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Otolith barium profiles verify the timing of settlement in a coral reef fish

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ABSTRACT: Otolith microstructure has been shown to record valuable information about fishes including age, growth, and the timing of life history transitions, while microchemical analysis can reveal information about environmental history, dispersal, and migration. For the bluehead wrasse *Thalassoma bifasciatum*, a common coral reef fish on an oceanic island, we examined whether otolith chemistry could be used to identify the timing of settlement from the pelagic larval phase to the reef-based juvenile phase. This species has a distinct settlement mark visible in its otolith microstructure. Using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS), we found that Ba:Ca ratios increased abruptly at the time of settlement. On average, Ba:Ca ratios were 6.5× greater in the juvenile than the larval phase. Other elements (Mg, Mn, and Sr) also displayed ontogenetic changes in concentration; however, those changes were not associated with the settlement mark. We demonstrate the potential utility of otolith chemistry as a method to identify the timing of settlement (and thereby the larval duration) in other marine fishes with similar early life histories, whose otoliths may not produce distinct settlement checks or those whose settlement stage larvae may not be captured by other means.

KEY WORDS: Larval–juvenile transition · Laser ablation inductively coupled plasma mass spectrometry · LA-ICPMS · Otolith chemistry · Otolith microstructure · Pelagic larval duration

INTRODUCTION

Calcified structures of marine organisms, such as fish otoliths or gastropod statoliths, are primarily produced for aiding biological functions such as balance or hearing. By analyzing the internal matrix of these structures, biologists have discovered that they contain a wealth of information about species' life histories (Campana & Thorrold 2001, Arkhipkin 2005). Otoliths grow throughout the life of a fish, differentially depositing calcium carbonate and protein (Campana & Neilson 1985), which results in the formation of thin daily increments (Pannella 1971). These growth rings provide information about age, in addition to growth rates, because the distances between increments are proportional to somatic growth for many fish species (Campana & Neilson 1985). Abrupt changes in otolith microstructure may reflect changes in environmental conditions or in life history, including hatching, settlement, metamorphosis, sex change, or habitat shifts (Victor 1982, 1986, Brothers et al. 1983, Wilson & McCormick 1997, 1999, Walker & McCormick 2004).

One of the most valuable properties of otoliths for fish scientists is the record of larval duration, because the timing of settlement, i.e. habitat transition from a pelagic to a benthic stage, is often indicated by changes in otolith microstructure, which are often associated with metamorphosis (Wilson & McCormick 1999). Knowledge of the larval duration may allow researchers to reconstruct the temporal patterns of larval production and recruitment (Victor 1982, Wilson & McCormick 1997), in addition to addressing dispersal potential, by using larval duration as a proxy (Lester & Ruttenberg 2005). However, some species of fish do not possess distinct settlement marks (e.g. tropical parrotfish, temperate rockfish), and researchers have to infer larval durations from the otoliths by capturing newly settled fishes on artificial reefs or settlement-stage fish with devices such as light traps, channel nets, or towed nets (Victor 1991). These techniques

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may potentially underestimate larval duration because presettlement-stage fishes may also be captured. In addition, many species cannot be adequately sampled at the settlement stage because of avoidance, rarity, or depth. Analysis of chemical otolith composition may help to alleviate these problems by resolving the timing of settlement in species lacking abrupt microstructural changes and/or those species that may only be captured as older juveniles or adults.

Otolith chemistry has the potential to reveal the environmental history experienced by fishes, because the incorporation of certain elements into the calcium carbonate matrix depends on the chemical and physical properties of the surrounding waters, e.g. temperature and salinity (Campana 1999, Bath et al. 2000, Elsdon & Gillanders 2002, 2003a). This includes information about larval dispersal (Swearer et al. 1999), migration (Secor et al. 1995, Elsdon & Gillanders 2003b), and geographic separation or mixing of fish stocks (Campana et al. 1994, 1999). For example, Bath et al. (2000) showed experimentally that Sr:Ca and Ba:Ca ratios in otoliths correspond to their ratios in ambient waters, and that temperature significantly influences incorporation of Sr, but not Ba. Abrupt changes in the chemical composition of otoliths, especially Sr:Ca and Ba:Ca ratios, are often attributed to distinct changes in habitat, such as migrations from seawater to freshwater (Elsdon & Gillanders 2003b). For fish inhabiting open coasts, if the chemical signature of waters bathing nearshore reefs differs from those experienced by larvae just a short distance offshore, then otolith chemistry combined with the chronological record of the otolith may serve to identify the timing of settlement. Hale & Swearer (2008) recently verified for a diadromous fish that the timing of settlement (adoption of freshwater residency) correlated with (1) distinct changes in otolith microstructure, (2) a spike in Ba:Ca ratio, (3) a precipitous drop in Sr:Ca ratio, and (4) a change in Sr isotope ratios.

