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Otolith microchemistry of the amphidromous *Galaxias maculatus* shows recruitment to coastal rivers from unstructured larval pools

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ABSTRACT: Marine larval development gives amphidromous fishes a powerful ability to disperse, but the low directional swimming ability of small amphidromous juveniles returning to freshwater increases the risk of expatrial dispersal. We used otolith microchemistry to investigate philopatry in *Galaxias maculatus*, whose juveniles constitute an important but declining commercial and recreational fishery in New Zealand. Using laser ablation inductively coupled plasma mass spectrometry, we analysed the elemental signature of embryonic otoliths from *G. maculatus* hatchlings from 12 rivers on the east and west coasts of the South Island of New Zealand. We then analysed the core region of the otoliths of recruits from the same cohort as they enter these rivers 6 mo later. The multi-elemental signatures of hatchling otoliths produced a high degree of differentiation among the 12 rivers. Using a multivariate approach, the streams tended to separate into east coast or west coast categories, driven largely by the influence of the relative values of Al and Fe in the otoliths. When the resulting discriminant model was used to classify the multi-elemental signature at the core of recruit otoliths, very few (2.8%) appeared to have returned to their natal river. Connection of *G. maculatus* populations within ecological time frames seems to be a common occurrence, especially within broad regions of the coast. Our findings imply 'leaky borders' with respect to larval pools, especially within coastlines. We highlight the importance of regional larval pools and the need to think about conservation efforts at both local and regional scales.

KEY WORDS: Inanga · Natal homing · New Zealand · Philopatry · Whitebait

INTRODUCTION

Diadromy is a migratory strategy that enables fishes and some aquatic invertebrates to occupy more favourable trophic, climatic or spawning conditions during critical life history stages (Dingle & Drake 2007). Amphidromous fishes (Myers 1949) typically spawn and hatch in freshwater, but their larvae move immediately to sea to develop in a biome that has increased food availability and reduced predation pressure (McDowall 2007). Amphidromous fishes return to freshwater as small juveniles and do most of their feeding and growing in freshwater before maturing and spawning there. Apart from improving growth and survival during the larval stage, amphidromy may also increase fecundity by allowing for the production of many smaller eggs, because the resulting tiny larvae have access to the trophically rich marine plankton (McDowall 2007, Closs et al. 2013).

Marine larval development gives amphidromous fishes a great ability to disperse. Although this dispersal may facilitate reestablishment of populations following localised extirpation (McDowall 2010), it also increases the risk of populations becoming isolated or suffering sporadic recruitment and means that such

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fishes are excluded from freshwater habitats upstream of hindrances to migration (Nehlsen et al. 1991). Furthermore, the directional swimming ability of small amphidromous juveniles returning to freshwater is substantially less than that of anadromous fishes such as salmonids, which are always much larger (McDowall 2010). Amphidromy, therefore, increases the risk of expatrial dispersal and the concomitant risk of not successfully returning to freshwater.

McDowall (2010) suggested several possible strategies that would minimise the risk of expatrial dispersal in amphidromous fishes. These included increased size at migration to sea, reduced planktonic larval duration and natal philopatry. Natal homing has been reported from some anadromous fishes, including salmonids, pikes, sturgeons and shads (Phelps et al. 2012, Engstedt et al. 2014, Keefer & Caudill 2014, McBride et al. 2014). Natal philopatry clearly provides an adaptive advantage (Cury 1994), because locations that support successful reproduction (sources) will tend to have more progeny returning to reproduce than locations that are less productive (sinks). However, natal homing seems unlikely for amphidromous fishes given the small size of larvae and their very brief residency in freshwater before migrating to the ocean.

Galaxias maculatus, known as inanga, is an amphidromous (or marginally catadromous) fish species that is native, but not endemic, to New Zealand (McDowall 1968). Juvenile *G. maculatus* (~55 mm total length) returning to rivers from their marine larval phase comprise the majority of New Zealand's whitebait fishery, which is of significant commercial, recreational and cultural importance. Recruiting *G. maculatus* juveniles lack the swimming/climbing ability of other diadromous galaxiids (McDowall 1993). Therefore, they mature in lowland waterways for ~6 mo before returning to upper estuarine areas to spawn in tidally inundated riparian vegetation (Hickford & Schiel 2011). The spawning season extends from late summer to early winter with discrete events around full and new moon spring tides (Taylor 2002). Eggs develop supratidally for 3 to 4 wk before hatching on the next series of spring tides (Benzie 1968). The hatchlings (6–7 mm total length) are washed out to sea with the retreating tide to begin a 6 mo larval phase (Rowe & Kelly 2009).

Although few historical catch data exist, there is anecdotal evidence that the New Zealand whitebait fishery is in decline (McDowall & Eldon 1980, McDowall 1984, Jowett et al. 1998). Rather than introducing quotas or further restricting the fishery, initial conservation efforts have focussed on the protection and remediation of spawning habitats as a means of maintaining or enhancing whitebait stocks. However, understanding the connections between the various habitats used for growth and spawning is fundamental to conservation efforts (Fausch et al. 2002). An understanding of metapopulation dynamics and thus protection of the supply of recruits that comprise the whitebait fishery is impossible if the natal areas that contribute recruits to adult stocks, as well as fish migration and dispersal patterns from these natal environments, are unknown (Hanski & Simberloff 1997). Furthermore, the benefits of spawning habitat rehabilitation efforts are difficult to promote with little knowledge of source/sink dynamics (Hickford & Schiel 2011) and thus the likely destination or fate of any enhanced larval production.

