

Otolith microchemistry of tropical diadromous fishes: spatial and migratory dynamics

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Otolith microchemistry was applied to quantify migratory variation and the proportion of native Caribbean stream fishes that undergo full or partial marine migration. Strontium and barium water chemistry in four Puerto Rico, U.S.A., rivers was clearly related to a salinity gradient; however, variation in water barium, and thus fish otoliths, was also dependent on river basin. Strontium was the most accurate index of longitudinal migration in tropical diadromous fish otoliths. Among the four species examined, bigmouth sleeper *Gobiomorus dormitor*, mountain mullet *Agonostomus monticola*, sirajo goby *Sicydium* spp. and river goby *Awaous banana*, most individuals were fully amphidromous, but 9–12% were semi-amphidromous as recruits, having never experienced marine or estuarine conditions in early life stages and showing no evidence of marine elemental signatures in their otolith core. Populations of one species, *G. dormitor*, may have contained a small contingent of semi-amphidromous adults, migratory individuals that periodically occupied marine or estuarine habitats (4%); however, adult migratory elemental signatures may have been confounded with those related to diet and physiology. These findings indicate the plasticity of migratory strategies of tropical diadromous fishes, which may be more variable than simple categorization might suggest.

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Key words: amphidromy; Caribbean; Gobiidae; migratory diversity; partial migration; semi-amphidromous.

INTRODUCTION

The larvae of many diadromous tropical fishes spend a period of time in marine habitats before returning to freshwater habitats as metamorphosing post-larvae (Keith, 2003), consistent with an amphidromous or catadromous life history (Myers, 1949). Amphidromous fishes spend the majority of their lives and spawn in fresh water, and larvae are passively transported to marine waters before migrating back to freshwater habitats as post-larvae. In contrast, catadromous fishes undergo downstream migrations, leaving juvenile and adult freshwater habitats to spawn at sea, and larvae hatch in the marine environment before returning to streams.

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The distinction between amphidromy and catadromy is based on the occurrence of adult migration to marine habitats (Myers, 1949), is somewhat subjective and can render distinguishing these two migratory types difficult (Thibault *et al.*, 2007). Diadromous spawning migrations may not occur or could be completed entirely within fresh water (amphidromous), between fresh and estuarine waters (semi-amphidromous), or between fresh and marine waters (catadromous). Further complicating the distinction between these reproductive strategies, diadromous populations may follow multiple migratory forms (Kerr *et al.*, 2009). Reproductive adult migration to marine, estuarine or downstream freshwater reaches, and recruitment from marine to fresh water or from downstream to upstream freshwater habitats could all occur within a single partially amphidromous fish population (Closs *et al.*, 2003).

Analysis of otolith microchemistry is a relatively recent and useful technique for characterizing the dispersal patterns of diadromous fishes between aquatic habitats (Limburg, 1995; Secor & Rooker, 2000; Kerr *et al.*, 2009; Lord *et al.*, 2011; Tsunagawa & Arai, 2011). Fish otoliths record environmental chemistry information throughout an individual's life, and trace element signatures within the otolith have been used to determine natal origins (Thorrold *et al.*, 1998, 2001; Rooker *et al.*, 2008), quantify metapopulation dynamics (Thorrold *et al.*, 2001; Chang *et al.*, 2008) and identify freshwater–marine migratory forms (Tsunagawa & Arai, 2008; Chino & Arai, 2009; Kerr *et al.*, 2009). The advantage of the indirect, otolith microchemistry approach compared to direct approaches to estimate fish dispersal rates, such as mark–recapture, is that otoliths store a record of the entire environmental history of a fish (Campana, 1999). Therefore, the otolith integrates a wealth of information relative to that possible by most direct approaches. Methods that integrate information over a long duration are especially advantageous when dispersal is temporary or episodic (Schilthuizen & Lombaerts, 1994; Wilson *et al.*, 2004). Furthermore, direct methods are often logistically limited to small spatial scales and sample sizes (Koenig *et al.*, 1996), whereas otolith samples can be easily obtained over broad spatial scales with large samples.

The migratory dispersal patterns of tropical diadromous stream fishes are largely unquantified, and evidence to support the categorization of Caribbean diadromous fishes as amphidromous or catadromous has been largely anecdotal (Anderson, 1957; Nordlie, 1981; Phillip, 1993; Winemiller & Ponwith, 1998). Here, otolith microchemistry techniques are applied to describe and quantify the recruit and adult migratory forms that occur within and among Caribbean diadromous fish populations.

MATERIALS AND METHODS

Native stream fish populations were sampled in each of three river basins across the Caribbean island of Puerto Rico, U.S.A., the Grande de Manatí, Sabana and Cañas basins (Fig. 1). Two of the rivers (Grande de Manatí and Sabana) were located on the north side of the island and flow into the Atlantic Ocean, and the third was on the south side of the island (Cañas) and flows into the Caribbean Sea. These rivers were selected to represent a broad geographic distribution across Puerto Rico and because they each contained the full complement of native Puerto Rico stream fish species (Kwak *et al.*, 2007). Fishes at sites within at least two physiographic regions per basin were sampled by pulsed-DC backpack electrofisher (Smith-Root Model LR-24; www.smith-root.com) over 1.5 years from July 2008 to August 2009 (Table I). Four of the most common native stream fishes were collected from each river: bigmouth sleeper *Gobiomorus dormitor* Lacépède 1800, mountain mullet

