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Spatial variation in otolith chemistry of *Lutjanus apodus* at Turneffe Atoll, Belize

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Abstract

Lutjanus apodus (Schoolmaster) were collected from several mangroves and coral reefs at Turneffe Atoll, Belize, in order to investigate whether elemental concentrations from the otolith edge could be used as a means to identify the habitat (mangrove or coral reef) and site (9 mangrove sites and 6 reef sites) from which they were collected. Results of a two factor nested MANOVA (sites nested within habitat) indicated significant differences in elemental concentrations between habitats (i.e., mangrove versus reef) as well as among sites. When separate Linear Discriminant Function Analyses (LDFA) were used to assess whether the spatial variability in otolith chemistry was sufficient to differentiate individuals to their respective habitats or sites, the results indicated that fish were classified (jackknife procedure) with a moderate to poor degree of accuracy (i.e., on average, 67% and 40% of the individuals were correctly classified to the habitat and site from which they were collected, respectively). Using a partial Mantel test we did not find a significant correlation between the differences in otolith elemental concentrations between sites and the distance between sites, while controlling the effect of habitat type (mangrove or reef). This suggests that for mangrove and reef sites at Turneffe Atoll, Belize, the overlap in terms of *L. apodus* otolith elemental concentrations is too high for investigations of fish movement. Finally, by comparing previously published *Haemulon flavolineatum* otolith chemistry to that of *L. apodus* we assessed whether these species showed similar habitat and/or site specific patterns in their otolith chemistry. Although both species were collected from the same sites our results indicated little similarity in their elemental concentrations, thus suggesting that habitat and site elemental signatures are species specific.

Keywords: otoliths; ablation; *Lutjanus apodus*; mangroves; reefs; Belize; Turneffe Atol

1. Introduction

Since the direct observation of the immigration or emigration of fish is unlikely, methods that involve the tagging of fish have been developed (some of which date back to the 1600's) to help identify the movement patterns of fish (Guy et al., 1996). However, in order to obtain accurate information on the movement of organisms the use of a tag must not distort an individual's natural behaviours or increase its chance of being killed, it must be retained for the period of time in question, be easily detectable, and it must be inexpensive and easy to administer (see reviews by Guy et al., 1996; Thorrold et al., 2002). Fortunately, a variety of tags and tagging methods exist that are categorized depending upon whether the organisms are tagged by the researcher (referred to as artificial tags, e.g., floy and coded wire tags) or tagged through the natural variation in gene frequencies or chemical differences in the environment (referred to as natural tags) (Thorrold et al., 2002). Because artificial tags are applied to the organism by the researcher, a substantial effort and cost is required to collect a reasonable sample of individuals (likely from several locations), tag, release, and eventually recapture some of them.

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Alternatively, natural tags eliminate the need for the labour intensive collection and tagging of fish since they take advantage of the natural variation in elemental concentrations or gene frequencies to distinguish fish (see Hellberg et al., 2002; Planes, 2002; Thorrold et al., 2002; Elsdon and Gillanders, 2003; Taylor and Hellberg, 2003).

To investigate population structure and the movement of individuals, environmentally-derived markers, mainly from calcified structures like bone (see Pollard et al., 1999), scales (see Wells et al., 2000a,b, 2003a,b), and otoliths (see reviews by Campana, 1999; de Pontual and Geffen, 2002; Thorrold et al., 2002), have been used (see work using non-calcified structures such as eye lenses; Dove and Kingsford, 1998). Although these calcified structures all grow continuously and record chemical aspects of the environment, the otolith is preferentially used in chemical investigations because it is metabolically inert (bones and scales have been shown to degrade during periods of stress; see Campana and Thorrold, 2001; de Pontual and Geffen, 2002; Wells et al., 2003a), and thus useful in terms of retrospective analyses (see Edmunds et al., 1989; Dove and Kingsford, 1998; Patterson et al., 1999; Kingsford and Gillanders, 2000; Thorrold et al., 2001; Forrester and Swearer, 2002; Rooker et al., 2003; Elsdon and Gillanders, 2005). But the successful use of otolith chemistry in studies of population structure and connectivity first requires a detectable level of chemical variation at biologically relevant spatial scales (Hamer et al., 2003). Because of this we chemically examined the otoliths of *Lutjanus apodus* (Schoolmaster) collected from mangroves and coral reefs throughout Turneffe Atoll, Belize, in order to begin to understand the role shallow water habitats such as mangroves play in maintaining nearby adult populations on coral reefs.