Galaxias maculatus has a very broad distribution and occurs throughout the southern hemisphere. Populations in New Zealand, Australia and Chile show strong inter-continental structuring in mtDNA, but there is evidence of recent trans-Tasman dispersal (Waters et al. 2000a) and possible ongoing trans-Pacific dispersal (Berra et al. 1996, Waters et al. 2000a). Waters et al. (2000a) found a lack of genetic differentiation among New Zealand populations of *G. maculatus*, suggesting recent (possibly ongoing) marine dispersal in this species. This finding was consistent with previous genetic analyses based on isozyme data (Barker & Lambert 1988, Allibone & Wallis 1993).

Waters et al. (2000a) suggested that the marine larvae of *G. maculatus* lack the strong natal philopatry that facilitates genetic differentiation among riverine populations of other diadromous fishes (e.g. Nielsen et al. 1994, Waters et al. 2000b). However, the absence of genetic differentiation does not preclude at least limited philopatry in *G. maculatus*. For example, Thorrold et al. (2001) used otolith chemistry to calculate the natal homing rate of weakfish (*Cynoscion regalis*) in estuaries along the Atlantic coast of the United States. Their estimates of philopatry ranged from 60 to 81%, with most strays going to adjacent estuaries. Despite this degree of philopatry, previous genetic analyses had indicated only a single weakfish stock (Graves et al. 1992).

Otolith microchemistry has proven to be useful in investigating philopatry in fish species that have been difficult to assess with genetic analyses (e.g. Thorrold et al. 2001, Patterson et al. 2004). We used otolith microchemistry to test 4 hypotheses regarding *G. maculatus*: $H_0(1)$: there is no consistent difference in elemental signature in the otoliths of hatchlings among rivers; $H_0(2)$: elemental signatures in the

otoliths of hatchlings are not consistent among spawning events within a year; $H_0(3)$: there is no affinity between the elemental signatures at the core of recruit otoliths and any of the established signatures of hatchling otoliths; $H_0(4)$: expatrial dispersal precludes natal philopatry.

MATERIALS AND METHODS

Sample collection

Hatchlings

In both May and June 2009, *Galaxias maculatus* eggs were collected from spawning sites in 12 rivers on the east and west coast of the South Island of New Zealand (Table 1, Fig. 1). The rivers ranged from 3rd to 6th order and in most cases the spawning site was within 5 km of the river mouth. Multiple clutches of eggs were sampled at each spawning site to account for any variability among spawning females. On each occasion, 50 to 100 fertilised eggs were removed from 2 to 4 clutches. Eggs were transported to the laboratory in plastic bags, together with soil and vegetation from their immediate surroundings, and incubated in a constant environment chamber (15°C, 87% relative humidity). Egg development was monitored, and when the embryos were ready to hatch (Benzie 1968) the eggs were concentrated on 250 µm nybolt mesh, rinsed with ultra-pure water (18.2 MΩ cm resistivity at 25°C) and transferred to 70 ml vials filled with ultra-pure water. The vials were placed on a low speed orbital shaker table and agitated gently.

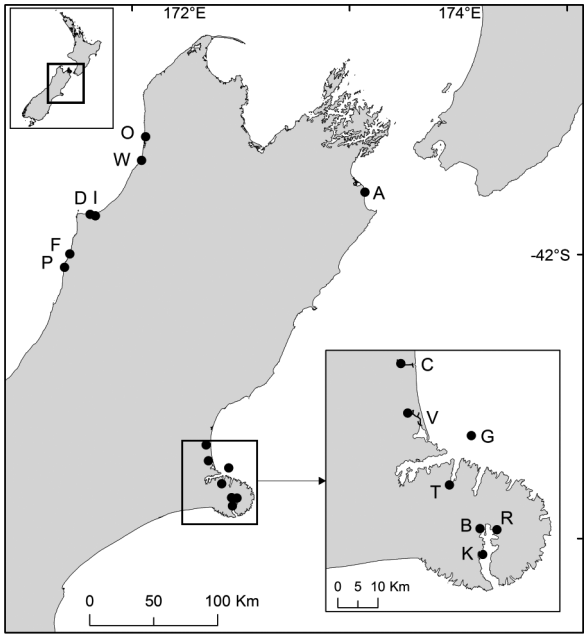

Fig. 1. Locations of 12 rivers on the east and west coasts of the South Island, New Zealand, from which *Galaxias maculatus* eggs and recruits were collected, and 2 marine sites where larvae were collected. West coast rivers: (O) Oparara River, (W) Little Wanganui River, (I) Orowaiti River, (D) Bradshaws Creek, (F) Fox River, and (P) Punakaiki River. East coast rivers: (A) Awatere River, (C) Courtenay Stream, (V) Avon River, (T) Te Kawa Stream, (B) Barrys Bay Stream, and (R) Robinsons Bay Stream. Marine sites: (G) Pegasus Bay and (K) Akaroa Harbour

Once the majority of embryos had hatched (<2 h), individual hatchlings were transferred to 0.6 ml Eppendorf tubes containing 0.5 ml of ultra-pure water, and snap frozen.