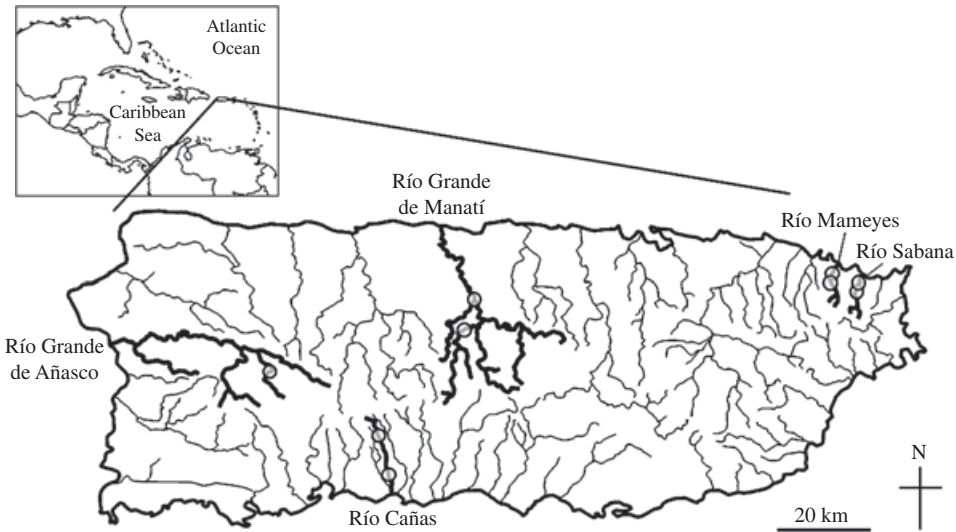


FIG. 1. Map of Puerto Rico, U.S.A., depicting river basins sampled for fish otolith microchemistry. ○, Locations of sampling.

Agonostomus monticola (Bancroft 1834), sirajo goby *Sicydium* spp. and river goby *Awaous banana* (Valenciennes 1837). Additional samples of *G. dormitor* were collected from Río Mameyes, and additional samples of *A. banana* were collected from the Río Grande de Añasco basin (Fig. 1). Samples of only one species were collected in each of these two additional basins (Mameyes and Grande de Añasco) to supplement the more extensive sampling of native assemblages in the primary sampling locations (Grande de Manatí, Sabana and Cañas). Only the otoliths of the largest fishes collected at each site were examined, as these older fishes possessed the longest records of environmental histories within their otoliths. Sagittal otoliths were extracted, cleaned with deionized water to remove all soft tissues and dried. Cleaned otoliths were mounted in epoxy resin (Struers EpoFix; www.struers.com) and sectioned transversely with a diamond blade (Buehler series 15 HC diamond; www.buehler.com). Each sectioned otolith was mounted to a petrographic slide with thermoplastic glue (Crystalbond 509; www.crystalbond.com) and polished with 3 μm diamond slurry to reveal the core. Prepared otoliths were sonicated for 15 min in ultrapure water to remove surface contamination.

The concentrations of Ba, Sr and Ca in each otolith were analysed using a laser ablation inductively coupled plasma mass spectrometer (LA-ICPMS; Cetac LSX-213; 213 nm laser; www.cetac.com) located at the GeoMed Analytical Laboratory at University of Massachusetts. A blank carrier gas mixture of He and Ar was used to introduce ablated material into the inductively coupled Ar plasma. A linear scan ablation (width = 25 μm , rate = 5 $\mu\text{m s}^{-1}$; Chang *et al.*, 2012) was completed for each otolith from the core to the edge, measuring the concentrations of each element. Concentrations of Ba and Sr were expressed as ratios to Ca (Ba:Ca and Sr:Ca) to account for variation in ablation profile and instrument drift. The calcium carbonate standard, MACS-3 (U.S. Geological Survey; crustal.usgs.gov; Wolf & Wilson, 2007), was used to calibrate the LA-ICPMS and convert blank-corrected counts to molar ratios. Calibrations were completed at the beginning and middle of each day to adjust for instrument drift.

Variation in otolith microchemistry may indicate transitions between marine and freshwater habitats, or simply a change in environmental conditions unrelated to fish movement (Elsdon & Gillanders, 2002, 2006; Gillanders, 2002). A difference between the variation in Ba:Ca and Sr:Ca profiles could indicate that environmental factors altered water chemistry (and thus otolith microchemistry) in addition to changes in ambient salinity. To account for water

TABLE I. Number of each fish species sampled in Caribbean insular streams for otolith microchemistry according to physiographic region and river basin

