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Spatial and temporal variability in the otolith chemistry of the Brazilian snapper *Lutjanus alexandrei* from estuarine and coastal environments

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Otolith chemistry of juvenile and adult individuals of the Brazilian snapper *Lutjanus alexandrei* was measured to assess the utility of natural markers for investigating individual movements. Individuals were collected over a 3-year period (2010–2012) along the north-eastern coast of Brazil from both estuarine (juvenile to sub-adult stages) and coastal (sub-adult to adult stages) areas. Six elements (^{7}Li, ^{24}Mg, ^{55}Mn, ^{59}Co, ^{88}Sr and ^{137}Ba) were measured in sectioned otoliths of *L. alexandrei* using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Edge composition analysis indicated that element:Ca ratios in the otoliths of juvenile and sub-adult *L. alexandrei* from estuaries were not significantly different among the three consecutive years (2010, 2011 and 2012), suggesting that physicochemical conditions within the nursery area investigated were temporally stable. Similarly, apart from two elements (Ba and Co), element:Ca ratios for larger *L. alexandrei* inhabiting coastal waters were also similar. In contrast, otolith chemistry of similar sized *L. alexandrei* from estuarine and coastal areas was significantly different (based on recently accreted material). Otolith Mn:Ca and Ba:Ca were both significantly higher for *L. alexandrei* collected in estuaries compared to fish from adjacent coastal reefs, while the opposite trend was observed for Sr:Ca. Given the pronounced differences in otolith chemistry between estuarine and coastal areas, element:Ca transects were constructed from the core to margin of the otoliths for adults (age 7+ years) collected on reefs to determine the timing of movement (ontogenetic migration) from estuarine to coastal areas. Based on observed patterns of decline for both Mn:Ca and Ba:Ca, it appears that *L. alexandrei* begin the move to more coastal habitats (*i.e.* lower element:Ca ratios) after age 2 years. The patterns observed for this species highlight the importance of conserving connectivity between coastal habitats to maintain sustainable fish stocks exploited by artisanal fisheries.

Key words: connectivity; movement between estuarine and coastal habitats; natural markers; ontogeny.

INTRODUCTION

Otoliths can serve as natural tags in fishes due to their acellular and metabolically inert structure which can permanently retain elements that are accreted onto a growing surface (Campana, 1999). Among the six most common elements routinely used in otolith michemistry studies in fishes (Li, Mg, Mn, Cu, Zn, Sr and Ba) (Sturrock *et al.,* 2012) the last two (Sr and Ba) have been widely used and accepted as useful markers of environmental conditions in both estuarine and marine systems, as both reflect environmental conditions and are associated with salinity and temperature (Walther

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& Thorrold, 2006; Elsdon *et al.,* 2008). Other elements seem to be regulated by physiological conditions (*e.g.* Mg and Cu) (Sturrock *et al.,* 2012; Woodcock *et al.,* 2012) or more recently as a viable hypoxia/redox condition markers as seems to be the case for Mn (Limburg *et al.,* 2015). Analyses of either whole otoliths or small areas within the otolith have been used to distinguish stocks or sub-populations and retrospectively determine migratory histories (Rooker *et al.,* 2008, 2010) including movement between estuarine and marine waters (Thorrold *et al.,* 1997; Secor *et al.,* 2001; Gillanders, 2005). Despite their promise for describing movement and population connectivity, the use of otolith chemistry is challenging due to the fact that these chemical tags are species specific and vary spatially and temporally within an environment (Gillanders, 2005).

The use of multiple habitats during early life stages is a key ecological feature of many reef fishes (*e.g.* snappers, Sheaves, 1995 and groupers, Nakamura *et al.,* 2008). Adults inhabit the marine environment and, after spawning, larvae and juveniles move into shallow coastal areas and estuaries, where they spend the first months or years of life (Gillanders, 2002*a*; Jones *et al.,* 2010; Reis-Santos *et al.,* 2012). Increased food availability and refuge from predators are benefits often associated with inshore or estuarine habitats (Miller *et al.,* 1985; Gibson, 1994; Mateo *et al.,* 2010). Key information for managing fish stocks and designing marine protected areas relies on understanding ontogenetic movements of different life-history stages as well as the timing of such movements, particularly the transition from juvenile to adult populations (Sheaves, 1995; Gillanders, 2002*a*, 2005).

Movement between estuarine and coastal habitats has been described for several species of dominant taxa on reefs, including snappers (family Lutjanidae) in the western Atlantic Ocean (Nagelkerken *et al.,* 2000; Chittaro *et al.,* 2006; Moura *et al.,* 2011). One of the most common lutjanids on reefs in the south-western Atlantic Ocean is the endemic, and recently described Brazilian snapper *Lutjanus alexandrei* Moura & Lindeman 2007. Similar to congeners in the Caribbean Sea, this species resides in estuarine or nearshore nurseries before moving to coastal reef environments later in life (Aschenbrenner & Ferreira, 2015). Despite its ecological and economic importance, basic life history information for *L. alexandrei* is scarce (Fernandes *et al.,* 2012; Aschenbrenner & Ferreira, 2015).

The aim of the current study was to evaluate the effectiveness of using natural chemical tags in otoliths of *L. alexandrei* for improving understanding of habitat use and associated ontogenetic shifts for this endemic yet poorly understood snapper. Specific objectives of this study were first, to assess spatial and temporal variability in the otolith chemistry (element:Ca ratios) of *L. alexandrei* from estuarine and coastal habitats to determine the potential value of the approach. Next, element: Ca life-history transects (from the core to margin of the otolith) were used to determine the timing of movement (ontogenetic migration) from estuarine to coastal areas.