Table 1. Attributes of the 12 rivers on the west and east coast of the South Island, New Zealand, used for *Galaxias maculatus* collections. ER: eggs and recruits; E: eggs

River	ID code	Catchment area (km^2)	Strahler stream order	Mean discharge ($m^3 s^{-1}$)	Spawning site (distance from mouth, km)	Sample type
West coast						
Oparara River	O	133.9	5	4.3	1.7	ER
Little Wanganui River	W	182.2	5	5.8	2.3	ER
Orowaiti River	I	47.3	4	1.5	6.4	ER
Bradshaws Creek	D	47.7	4	0.8	5.0	E
Fox River	F	104.1	5	3.3	0.3	E
Punakaiki River	P	63.3	4	2.0	0.5	ER
East coast						
Awatere River	A	1576.8	6	49.9	0.9	ER
Courtenay Stream	C	13.7	3	0.4	4.0	ER
Avon River	V	166.1	4	5.4	11.9	E
Te Kawa Stream	T	14.7	3	0.5	0.1	E
Barrys Bay Stream	B	11.2	3	0.4	0.1	ER
Robinsons Bay Stream	R	11.7	3	0.4	0.1	ER

Recruits

In November 2009, returning *G. maculatus* recruits (whitebait, <60 mm total length) were collected as they entered the mouth of 8 of the original 12 rivers (Table 1). In addition, larvae were collected at 2 marine sites (Fig. 1) in neuston tows using a box-pyramid plankton net (250 µm mesh) with a 1 m^2 mouth. The plankton net was towed beside a 7.8 m boat with the uppermost edge of the net frame fixed at 0.1 m above the water surface. The 2 marine sites were included to attempt to capture larvae from nearby streams that had been retained in the coastal eddy within Pegasus Bay or the semi-enclosed Akaroa Harbour (Reynolds-Fleming & Fleming 2005). In the laboratory, 50 recruits from each location were placed individually in 1.5 ml Eppendorf tubes and snap frozen.

Sample preparation

We performed all of the otolith isolation steps in a clean laboratory equipped with HEPA-filter class 100 laminar flow hoods. All glassware used in the isolation steps was cleaned with Citronox®, rinsed 5 times with distilled water (>2 MΩ cm resistivity), soaked in 1 N trace-metal grade HCl overnight, and then rinsed 5 times with ultra-pure water.

Hatchlings

The sagittae of *G. maculatus* hatchlings were removed and prepared according to the methods developed by Barbee & Swearer (2007). With a dissecting microscope at 60× magnification, the sagittae were visible inside the saccules of the hatchlings. Thawed larvae were placed in a drop of ultra-pure water on an acid-cleaned glass cavity slide. The left sagitta was removed from each hatchling using a crook-tipped tungsten needle. Sagittae were transferred using the bristles of an acid-cleaned fine-tipped paintbrush to a droplet of semiconductor grade 30–32 wt% H_2O_2 in water (buffered with Suprapur 0.1 N NaOH) for cleaning. After 15 min of cleaning, each otolith was rinsed by transitioning it through 3 droplets of ultra-pure water. The cleaned otoliths were placed into a grid that had been etched into the surface of an acid-cleaned glass microscope slide. The microscope slide had been pre-coated with a thin layer of low-viscosity epoxy resin (Buehler EpoThin™). Each otolith was embedded in a tiny droplet of the same epoxy resin. Otoliths were successfully removed from 25 to 50 hatchlings from each river, and hatchlings were sampled from all available clutches.

Recruits

The sagittae of thawed recruits were removed, cleaned of adhering tissue in ultra-pure water and air-dried. Left sagittae were mounted sulcal side down onto 20 × 20 mm plastic slides using low-viscosity epoxy resin (Buehler EpoThin™). The sagittae were polished with a lapping wheel and 9 and 3 µm 3M® diamond lapping film to expose the growth layers and the core. Sagittae were polished to within 5 to 15 µm above the visible core. We prepared recruit otoliths for chemical analysis following the procedures of Ruttenberg et al. (2005). Mounted otoliths were cleaned of surface contaminants and organic material by rinsing in ultra-pure water and then soaking for 1 h in semiconductor grade 30–32 wt% H_2O_2 in water (buffered with Suprapur 0.1 N NaOH) in acid-cleaned plastic trays. Each otolith was rinsed with ultra-pure water and then soaked and sonicated 3 times in ultra-pure water for 5 min. Samples were then rinsed in a final ultra-pure water wash and air-dried in a HEPA-filter class 100 laminar flow hood.

Right sagittae were mounted sulcal side up onto glass microscope slides using CrystalBond™ 509 adhesive. The sagittae were polished longitudinally using 12 and 0.3 µm South Bay Technology aluminium oxide lapping film. When necessary, sagittae were remounted and polished sulcal side down until a complete transect from core to edge was visible. Polished sagittae were soaked in immersion oil for 24 h before photomicrographs were captured with an AxioCamHRc CCD camera attached to a Zeiss compound microscope (Axio IMager.M1) equipped with differential interference contrast (DIC). Images were imported into Image-Pro Premier 9.1 and counts of daily increments (McDowall et al. 1994) were used to derive age estimates using the Otolith App.