River basin	Coastal plain (0–20 m above sea level)	Foothills (21–70 m above sea level)	Mountains (>70 m above sea level)	Total sampled per basin
<i>Gobiomorus dormitor</i>				
Cañas	10	2	10	22
Grande de Manatí	0	10	9	19
Mameyes	9	10	0	19
Sabana	10	11	0	21
<i>Agonostomus monticola</i>				
Cañas	13	6	11	30
Grande de Manatí	0	8	10	18
Sabana	11	12	0	23
<i>Sicydium</i> spp.				
Cañas	7	5	10	22
Grande de Manatí	0	8	15	23
Sabana	13	12	0	25
<i>Awaous banana</i>				
Cañas	0	6	9	15
Grande de Manatí	0	7	16	23
Grande de Añasco	0	0	16	16
Sabana	1	1	0	2

chemistry and the potential for basin-specific effects in water chemistry, water samples were analysed along a salinity gradient in each basin and tested for basin-specific patterns in otolith microchemistry variation. Triplicate water samples were collected along a salinity gradient from completely fresh water (<0.5 salinity) to at least half sea water (15–30 salinity) at three points in each river basin sampled. Water samples were filtered with a 0.45 µm membrane, acidified to pH < 2 with ultrapure nitric acid and analysed following the procedures described by Dorval *et al.* (2005) and using a dynamic reaction cell ICPMS to quantify concentrations of Ba and Sr. The relationship between concentrations of Ba and Sr (dependent variables) and the corresponding salinity at each sampling location (independent variable) was analysed with linear regression. Each river basin was modelled separately, and significant slope parameters ($P < 0.05$) indicated that Sr and Ba concentrations were related to salinity gradients and that otolith Sr and Ba could be applied as indices of a fish's salinity environment. Intercept parameters indicated regression model predictions in fresh water (*i.e.* 0 salinity), so that the 95% c.i. of intercept parameters were compared to evaluate the potential for basin-specific patterns in freshwater chemistry. Non-overlapping intercept parameter 95% c.i. were interpreted as significant differences.

Each individual fish's profile was stratified into a recruitment period and an adult period, and recruitment and adult patterns were analysed separately. Amphidromous recruitment from marine or estuarine to fresh waters is characterized by a rapid decline in Sr:Ca near the otolith core (Tsunagawa & Arai, 2008; Lord *et al.*, 2011), so that the lowest Sr:Ca value near the core was assumed to represent the point of recruitment to fresh water and this point was used to stratify each individual profile into recruit and adult periods. Individuals with no decline in Sr:Ca near the core were stratified into recruit and adult periods using the average core radius measured in individuals that experienced a Sr:Ca decline in early life stage. Tropical diadromous larvae may move between catchments (Cook *et al.*, 2009, 2010) and use marine habitats (Anderson, 1957; McDowall, 1988; Keith, 2003), where they are less affected by freshwater chemistry, so only the adult periods of otolith microchemistry profiles accurately

represent environmental conditions experienced in riverine habitats. Thus, the analysis of the relationships between otolith microchemistry variation and river basin was restricted to adult periods.

Variation in otolith microchemistry profiles was quantified by calculating the range of Ba:Ca and Sr:Ca for each individual's adult period. Range was selected as an index of profile variability, because it is sensitive to outlying data, such as that produced in otolith microchemistry by episodic movement into varying salinity habitats. The hypothesis that mean Ba:Ca and Sr:Ca ranges were different among basins was tested using a one-way analysis of variance (ANOVA) ($\alpha = 0.05$). The normality of each response variable was tested using the Shapiro–Wilk test, and non-normal variables were transformed with the Box–Cox power transformation. If ANOVA results revealed significant differences, mean Ba:Ca and Sr:Ca ranges were compared among basins using Tukey's test for multiple comparisons. All statistical tests were performed in R (R Development Core Team; www.r-project.org). Agreement between tests on Ba:Ca and Sr:Ca variation was interpreted to support the conclusion that variability was a valid migratory signal, but disagreement between tests on Ba:Ca and Sr:Ca variability supported the conclusion that some unaccounted environmental factor influenced otolith microchemistry.

Recruitment periods were classified as amphidromous if a peak in Sr:Ca was found at the otolith core and Sr:Ca declined by at least 2 mmol mol^{-1} or semi-amphidromous if the change in Sr:Ca was $< 2 \text{ mmol mol}^{-1}$. An absence of high Sr:Ca values could indicate that an individual completed its life cycle within fresh water or that the core was missed during otolith preparation. Therefore, for each individual classified as a semi-amphidromous recruit, the polished and ablated otolith was microscopically re-examined to confirm that the core was sampled by the LA-ICPMS. Only individuals with a confirmed core sample were included in estimates of relative proportions of amphidromous and semi-amphidromous recruits.

The proportion of adults with microchemistry indicating a return to marine or estuarine waters (semi-amphidromy or catadromy) was also quantified. For each species, the threshold marine or estuarine signature was defined as the minimum Sr:Ca value found in otolith cores of the adults identified as experiencing an amphidromous recruitment pattern, and adult periods were classified as semi-amphidromous when two adjacent Sr:Ca data points were greater than the threshold and amphidromous when fewer than two adjacent Sr:Ca data points were greater than the threshold.

RESULTS

A total of 278 diadromous fish otoliths were sampled and analysed for microchemistry, and water samples were collected and analysed from each basin sampled for otolith microchemistry, except from Río Grande de Añasco (36 water samples; Table I). Based on three times the s.d. of the blank He and Ar carrier gas mixture used by the LA-ICPMS, the detection limits for Ba:Ca and Sr:Ca were $0.16 \text{ } \mu\text{mol mol}^{-1}$ and $0.19 \text{ mmol mol}^{-1}$, respectively. Among all species, measured otolith Ba:Ca values ranged from below the detection limit during recruitment periods to $3.18 \text{ } \mu\text{mol mol}^{-1}$ during adult periods, and Sr:Ca values ranged from $11.57 \text{ mmol mol}^{-1}$ during recruitment to below the detection limit during adulthood (Table II). Among all river basins sampled for water chemistry, freshwater Ba concentrations ranged from 0.008 to 0.096 mg l^{-1} , and freshwater Sr concentrations ranged from 0.03 to 0.27 mg l^{-1} . Estuarine Ba concentrations ranged from 0.006 to 0.056 mg l^{-1} and estuarine Sr concentrations ranged from 0.55 to 1.84 mg l^{-1} .