For this study, we used laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) to investigate whether trace metal profiles can be used to identify the timing of settlement in a coral reef fish that spends its entire life cycle in saltwater. Patterson et al. (2005) reported preliminary evidence for a tropical damselfish suggesting that increased Ba:Ca ratios may help to identify migration into nearshore waters at the end of the larval phase. Here we show for juvenile bluehead wrasse *Thalassoma bifasciatum* that the timing of settlement, as indicated by a previously verified settlement mark (Victor 1982), is associated with a significant increase in Ba:Ca ratio that remains elevated for over a month during the early juvenile phase on nearshore reefs. Temporal changes in the profiles of other elements were not associated with the larval–juvenile transition. Therefore otolith chemistry may provide a useful technique in identifying the timing of settlement in other purely marine fishes with similar early life histories.

MATERIALS AND METHODS

Species background. The bluehead wrasse *Thalassoma bifasciatum* is a carnivorous labrid fish common on shallow reefs throughout the Caribbean. Settlement occurs after a pelagic larval duration (PLD) of approximately 47 d (range 35 to 78 d) (Caselle & Warner 1996, Sponaugle & Cowen 1997). Upon settlement, bluehead wrasse undergo metamorphosis over a 3 to 5 d nonfeeding period while sequestered in the sand or reef substrate (Sponaugle & Cowen 1997). This metamorphic period coincides with the interruption of distinct daily otolith increment formation resulting in a visible settlement mark and metamorphic band of faint increments (Type II settlement-mark structure sensu Wilson & McCormick 1999) (Fig. 1a). Upon the completion of metamorphosis, newly emerged recruits prefer benthic, low-relief rubble or coral crevices and initially remain solitary or in small groups for several weeks before joining juveniles to shoal in the water column. Adults show site fidelity, although some individuals will migrate up to 1.5 km from their sleeping sites for mating purposes (Warner 1995).

Sampling sites. St. Croix, US Virgin Islands (17.75° N, 64.75° W) is a relatively small (40 × 7 km) island in the northeast Caribbean Sea, 90 km southeast of Puerto Rico. We used aquarium dipnets to collect approximately 1 mo postsettlement-stage juveniles from 2 opposite fringing reefs on the leeward (Butler Bay) and the windward shore (Jacks Bay). Both sites were located near the outer reef slope and were primarily composed of flat coral pavement with sparse patches of living and dead coral, interspersed with patches of rubble and sand (for details see Caselle & Warner 1996). Collections occurred within 100 m of shore. Water depth increased rapidly just offshore. These sites were chosen because they receive consistent levels of recruitment (Hamilton et al. 2006) and are located at opposite ends of the island, potentially bathed by waters with distinct chemical signatures (Swearer 2000). Juveniles were collected from continuous reef at 6 to 10 m depth by targeting the appropriate size classes using known size-at-age relationships (Caselle et al. 2003). Collections occurred in July and August of 2001.

