiomorphus (Perciformes: Eleotridae). Sr/Ca has been used to determine the migratory history of common bullies (*Gobiomorphus cotidianus*) in New Zealand.[4]

In rivers without barriers to migration, the five species of diadromous galaxiids are generally assumed to make use of a marine rearing phase. Diadromous galaxiids spawn in freshwater, migrate to sea as larvae, and then migrate back into freshwater 4-5 months later as 50-55 mm juveniles.[3] Some galaxiids have prodigious climbing abilities as small juveniles, which allows them to scale vertical faces that are covered by a film of water,[5] and penetrate far upstream,[6,7] Koaro (*Galaxias brevipinnis*) is a climbing species, so its presence upstream of dams and waterfalls that are barriers to the migration of swimming fish cannot be assumed to preclude a diadromous origin. Koaro can also form land-locked populations that have their larval stage in a lake and do not need to go to sea, and thus the migratory history of koaro upstream of dams and waterfalls cannot be assumed. On the other hand, inanga (*Galaxias maculatus*) occupy lowland freshwater habitats, and cannot climb. Inanga generally live for about 1 year, whereas diadromous koaro can live for 8 years or more (DWW, unpubl. data).

We used high-resolution nuclear microscopy to measure concentrations of Sr and Ca in the otoliths of inanga and koaro to test the assumptions of migratory history.

2. Methods

2.1. *Sampling sites*

Inanga and koaro were caught at five sites in the North Island and one site in the South Island. In the North Island, juvenile inanga at the whitebait (upstream migrant) stage were caught in the lower Waikato River in October near Te Kohanga 11 km from the sea (Table 1). Adult inanga were caught in November 2000 at a distance of 45 km from the sea in the Whangamarino River. This river drains the Whangamarino Wetland, which is a major inanga rearing habitat.

Adult koaro were collected from the Oparau River, a short, steep river that drains from the slopes of Mt Pirongia to the Kawhia Harbour on the West Coast of the North Island. The collection site had open access for fish to and from the sea.

Juvenile koaro were collected in October 2001 below the spillway of the Upper Mangatawhiri Dam, which has formed the Upper Mangatawhiri Reservoir (1.2 km^2 in area)[8] on the Mangatawhiri River since 1965 (http://www.watercare.co.nz). Adult koaro were collected from the Lilburne Stream that feeds into the Upper Mangatawhiri Reservoir upstream of the dam in June 2002. Adult koaro were also caught in the South Island of New Zealand from three unnamed tributaries of Lake Matiri (0.6 km^2 in area), which was formed upstream of a natural dam created by a landslide that occurred 290-360 years before present.[8]

2.2. *Otolith analysis*

Analysis of Sr/Ca concentrations was performed on otoliths dissected from freshly killed and frozen fish. After removal, the otoliths were dried and embedded in a gel formulation of cyanoacrylate glue on a glass microscope slide. After 24 h, the otoliths were ground sequentially with 1200, 2400, and 4000 grit wet and dry sanding paper to reveal the nucleus.

Sr/Ca ratios were measured by particle-induced X-ray emission (PIXE)[9] under a proton beam produced by a 3 MV van de Graaff accelerator at the Institute of Geological and Nuclear Sciences, Lower Hutt. The X-ray energy spectra were measured with a Si(Li) detector; using Ca Kα X-ray lines at 3.69 keV and Sr Kα X-ray lines at 14.14 keV.[10] The Sr X rays were quantified with a 1000 mm^2, 1-mm thick NaI(Tl) detector which was at least thirty times more efficient than the Si(Li) detector, thus enabling shorter data acquisition times. The NaI(Tl) detector is not normally used for PIXE analysis because of its low energy resolution, i.e., its limited ability to distinguish between elements. In this case however, the spectra from the Si(Li) detector confirmed the lack of interfering elements. A 75 μm aluminium window was placed in front of the detector to improve the signal to noise ratio of the Sr:Ca measurements. Area scans and line scans through the otolith cores were performed using integrated charges of typically 10 μC. Pellets of $CaCO_3$ (NIST 610 standards) containing 1% and 10% $SrCO_3$ were used as standards. All Sr/Ca ratios are reported here as molar ratios (MR).

Table 1. Sources and lengths of inanga and koaro sampled for otolith Ca and Sr.