Linear regression results confirmed significant positive Sr and negative Ba relationships between elemental concentration and salinity in water sampled from all rivers (Fig. 2). Therefore, otolith concentrations of these two elements may serve as suitable indices of a fish's movement through salinity gradients. No intercept

TABLE II. Mean radii of otolith recruitment period and whole otolith and threshold marine or estuarine Sr:Ca signatures, estimated from the minimum otolith core values of fully amphidromous recruits. Values greater than the threshold indicate that a fish experienced a marine or estuarine environment when the corresponding otolith section was formed. Otolith recruitment period radii were estimated as the distance from the start of the laser ablation inductively coupled plasma mass spectrometer path to the minimum otolith core Sr:Ca value

Species	Mean recruitment period radius (μm)	Mean whole otolith radius (μm)	Threshold marine Sr:Ca value (mmol mol^{-1})	Range of otolith Ba:Ca ($\mu\text{mol mol}^{-1}$)	Range of otolith Sr:Ca (mmol mol^{-1})
<i>Gobiomorus dormitor</i>	461	1594	3.0	BD–0.71*	BD–6.22*
<i>Agonostomus monticola</i>	479	1138	3.3	BD–3.18*	0.40–11.57
<i>Sicydium</i> spp.	232	590	3.1	BD–1.17*	0.28–11.00
<i>Awaous banana</i>	186	1138	2.8	BD–0.98*	0.41–9.58

*BD, below detection limit; Ba:Ca detection limit = $0.16 \mu\text{mol mol}^{-1}$; Sr:Ca detection limit = $0.19 \text{mmol mol}^{-1}$.

parameters for Sr regression were significantly different among rivers, indicating similar Sr levels in the freshwater habitats of the rivers sampled. The regression intercept parameter fit to Río Cañas Ba values, however, was significantly lower than all other rivers, indicating that fresh water in Río Cañas was reduced in Ba compared to other sampled rivers.

Water chemistry differences were reflected in otolith microchemistry signatures; ANOVA results indicated that variation in otolith Ba:Ca was associated with river basin, whereas variation in otolith Sr:Ca was not (Table III). Further, graphical comparison of otolith Ba:Ca and Sr:Ca temporal profiles showed that dramatic oscillations in Ba:Ca were not reflected in Sr:Ca, and conversely, changes in Sr:Ca did not correspond well with changes in Ba:Ca (Fig. 3). Mean Río Cañas otolith Ba:Ca ranges for adult periods were significantly lower than those of all other basins, and Río Sabana Ba:Ca ranges for adult periods were significantly higher than all other basins. This pattern was consistent among fish species, and indicated that some unknown environmental factor, specific to each river basin, produced variation in otolith Ba:Ca but not in Sr:Ca. Given the extensive literature documenting the relationship between Sr:Ca and ambient water salinity across taxa (Radtke, 1989; Farrell & Campana, 1996; Secor & Rooker, 2000; Walther & Limburg, 2012) and the conclusion of other researchers that Ba in water may cycle temporally, producing false marine Ba signatures in otoliths (Elsdon & Gillanders, 2006), all further otolith data analyses were restricted to Sr:Ca results to elucidate fish migratory patterns.

All species except *A. monticola* appeared to include a small proportion of individuals within populations that never experienced marine conditions during early life stage (Fig. 4). A total of 75 of 81 *G. dormitor*, 58 of 70 *Sicydium* spp. and 45 of 56 *A. banana* otoliths were confirmed to include microchemistry samples from the core, and of those, 9.3–12.1% had an entirely freshwater Sr:Ca recruitment signature (semi-amphidromous recruits) and 87.0–92.7% had an amphidromous recruitment signature (Table IV and Fig. 4). All *A. monticola* otoliths ($n = 72$) had a marine or estuarine amphidromous signature at the core, and core samples were confirmed for all *A. monticola*.