Analytical methods

Hatchlings

Elemental analyses of otoliths were done with a Finnigan Element 2 High Resolution Double Focusing Magnetic Sector inductively coupled plasma

mass spectrometer (ICP-MS) fitted with a New Wave UP-213 laser ablation system. Calibration solutions with known analyte to ^{48}Ca ratios were used to calculate the isotope ratio mass bias correction. NIST (National Institute of Standards and Technology) glass standard (612) doped with trace elements at known concentrations was used to estimate accuracy and precision (see Warner et al. 2005 for more details). Hatchling otoliths were run in blocks of 15 to 18 samples selected randomly from all sites and bracketed by analyses of the standards. A 1% HNO_3 blank sample (30 s) was acquired prior to each hatchling sample and the standards. Hatchling otoliths were analysed with a series of 8 laser pulses of 0.1 mJ at 3 Hz and a nominal spot diameter of 30 µm. Due to the small size of hatchling otoliths (~26 µm diameter), all material from an otolith was consumed during acquisition. Counts were collected for ^{24}Mg, ^{27}Al, ^{48}Ca, ^{55}Mn, ^{56}Fe, ^{66}Zn, ^{86}Sr, ^{138}Ba and ^{208}Pb at medium resolution (we were restricted to using medium resolution because of the requirements for finding the core in recruit otoliths; see next paragraph).

Recruits

Recruit otoliths were analysed for the chemical composition of the core. The core was located on the mounted otolith in the sample cell of the laser ablation system using a 400× objective and video imaging system. We define the otolith core as the otolith primordium and other material deposited during gestation before exogenous feeding begins (Ruttenberg et al. 2005). We used a series of successive small ablation pits to isolate the material associated with the core. Each pit comprised 8 laser pulses (0.1 mJ at 3 Hz), in a vertical transect from the surface of the otolith through the visible core (see Ruttenberg et al. 2005 for details). The diameter of the resulting pit (25.7 ± 0.3 µm; x ± SE, n = 9) was not significantly different (ANOVA, $F_{1,32}$ = 1.227, p = 0.277) from the diameter of hatchling otoliths (26.4 ± 0.4 µm, n = 24). Previous work found that the cores of otoliths in general contain elevated Mn (Brophy et al. 2004, Macdonald et al. 2008), which has been shown to be an accurate indicator of the location of the core of the otolith for *G. maculatus* recruits (Ruttenberg et al. 2005). We identified the specific pit containing the core material using elevated concentrations of Mn (at least 3× higher than surrounding material) as a proxy to identify the specific pit containing the core material in the vertical transect. Elements associated with the identified pit were used to characterize the core elemental composition. When analysing the core of the recruit otolith, we were constrained to using only 1 resolution for all elements, because the core of the otolith is very small and is contained within one or two 8-pulse ablation events. Because we used a spike in Mn as an indicator of the location of the core, and Mn is collected at medium resolution to avoid interferences, all elements analysed for recruit cores were acquired using medium resolution.

Limits of detection for each of the elements, calculated as 3× the SD of a 1% HNO_3 blank sample analysed after every otolith and expressed as ratios of the isotope intensity and mean otolith Ca intensity, were: 0.04 mmol mol^{-1} (Mg), 5.56 µmol mol^{-1} (Al), 0.96 µmol mol^{-1} (Mn), 6.72 µmol mol^{-1} (Fe), 2.78 µmol mol^{-1} (Zn), 0.07 mmol mol^{-1} (Sr), 0.62 µmol mol^{-1} (Ba) and 0.84 µmol mol^{-1} (Pb). Because we analysed recruit cores at medium resolution, the Pb signal strength was low and values were consistently below detection limits. As a result, we excluded this element from all hatchling–recruit comparisons. We analysed solid glass standard reference material (NIST 612) at the beginning and end of each workday to provide external estimates of precision. Mean percent relative standard deviations (%RSD, n = 18 workdays) were: 9.8% (Mg), 3.5% (Al), 3.5% (Mn), 10.5% (Fe), 9.8% (Zn), 5.9% (Sr), 4.3% (Ba) and 6.4% (Pb). These RSD values reflect instrument precisions previously reported using this method (Ruttenberg et al. 2005, Warner et al. 2009).

Statistical analysis

Differences among rivers (nested within Coast) in the concentration of individual elements in the otoliths of hatchlings were examined using nested ANOVA with River as a random factor. Post hoc Tukey's Honest Significant Difference (HSD) tests were used to investigate which rivers differed significantly from each other. Prior to all analyses, Cochran's tests were used to check for homogeneity of variances and data were log-transformed when necessary.

To investigate whether inter-clutch variability within rivers was a major component of the overall variation in multi-element otolith signatures, we ran a nested ANOVA for each element with River, Month and Clutch(Month) as random factors. This analysis was limited by sample sizes to a subset of 5 rivers

(Oparara, Bradshaws, Punakaiki, Barrys and Robinsons) and we report the variance components (factor SS as a percentage of the total SS) for each factor. All ANOVA tests were completed with Statistica v12.