Traditionally, mangroves (along with other shallow water habitats) have been regarded as areas that provide food and shelter for developing fish and crustaceans, as well as sources of recruits for nearby coral reefs (see reviews by Beck et al., 2001; Gillanders et al., 2003; Sheridan and Hays, 2003). The extent of connectivity (i.e., the demographic link between populations of a species due to the movement of individuals; Mora and Sale, 2002) between potential nursery (e.g., mangrove) and adult (e.g., coral reef) habitat is relevant to fisheries conservation and management throughout the world, yet its direct quantification remains a significant gap in our understanding (but see Yamashita et al., 2000; Thorrold et al., 2001; Gillanders, 2002; Gillanders et al., 2003; Chittaro et al., 2004; Hamer et al., 2005). In this study we expand on previous work of Chittaro et al. (2005) to examine the utility of using otolith chemistry of *Lutjanus apodus* to classify individuals to their habitat and site of collection; which is a necessary step towards understanding the movement of individuals between potential nursery and adult habitats. Specifically, we investigate whether it is possible to identify the habitat (i.e., mangrove or coral reef), and at a finer resolution, the sites from which individuals were collected. In addition, we compare previously published data on otolith chemistry of *Haemulon flavolineatum* to that of *L. apodus*, which were collected from the same place and time, to assess whether both species show similarities in their spatial patterns of elemental concentrations.

2. Materials and methods

2.1. Site description

Our sampling location, Turneffe Atoll, Belize, is a large (approximately 60 km long and 16 km wide) complex of cayes that are isolated from the mainland (51 km) and the Belize barrier reef (14 km) by a 275–300 m deep channel (Fig. 1). Turneffe Atoll is composed of numerous cayes, the majority of which are covered with mangrove forest (covering 74.2 km^2), while the perimeter of Turneffe Atoll consists of a barrier reef.

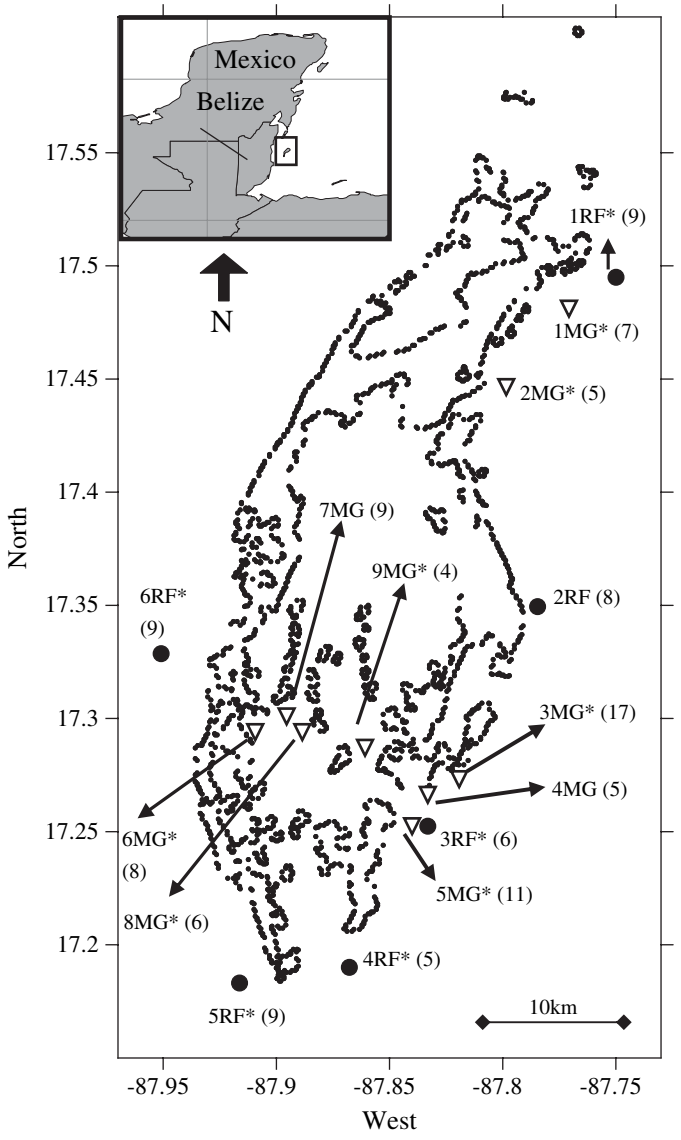

Fig. 1. Geographic position (in decimal degrees) of mangrove (MG; triangles) and coral reef (RF; circles) sites throughout Turneffe Atoll, Belize. Site sample sizes are provided in parentheses and * indicates sites where both *Lutjanus apodus* and *Haemulon flavonineatum* were collected.