Source (km from the sea)	Species and life stage	*N*	Fork length (mm)	Capture date	Latitude S	Longitude E	Barriers to swimming migration
Waikato River (11)	Inanga juvenile	5	48-51	26 Oct 2002	37° 18.280'	174° 49.852'	No
Whangamarino River (45)	Inanga adult	2	119-125	Nov 2000	37° 17.481'	175° 7.514'	No
Oparau River (9)	Koaro adult	6	102-180	4 May 1989	38° 1.709'	174° 58.025'	No
Mangatawhiri River (75)	Koaro juvenile	6	41-45	24 Oct 2001	37° 5.275'	175° 9.406'	No
Lilburne Stream (77)	Koaro adult	7	90-140	11 Jun 2002	37° 4.620'	175° 10.398'	Yes
Lake Matiri tributaries (117)	Koaro adult	13	66-114	21 Jul 2004	41° 39.337'	172° 19.785'	Yes

3. Results

Otoliths of all inanga (*G. maculatus*) tested had a central zone of 100-200 μm radius with elevated strontium (Sr/Ca ~0.008), indicating early rearing in the sea. Inanga juveniles caught in freshwater but recently arrived from the sea (whitebait; 48-50 mm long) had Sr/Ca ~0.008 without reduced values of Sr/Ca at the edges typical of freshwater residence (Fig. 1). A 119-mm long adult inanga had a central zone with elevated Sr/Ca (0.008-0.009) characteristic of marine residence. Around this was a zone with lower Sr/Ca values (0.001-0.002) showing freshwater residence.

Of the six adult koaro examined from the Oparau River, five showed central cores with elevated Sr/Ca molar ratios of 0.008-0.012, surrounded by values of ~0.002 indicating freshwater residence (e.g., Fig. 2). However, one fish had uniform Sr/Ca values across the otolith of ~0.002 with no elevated central core, indicating that it had not been to sea.

All juvenile koaro examined from the Mangatawhiri River immediately downstream of the Upper Mangatawhiri Dam showed low Sr/Ca values in the central core (0.002-0.004), and from this we infer that the fish had resided only freshwater (Fig. 3). Adults from the Lilburne Stream above the Upper Mangatawhiri Dam had similarly low Sr/Ca values (Fig. 3), indicating that the Upper Mangatwhiri Reservoir was the most likely source of the juveniles. Adult koaro from tributaries of Lake Matiri also had low Sr/Ca values in the central core, indicating that the population is lake locked (Fig. 4).

A useful way to summarize the life history forms of inanga and koaro is to examine the means and ranges of the Sr/Ca values across the otolith line scans (Fig. 5). Fresh-run inanga showed a high mean value characteristic of marine residence, but a small range because of the lack of low values indicative of freshwater residence. River-resident inanga and koaro have a higher range of Sr/Ca (0.007-0.008) but a lower mean Sr/Ca than fresh-run inanga because of the difference between the marine and freshwater phases. Non-diadromous koaro showed both low means for Sr/Ca values (0.001-0.004), and a low range (0.0005-0.003; Fig. 5).

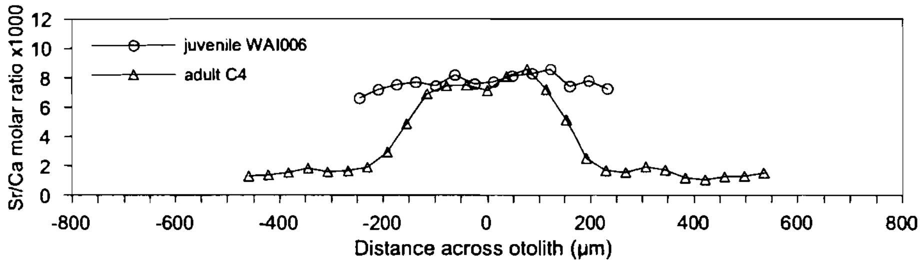

Fig. 1. PIXE line scans of Sr and Ca molar ratios across typical otoliths from diadromous (sea-migrant) inanga (*Galaxias maculatus*) from the Waikato River showing a 49-mm juvenile and a 119-mm adult. The distance 0 µm defines the center of the otolith.

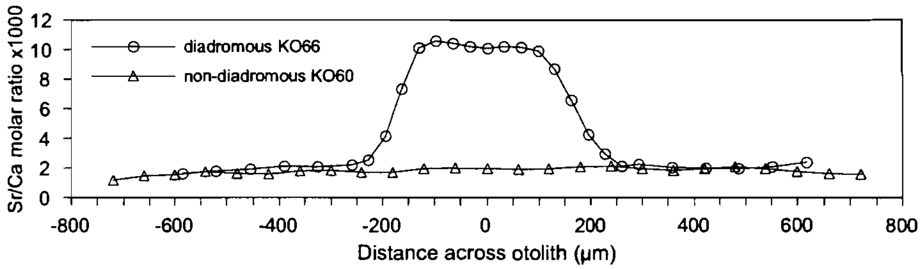

Fig. 2. PIXE line scans of Sr and Ca molar ratios across otoliths from typical adult koaro (*Galaxias brevipinnis*) from the Oparau River showing scans of a 180-mm diadromous (sea-migrant) fish (N=5), and a 156-mm non-diadromous fish (N=1). The distance 0 µm defines the center of the otolith.