We used SPSS Statistics v17 to complete discriminant function (DF) analysis of the multi-elemental signature in otoliths of hatchlings from each of the 12 rivers. Prior to this analysis, individual elemental ratios were standardised. We plotted centroids with 95% confidence ellipses against the first 2 discriminant functions to visualise the spatial differences between rivers. We used leave-one-out cross-validation to test the reclassification success of hatchlings to rivers of known origin by classifying each hatchling with the discriminant functions derived from all other hatchlings.

The discriminant model derived from the hatchling data was used to classify the multi-elemental signature at the core of recruit otoliths and, where possible, to identify natal origin. Individual Mahalanobis distances (the distance between individual data points and the centroid) were examined and only recruits inside the 95% confidence ellipsoid of a river were determined to have originated from that river.

RESULTS

Hatchling otoliths from west coast rivers generally had higher concentrations of Al, Mn and Fe than east coast rivers (Table 2, Fig. 2). There were significant differences among the 12 rivers in the hatchling otolith concentrations of all elements. Pair-wise comparisons showed that differences among rivers varied for the 7 elements (Table 2). For the subset of 5 rivers where sample sizes allowed further analyses, most of the variation in elemental concentrations was attributable to differences between rivers (Table 2). Notable exceptions to this were Mg and Mn, which showed high residual variability. Differences between months (May and June) only accounted for 0.001 to 1.68% of the variation observed in the otolith concentration of elements (Table 2). Differences among egg clutches accounted for 0.3 to 4.36% of the total variation.

The multi-elemental signatures of hatchling otoliths produced a high degree of differentiation among rivers, as illustrated by the discriminant function (DF) analysis plot (Fig. 3), which shows minimal overlap of the 95% confidence ellipses around the centroids. The first DF accounted for 50.3% of the

Table 2. Results from nested ANOVA testing for differences among 2 coasts and 12 rivers in the concentration of 7 elements (relative to Ca concentration) in hatchling otoliths; $^{**}p < 0.01$, $^{***}p < 0.001$, ns = not significant. Pair-wise comparisons: lines under ID codes (see Table 1) join rivers that were not significantly different from each other (Tukey's HSD, $p > 0.05$); **bold** = West Coast rivers; Variance components: factor SS as % of total SS for 3 factors in nested ANOVA done on a subset of 5 rivers (Oparara, Punakaiki, Bradshaws, Barrys and Robinsons).

Element	Source	df	SS	*F*	Pair-wise comparisons	Variance components		
						River	Month	Clutch(Month)
^{24}Mg	Coast	1	2.703	1.386^{ns}	**W P** R A **D O** B **F** C T **I** V	8.34	0.01	0.30
	River(Coast)	10	22.673	44.511***				
	Error	330	16.809					
^{27}Al	Coast	1	19.609	41.506***	T R A V C B **F O P D W I**	59.01	0.07	0.82
	River(Coast)	10	5.495	46.698***				
	Error	330	3.883					
^{55}Mn	Coast	1	2.290	11.370**	A B C R T **F** V **P W I O D**	10.84	1.34	4.36
	River(Coast)	10	2.126	1.590^{ns}				
	Error	330	44.067					
^{56}Fe	Coast	1	6.213	12.002**	R C A **F** V T B **W I P O D**	65.18	0.001	0.77
	River(Coast)	10	6.012	33.466***				
	Error	330	5.929					
^{66}Zn	Coast	1	0.547	0.923^{ns}	C A **D** T V **P O W F** B R **I**	43.85	0.14	1.52
	River(Coast)	10	6.888	35.959***				
	Error	330	6.321					
^{86}Sr	Coast	1	0.116	0.784^{ns}	C **O** V **F D P W** A **I** B R T	53.82	1.68	0.31
	River(Coast)	10	1.720	34.958***				
	Error	330	1.623					
^{138}Ba	Coast	1	0.305	0.159^{ns}	C **W** T A **O F D** R **P** V **I** B	63.64	0.001	0.56
	River(Coast)	10	22.418	103.380***				
	Error	330	7.156					