2.2. Study organism and sampling

Our study organism is *Lutjanus apodus*, an abundant commercially important Caribbean fish (Class Actinopterygii, Order Perciformes, Family Lutjanidae) known to occupy mangrove and reef habitat and suspected to move between them (Rooker, 1995; Nagelkerken et al., 2000; Cocheret de la Moriniere et al., 2003; Dorenbosch et al., 2004). Individuals of *L. apodus* (5 to 21 per site; average standard length of 17 ± 6 cm) were collected at 15 sites (9 mangrove and 6 reef sites), which were separated by 1–54 km, at Turneffe Atoll over 20 days (July 21–August 9, 2003) (Fig. 1), using both hand spear and gill net (5 m by 2 m monofilament barrier net, 1 cm stretched mesh). Coral reef sites (referred to as RF) were located on back reef sections of the barrier reef and were at a depth of approximately 0.75 to 1.5 m. Mangrove sites (referred to as MG) were chosen based on their accessibility as well as their proximity to coral reef sites (although desired, paired mangrove and coral reef sites were not always possible; e.g., 2RF, 4RF and 5RF did not have a corresponding mangrove site) and were approximately 1 to 3 m deep (each site encompassed a total area of *c.* 200 m^2). Immediately after *L. apodus* collection, we removed sagittal otoliths, which were then stored dry in individual vials.

2.3. Otolith chemical analysis

At the University of Windsor, we embedded sagittal otoliths in epoxy resin (Gougeon®) and sectioned them in a transverse plane, using a low speed diamond saw (Buehler®), to a width of 350 μm. In a class 100 clean room we mounted multiple otolith sections (up to 30) to a microscope slide (otoliths from each site were randomly distributed among the slides). Each slide was then sonicated in a milli-Q water bath for 2.5 min, triple rinsed in 95% ethanol, triple rinsed in milli-Q water, and dried in a laminar flow HEPA filtered fume hood. Otoliths were chemically analyzed at the Great Lakes Institute of Environmental Research, University of Windsor, using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). A Thermo Elemental X7 ICP-MS was operated at low resolution using argon as the carrier gas. The laser sampling system is a purpose-built system (Fryer et al., 1995) based on a non-homogenized, high power, frequency quadrupled (266 nm) Nd:YAG (neodymium-doped yttrium aluminum garnet) laser. The laser beam is focused onto the sample using an Olympus® BX-51 petrographic microscope and an Optics For Research® 266 nm 10× objective lens. A 1.5 mm pinhole beam constrictor was used to increase the spatial resolution of the laser sampling (beam diameter was approximately 15–20 μm). The sampling system is more fully described in Crowe et al. (2003).

The otolith edge was targeted using an automated microscope stage resulting in a contour of approximately 80 to 120 μm in length (speed of the stage varied between 3–5 μm/s) (all aspects of otolith chemical analysis used by Chittaro et al. (2005) were followed in this study in order to facilitate comparisons). Data acquisition lasted 100 s with 60 s of background acquisition at the start of each ablation. Trace element doped glass standards (National Institute of Standards and Technology, NIST, 610) were analyzed twice at the beginning and end of each sample set (which consisted of up to 16 otoliths) to correct for instrument drift. Calcium was used as an internal standard to compensate for signal variation caused by differences in the amount of ablated material. Limits of detection for each isotope were determined as the average background plus three standard deviations.