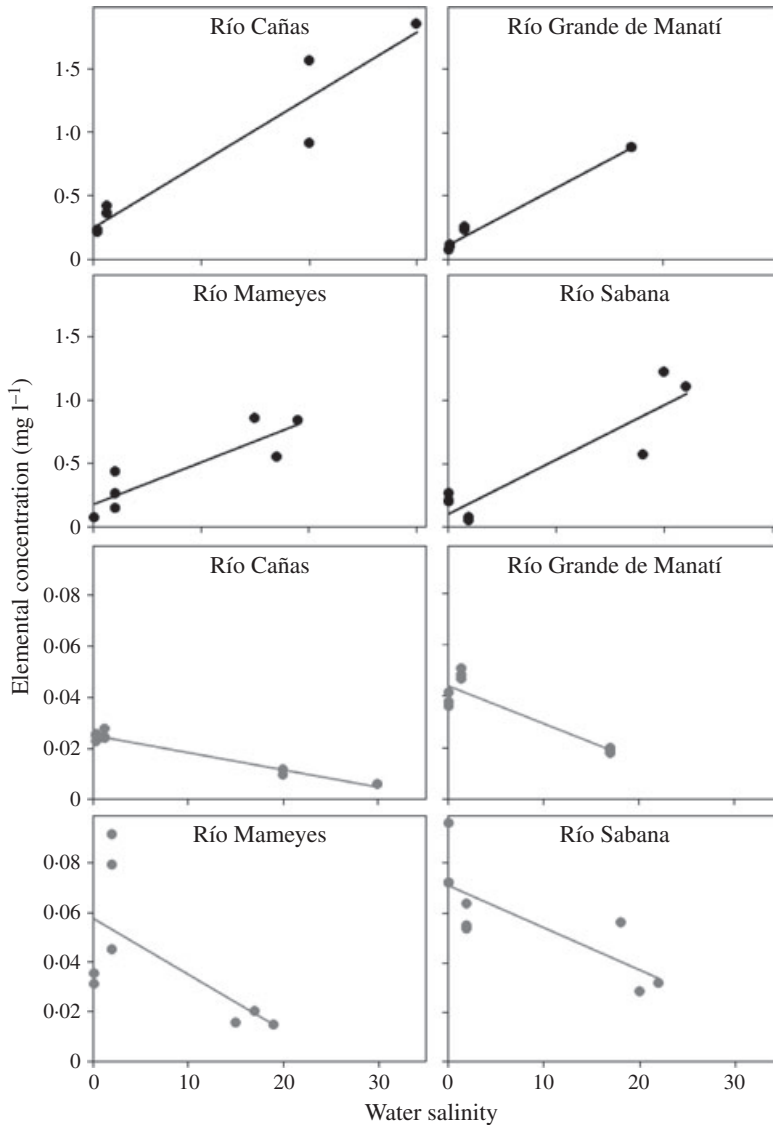


FIG. 2. Strontium (Sr; ● and —) and barium (Ba; ○ and —) water chemistry along a salinity gradient in four of the river basins sampled for fish otolith microchemistry.

Applying the threshold marine Sr:Ca value estimated from otolith core samples (Table II), only *G. dormitor* otoliths, among all species examined, contained evidence that adults experienced marine or estuarine conditions (semi-amphidromous adults). The adult Sr:Ca periods of *G. dormitor* included a continuum of oscillatory to flat profiles, but all *A. monticola*, *Sicydium* spp. and *A. banana* adult periods were relatively flat with no evidence of a return to marine or estuarine conditions. Three of 81 (3.7%) adult *G. dormitor* periods contained marine or estuarine signatures (Fig. 5). Visual assessment of contingency tables revealed no obvious associations

TABLE III. Results of an analysis of variance of adult otolith Ba:Ca and Sr:Ca ranges, testing the association between otolith microchemistry and the basin in which fishes were captured

Species	<i>P</i> -value of the <i>F</i> -statistic	
	Ba:Ca	Sr:Ca
<i>Gobiomorus dormitor</i>	<0.001	0.35
<i>Agonostomus monticola</i>	<0.001	0.22
<i>Sicydium</i> spp.	<0.001	0.42
<i>Awaous banana</i>	<0.001	0.17

between river basins and the fraction of recruit and adult migratory forms among basins.

DISCUSSION

The findings of this study are the first evidence of variation in migratory strategies within Caribbean amphidromous fish assemblages. To be considered amphidromous, fishes must conform to two criteria: (1) occupy and spawn in freshwater habitats during their adult life stage and (2) inhabit marine or estuarine environments during their early life stages (Myers, 1949; McDowall, 1988). A small proportion of semi-amphidromous *G. dormitor*, *Sicydium* spp. and *A. banana* (9–12%) did not show marine signatures in their otolith cores, indicating that they completed early life stages within fresh waters. All *A. monticola*, *Sicydium* spp. and *A. banana* otolith microchemistry adult periods were flat, with no evidence of a return to marine or estuarine waters; however, oscillatory microchemistry patterns were observed in 4% of *G. dormitor* otoliths, indicating periodic movement into higher salinity habitats. These results are evidence that the fish assemblages examined are composed of a range of migratory contingents from amphidromous to semi-amphidromous, and that varying degrees of plasticity exist in amphidromous fish migratory patterns.

Previous definitions of the amphidromous life cycle (Myers, 1949; McDowall, 1988, 2010) suggest that amphidromy is distinguished from catadromy by the lack of a return migration to marine waters for spawning. This definition fails to capture the more nuanced migratory life-history patterns documented here, in which a small proportion of otherwise amphidromous populations may experience marine or estuarine conditions as adults or never experience a fully marine environment before recruitment. Thus, the term ‘semi-amphidromous’ is introduced, after Cronin & Mansueti (1971), Whitfield (2005) and Secor & Kerr (2009), to describe individuals that deviate from the modal pattern within a partially amphidromous population. To be semi-amphidromous as an adult, a fish must occupy and spawn in fresh water as an adult [criterion (1) described above] and inhabit marine or estuarine waters as larvae [criterion (2) described above], with the modification to criterion that adults remain in riverine systems but periodically occupy higher salinity, lower river reaches. To be semi-amphidromous as a recruit, a fish must conform to criteria (1) and (2), with the exception to criterion (2) that recruits never reach higher salinity, lower river reaches before metamorphosis and recruitment into upstream riverine habitats.