Otolith elemental analysis. Otoliths were removed and cleaned of adhering tissue and stored dry. For 90 fish (Butler Bay: n = 44; Jacks Bay: n = 46), the left sagitta was mounted sulcus side up on plastic slides using low viscosity resin (Epo-Thin epoxy resin,

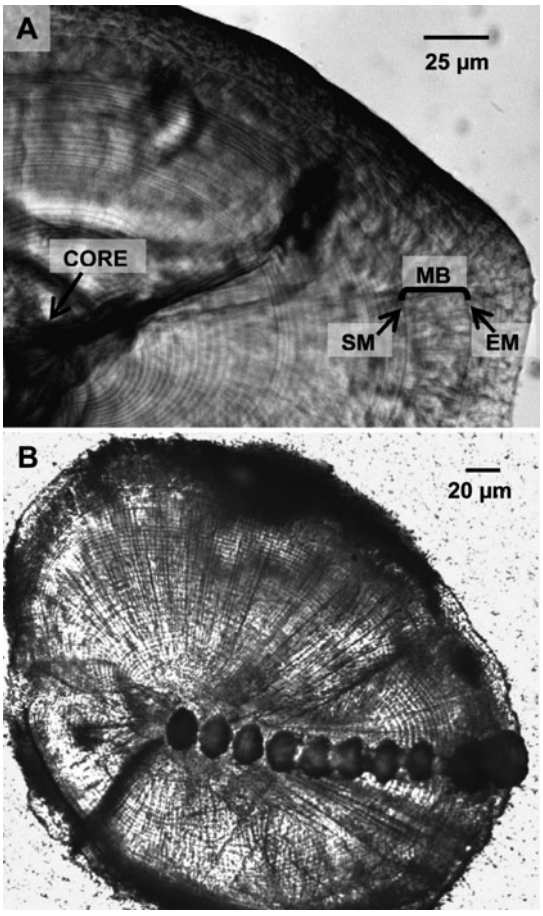

Fig. 1. *Thalassosoma bifasciatum.* Otoliths of recently settled juveniles. Concentric rings in the otolith matrix represent daily increments used to determine age and measure growth trajectories. (A) Ca. 1 wk postsettlement otolith showing the location of the core, settlement mark (SM), metamorphic band (MB), and emergence mark (EM). (B) Series of pits (approximately 20 × 30 µm in diameter) ablated by laser to excavate material for LA-ICPMS chemical analysis

Buehler). Sagittae were polished to within 5 to 15 µm of the core using a lapping wheel and diamond polishing film (9 and 3 µm grain size, 3M®) to expose inner growth layers. To remove contaminants and organic material from the otolith surface prior to analysis, sagittae were rinsed in ultra pure water (N-pure, resistivity >18.1 MΩ), soaked in semiconductor grade 15% H_2O_2 buffered with Suprapur 0.05 N NaOH in acid-leached plastic trays for 1 h, rinsed again in N-pure, soaked and sonicated 3 times in N-pure for 5 min, rinsed a final time with N-pure and air dried in a class-100 (ISO 5) flow bench. We used a Finnegan MAT Element 2 sector field ICP-MS for chemical analysis with VG-UV microprobe Nd:YAG 266 nm laser ablation system for sample induction into the ICPMS, for details see Warner et al. (2005).

We ablated and analyzed the composition of individual pits along the longest axis from the core to edge of the otolith (Fig. 1). For ablation, 8 laser pulses of 0.1 mJ at 3 Hz were used, which created a pit ~30 µm in diameter and ~10 µm deep. Before acquiring data, we pre-ablated using 2 laser pulses to remove any surface contamination. For each sample, 6 to 9 pits were ablated between the core and the settlement mark and each pit was categorized into one of 6 developmental periods (core, beginning, early, middle, late and end) based on the fraction of PLD elapsed. Fig. 1B shows an otolith of a recently settled individual. We determined the PLD of each individual, as well as the age range overlapping each pit, by counting daily growth rings using a compound microscope at 400× and an image analysis system (ImagePro 4.5, Media Cybernetics). If several pits fell within one developmental period, the concentrations of each metal were averaged. Another 4 to 8 pits were ablated between the settlement mark and edge of the otolith, corresponding to the reef-associated juvenile period. Otolith age determination revealed that juveniles had spent approximately 40 ± 1.5 d (range: 20 to 77 d) on the reef at the time of collection.