In total, 20 isotopes were analyzed by LA-ICP-MS and chemical concentrations and detection limits (parts per million) were calculated using Lamtrace software (van Achterbergh et al., 2001). Isotopes that met the following two criteria were included in statistical analyses: 1) an isotopes' concentration in the NIST samples was determined with a satisfactory precision (coefficient of variation less than 10%) and 2) an isotopes' concentration in otoliths was greater than detection limit for more than 50% of otoliths analyzed. Of these isotopes, if an otoliths' isotopic concentration was below detection limit we used an average concentration of otoliths from the same site and time. For isotopes meeting the above criteria, outlier analysis was performed for each element such that any value that was three times the interquartile distance was removed (see Fowler et al., 1995; StatSoft, 2001). Unless otherwise reported, data were $\log_{10}$ transformed to improve normality for multivariate analyses (see below).

2.4. Spatial variability

To investigate patterns of elemental concentrations between mangrove and reef habitats we utilized a multivariate approach and therefore tests of homogeneity of variance and normality were required. If assumptions were met then a nested MANOVA was used with sites (9 mangrove sites and 6 reef sites) nested within habitats. Dependent variables were the elemental concentrations and independent variables were habitat (mangrove or reef) and sites. If significant differences were detected among sites within habitats, a Tukeys HSD post hoc test for unequal sample sizes was used to determine which sites were significantly different from each other.

We performed two Linear Discriminant Function Analyses (LDFA); one at the level of habitat and the other at the level of sites. Specifically, one LDFA tested whether otolith chemical differences were substantial enough to differentiate amongst fish collected from mangrove and coral reef habitats, while the other determined if there was sufficient variation in elemental concentrations to identify the sites from which fish were collected. A jackknifed classification matrix and partial Wilks' Lambda statistic were determined for both LDFAs; the former indicates the percent of fish that were correctly identified to the habitat/site from which they were collected, while the latter indicates the element(s) that explained the greatest degree of separation between/among habitats/sites (StatSoft, Inc., 2001).

2.5. Spatially explicit analysis

To assess the relative effect, if any, of distance and habitat on otolith elemental concentrations a partial Mantel test was conducted (using zt software program; Bonnet and Van de Peer, 2005). The goal of the partial Mantel test is to test the correlation between two matrices while controlling the effect of a third matrix, and thus remove spurious correlations (Bonnet and Van de Peer, 2005). Specifically, this analysis tests the significance of the correlation between matrices by assessing the result from repeated randomizations (McCune and Grace, 2002). If randomization (i.e., shuffling the order of the rows and columns of one matrix) results in correlations between matrices (evaluated by the standardized Mantel statistic, r, and P value) that are as strong as the non-randomized data, then there is little correlation between matrices (McCune and Grace, 2002). Alternatively, if a strong correlation exists between matrices, then a randomization of one matrix will result in the loss of this correlation. The three matrices (each 15×15) used in this analysis were, 1) dissimilarity matrix based on the mean differences in concentrations for all elements between each pair of sites, 2) distance matrix based on the distance between pairs of sites, and 3) a habitat matrix based on whether pairs of sites were of similar habitats (MG-MG or RF-RF) or different habitats (MG-RF). Because of the relatively small sample sizes the method of permutation of raw values was used (Bonnet and Van de Peer, 2005) and 100,000 randomizations were performed.

2.6. Species comparison

Finally, to determine the extent to which two species of reef fish have similar spatial patterns in their elemental signatures, we compared otolith chemical signatures of *Lutjanus apodus* to that of previously published data on *Haemulon flavolineatum* (Chittaro et al., 2005) that were collected at 12 of the 15 sites (Fig. 1) over the same sampling period. Specifically, a MANOVA model was run with elemental concentrations as dependent variables and species (*L. apodus* and *H. flavolineatum*) and habitat (MG and RF) as main effects together with a nested term of sites nested within habitat, and two interaction terms; species-habitat and species-sites nested within habitat. Since we were primarily interested in whether both species showed similar elemental concentrations among sites and habitats we focused on the interaction terms. If significant multivariate differences were detected then univariate analyses were also investigated.

3. Results

3.1. Elements used in statistical analyses

Based on the two criteria (an isotopes' concentration in NIST has a coefficient of variation less than 10%, and an isotopes' concentration in otoliths was greater than the detection limit for more than 50% of otoliths analyzed), several elements were retained for statistical analysis. Specifically, Li, Mg, Cu, Zn, Rb, Sr, Sn, Ba, and Pb were at concentrations sufficiently above detection limit to permit meaningful interpretations (Table 1). Outlier analysis removed 26 fish from the 144 that were collected, resulting in 72 and 46 fish analyzed for the mangrove and coral reef sites, respectively.