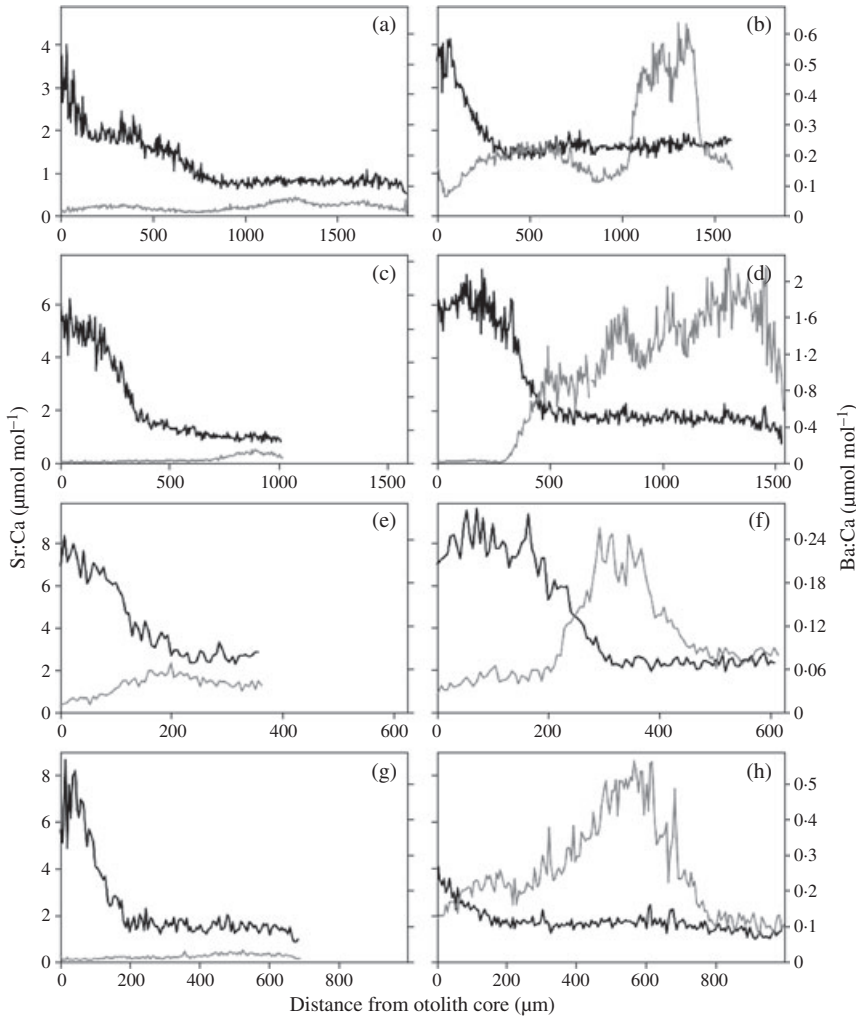


FIG. 3. Characteristic otolith microchemistry profiles for (a, b) *Gobiomorus dormitor*, (c, d) *Agonostomus monticola*, (e, f) *Sicydium* spp. and (g, h) *Awaous banana*, demonstrating discrepancies between Ba:Ca (—) and Sr:Ca (—) profiles. (a, c, e, g) Instances of patterns in Sr:Ca that were not reflected in Ba:Ca and (b, d, f, h) instances of patterns in Ba:Ca that were not reflected in Sr:Ca.

Agonostomus monticola is amphidromous, with all marine recruits and no adult return to the estuary or ocean. *Sicydium* spp. and *A. banana* populations contain contingents that recruited from both marine and freshwater habitats (amphidromous and semi-amphidromous recruitment) but no contingent of migratory adults. *Gobiomorus dormitor* populations consisted of both amphidromous and semi-amphidromous recruit contingents and amphidromous and semi-amphidromous adult contingents; however, marine signals in the migratory adult contingent may have been confounded with dietary or physiological signals. It is noteworthy that only small proportions of populations deviated from an amphidromous migratory pattern, and all populations examined in this study were predominantly amphidromous.

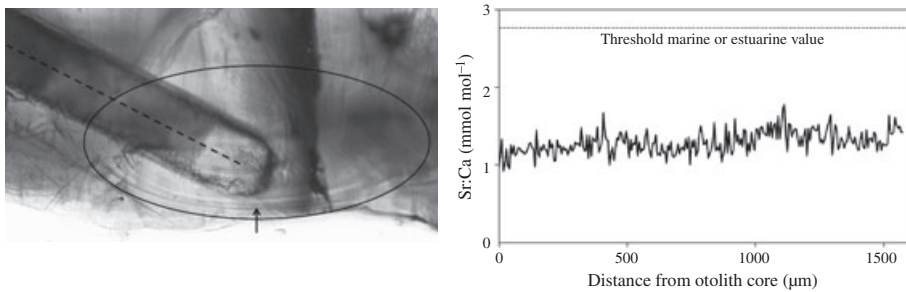


FIG. 4. Example of an amphidromous *Awaous banana* otolith microchemistry profile with no marine or estuarine signature at the core, indicating that the life cycle was completed entirely in fresh water, and corresponding otolith image, showing the location of the otolith core that was sampled by laser ablation inductively coupled plasma mass spectrometer (LA-ICPMS). \circ , the area of the otolith core; \rightarrow , the metamorphosis mark that occurs at recruitment and delineates the otolith core; ----, the path of the LA-ICPMS scan. See Fig. 3 for typical amphidromous *A. banana* otolith microchemistry profiles that include marine or estuarine signals at the core.