MATERIAL AND METHODS

SAMPLE COLLECTIONS

Juvenile *L. alexandrei* were sampled at two sites, Formoso and Ariquinda Rivers, located in the Formoso River basin. Samples were collected from traditional corral fisheries landings operating over estuarine margins and parallel to mangrove prop roots. Specimens were collected during three consecutive years (2010, 2011 and 2012) in the dry

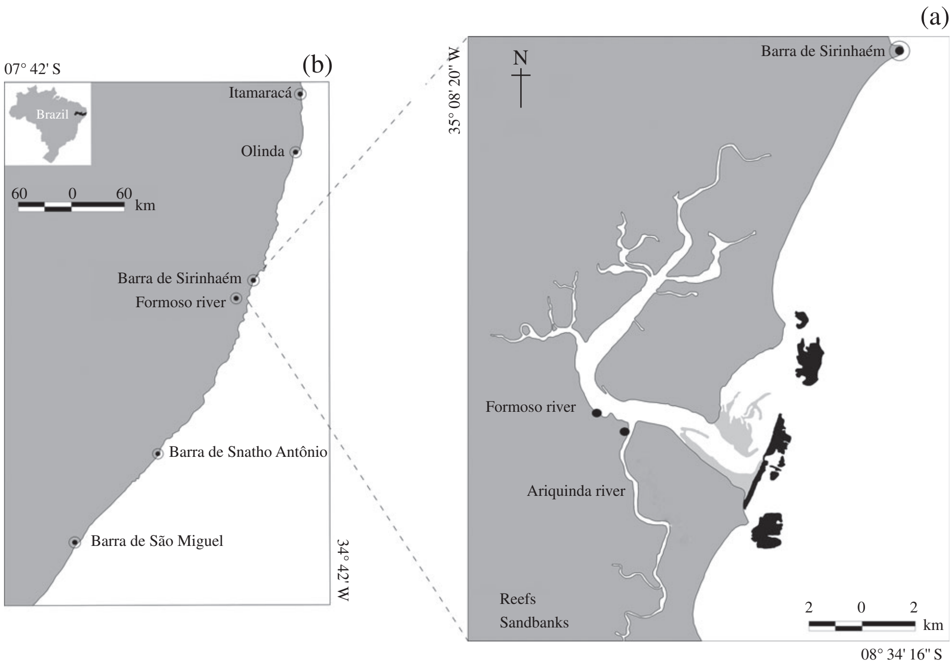

Fig. 1. Location of collection sites of adult *Lutjanus alexandrei* (●, sites where fish were collected): (a) estuarine area: Formoso River basin (FR) and (b) coastal areas Itamaracá (IT), Olinda (OL), Barra de Sirinhaém (SI), Barra de Santo Antonio (SA) and Barra de São Miguel (SM). ■, reefs; ■, sandbanks.

season (from November to April). As the collection sites were both at the mid part of the estuarine system and located <2 km apart [Fig. 1(a)], samples were considered as from the same source and pooled for analyses. Additionally salinity and temperature were measured on a monthly basis at each fishing site on board fishing boats during 2012. Adult individuals were either collected by commercial fisheries landings using gillnets or fish traps operating over the continental shelf in depths between 30 to 60 m. Fish were collected at two sites in the coastal portion of Pernambuco State: Itamaracá (IT) and Olinda (OL) considered as the northern location, another two sites Santo Antonio (SA) and São Miguel (SM) in the coastal part of Alagoas State, considered as southern locations [Fig. 1(b)]. Distances between southern sites were <30 km and distances between northern sites were <50 km. Northern and southern locations were <230 km apart. All coastal individuals were collected in 2012.

In order to evaluate temporal variability in otolith chemistry within the estuarine area, 10 juvenile individuals of *L. alexandrei* were randomly selected from each of the three consecutive years (2010, 2011 and 2012). Fish ranged in total length (L_T) from 16 to 17 cm. Spatial variability patterns of otolith chemistry in coastal areas was estimated using 10 adult individuals randomly selected from each site from north and south areas ($n = 40$ in total) ranging from 28 to 29 cm L_T.

Differences in element:Ca ratios between habitats, estuary and the adjacent coastal site were also tested using 10 individuals each from the Formoso River basin and the nearest coastal site from the estuary Barra de Sirinhaém ($n = 20$ in total). All samples were collected in the same year (2011) during the dry season. Samples ranged in L_T from 20 to 24 cm, comprising the larger individuals from estuarine and smallest from coastal areas, though there was some size overlap between the two habitat types.

Trace element life-history transects were then performed from ages 1 to 7 years in order to identify possible migration patterns from estuarine to coastal habitats. Ten individuals of age

Table I. Overview of type of analyses, sampling habitats, sites, sampling code, sampling year, number of samples analysed (n) and total length (L_T) ranges of *Lutjanus alexandrei*

Type of analysis	Habitat	Sampling site	Sampling code	Year	n	L_T (cm)
Temporal variability	Estuarine	Formoso river basin	FR	2010–2012	30	16–17
Spatial variability	Coastal	Itamaracá	IT	2012	10	28–29
Spatial variability	Coastal	Olinda	OL	2012	10	28–29
Spatial variability	Coastal	Barra de Santo Antônio	SA	2012	10	28–29
Spatial variability	Coastal	Barra de São Miguel	SM	2012	10	28–29
Habitat signatures	Estuarine	Formoso river basin	FR	2011	10	20–22
Habitat signatures	Coastal	Barra de Sirinhaém	SI	2011	10	22–24
Life transect	Coastal	Itamaracá	IT	2012	4	26–28
Life transect	Coastal	Barra de Santo Antônio	SA	2012	3	26–28
Life transect	Coastal	Barra de São Miguel	SM	2012	3	26–28

FR, Formoso River basin; IT, Itamaracá; OL, Olinda; SA, Santo Antonio; SI, Sirinhaém; SM, São Miguel.

7 years, between 26 and 28 cm L_T, were randomly selected from all coastal areas. A summary of data collections is provided in Table I.