3.2. Spatial variability

A traditional nested MANOVA was performed since assumptions of homogeneity of variance and covariance and normality were met (Zn and Ba failed to meet assumptions of normality even after transformation, and therefore were excluded from this analysis). Overall, there were significant differences in elemental concentrations of fish from mangrove and coral reef habitats (Wilks' Lambda $= 0.79$; df $= 7, 97$; $F = 3.59$; $P < 0.01$). We observed statistical significance of the univariate analyses between habitats for Mg and Sn (Table 2), such that concentrations of both elements were greater in otoliths from mangroves than in those from reefs. In addition, we observed significant variation in the concentrations of most elements (Li, Cu, Rb, Sr, Sn, and Pb) among sites within habitats (Table 2). Tukeys HSD post hoc tests revealed that only a relatively small number of pair-wise comparisons were significant. For Li, Cu, Rb, and Sn, 8, 33, 5, and 25 pair-wise comparisons, respectively, were significantly different out of 171 possible comparisons, while Sr and Pb showed only one significant pair-wise comparison. Furthermore, there was an absence of habitat specific patterns (Fig. 2). In other words, sites that had significantly greater or lower concentrations were not necessarily associated with a particular habitat type. For instance, Li and Rb concentrations at the North East mangrove site (2MG) were significantly lower than several mangrove and coral reef sites (1MG, 4–7MG, 4–6RF, and 4–5MG, 7–8MG, 2RF, respectively). Also, concentrations of Cu and Sn were significantly higher at central sites on the west (6RF and 6MG) and east (4MG) side of Turneffe Atoll, and differed significantly from the

Table 1
Average coefficient of variation (CV) of NIST and the percentage of samples greater than detection limit for those elements that met two criteria; the CV of NIST samples was <10% and >50% of otolith samples had concentrations that were greater than detection limit

Isotope measured (atomic mass)	Average. CV (std. dev.)	Percentage of samples > detection limit	Average detection limit by sample (std. dev.)	Average concentration (std. dev.)
Li (7)	4.57 (0.46)	68	0.09 (0.03)	0.13 (0.04)
Mg (25)	2.47 (0.19)	100	0.86 (0.23)	15.82 (8.27)
Cu (65)	3.42 (0.08)	97	0.27 (0.07)	8.01 (15.91)
Zn (66)	4.13 (0.24)	97	0.05 (0.02)	1.31 (3.32)
Rb (85)	3.17 (0.26)	59	0.05 (0.02)	0.12 (0.08)
Sr (86)	1.53 (0.13)	100	0.42 (0.17)	2783 (379)
Sn (120)	3.98 (0.22)	100	0.05 (0.01)	2.14 (2.56)
Ba (138)	1.68 (0.13)	100	0.02 (0.01)	3.62 (2.53)
Pb (208)	4.54 (0.26)	80	0.01 (0.01)	0.03 (0.02)

For each element, average (standard deviation) detection limit and concentration (both measured as parts per million) are provided. Average CV is the mean of 11 different sets of otolith ablations.

Table 2
Univariate results of the nested MANOVA (using otolith edge concentrations of *Lutjanus apodus*), whereby site was nested within habitat

Effect	DF	MS	*F*	*P*<	MS	*F*	*P*<
		Li			Mg		
Habitat	1	0.01	0.26	NS	0.76	19.8	0.001
Site (Habitat)	13	0.08	3.53	0.001	0.04	1.09	NS
Error	103						
		Cu			Rb		
Habitat	1	0.25	1.65	NS	0.01	0.01	NS
Site (Habitat)	13	1.77	11.9	0.001	0.17	3.55	0.001
Error	103						
		Sr			Sn		
Habitat	1	0.01	0.3	NS	0.59	6.66	0.001
Site (Habitat)	13	0.01	3.2	0.001	0.77	8.67	0.001
Error	103						
		Pb					
Habitat	1	0.01	0.05	N.S.			
Site (Habitat)	13	0.27	3.23	0.001			
Error	103						

N.S. indicates non-significance ($P > 0.05$).

majority of sites ([Cu] at 6RF > 2–3MG, 5MG, 8–9MG, 2–3RF, 5RF; [Cu] and [Sn] at 4MG > 1–3MG, 5MG, 7–9MG, 1–5RF; [Cu] at 6MG > 1–3MG, 5MG, 8–9MG, 2–5RF; [Sn] at 6RF > 2–3MG, 5MG, 1–5MG; [Sn] at 6MG > 2–3MG, 2RF, 5RF).