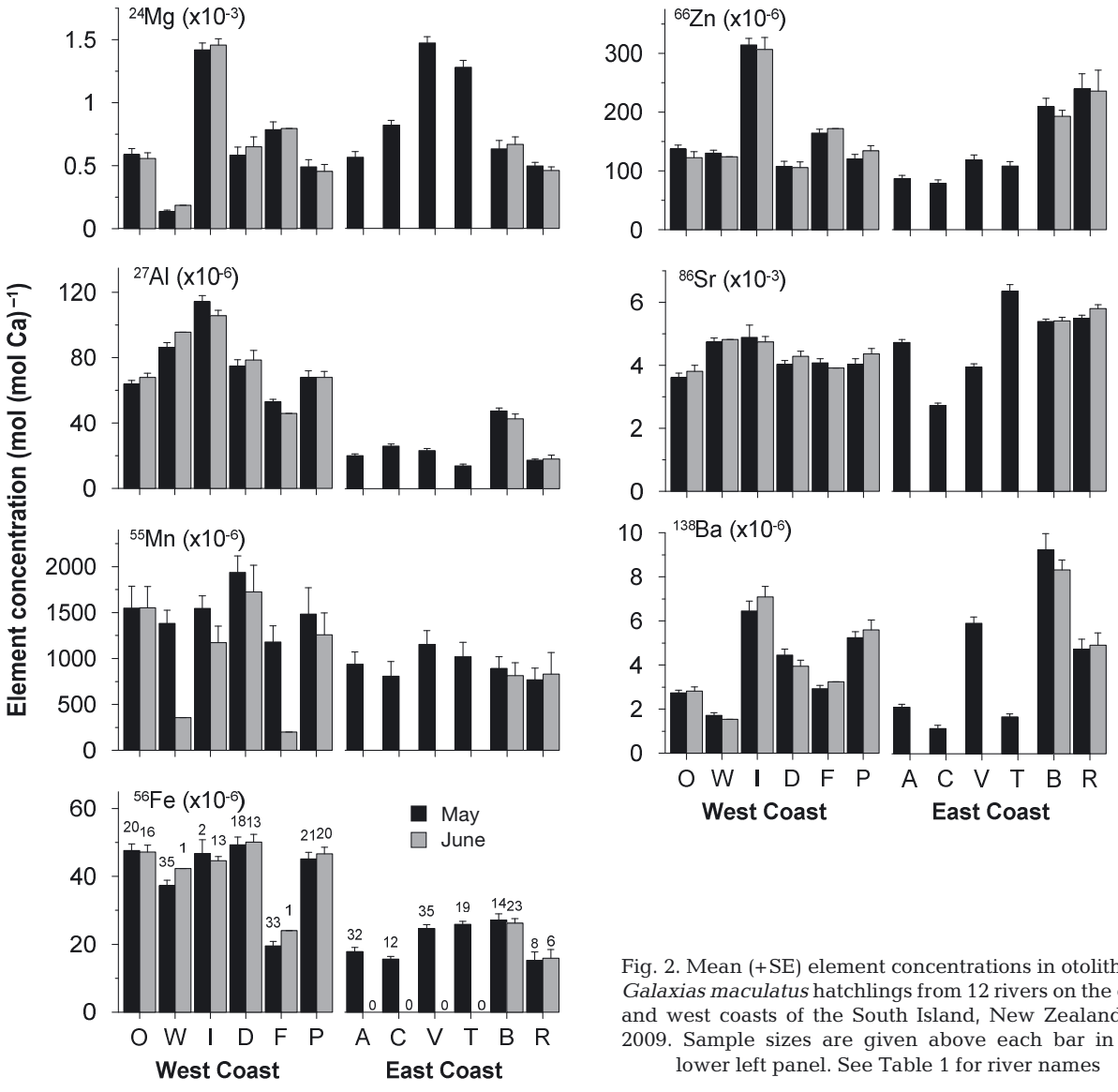

Fig. 2. Mean (+SE) element concentrations in otoliths of *Galaxias maculatus* hatchlings from 12 rivers on the east and west coasts of the South Island, New Zealand, in 2009. Sample sizes are given above each bar in the lower left panel. See Table 1 for river names

discriminating ability of the discriminating variables and separated most west coast sites from the east coast sites, with Al contributing most to coast differentiation. DF2 (24.5%) and DF3 (12.1%) separated rivers within coasts with Ba contributing most to DF2 (see Fig. 3 caption) and Mg contributing most to DF3. The overall reclassification rate was high (85%), with some rivers showing 100% successful reclassification (e.g. Orowaiti, Awatere, Courtenay and Te Kawa; see Fig. 3 caption) and others showing less successful reclassification rates (e.g. Bradshaws = 45%).

Counts of daily increments in the otoliths of November recruits showed that all fish had hatched in either June or July from eggs that were spawned in May or June respectively. Planktonic larval durations ranged from 119 to 168 d.

When the discriminant model was used to classify the multi-elemental signature at the core of recruit otoliths (Table 3), very few (2.8%) appeared to have returned to their natal river. Only Little Wanganui (3.8%), Punakaiki (4.2%), Courtenay (3.8%), Barrys (3.0%) and Robinsons (8.7%) had recruits with otoliths that had a core signature indicative of them originating from the same river they had re-entered. Most rivers had very few recruits (<10%) that originated from one of the sampled rivers, but when the natal origin was identified, the recruits were frequently from the same coast and nearby rivers (Table 3, Fig. 1). The exception to this was Awatere (on the northern east coast), where the identifiable recruits all originated from west coast rivers.