Individuals collected from artisanal fishery landings in the estuarine and coastal areas were placed into labelled plastic bags, stored on ice and processed within 24 h. Both sagittae were removed using plastic forceps, rinsed with distilled water and cleaned of adhering tissue. Otoliths were then air dried and stored in plastic vials. A single otolith (right) were used for trace element analysis, as age had previously been determined in otolith (left) sections and used for age determination for stock assessment work [for details on age determination see Aschenbrenner & Ferreira (2015)]. Measurements for annuli distances were performed for ages 1–7 years ($n = 170$) parallel to the sulcus acusticus from the nucleus to the proximal face using a stereomicroscope with reflected light on a black background (Fig. 2). Otolith images were captured, treated and measurements for the first seven annuli performed using the IMAGE Pro Plus V. 4.5 computer programme (SPSS Science; www-01.ibm.com).

OTOLITH PREPARATION AND ANALYSIS

One otolith from each fish was selected and prepared for chemical analysis. Each otolith was first embedded in epoxy resin and a transverse thin section (1·5 mm) was removed from the core region using a IsoMet low-speed saw (Buehler; www.buehler.com). Each otolith section was glued to a glass slide with crystal bond thermoplastic cement and thin sections were hand polished to the core on one side using 1200 grit wet-dry sandpaper and 3 μm aluminium oxide lapping film rinsed with Milli-Q water. Polished sections were triple rinsed in Milli-Q water and air dried for 12 h under a laminar flow hood before being stored in a slide box.

Trace element chemistry of thin sections was determined using a laser ablation inductively coupled plasma mass spectrometer (LA-ICPMS) at Texas A&M University at Galveston. The system consists of an ultraviolet laser ablation with a high-resolution digital camera (NWR 213, New Wave Research; www.esi.com) and quadrupole inductively coupled plasma mass spectrometer with a Xs cone (ICPMS, XSeries II, Thermo Scientific; www.thermoscientific.com). Ablation diameters for points sampled on each otolith were 50 μm, and the location of annuli

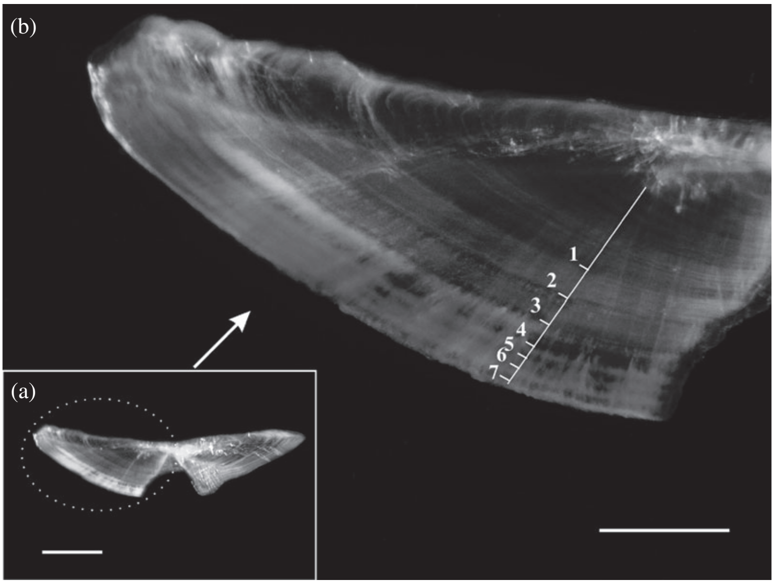

Fig. 2. Sectioned otolith of (a) *Lutjanus alexandrei*, scale bar = 0·5 mm. Details of sectioned otolith (b) showing measurements performed in parallel to the sulcus acusticus from the nucleus to the proximal face of a 8-year-old individual, scale bar = 0·4 mm

was determined using otolith microstructure analysis. For assessments of spatial and temporal variability, replicate points were ablated on the edge of each otolith to ensure that the analysed material was recently accreted or from the capture location. Life-history transects were performed by ablating multiple points at each annulus from ages 1 to 7 years. All elemental concentrations were based on an average of four ablations on the otolith edges and an average of three ablations for each annulus on the life-history transects. Before acquiring data, otoliths were pre-ablated using two laser pulses to remove any surface contamination. Six elements were measured using LA-ICPMS: ^{7}Li, ^{24}Mg, ^{55}Mn, ^{59}Co, ^{88}Sr and ^{137}Ba. NIST 614 (National Institute of Standards and Technology; www.nist.gov) was used as the external standard for calibration. Calcium ^{44}Ca was used as the internal standard to correct variations in ablation yield. Calcium concentration was assumed to be evenly distributed across the otolith sections at 38% or 388 000 μg g^{-1}, a published value for certified reference material of otoliths (Yoshinaga *et al.,* 2000). For an ablated point size of 50 μm, estimated detection limits (in μg g^{-1}) for examined elements were estimated as the quantity of analyte required to produce a signal equivalent to three times s.d. of the NIST 614 ($n = 30$), and were: ^{7}Li: 0·48, ^{24}Mg: 2·08, ^{55}Mn: 0·33, ^{59}Co: 0·25, 0·52, ^{88}Sr: 3·71 and ^{137}Ba: 1·24. A calibration curve was generated for each otolith sample and one standard run (NIST 614) was added every 10 ablations in each queue to monitor and correct the potential instrumental drift. After elemental analysis on the LA-ICMPS, otolith sections were examined under a microscope and a digital image of the ablated section was taken using IMAGE Pro Plus V. 4.5 computer program (SPSS Inc., 1999) to confirm the laser ablation locations (Fig. 3).