Although Zn and Ba did not meet assumptions of normality we included them in the LDFA because this analysis is relatively robust with respect to skew (McCune and Grace, 2002). Significant discrimination (Wilks' Lambda = 0.70; df = 9,108; $F = 5.06$; $P < 0.001$) was observed for the LDFA comparing elemental concentrations of fish from mangrove and coral reef habitats, such that one significant function (chi-squared statistic = 39.24, df = 9, $P < 0.001$) was produced explaining 55% of the variation. The partial Wilks' Lambda was relatively high for all elements (the lowest value was 0.79 for Mg indicated that it was most important in the discrimination, followed by Zn, Ba, Rb, Sn, Cu, Sr, Pb, and Li), suggesting that most elements contributed relatively weakly to the discrimination of fish between mangrove and reefs sites. Overall, the LDFA indicated that otolith chemistry varied sufficiently between habitats to permit the correct classification of 67% of the fish to mangrove and coral reef habitats.

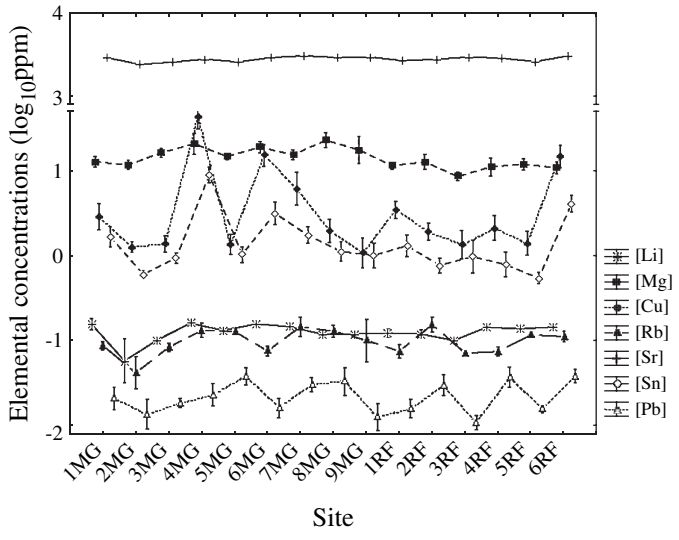

Fig. 2. Log_{10} transformed otolith elemental concentrations (ppm) of lithium (Li), magnesium (Mg), copper (Cu), rhubidium (Rb), strontium (Sr), tin (Sn), and lead (Pb) per site. Mean and standard error are plotted. Significant pair-wise comparisons were not displayed, but see text for results of Tukeys HSD post hoc test.

Results of the LDFA comparing otolith chemistry among sites indicated significant discrimination (Wilks' Lambda = 0.02; df = 126, 740; $F = 4.05$; $P < 0.001$), such that six significant functions (chi-square statistic for the entire model = 420.44, df = 162, $P < 0.001$) were produced explaining 88% of the variation (first two functions accounted for 58% of the variation). The greatest discriminatory ability for this model (i.e., partial Wilks' Lambda) resulted from Mg, Zn, and Ba (0.46–0.65). Overall, the LDFA indicated a poor ability to correctly classify fish to the site from which they were collected (average correct classification of 40%; and a range of 0–75%). Misclassified fish collected from mangroves and reefs were more likely to be classified to mangrove sites than reef sites (55% and 62% of misclassified mangrove and reef fish, respectively, were classified to mangrove sites). Although larger sample sizes are ideal (average number of fish per site was 7.7 and 8.0 for coral reefs and mangroves, respectively) no significant relationship between sample size and discriminatory ability was detected ($R^2 = 0.17$; df = 1,13; $F = 2.7$; $P = 0.12$).

3.3. Spatially explicit analysis

The analysis to determine whether differences in otolith elemental concentrations between sites were related to distance (when controlling for habitat) did not reveal a significant correlation. This suggests that regardless of the distance between sites from which fish were collected at Turneffe Atoll there is sufficient overlap in otolith elemental concentrations that resulted in the poor discriminatory ability among sites.