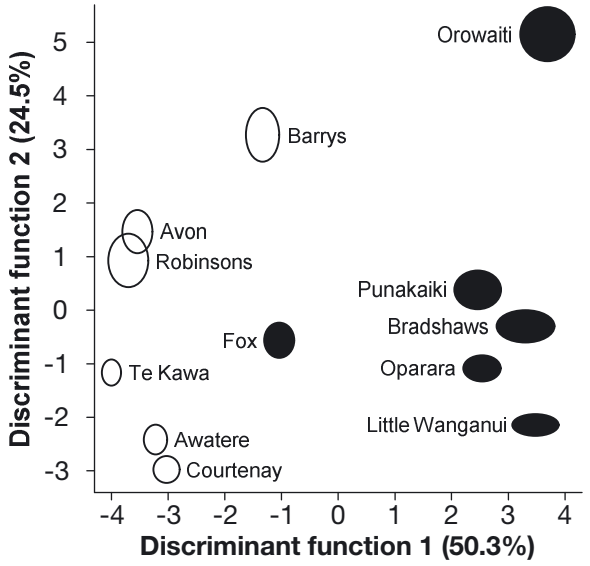

Fig. 3. Discriminant function (DF) analysis of element concentrations in otoliths of *Galaxias maculatus* hatchlings from 12 rivers on the west (filled symbols) and east (open symbols) coasts of the South Island, New Zealand, with 95% confidence ellipse around each centroid. DF1 canonical structure — Al: 0.72, Fe: 0.50, Mg: –0.27, Sr: –0.11, Mn: 0.09, Zn: 0.05, Ba: –0.02. DF2 canonical structure — Ba: 0.78, Zn: 0.46, Mg: 0.36, Sr: 0.15, Al: 0.14, Fe: 0.08, Mn: –0.03. Reclassification success rates using cross-validation — Oparara: 80.6%, Little Wanganui: 86.1%, Orowaiti: 100%, Bradshaws: 45%, Fox: 97%, Punakaiki: 61%, Awatere: 100%, Courtenay: 100%, Avon: 97%, Te Kawa: 100%, Barrys: 89%, Robinsons: 92%

Samples of marine larvae from Pegasus Bay and Akaroa Harbour also contained very few fish from one of the sampled rivers, but there was some evidence of local retention (Table 3). The 3 larvae (17.6%) in the Pegasus Bay sample with identifiable natal origins came from the nearby Avon River (Fig. 1), while the one identifiable larva (2.9%) from Akaroa Harbour originated from Robinsons Bay Stream, which flows into the harbour (Fig. 1).

Mahalanobis distances (D^2_{crit} = 14.07) indicated that most recruits (>80%) were outside the 95% ellipsoid of any of the 12 rivers, so their natal origin was unidentified. However, it was possible to estimate likely origins of some of these recruits by using the observed differences between coasts in concentrations of Al and Fe in hatchling otoliths (Table 2, Fig. 2). Recruits with Al and Fe core signatures above the 99th percentile of those observed for east coast hatchlings (61.1 and 38.7 µmol mol^{-1} respectively) were likely to have originated from an unknown west coast river (Table 3). Recruits with Al and Fe core signatures below the 1st percentile of those observed for west coast hatchlings (40.7 and 10.77 µmol mol^{-1}) were likely to have originated from an unknown east coast river. No west coast recruits had core signatures that clearly indicated that they originated from an east coast river (Table 3). However, all east coast rivers had some recruits (> 7%) that appeared to originate from west coast rivers (Table 3).

Table 3. Classification of the likely natal origin of *Galaxias maculatus* recruits from 8 rivers and 2 marine sites using a discriminant model developed from the multi-elemental signatures of otoliths from hatchlings from 2 coasts and 12 rivers. n: sample size of recruits; TL: total length. Observed differences in univariate otolith elemental signatures (Al and Fe; Fig. 2) were used to broadly classify some recruits (west coast river and east coast river) that were outside the 95% ellipsoids of the discriminant model, but the natal origin of the remaining recruits was unidentified

					Natal origin (% return)														
					West coast							?	**East coast**						
			n	Mean TL ± SE	Oparara	L. Wanganui	Orowaiti	Bradshaws	Fox	Punakaiki	West coast river	Unidentified	Awatere	Courtenay	Avon	Te Kawa	Barrys	Robinsons	East coast river
Destination	West coast	Oparara	28	54.4 ± 0.4		3.6			3.6	3.6	42.9	46.4							
		L. Wanganui	26	55.1 ± 0.4	3.8	3.8					65.4	26.9							
		Orowaiti	30	55.0 ± 0.4					3.3	3.3	46.7	46.7							
		Punakaiki	24	54.9 ± 0.3						4.2	29.2	66.7							
	East coast	Awatere	27	51.8 ± 0.3	3.7				3.7		22.2	70.4							
		Courtenay	26	52.1 ± 0.4							7.7	73.1	3.8				3.8	3.8	7.7
		Barrys	33	52.4 ± 0.3							9.1	75.8					3.0	6.1	6.1
		Robinsons	23	52.0 ± 0.4							8.7	73.9						8.7	8.7
	Marine	Pegasus	17	49.7 ± 0.3								70.6		17.6					11.8
		Akaroa	35	50.4 ± 0.3								74.3						2.9	22.9

DISCUSSION

There has been some debate about the early life history of *Galaxias maculatus* with respect to offshore larval pools and natal philopatry of returning post-larvae (e.g. Barker & Lambert 1988, Waters et al. 2000a). Here we showed that there are clear elemental signatures in the otoliths of hatchling fish, that these are stable between months in the spawning season, that these are capable of distinguishing west coast and east coast fish, and that some sites within these regions also have distinguishable elemental signatures. It appears that most fish with identifiable elemental signatures returned to streams within the regions where they were hatched, but there is very little support for fidelity to natal streams.