DATA ANALYSIS

Element:Ca ratios (Li:Ca; Mg:Ca; Mn:Ca; Co:Ca; Sr:Ca and Ba:Ca) were first examined for within-group normality (Kolmogorov-Smirnov test) and for homogeneity of variances among groups (Levene's test). When both assumptions were met, multivariate analysis of variance (MANOVA) was used to test for spatial and temporal differences in element:Ca ratios. Pillai's trace (V) was chosen as the test statistic because it is the most conservative test in MANOVA

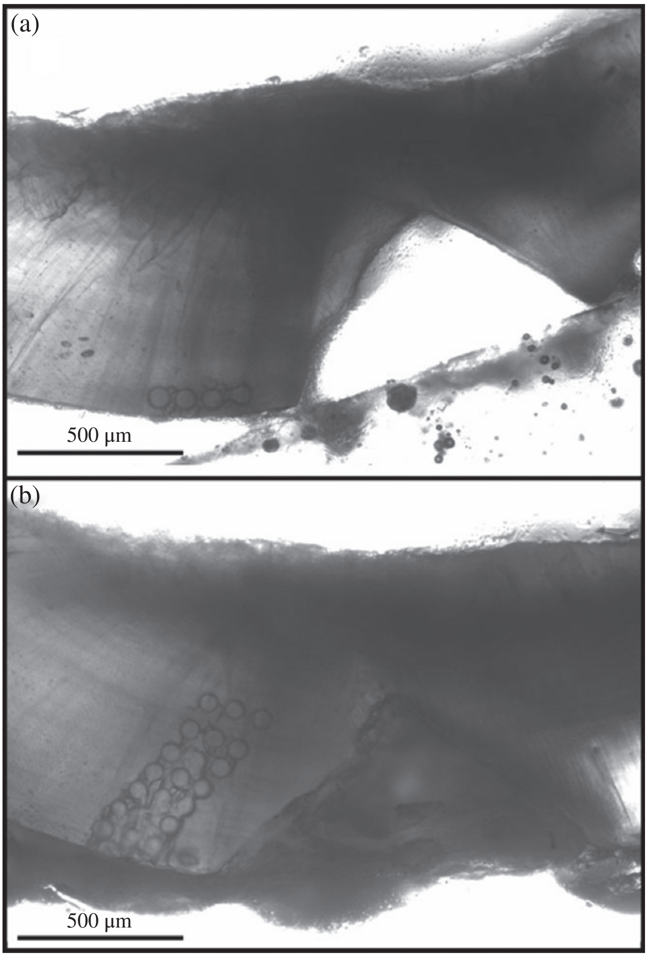

Fig. 3. Images of *Lutjanus alexandrei* otolith sections showing the laser ablation points for (a) edge analysis and (b) life-history transects of 7-year-old individuals for chemical analysis using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS).

(Wilkinson *et al.,* 1996). ANOVA based on ablation means and *post hoc* comparisons using Tukey's honestly significant difference (HSD) test were subsequently applied to detect any significant differences in element:Ca ratios among factor levels (*i.e.* years, coastal locations and habitats). For edge composition analyses the following hypothesis were tested that: (1) there was no variation in trace elements composition among the 3 years of collection for the estuarine area, (2) there were no differences in mean element concentrations among coastal locations and (3) there were no differences between estuarine and adjacent coastal areas in otolith element concentrations. Once concentrations of element:Ca were detected to be relatively similar among coastal locations and variation for estuarine and adjacent coastal area were observed for three elements, elemental profile was tested according to the last hypothesis: (4) there was no variation in trace elements profiles across individual otoliths (*i.e.* from core to age 7 years).

The elemental profile of three elements that previously displayed significant differences between estuarine and coastal areas (Mn, Ba and Sr) were matched to fish age based on average annuli measurements (1–7) in the otolith macrostructure as temporal references. Comparisons of elemental profiles across otoliths were made with repeated measures ANOVA. As Mauchly's test for sphericity was violated for all three elements, the Huynh and Feldt correction computed in SPSS, was used to adjust *F*-critical values to protect against inflated type I errors in repeated-measures ANOVA tests (FitzGerald *et al.,* 2004). Tukey's HSD tests were used *post hoc* detect any significant differences in element:Ca ratios among factor levels (*e.g.* years).

RESULTS

All six elements (Li, Mg, Mn, Co, Sr and Ba) were regularly detected at all sites and were therefore included in all statistical analyses. In general, element:Ca ratios met both within-group normality (Kolmogorov-Smirnov's test, $P > 0{\cdot}05$ in all cases) and homogeneity of variances among groups (Levene's test, $P > 0{\cdot}05$ in all cases); however, when assumptions were violated (*e.g.* Mn:Ca and Li:Ca among years), element:Ca ratios were transformed $\ln(x + 1)$ prior to statistical analyses.

Temporal variability of element:Ca: ratios were not significantly different among years (MANOVA: $P > 0{\cdot}05$). Although, higher variation in elements:Ca concentrations were observed for Mn:Ca and Co:Ca (Fig. 4), Mn:Ca presented a slightly higher