In each sample pit, we collected counts for the isotopes ^{24}Mg, ^{48}Ca, ^{55}Mn, ^{88}Sr, and ^{138}Ba. Mg and Mn were collected in medium resolution mode (R = 3000) because of molecular interferences, while Sr and Ba were collected in a second vertical ablation pit in low resolution mode (R = 300). Molar ratios of analyte to Ca were calculated using the ratio of each isotope to ^{48}Ca and an elemental mass bias correction calculated from calibration standards (repeated after every 3 to 5 otoliths) with known analyte-to-Ca ratios. We analyzed solid glass standard reference material from the National Institute of Standards and Technology (NIST 612) along with the samples to maintain instrument analytical precision; estimates of precision and limits of detection are in Table 1.

Data analysis. We plotted the temporal trajectories for ^{24}Mg, ^{55}Mn, ^{88}Sr, and ^{138}Ba from the beginning of the larval period, through the larval–juvenile transition, and up to 1 mo postsettlement. We looked for abrupt changes in metal concentrations at the larval–juvenile transition to assess whether any of the 4 metals could serve as an indicator of settlement from the pelagic larval to the benthic-associated juvenile stage. To compare differences in the metal concentrations of the larval and juvenile periods, we used linear mixed-effects models (using the lme4 package in R) (Bates et al. 2008). We used this approach because data from the larval and juvenile periods came from the same individual and were therefore non-independent. A linear-mixed model approach allowed us to include a random effect for each fish with an independent covariance structure (similar to repeated-measures analysis). For each element, we compared

Table 1. Estimates of precision (%RSD; analyzing glass standard reference material NIST 612) and limits of detection (LOD; analyzing a blank solution) for the analyzed metals in proportion to calcium. LOD were calculated as 3 × SD of blanks

Metal	RSD (%)	LOD (ratio of molar concentrations metal:Ca)
Mg	4.8	0.027 mmol mol^{-1}
Mn	4.7	0.738 µmol mol^{-1}
Sr	8.4	0.059 mmol mol^{-1}
Ba	4.6	0.167 µmol mol^{-1}

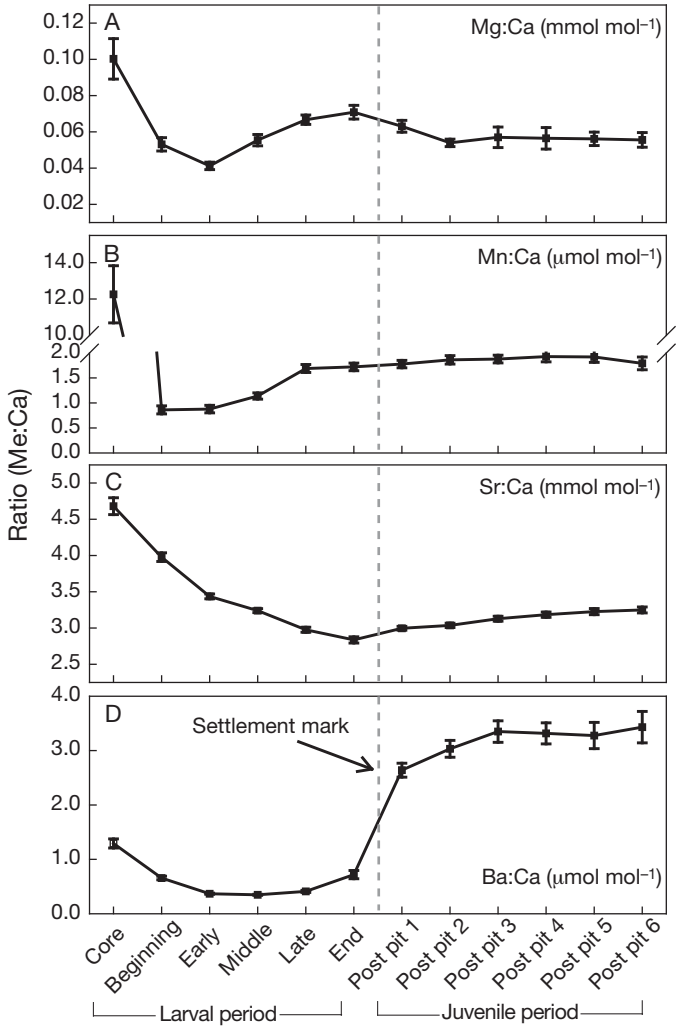

Fig. 2. *Thalassosoma bifasciatum.* Metal concentration profiles obtained from LA-ICPMS analysis extending across the larval and juvenile otolith section. Mean metal concentrations given as molar ratios of metal:calcium (Me:Ca) ± 1 SE for each ablation pit from larval to juvenile section. Dashed gray line represents the settlement mark, i.e. the timing of transition from the pelagic larval to the reef-associated juvenile phase. (A) Magnesium (Mg), (B) manganese (Mn), (C) strontium (Sr), and (D) barium (Ba)