Basin-specific patterns were evident in water Ba but not Sr, validating the finding that Ba levels in otoliths were also associated with a fish's river basin. Low Ba in the fresh waters of Río Cañas produced false marine Ba signatures in the otoliths of Río Cañas fishes, and without an understanding of Río Cañas water chemistry from spatially stratified water sampling, these patterns might have been erroneously interpreted as basin-specific migration patterns in diadromous fish assemblages. The large, basin-specific oscillations in otolith Ba:Ca that were observed have been noted by other investigators studying tropical diadromous fishes (Miles *et al.*, 2009; Lord *et al.*, 2011). Previous researchers (Lord *et al.*, 2011), however,

TABLE IV. Per cent of each fish species with otolith microchemistry (Sr:Ca) indicating amphidromous or semi-amphidromous recruit and adult life-history patterns. Amphidromous recruitment included a diadromous migration from marine or estuarine to fresh waters, but semi-amphidromous recruitment did not include a marine larval phase (only a freshwater Sr:Ca signature). Amphidromous adults did not migrate to marine or estuarine waters after recruitment; semi-amphidromous adults periodically returned to marine or estuarine waters. Semi-amphidromous recruits and adults would not include a marine larval phase and adults would periodically return to marine or estuarine waters

Species	Migratory pattern			
	Fully amphidromous (%)	Semi-amphidromous recruits (%)	Semi-amphidromous adults (%)	Semi-amphidromous recruits and adults (%)
<i>Gobiomorus dormitor</i>	87.0	9.3	3.7	0
<i>Agonostomus monticola</i>	100.0	0	0	0
<i>Sicydium</i> spp.	87.9	12.1	0	0
<i>Awaous banana</i>	93.3	6.7	0	0

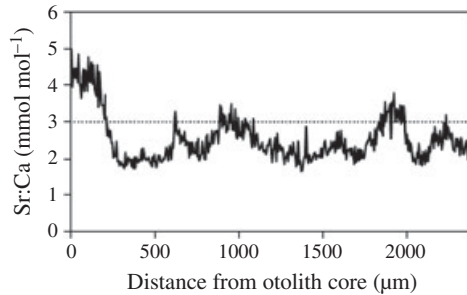


FIG. 5. *Gobiomorus dormitor* otolith microchemistry profile indicating multiple returns to marine or estuarine water., the threshold marine or estuarine Sr:Ca value.

concluded that inconsistency in Ba:Ca within basins was evidence of migratory diversity within populations. In contrast, within species and among fish assemblages, this study documented high within-basin agreement in the magnitude of Ba:Ca ranges. Almost all fishes captured in Río Cañas spanned low Ba:Ca ranges, and almost all fishes captured in Río Sabana showed high Ba:Ca ranges. The patterns in Ba:Ca were clearly related to the basin in which a fish was captured and not necessarily longitudinal fish movements. Further complicating interpretation of otolith Ba:Ca as an index of salinity, Ba has a non-linear relationship with salinity, and intensive spatial sampling of water chemistry is required to identify mid- to low-salinity peaks in ambient Ba concentration and thus interpret variation in otolith Ba:Ca (Walther & Limburg, 2012). Basin-specific patterns in otolith microchemistry are commonly used to identify natal source populations (Thorrold *et al.*, 1998, 2001; Walther *et al.*, 2008) and are related to complex interactions of geological and environmental factors (Walther & Limburg, 2012). Puerto Rico is composed of many geological formations, including volcanic material in the central mountains and karst limestone (carbonate bedrock) near the coast (Kaye, 1957), each of which undergoes different weathering patterns and carries different trace element signatures. Further, seasonal weathering patterns on the hydric north side of the island differ from those of the xeric south side, which falls in the rain shadow of the Puerto Rico Cordillera Central mountain range (Hunter & Arbona, 1995). Thus, Ba:Ca is not always a reliable indicator of freshwater–marine movements in the otolith microchemistry of tropical insular fish assemblages. Validation by intensive spatial and temporal sampling of water chemistry, accounting for both seasonal and longitudinal variation in Ba would enhance interpretation of Ba:Ca microchemistry profiles in future research. An accurate model of water chemistry variation is central to the interpretation of otolith microchemistry (Rieman *et al.*, 1994; Campana, 1999; Elsdon & Gillanders, 2006; Elsdon *et al.*, 2008).

Many *G. dormitor* adult microchemistry periods showed evidence of a return to a moderately higher salinity environment, but only 4% of *G. dormitor* adult periods contained values that might be interpreted as a return to a fully marine or estuarine environment. All *A. monticola* and gobiid adult periods were flat with no return to high Sr:Ca levels. Two general hypotheses may explain these patterns. (1) *Gobiomorus dormitor* populations contain contingents that either remain in fresh water (dominant contingent) or periodically migrate to marine or estuarine habitats

(marginal contingent). The function of such a migration may be related to feeding, reproduction, density-dependent mechanisms or displacement from freshwater habitat by disturbance (e.g. drought or flood). *Agonostomus monticola*, *Sicydium* spp. and *A. banana* never experience higher salinity environments after recruitment to fresh water. Hypothesis 1 is consistent with a semi-amphidromous adult life history for *G. dormitor* and a fully amphidromous adult life history for *A. monticola* and the gobiids. (2) No species in the fish assemblages studied occupy marine or estuarine environments after recruitment into fresh water, but a contingent of *G. dormitor* populations feeds on a periodically available prey source that is enriched in marine elements, such as Sr. Hypothesis 2 is consistent with an amphidromous adult life history for all fishes sampled.