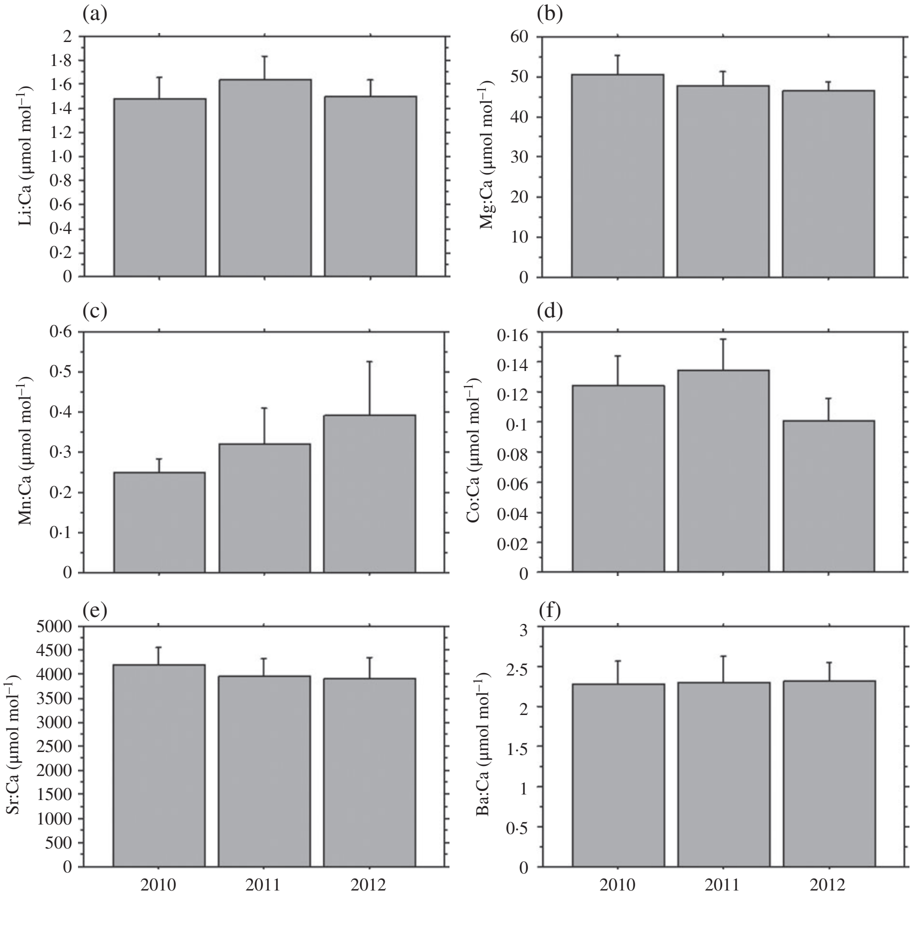

FIG. 4. *Lutjanus alexandrei* mean + S.E. (a) Li:Ca, (b) Mg:Ca, (c) Mn:Ca, (d) Co:Ca, (e) Sr:Ca and (f) Ba:Ca ratios for different years (2010, 2011 and 2012) in the same estuarine area (RF).

TABLE II. Results of one-way ANOVA test for differences in element:Ca concentrations of (Li, Mg, Mn, Co, Sr and Ba) in *Lutjanus alexandrei* otoliths collected among coastal locations IT, OL, SA and SM ($n = 40$) and between estuarine and adjacent coastal area RF and SI ($n = 20$)

Otolith area	Source	Element	d.f.	MS	*F*	*P*
Edges	Coastal locations	Li:Ca	3	0·11	1·12	>0·05
		Mg:Ca	3	49·03	0·49	>0·05
		Mn:Ca	3	0·00	0·56	>0·05
		Co:Ca	3	0·01	3·95	<0·05
		Sr:Ca	3	1612423	1·96	>0·05
		Ba:Ca	3	1·32	6·33	<0·01
Edges	Estuarine *v.* coastal	Li:Ca	1	0·15	0·97	>0·05
		Mg:Ca	1	289·71	1·38	>0·05
		Mn:Ca	1	0·22	15·89	<0·001
		Co:Ca	1	0·01	1·20	>0·05
		Sr:Ca	1	1823971	12·19	<0·01
		Ba:Ca	1	2·93	5·42	<0·05

IT, Itamaracá; OL, Olinda; SA, Santo Antonio; SI, Sirinhaém; SM, São Miguel.

trend for means (0·25, 0·32 and 0·39 μmol mol^{-1}) in consecutive years (2010, 2011 and 2012). Co:Ca ratios also displayed a large variability among the consecutive years (0·12, 0·13, 0·10 μmol mol^{-1}) though no trend was observed.

Multi-elemental fingerprints differed significantly among coastal sampling locations (MANOVA: $P < 0·05$). Univariate contrasts indicated that Co:Ca and Ba:Ca ratios varied significantly (ANOVA, $P < 0·05$; Table II) among the four coastal sampling sites. Co:Ca ratios were significantly higher for OL compared to IT and SM, but not for SA, while Ba:Ca ratios OL site were significantly higher when compared to the other three coastal locations (Tukey's HSD test, $P < 0·05$, Fig. 5).

Multi-elemental fingerprints of similar sized *L. alexandrei* collected in estuarine and coastal waters were significantly different (MANOVA: $P < 0·05$). Univariate contrasts revealed that element:Ca ratios of Mn, Sr and Ba were significantly different (ANOVA, $P < 0·05$, Table II) between the two regions. Mean Mn:Ca and Ba:Ca ratios of *L. alexandrei* from estuarine areas (0·32 and 2·31 μmol mol^{-1}) were markedly higher than observed for coastal reefs (0·11 and 1·54 μmol mol^{-1}). In contrast mean Sr:Ca for *L. alexandrei* from the estuary (3552 μmol mol^{-1}) was significantly lower than observed for individuals from the adjacent coastal area (4156 μmol mol^{-1}) (Fig. 6).

Ontogenetic variability of (Mn and Ba) concentrations for *L. alexandrei* was observed across age classes (*i.e.* material accreted during each age interval), although for Sr concentrations no trend was observed. Element:Ca ratios of Mn and Ba were significantly different across portions of the otolith that corresponded to different years (ANOVA $P < 0·05$, Table III), however, Sr concentrations were not significantly different across otolith portions (ANOVA, $P > 0·05$, Table III). Mn:Ca ratios in the age-1 region of the otolith were significantly higher than all other ages (2–7 years), while age 2 years differed significantly from ages 5 to 7 years (Tukey's HSD test $P < 0·05$, Fig. 7). Similarly, age 1 year for Ba:Ca ratios were also significantly higher than ages (4–7 years) while age 2 years differed significantly of ages 6 and 7 years (Tukey's HSD test $P < 0·05$, Fig. 8). Additionally means and 95% C.I. of Mn:Ca and Ba:Ca ratios observed in earlier trials from coastal sites were plotted as a reference