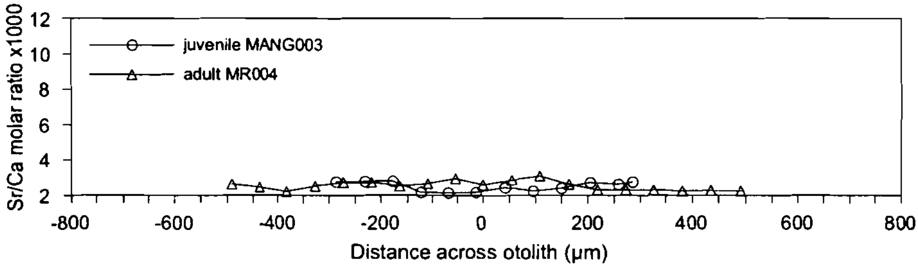

Fig. 3. PIXE line scans of Sr and Ca molar ratios across the otoliths of typical non-diadromous koaro (*Galaxias brevipinnis*) showing a 41-mm juvenile from the Mangatawhiri River below the dam and a 106-mm adult from a tributary of the Upper Mangatawhiri Reservoir above the dam. The distance 0 μm defines the center of the otolith.

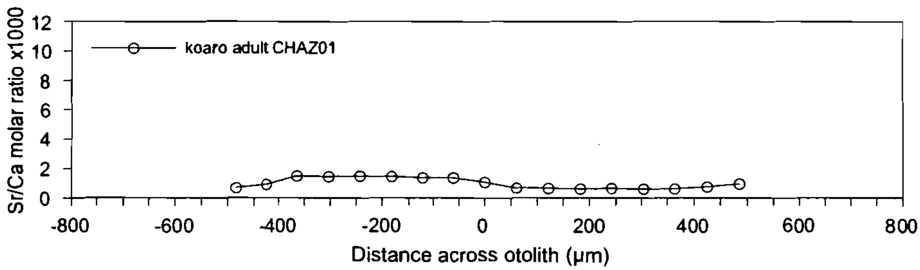

Fig. 4. PIXE line scans of Sr and Ca molar ratios across the otolith of a typical non-diadromous adult koaro (*Galaxias brevipinnis*) from a tributary of Lake Matiri upstream of a natural landslide. The distance 0 μm defines the center of the otolith.

4. Discussion

The highly elevated Sr/Ca values in the central core of inanga otoliths show that quantitative analysis of Sr and Ca concentrations yield a chronosequence that can be used to evaluate migratory life history of galaxiids. All inanga in this study had inhabited the sea during early rearing. Within a short time of entry into freshwater, the otoliths of juvenile inanga reflected the Sr/Ca ratios of their new freshwater habitat.

Koaro, however, displayed more flexible life histories. Five out of six individuals from the short, steep, coastal Oparau River had gone to sea, but one fish had not. This lack of complete reliance on a sea-migrant rearing phase explains the ability of koaro to abandon its usual migratory habits when aggressively colonizing new habitats,[11] and to form a species complex of non-migratory galaxiids as it probably did in Central Otago.[12] Coastal populations of giant kokopu (*Galaxias argenteus*), usually assumed to be a migratory species where fish have unrestricted upstream and downstream access to the sea, also exhibited variable recruitment. Sr/Ca patterns in otoliths suggested that freshwater or estuarine recruitment is a significant life history strategy among giant kokopu populations in the southeast of the South Island, New Zealand.[13]