whether average elemental signatures differed between periods, sites, and their interaction (i.e. whether the magnitude of difference in elemental signatures between the larval and juvenile periods differed for fish that settled to the 2 sites). We tested for significant differences among sites to account for this level of variation and to assess whether the changes in elemental concentrations were consistent at 2 sites.

RESULTS

Compared to the Ca concentrations of the non-core larval portions of the otolith, the other 4 metals were enriched in the core. In particular, Mn:Ca ratios were 10× greater in the core than in the adjacent early larval phase pits (see Ruttenberg et al. 2005 for discussion of core enrichment). We excluded the core (i.e. natal) pit from the calculations of average elemental concentrations in the larval phase in order to preclude potential maternal influences on the chemical signature of the pelagic larval phase.

We examined temporal profiles of the 4 metals through time, seeking evidence of abrupt changes in concentrations that coincided with the settlement mark (Fig. 2). Profiles of Mg:Ca indicated no consistent change in concentration between the larval and juvenile periods. Mn:Ca ratios peaked at the core and then decreased precipitously in the beginning and middle of the larval period, before rising to constant levels in the late larval through the juvenile periods (this increase happened well before settlement). Sr:Ca ratios changed throughout ontogeny, decreasing from the core to the settlement mark and then gradually increasing. Ba:Ca ratios indicated a pattern of cross-shore larval development with elevated concentrations at the beginning and end of the larval phase. At the end of the larval phase, Ba:Ca ratios spiked abruptly corresponding with the transition from pelagic larval life to the demersal juvenile phase (Fig. 2d).

Linear mixed-effects models detected significant differences in the average ratios of Mn:Ca, Sr:Ca, and Ba:Ca between the larval and juvenile portions (Table 2). On average, concentrations of Mn:Ca and Ba:Ca were significantly higher in the juvenile than the larval portions (excluding the core), while ratios of Sr:Ca were significantly lower (Fig. 3); however, these patterns masked the interesting temporal trajectories (Fig. 2). Ba:Ca ratios were 6.5× higher on average in the juvenile portion than the larval portion and 3.7× higher in the first postsettlement pit than the last presettlement pit. In addition, approximately 95% of the individuals we sampled showed a minimum of a doubling in the Ba:Ca ratio between the end of the larval phase and the start of the juvenile phase.

We also detected site-level differences in Sr:Ca and Ba:Ca ratios in the juvenile portion, with significantly higher concentrations of both elements in the otoliths of fish collected at Jacks Bay (Table 2). However, site-level differences were much lower than the differences between the larval and juvenile periods (Fig. 3). Given the profile of Ba and the dramatic spike in Ba:Ca ratios coincident with the microstructural settlement mark, Ba appears to be particularly good for discriminating the timing of settlement and the transition from an open ocean to an on-reef chemical signature in the otoliths of bluehead wrasse.