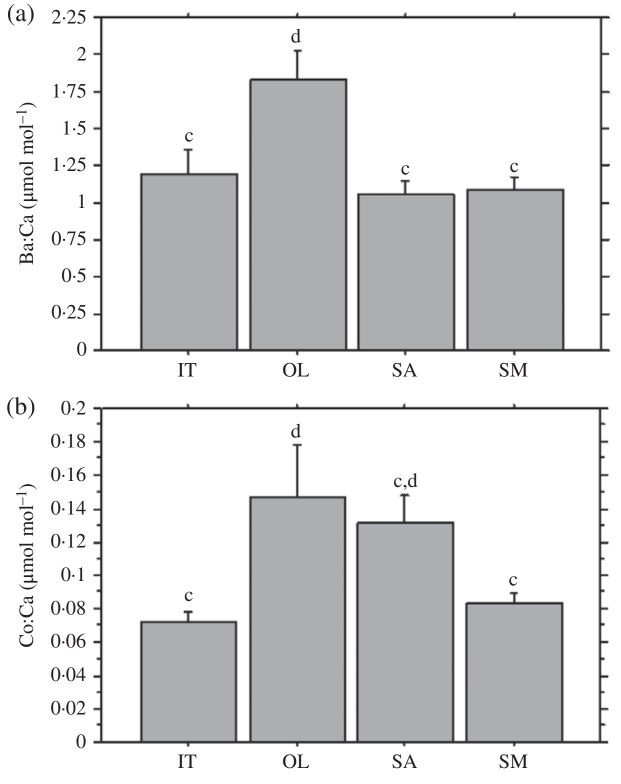

FIG. 5. *Lutjanus alexandrei* mean + S.E. elemental concentrations ratio for (a) Ba:Ca and (b) Co:Ca in otolith edges that differed significantly among coastal sampling locations (see Table I for location codes). Bars with different lowercase letters are statistically different from each other [Tukey's honestly significant difference (HSD) test; $P < 0{\cdot}05$].

TABLE III. Results of one-way repeated-measures ANOVA test for differences of otolith profile of Mn, Sr and Ba across seven increments of same aged *Lutjanus alexandrei* individuals collected in coastal locations (IT, SA and SM, $n = 10$)

Otolith area	Source	Element	d.f.	MS	*F*	*P*
Core to edges	Coastal locations	Mn:Ca	13·98	0·14	21·72	<0·001
		Error	1·94	13·98		
		Sr:Ca	2·78	375488	1·51	>0·05
		Error	22·20	248160		
		Ba:Ca	1·63	58·16	6·91	<0·05
		Error	13·04	8·41		

IT, Itamaracá; SA, Santo Antonio; SM, São Miguel

values for coastal Mn and Ba concentrations, representing a habitat break point (Figs 7 and 8).

Measurements of temperature and salinity at the two sites were pooled (representing a proxy of estuarine conditions where fish were collected) and varied from a mean of 28·4 (26·5–30·3) to 32·3 (22·6–41) (Fig. 9).

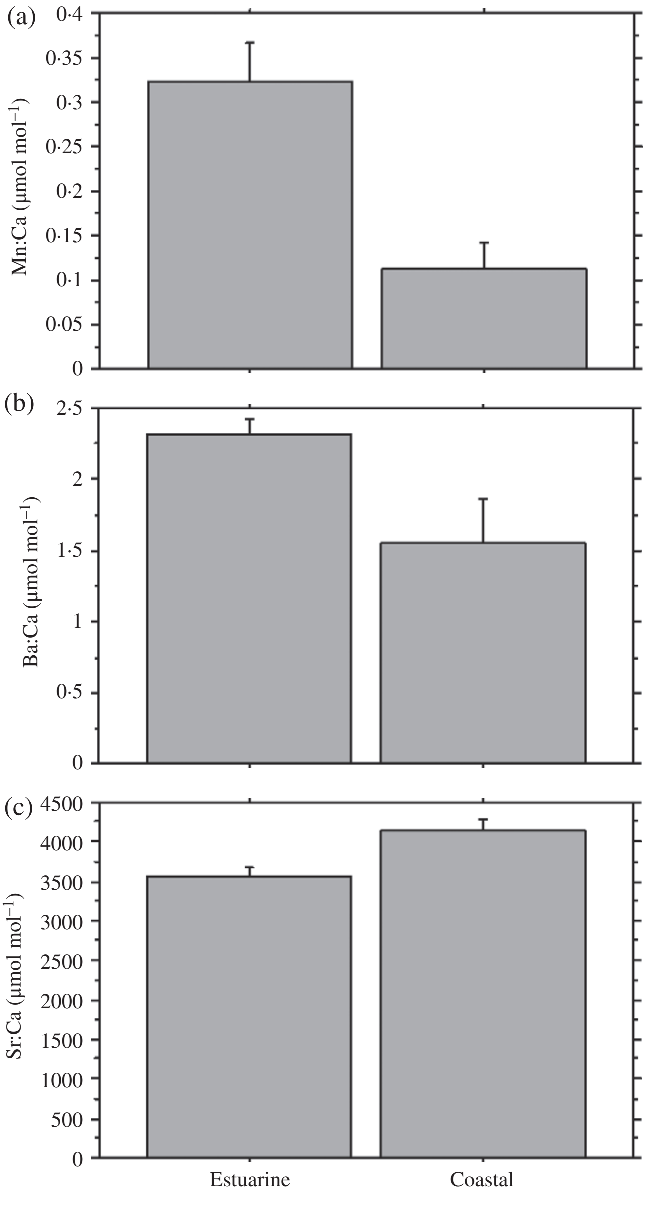

FIG. 6. *Lutjanus alexandrei* mean + S.E. elemental concentration ratios of (a) Mn:Ca, (b) Ba:Ca and (c) Sr:Ca in otolith edges that differed significantly between estuarine (RF) and adjacent coastal area (SI) (see Table I for location codes).