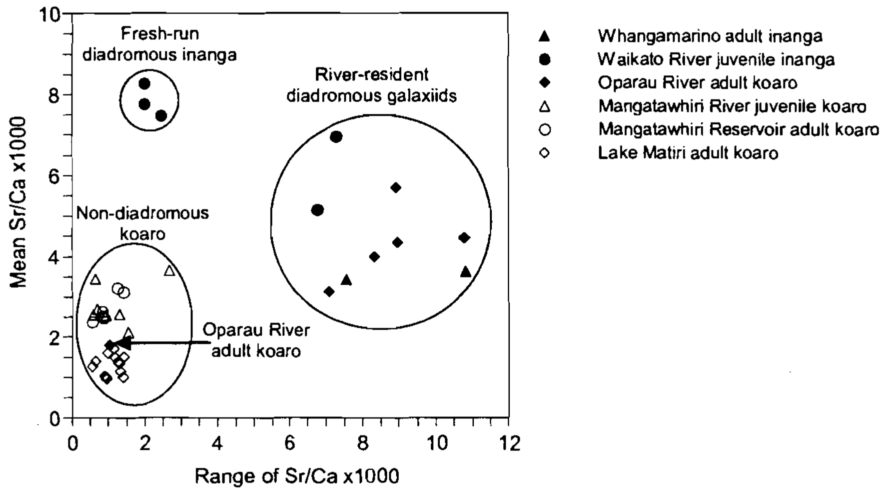

Fig. 5. Discrimination of diadromous (sea-migrant) from non-diadromous inanga (*Galaxias maculatus*) and koaro (*Galaxias brevipinnis*) by means and range of Sr/Ca molar ratios in otoliths.

Evidence of non-diadromous juvenile koaro in the Mangatawhiri River below the dam (Fig. 3) suggests that larvae washed out from the reservoir upstream have recruited to the river. These juveniles had congregated below the dam spillway, and were possibly attracted to the odour of adults upstream in the reservoir.[14] Thus dispersal of koaro to downstream habitats from lake-locked populations might be as important as upstream dispersal from the sea. Our results suggest that the incidence of non-diadromous individuals of galaxiids in situations where they would usually presumed to be diadromous should be further explored.

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References

1. R. L. Radtke, Strontium-calcium concentration ratios in fish otoliths as environmental indicators. *Comp. Biochem. Physiol.* **92A**, 189-193 (1989).
2. T. S. Elsdon and B. M. Gillanders, Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. *Reviews in Fish Biology and Fisheries* **13**, 219-235 (2003).
3. R. M. McDowall, The Reed field guide to New Zealand freshwater fishes. Reed, Auckland (2000).

4. G. P. Closs, M. Smith, B. Barry and A. Markwitz, Non-diadromous recruitment in coastal populations of common bully (*Gobiomorphus cotidianus*). *New Zealand Journal of Marine and Freshwater Research* **37**, 301-313 (2003).
5. J. Boubée, I. Jowett, S. Nichols and E. Williams, Fish passage at culverts: a review, with possible solutions for New Zealand indigenous species. Department of Conservation, Wellington (1999).
6. R. M. McDowall, Implications of diadromy for the structuring and modelling of riverine communities in New Zealand. *New Zealand Journal of Marine and Freshwater Research* **27**, 453-462 (1993).
7. R. M. McDowall, Fighting the flow: downstream-upstream linkages in the ecology of diadromous fish faunas in West Coast New Zealand rivers. *Freshwater Biology* **40**, 111-122 (1998).
8. D. J. Lowe and J. D. Green, Origins and development of the lakes, in *Inland waters of New Zealand,* ed. A. B. Viner (Science Information Publishing Centre, New Zealand Department of Scientific and Industrial Research, Wellington, 1987).
9. A. R. Markwitz, W. Gauldie, J. Pithie, S. Sharma and D. J. Jamieson, Nuclear microprobe and Raman investigation of the chemistry of the shell of the Pacific oyster, *Crassostrea gigas. International Journal of PIXE* **9**, 345-352 (1999).
10. A. D. Markwitz, Grambole, F. Herrmann, W. J. Trompetter, T. Dioses and R. W. Gauldie, Reliable micro-measurement of strontium is the key to cracking the life-history code in the fish otolith. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* **168**, 109-116 (2000).
11. J. M. Waters, M. Shirley and G. P. Closs, Hydroelectric development and translocation of *Galaxias brevipinnis*: a cloud at the end of the tunnel? *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 49-56 (2002).
12. J. M. Waters and G. P. Wallis, Cladogenesis and loss of the marine life-history phase in freshwater galaxiid fishes (Osmeriformes : Galaxiidae). *Evolution* **55**, 587-597 (2001).
13. B. David, L. Chadderton, G. Closs, B. Barry and A. Markwitz, Evidence of flexible recruitment strategies in coastal populations of giant kokopu (*Galaxias argenteus*). DOC Science Internal Series 160, New Zealand Department of Conservation, Wellington (2004).
14. C. F. Baker and B. J. Hicks, Attraction of migratory inanga (*Galaxias maculatus*) and koaro (*Galaxias brevipinnis*) juveniles to adult galaxiid odours. *New Zealand Journal of Marine and Freshwater Research* **37**, 291-299 (2003).