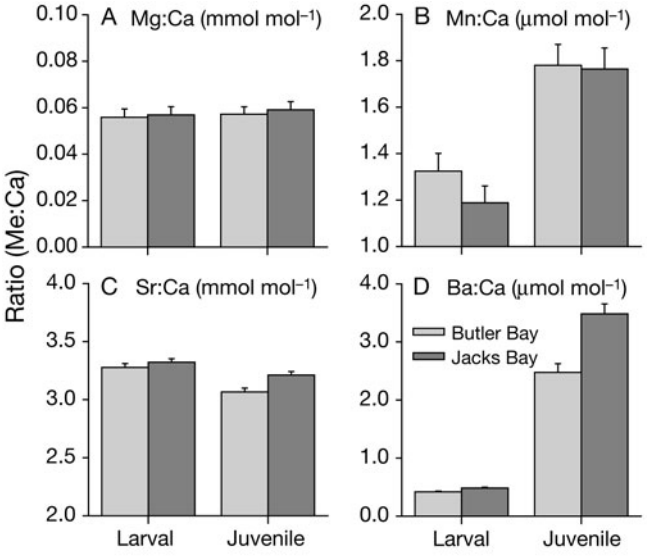

Fig. 3. *Thalassosoma bifasciatum.* Mean metal concentrations in the larval and juvenile otolith portions given as molar ratios of metal:calcium (Me:Ca) ± 1 SE. Comparison by sampling site: Butler Bay (n = 46) and Jacks Bay (n = 44). (A) Magnesium (Mg), (B) manganese (Mn), (C) strontium (Sr), and (D) barium (Ba)

DISCUSSION

Distinct changes in otolith microstructure are often associated with life history transitions, and structural marks are commonly used to estimate larval durations (Victor 1982, Brothers et al. 1983, Wilson & McCormick 1997, 1999). Otolith chemistry, paired with daily increment analysis, may provide a unique means to pinpoint the timing of settlement for species that do not have distinct structural settlement marks in their otoliths, or those whose putative settlement marks have not been validated. (Very few settlement marks have been validated, see Victor 1982, 1983, Wilson & McCormick 1997 for examples.) For those species that are not readily captured at the end of their larval period or that may only be captured and identified as juveniles or adults, techniques in otolith chemistry may help to resolve the timing of movement from the offshore-pelagic to the nearshore-demersal phase.

Otolith Ba:Ca profiles appear to be well suited to identify the timing of settlement in reef-associated fishes inhabiting open coasts, even on oceanic islands.

Table 2. *Thalassosoma bifasciatum.* Juveniles collected at Butler Bay and Jacks Bay, St. Croix, USVI. Results of linear mixed-effects models comparing average otolith metal concentrations from the larval to the juvenile portion. Models include a random effect for Fish (random effect on intercept) and Fish × Period (time effect varies within an individual) to allow for an independent covariance structure similar to repeated-measures analysis. Average metal concentrations over the larval portion do not include the natal core part of the otolith, i.e. the core pit. Data were log transformed to meet model assumptions of normal residuals

Metal	Fixed effects	Estimate	SE	*t*-value	p	Random effect	Variance	SE
Mg	Intercept	−2.776	0.0585	−47.49	**<0.001**	Fish	0.121	0.348
	Period	0.001	0.0464	0.03	0.98	Fish × Period	0.037	0.192
	Site	0.062	0.0818	0.76	0.45	Residual	0.029	0.170
	Period × Site	−0.028	0.0650	−0.43	0.668			
Mn	Intercept	1.780	0.0917	19.41	**<0.001**	Fish	0.290	0.538
	Period	−0.456	0.0775	−5.88	**<0.001**	Fish × Period	0.104	0.323
	Site	−0.015	0.1282	−0.12	0.90	Residual	0.079	0.282
	Period × Site	−0.120	0.1083	−1.11	0.27			
Sr	Intercept	1.121	0.0099	113.55	**<0.001**	Fish	0.002	0.049
	Period	0.066	0.0128	5.15	**<0.001**	Fish × Period	0.004	0.061
	Site	0.047	0.0138	3.38	**<0.01**	Residual	0.002	0.042
	Period × Site	−0.033	0.0180	−1.83	0.07			
Ba	Intercept	0.832	0.0585	14.22	**<0.001**	Fish	0.118	0.344
	Period	−1.708	0.0562	−30.36	**<0.001**	Fish × Period	0.075	0.274
	Site	0.356	0.0819	4.35	**<0.001**	Residual	0.032	0.179
	Period × Site	−0.208	0.0787	−2.646	**<0.01**			