DISCUSSION

The element:Ca ratios in otoliths of juvenile *L. alexandrei* collected in the estuary showed a consistent pattern among the 3 years of this study, as no significant temporal variations were found. Although interannual variations in otolith chemistry are frequently observed for fishes occupying estuaries and coastal environments

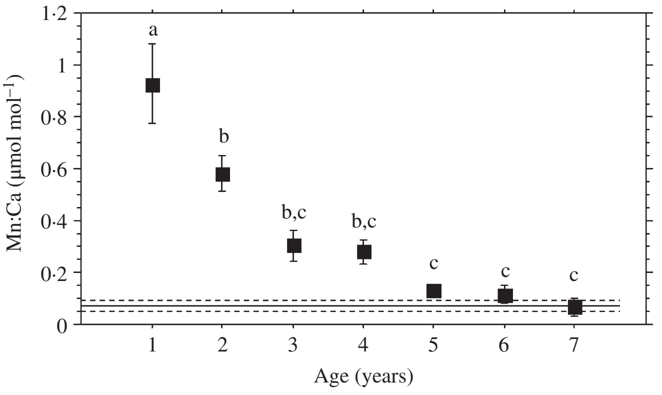

Fig. 7. *Lutjanus alexandrei* mean + s.e. Mn:Ca elemental ratios for age groups (1–7 years) in otoliths of individuals collected in coastal areas [Itamaracá (IT), Santo Antonio (SA) and São Miguel (SM)]. Different lower case letters are statistically different from each other [Tukey's honestly significant difference (HSD) test $P < 0{\cdot}05$]. - - - - - - represents a habitat break point of mean Mn:Ca concentrations (0·07 µmol mol^{-1}) and ——— (95% c.i.) observed for individuals previously sampled in coastal areas ($n = 30$).

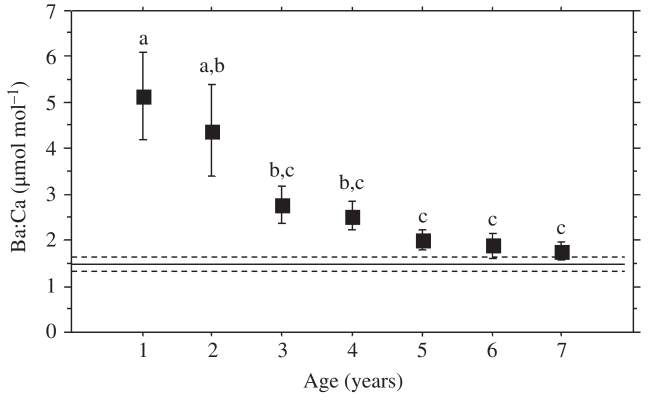

Fig. 8. *Lutjanus alexandrei* mean + s.e. Ba:Ca elemental ratios for age groups (1–7 years) in otoliths of individuals collected in coastal areas [Itamaracá (IT), Santo Antonio (SA) and São Miguel (SM)]. Different lower case letters are statistically different from each other [Tukey's honestly significant difference (HSD) test $P < 0{\cdot}05$]. - - - - - - represents a habitat break point of mean Ba:Ca concentrations (1·49 µmol mol^{-1}) and ——— represent (95% c.i.) for individuals previously sampled in coastal areas ($n = 30$).

(Gillanders & Kingsford, 2000; Walther & Thorrold, 2009; Reis-Santos *et al.,* 2012), several studies have also reported elemental stability across years (Gillanders, 2002*b*; Hamer *et al.,* 2003), suggesting for these cases that physicochemical conditions within the study area were relatively stable over sampled time. Relative high salinity values found in the sampled estuarine sites suggest that the sea water might be an important physical factor, influencing estuarine water, thus this tropical estuary seems to behave as a branch of the sea based on salinity and temperature values, different from previous studies in Brazil that have explored regions with higher freshwater discharges and stronger salinity gradients, such as observed on the South Brazilian coast (Albuquerque *et al.,* 2012). Nevertheless, variations in elemental signatures of estuarine

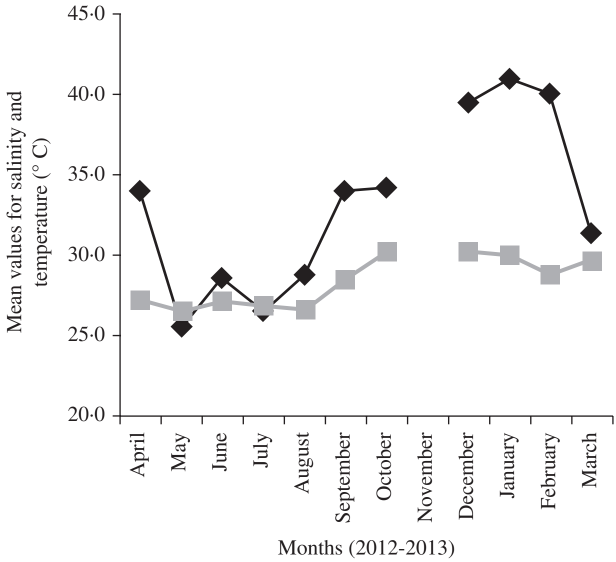

FIG. 9. Monthly mean temperatures (■) and salinities (◆) in estuary sites where *Lutjanus alexandrei* were collected from April 2012 to March 2013.

fishes are likely to be system dependent, based on tides, water movements, hydrology, precipitation, upwelling and geochemical composition of the estuarine drainage basin (Elsdon *et al.,* 2008). Also, anthropogenic influence and type of species (as element concentrations are species dependent) may influence chemical composition of fish otoliths (Gillanders & Kingsford, 2003; Chittaro *et al.,* 2005, 2006).

Spatial variations in element:Ca ratios in otoliths of *L. alexandrei* from the four coastal collection sites were negligible. Apart from Co:Ca and Ba:Ca being significantly different at only one site (OL), present results suggest that element:Ca ratios in the coastal areas are relatively similar even though certain sites are separated by over 200 km. Chemical similarity was also observed among otoliths of fishes sampled over small spatial scales by Gillanders *et al.* (2001) and Chittaro *et al.* (2005, 2006) in oligotrophic coastal areas. Alternatively, differences over broader spatial locations were found up to 1200 km (Secor & Zdanowicz, 1998; Thorrold *et al.,* 1998), while other studies have found no differences in otolith chemical composition among locations separated by 3000 km (Proctor *et al.,* 1995; Kalish *et al.,* 1996). Thus, otolith chemistry is not necessarily a good predictor of distances among locations in coastal areas (Elsdon *et al.,* 2008), but to environmental water properties (*e.g.* temperature and salinity) and physiographic process (*e.g.* rainfall and associated runoff). Little variation in otolith chemistry found among sampled coastal areas may be related to relatively homogeneous environmental conditions or alternatively movement of fishes among areas (Gillanders *et al.,* 2001).