From earlier work, we surmised that natal philopatry of recruits (post-larval juveniles) was highly unlikely. Some rivers with large whitebait fisheries have little to no effective spawning habitat and are sink populations (Hickford & Schiel 2011). In this study, however, we sought unique combinations of elements embedded in fish otoliths to reveal more about the nature of the dispersal of fish among rivers, regions and offshore larval pools. One basis for this is that acidic streams are common on the west coast of New Zealand (Collier et al. 1990) because of leaching of naturally occurring organic acids. The 5 diadromous *Galaxiid* species are more tolerant of acidic waters (pH < 5) than many other fishes, and these naturally acidic waterways have significantly higher concentrations of Al and Fe than circumneutral (pH ≈ 7) streams (Greig et al. 2010). This was largely substantiated in our study, with Al and Fe being generally more abundant in otoliths of west coast hatchlings compared to those on the east coast.

With respect to *G. maculatus*, the spawning environment in which hatchlings develop is more complex than just water chemistry. Eggs are laid during spring tides among tidally inundated riparian vegetation where they develop supratidally for 3 to 4 wk before hatching and being swept out to sea on the next sequence of spring tides (Benzie 1968). Furthermore, *G. maculatus* is a lowland species and does not move upstream through rapids and vertical obstacles as do most of the other diadromous galaxiids (McDowall 1998). Many lowland areas have swampy conditions, an accumulation of litter and tannins, as well as aquatic influences from upstream. Much of the variability in the elemental signatures of otoliths within and among streams, therefore, may well have been due to site-specific differences in these influences, right down to the particular qualities of the spawning habitats within individual streams. Using a multivariate approach, the streams tended to separate into east coast versus west coast categories (Fig. 3), driven largely by the influence of the relative values of Al and Fe in the otoliths. Orowaiti stood out as much different from other streams because of relatively high levels of Zn and Ba in hatchling otoliths, and Fox appeared to be intermediate between the west coast and east coast streams.

There were major differences between regions in the natal origins of recruits. Of the 108 otoliths examined from returning whitebait at 4 rivers on the west coast, 45% could be reliably ascribed to west coast origins and 55% could not reliably be ascribed to anywhere. Only 8 (7.4%) of these fish returned to natal streams. No fish with clear east coast origins were found returning to the west coast. These results are consistent with known current and transport mechanisms in the different regions (Chiswell & Rickard 2011). Fifteen returning east coast fish (13.8%) had natal origins on the west coast. There are strong flows through Cook Strait and a good possibility of northwestern areas of the South Island being connected to the east coast, particularly the Awatere River in the north (Chiswell & Rickard 2011). However, no currents flow directly between the east and west coasts of the South Island, making westward transport unlikely. Only 12.8% of the returning whitebait on the east coast could be reliably ascribed to east coast rivers. There are strong flows and many eddies along the east coast that may account for a diffuse offshore larval pool, considerable mixing and diverse origins along the entire coast. In particular, the Southland Current flows close to shore around southern New Zealand and up the east coast, deflecting off Banks Peninsula and eventually moving seaward from around Kaikoura on the north-east coast of the South Island (Chiswell & Rickard 2011). This provides a mechanism for extensive larval mixing and for considerable expatrial dispersal during the ~6 mo of larval development.

Connection of *G. maculatus* populations within ecological time frames seems to be a common occurrence, especially within broad regions of the coastline. Spatially separated populations interact to form a metapopulation as larvae move from one population to another. There are undoubtedly many 'leaky borders' with respect to the larval pools, especially within coastlines, that lead to a widely sourced propagule rain. Very little genetic

structure has been found in *G. maculatus* recruits sampled from around New Zealand (Waters et al. 2000a), again suggesting that there is considerable mixing of populations. This mixing makes it challenging to resolve the origins of fish. Earlier, we hypothesised that small streams may be providing a disproportionate number of the whitebait recruits, since many of the large rivers are effectively sink populations because of their compromised spawning habitat (Hickford & Schiel 2011). The present study showed that many unidentified streams were responsible for returning recruits. This provides at least circumstantial evidence that it is the composite of waterways within regions that contributes to the persistence of *G. maculatus* populations across the region. It also suggests that both local and more distant populations will benefit from increased hatchling production resulting from continuing efforts at in-stream habitat restoration (Hickford & Schiel 2014).

Much of the literature focuses on anadromous salmonids, which begin life in rivers, head to sea to develop and return to natal rivers as mature adults to spawn. *G. maculatus* are amphidromous, leaving natal streams as tiny hatchlings, developing through their larval phases in the sea (McDowall et al. 1975), and then returning to streams as post-larvae. Unlike salmonids, which enter the ocean as larger smolt, *G. maculatus* enter the sea only a few millimetres in length (McDowall 1968) and are largely dependent on tidal and current movement for food and transport in their early life stages (Rowe & Kelly 2009). Their concentrating mechanism is the occurrence of strong river plumes that guide *G. maculatus* recruits back to the coast (McDowall & Eldon 1980). These aggregations can be so dense that trawl fishers for nearshore species have reported their nets being fouled with whitebait. The work to date indicates that river plumes are drawing *G. maculatus* from across diverse larval pools and provide the cues for them to return to the coast (Hickford & Schiel 2011). In these plumes, the larvae may well disperse among the cues from smaller streams along a coastline. It seems to be the total larval pool that matters most for colonisation and persistence of *G. maculatus* populations within regions, regardless of the origins of these fish. Therefore, potential bottlenecks in populations, such as poor spawning habitats, are most likely incremental barriers contributing to the reduction in populations over time. Our study highlights the importance of regional larval pools and taking both local and regional scales into consideration for conservation efforts.

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