3.4. Species comparison

Finally, through the comparison of elemental concentrations of both *Lutjanus apodus* and *Haemulon flavolineatum* collected from the same sites, we determined that there was little similarity in the spatial patterns of their elemental signatures. Specifically, significant multivariate interactions were detected for species and habitat (Wilks Lambda = 0.83; df = 7,158; $F = 4.5$; $P < 0.001$) as well as species and site nested within habitat (Wilks Lambda = 0.25; df = 70,928; $F = 3.5$; $P < 0.001$). From univariate analyses, concentrations of Li and Rb showed a significant interaction between species and habitat, while Li, Cu, Rb, Sr, Sn, and Pb were significant between species and site nested within habitat (Table 3).

4. Discussion

The successful use of otolith chemistry to differentiate fish collected from multiple sites is directly related to detectable differences in their chemical signatures (that result from natural and/or anthropogenic influences present in the environment) at the investigated spatial scale. Several studies have reported substantial differences in otolith chemistry that has permitted successful investigations of the spatial arrangement of fish (e.g., Edmunds et al., 1989; Dove and Kingsford, 1998; Kingsford and Gillanders, 2000; Rooker et al., 2003), while others have highlighted difficulties (see Gillanders et al., 2001; Patterson et al., 2004; Chittaro et al., 2005). In this study, otolith elemental concentrations of *Lutjanus apodus* varied significantly between fish collected from mangroves and reefs, as well as among sites within habitats, but only at the habitat level of comparisons was there a consistent pattern that permitted a moderate level of accuracy in the classification of individuals. Specifically, fish from mangroves had significantly greater concentrations of Mg and Sn relative to those from reefs (Mg had higher average concentrations in 7 mangrove sites relative to all reef sites, while Sn had higher average concentrations in 4 mangrove sites relative to all but 1 reef site) (Fig. 2). Interestingly, work on *Haemulon flavolineatum* by Chittaro et al. (2005), in which individuals were collected from many of the same sites at Turneffe Atoll, also indicated that consistent chemical signatures were only apparent at the level of habitat. In fact, from their otolith chemical analyses, Chittaro et al. (2005) observed that concentrations of Li, Mg, Zn, and Rb were all significantly greater in fish from mangroves than those from reefs. Although the results by Chittaro et al. (2005) were based on 19 sites compared to the 15 of this study (and thus some of the differences between studies can be explained by differences in the datasets) the more likely explanation for the variation between studies in terms of the 'mangrove − reef signature' suggests that different species differentially record chemical aspects of the environment (see below).

Table 3
Univariate results of the MANOVA using otolith edge concentrations of *Lutjanus apodus* and *Haemulon flavolineatum*

Effect	DF	MS	*F*	*P*<	MS	*F*	*P*<
		Li			Mg		
Species	1	0.19	10.8	0.001	0.01	0.45	NS
Habitat	1	0.02	1.18	NS	1.14	34.9	0.001
Site (Habitat)	10	0.08	4.24	0.001	0.05	1.66	NS
Species × Habitat	1	0.26	14.3	0.001	0.00	0.11	NS
Species × Site (Habitat)	10	0.09	4.90	0.001	0.04	1.14	NS
Error		0.02			0.03		
		Cu			Rb		
Species	1	5.06	36.6	0.001	1.85	52.8	0.001
Habitat	1	0.02	0.14	NS	0.19	5.53	0.05
Site (Habitat)	10	0.71	5.14	0.001	0.31	3.73	0.001
Species × Habitat	1	0.46	3.30	NS	0.21	5.94	0.05
Species × Site (Habitat)	10	0.63	4.53	0.001	0.10	2.93	0.001
Error		0.14			0.40		
		Sr			Sn		
Species	1	0.17	66.9	0.001	4.20	46.5	0.001
Habitat	1	0.01	4.07	0.05	0.04	0.44	NS
Site (Habitat)	10	0.04	3.54	0.001	0.43	4.77	0.001
Species × Habitat	1	0.00	0.14	NS	0.06	0.65	NS
Species × Site (Habitat)	10	0.00	3.35	0.001	0.46	5.12	0.001
Error		0.00			0.09		
		Pb					
Species	1	14.1	131	0.001			
Habitat	1	0.20	1.88	NS			
Site (Habitat)	10	0.23	2.15	0.05			
Species × Habitat	1	0.29	2.67	NS			
Species × Site (Habitat)	10	0.70	6.52	0.001			
Error		0.11					

N.S. indicates non-significance ($P > 0.05$).