Mn:Ca and Ba:Ca ratios present in recently accreted otolith material of *L. alexandrei* collected in the estuary were significantly greater than those in fish sampled in adjacent coastal area, whereas Sr:Ca ratios showed the opposite trend with higher ratios for coastal area. These three elements follow the same pattern observed in the literature regarding comparisons among estuarine and coastal habitats (Gillanders & Kingsford, 1996; Rooker *et al.,* 2004; Chittaro *et al.,* 2005, 2006). Despite the fact that the relationship between environmental and physiological processes in otolith chemical composition is poorly understood (Campana, 1999; Thresher, 1999), elements that are under physiological regulation are less likely to reflect environmental variables, while elements that are incorporated into the otolith *via* substitution of calcium and included in interstitial spaces are likely to reflect environment parameters (*i.e.* Sr and Ba). These last two elements have been widely used to investigate the movement and connectivity of fishes between estuarine and coastal environments (Elsdon & Gillanders, 2004; de Vries *et al.,* 2005; Walther & Thorrold, 2006). Mn has been used as an environmental indicator, particularly in estuarine habitats (Gillanders & Kingsford, 2000, 2003; Reis-Santos *et al.,* 2012), however, caution is needed in the interpretation of Mn:Ca otolith ratios, as the mechanisms regulating incorporation are still not fully understood (Miller, 2009).

Life-history transects of several elements decreased with increasing age for adult (age 7 years) *L. alexandrei* inhabiting coastal areas, and observed changes in element:Ca ratios appear to track the movement of individuals from waters of higher (estuary) to lower (coastal) trace metal availability. Ba:Ca and Mn:Ca showed similar patterns, with higher element:Ca ratios in the first year, subsequent decrease until age 4 years, and similar values from age 5 to 7 years. Contrary to Ba and Mn, no difference was found for Sr:Ca along otolith transects, this pattern may be expected as the salinity in the studied estuary is depressed, but not low enough to overwhelm some physiologically-mediated variance given that mixing curves of Sr:Ca are generally curved with the largest change at lower salinities (*i.e.* lower than 20) (Albuquerque *et al.,* 2012). Although Sr has often been successfully used to infer habitat change along a salinity gradient (Thorrold *et al.,* 1997; Rooker *et al.,* 2004), care is necessary when a stronger salinity gradient between habitats is lacking (as in this study), suggesting that Sr:Ca might be influenced by endogenous factors such as ontogeny (Fowler *et al.,* 1995; Brown & Severin, 2009; Walther *et al.,* 2010), growth rates (Sadovy & Severin, 1994), or a mix of both (Walther *et al.,* 2010). Further work is necessary to assess the importance of these factors on otolith accumulation. Despite confounding factors, these results seem to confirm that *L. alexandrei* occupy estuaries during their first year of life, moving to areas influenced by coastal processes as early as age 2 years as inferred from age studies (Aschenbrenner & Ferreira, 2015). Yet for general conclusions related to emigration patterns, larger sample sizes may be required. Ontogenetic shifts from estuarine to coastal areas have been described directly (markers) (Chittaro *et al.,* 2006) and indirectly (UVC) for other snapper species in the western Atlantic Ocean (Faunce & Serafy, 2007, 2008; Jones *et al.,* 2010; Moura *et al.,* 2011). Similar to *L. alexandrei,* certain congeners [*e.g.* schoolmaster snapper *Lutjanus apodus* (Walbaum 1792)] use estuarine mangrove prop-root habitats during early life, moving out to coastal reef environments as sub-adults or adults (Rooker, 1995; Nagelkerken *et al.,* 2000; Cocheret de la Morinière *et al.,* 2003). Present results show a similar pattern for *L. alexandrei*. Examining habitat-specific and age-specific variation in element:Ca ratios allow inference of potential movement patterns of *L. alexandrei* between estuarine and coastal areas in Brazil. This life-history model is supported by a positive relationship between size and age, and depths for Lutjanidae on the north-eastern Brazilian coast (Frédou & Ferreira, 2005; Moura *et al.,* 2011; Aschenbrenner & Ferreira, 2015).

Along the north-eastern coast of Brazil, lutjanids are one of the main components of artisanal fisheries (Rezende *et al.,* 2003; Frédou *et al.,* 2009*a*, *b*). In the tropical south-west Atlantic Ocean, estuarine areas harbour mangrove forests that constitute essential habitats for juvenile fishes. In Brazil, despite the existence of various environmental laws to protect mangroves, this ecosystem has been affected by a variety of anthropogenic activities (Magrisi & Barreto, 2010). Although in Brazil there are not concrete estimates, the occupation of the coastal zone has dramatically increased, exerting diverse and numerous stresses on coastal ecosystems. The impacts that threaten the future of Brazilian mangroves include the diversion of freshwater flows, deterioration of water quality caused by pollutants and nutrients as well as conversion for development activities such as agriculture, aquaculture (mainly by shrimp farms), salt extraction and infrastructure, all of which contribute to the degradation and deforestation process (Santos *et al.,* 2014). The present results indicate that ontogenetic shifts between estuarine and coastal habitats may occur for *L. alexandrei* and that these habitats are essential for recruitment to the adult population (Aschenbrenner & Ferreira, 2015). Connectivity integrity between juvenile and adult habitats is thus an essential consideration for fishery managers and should be included in management plans as well as other customary fishery management measures.

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