The spike in Ba:Ca ratios upon settlement is probably not related to a change in diet following metamorphosis, because 99% of the Ba is absorbed from the water via the gills during normal respiration (Walther & Thorrold 2006). Therefore, the rapid increase in Ba:Ca upon settlement likely reflects a distinct change in water chemistry directly over nearshore reefs. Ba in seawater follows a nutrient-like distribution and its main sources are river runoff and upwelling of Ba-rich deep water (Chan et al. 1977). Consequently, terrestrial runoff, seepage of groundwater, or weathering and erosion may elevate Ba levels in the waters bathing reefs (e.g. Prouty et al. 2008). Localized upwelling may also occur in nearshore waters on oceanic islands due to processes such as internal waves and island wake eddies (Wolanski 1986). Finally, changes in elemental composition could be a result of either changes in the mineral:protein ratio or ontogenetic shifts in physiology during larval metamorphosis (e.g. Otake et al. 1997), which could influence the incorporation rate of elements within otoliths. Such effects would likely be strong for elements that are highly regulated, e.g. Mg (low distribution coefficient) (Swearer 2000), and we would expect stronger regulation (i.e. lower Ba:Ca) after metamorphosis. Given what we know about environmental effects on Ba incorporation into biogenic carbonates (Campana 1999, Bath et al. 2000, Elsdon & Gillanders 2003a), changing physiology at metamorphosis is a less likely explanation than differences in water chemistry between 2 distinct habitats.

We did not detect abrupt concentration changes in any other metal associated with the larval–juvenile transition. While Mn:Ca ratios were generally greater in the juvenile than the larval portion, the increase in Mn:Ca occurred prior to settlement, while individuals were still developing offshore. We also detected changes in the Sr:Ca profile with concentrations steadily decreasing during the larval portion and then increasing in the juvenile portion. However, this pattern may most likely be attributed to ontogenetic changes in otolith growth rates influencing the distribution coefficient or the rate of incorporation of metals that substitute for Ca in the aragonite matrix (Mugiya & Satoh 1995, see Swearer 2000 for similar patterns). Otolith growth increases throughout the larval period, and progressively decreases in the juvenile period for bluehead wrasse (Searcy & Sponaugle 2000, Hamilton 2008). This otolith growth pattern reflects the Sr:Ca profiles we detected in this study because Sr incorporation is inversely related to otolith growth. We do not expect environmental effects to produce this pattern because concentrations are fairly constant in seawater and Sr:Ca in otoliths generally reflects differences in temperature and salinity (Campana 1999, Bath et al. 2000, Elsdon & Gillanders 2002). Physical properties of St. Croix waters in the top 50 m are unlikely to differ greatly between nearshore reefs and locations further offshore (Swearer 2000).

The presence of a settlement mark and the chronological properties of otoliths allowed us to attribute the abrupt increase in Ba:Ca profiles to settlement. This rapid rise in Ba:Ca to stable levels at the beginning of the juvenile period represents a 6.5× increase on average. Ba:Ca ratios in the juvenile reef-based phase were ~3× higher than those of the otolith core, which is formed at site of hatching. This result is not surprising because bluehead wrasse are broadcast spawners and otolith formation occurs 2 d after fertilization (Victor 1982), when larvae are likely carried offshore by currents. Additionally, the strong maternal effects on natal otolith chemistry (Thorrold et al. 2006) suggest that core chemistry may not reliably reflect the environment (Warner et al. 2005).

Changes in trace element concentrations may be associated with important transitions in life history or habitat. There exists a long history of using changes in Sr:Ca concentrations to identify the timing of migration of anadromous fishes between oceanic and freshwater environments (e.g. Secor et al. 1995, see Elsdon & Gillanders 2003b for a review). In South Australia there is recent evidence for *Galaxias maculatus*—a diadromous fish with a sea-going larval phase that settles into rivers—that changes in otolith microstructure upon settlement correlate strongly with (1) rapid decreases in Sr:Ca ratio, (2) changes in Sr isotope ratios, and (3) abrupt increases in Ba:Ca ratio (Hale & Swearer 2008). While changes in Sr:Ca ratio, and the adoption of freshwater residency reliably correlated with changes in otolith microstructure, spikes in Ba:Ca ratio occasionally occurred prior to the microstructural settlement mark. These premature peaks may signify migration into nearshore waters prior to entering a river or estuary. We detected dramatic changes in Ba:Ca only upon settlement, but this occurred for an open coast fish in the Caribbean. Patterson et al. (2005) showed preliminary evidence of an abrupt increase in Ba:Ca ratios at the end of the larval phase for the damselfish *Pomacentrus coelestis* in the Great Barrier Reef. However, they analyzed only recently settled individuals and presented the Ba profile from only 1 fish at the time of settlement. Our study is the first to systematically characterize temporal changes in the profiles of 4 metals from the larval period through an equivalent duration of the juvenile period, and we found that Ba:Ca ratios increased and stabilized at a higher level upon settlement. Overall, 3 studies conducted on taxonomically different species in 3 different locations have shown similar spikes in Ba:Ca ratios at the larval–juvenile transition.