Several authors have concluded that *G. dormitor* and *A. monticola* are catadromous and make reproductive migrations to the mouths of rivers (Anderson, 1957; Phillip, 1993; Winemiller & Ponwith, 1998), but very little direct information about individual fish movements has been published. In related research, direct evidence from tagging fishes in Río Mameyes indicated that *G. dormitor* and *A. monticola* were quite sedentary, and estimated probabilities of moving from high to low elevations were minimal (Smith & Kwak, 2014). Even after a major flood disturbance during the spawning season, tagged fishes did not redistribute to lower reaches of the river. This is in contrast to the finding presented here that *G. dormitor* otoliths, including samples from Río Mameyes, contain marine or estuarine signals, indicating that a small proportion of populations may periodically occupy marine or estuarine habitats. Otolith microchemistry, however, reveals little about the timing of these movements, and it is likely that such a small proportion of migrants (4%) could be overlooked by monitoring tagged fishes only during short, discrete periods (spawning seasons). Thus, evidence from otolith microchemistry and tagging studies should be viewed as complementary sets of evidence. Movements from fresh to estuarine waters could be overlooked if they occurred in a very small fraction of the tagged population, were episodic or if a movement occurred when tagged fishes were not monitored (e.g. during flooding events).

Sicydiine post-larval gobies are known to recruit to fresh water in large pulse migrations of several millions of individuals (Erdman, 1961; Castellanos-Galindo *et al.*, 2011), and predatory fishes that aggregate with the post-larval migrations to feed attract local fishers in Puerto Rico (pers. obs.). *Gobiomorus dormitor* is predatory, and the most common items in its diet are decapods and small fishes (Winemiller & Ponwith, 1998; Bacheler *et al.*, 2004). It is highly probable that *G. dormitor* opportunistically prey on recruiting diadromous post-larvae as they migrate up the river, transporting marine elements such as Sr. Fish otoliths incorporate diet information, in addition to a record of environmental and physiological conditions (Kennedy *et al.*, 2000; Buckel *et al.*, 2004; Walther & Thorrold, 2006; Sturrock *et al.*, 2012); thus, *G. dormitor* could remain in fresh water and feed on a seasonally available prey enriched in marine elements, producing seasonal oscillations in otolith microchemistry that might otherwise be interpreted as a seasonal migration to a high-salinity environment. Finding this phenomenon (*i.e.* marine signatures in adult *G. dormitor*) in a small proportion of the individuals examined, however, may indicate a size-selective or behavioural feeding mode that may limit the number of *G. dormitor* that utilize the seasonal food source (Bacheler *et al.*, 2004) or it may further support an adult marine migratory hypothesis.

On the basis of direct evidence from tagging *G. dormitor* and *A. monticola* and the indirect otolith microchemistry patterns for the majority of the native stream fish assemblage, the native freshwater fishes in the Caribbean insular streams examined here are amphidromous, not catadromous, and adults generally do not return to marine or estuarine nursery habitats. No adult *A. monticola*, *Sicydium* spp. or *A. banana* otolith microchemistry periods showed marine or estuarine signatures, but a small fraction of *G. dormitor* adult periods did. The origins of the marine signatures in *G. dormitor* adult periods, whether migratory, dietary or physiological, remain uncertain; however, if the marine signatures are the result of a migratory pattern, the affected proportion of adults is relatively small, and the modal migratory life history of *G. dormitor* is amphidromous.

Recent findings indicate that many diadromous populations are composed of a diversity of migratory contingents that conduct full, partial or no migration between marine and fresh waters (Kerr *et al.*, 2009). Migratory diversity within populations can confer resilience to frequent disturbance (Secor, 2007) such as the flood and drought regimes that commonly affect diadromous assemblages on tropical islands (Covich *et al.*, 2006). Here too, plasticity in diadromous populations that follow multiple permutations of the typical migratory pattern was documented. Larvae (except *A. monticola*) may be amphidromous or semi-amphidromous, completing early life stages in either marine or fresh waters, and adult populations of *G. dormitor* may contain a small proportion of migratory, semi-amphidromous adults. Future research on dispersal and migration in amphidromous fish assemblages focusing on the relationships between amphidromous and semi-amphidromous recruitment and expatriation *v.* natal larval dispersal would elucidate the dynamics of the migratory plasticity revealed in this research. River basin-specific factors, such as habitat quality and the degree of connectivity between fresh and marine waters, substantially influence larval dispersal patterns (Cooney & Kwak, 2013). Although recruitment between rivers and islands is known to occur (Cook *et al.*, 2009), reduced streamflow from drought or water extraction might facilitate semi-amphidromous recruitment (natal dispersal), and the seasonal formation of terminal estuaries may promote larval retention and the closure of populations. The dynamics of larval movements between individual source and sink subpopulations within larger Caribbean metapopulations are the likely primary forces structuring assemblages of native diadromous fishes on tropical islands (McRae, 2007; Ramírez *et al.*, 2012), and a greater understanding of the factors that determine metapopulation, source-sink and migratory dynamics will inform enhanced conservation of tropical aquatic ecosystems and communities.

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