We also detected significant differences in Ba:Ca and Sr:Ca ratios in juveniles from the opposite ends of the island (leeward and windward shore). These may reflect environmental differences in water chemistry, from localized upwelling, runoff, or other inputs into nearshore waters. The elevated Ba:Ca and reduced Sr:Ca ratios at Jacks Bay are indicative of cooler, more nutrient rich waters on the windward shore of St. Croix. Swearer (2000) reported elevated concentrations of chl *a* off the windward shore during the summer of 1997, consistent with this interpretation. However, these site-specific differences in otolith chemistry, regardless of the environmental drivers, are much less than the differences between the larval and juvenile periods.

Utilizing changes in otolith elemental profiles to identify the timing of important life history events requires there to be a correlation between the timing of a particular event and (1) an abrupt change in the chemical and physical properties of the environment or (2) a distinct change in physiology. For elements like Sr and Ba, environmental effects on otolith chemistry are likely to be stronger than physiological changes in elemental incorporation or uptake rates, however this requires further study. Future studies should assess physiological changes in otolith chemistry in a controlled lab setting while holding water chemistry constant, using a species that can be reared throughout its life cycle. Consequently, this technique may be most useful for identifying major habitat transitions, such as movements from pelagic to benthic habitats upon settlement or migrations between freshwater and saltwater, and may not be ideally suited for identifying life history transitions (i.e. metamorphosis) if those transitions do not correspond with a change in habitat. For example, temperate rockfish *Sebastes* spp. metamorphose into juveniles while still pelagic, weeks prior to settling on nearshore reefs. This technique may therefore help to identify the date of settlement, but not of metamorphosis.

There are a number of important caveats that must be considered before employing the LA-ICMPS technique generally to identify the timing of settlement. The approach relies on the assumption that larvae occupy only a single water body, or, if they migrate between different water bodies, that differences in the chemical and physical properties are much less within the larval period than between the larval and juvenile periods. This is likely a fair assumption for bluehead wrasse in St. Croix because physical properties are similar in larval and juvenile habitats, e.g. water temperature does not vary cross shore and temperature profiles show little difference in the top 50 m in St. Croix, while bluehead wrasse larvae typically occur at 30 m depth (Swearer 2000, Cowen 2002). However, these assumptions may not hold for other species in other locations.

LA-ICPMS is a fairly costly procedure and requires collaboration with specialist laboratories. However, analyzing 10 to 20 individuals is not cost prohibitive and should yield a reliable estimate of the mean and variance in PLD for a chosen species.

For species whose otoliths lack clear settlement marks and when specimens cannot be captured easily by other means at the end of the larval period, investigators can use LA-ICMPS techniques to identify the timing of settlement by looking for rapid increases in Ba:Ca. PLDs may then be calculated by counting the number of increments that are deposited between the core and the spike in Ba:Ca ratio. This can be accomplished either by using discrete pits or a continuous ablation process (e.g. Patterson et al. 2005, Hale & Swearer 2008). Finer temporal-scale information may be gleaned from the continuous ablation approach, in contrast to the approach of discrete pits used in this study. The quality of the laser and the ablation spot size chosen by the researcher can influence the temporal resolution that is achieved when using discrete pits. In this study, each ablation pit overlaid ~5 d of growth. We have demonstrated that otolith chemistry may provide a reliable means of identifying the timing of settlement at the end of the larval period.

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