At the finer resolution of sites, although our analysis of otolith chemistry revealed significant variability in the concentrations of Li, Cu, Rb, Sr, Sn, and Pb (Table 2) the majority of sites showed substantial overlap in elemental concentrations (Fig. 2), which resulted in 60% of the individuals, on average, being misclassified away from their site of residence. Low levels of natural or man-made inputs of elements were suggested by Gillanders et al. (2001) and Patterson et al. (2004) to explain their relatively poor discriminatory ability for *Diplodus vulgaris* (Two-branded bream) in the Mediterranean (sites and locations separated approximately 0.1−10 km) and *Pomacentrus coelestis* (Neon damselfish) along the Great Barrier Reef (differentiation difficulty only among sites separated by approximately 3−12 km), respectively. In addition to the lack of terrigenous inputs, Chittaro et al. (2005) suggested that the movements of their study species (*Haemulon flavolineatum*) were likely to contribute to the overlap in edge elemental concentrations, which in turn lead to their reported poor discriminatory ability among sites. In short, they suggested that due to the nocturnal movements of *H. flavolineatum* (individuals have been observed to move up to 199 m; see Burke, 1995) the chemistry of the edge of otoliths from individuals collected from the same site would likely contain the elemental concentrations from multiple areas, thus increasing the variability in otolith elemental concentrations per site. Unfortunately, *Lutjanus apodus* has also been reported to move into adjacent areas at night (Rooker, 1995; Nagelkerken et al., 2000; Cocheret de la Moriniere et al., 2003), and although the extent of movement is unknown it likely contributed to the poor classification of individuals reported in this study.

Because of the likelihood of fish movements together with the relative lack of terrigenous inputs, the ability to differentiate individuals to their site of collection was suspected to improve as the distance between sites increased. If so, then for future work at Turneffe Atoll it would be valuable to know the spatial scale at which the discrimination of individuals would likely be maximized. Based on our investigation in which we assessed (using a partial Mantel test) the relative effect of distance (when controlling for habitat type) on otolith elemental concentrations, the results indicated that throughout Turneffe Atoll there was overlap in elemental concentrations regardless of distance. Consequently, we advice that otolith chemical investigations into the movement of individuals

among sites at Turneffe Atoll, Belize, are not feasible due to the lack of spatial variation in elemental concentrations.

Finally, having otolith chemistry of *Lutjanus apodus* collected at many of the same sites in which previously published work on *Haemulon flavolineatum* were also collected (see Chittaro et al., 2005) allowed us to investigate the extent to which elemental concentrations between habitats and among sites, are species specific. Results of this analysis suggested little similarity in otolith chemistry between these species since species and habitat interacted in their effect on elemental concentrations of Li and Rb, and species and site (which were nested within habitat) interacted in their effect on the concentrations of several elements (except for Mg; Table 3). Similar reports of interspecific differences in otolith chemistry have been noted by Gillanders and Kingsford (2003) and Patterson et al. (2004), such that Gillanders and Kingsford (2003) speculated whether the differences were due to differential microhabitat use by each species and/or age related differences that were incorporated when using solution-based analyses. In addition, Swearer et al. (2003) observed significant variability in otolith chemistry among five species (two were gobies, two were flatfish, and one was a smelt), whereby elemental concentrations were most similar between closely related species.

Overall, this study determined that *Lutjanus apodus* collected from mangrove and coral reefs at Turneffe Atoll are best identified by concentrations of Mg and Sn, and therefore suggests that these elements would be useful in examining movement between habitats at this location. However, due to the lack of terrigenous inputs at Turneffe Atoll and the likelihood of individual movement, site-specific otolith chemical signatures were insufficient to classify more than 60% of the individuals, on average, to their site of collection. Furthermore, we advice against the use otolith chemistry to investigate the movement of individuals among sites at this location because of a lack of sufficient elemental variability throughout Turneffe Atoll, Belize, even at increasing distances between sites. Finally, since interspecific comparisons of otolith elemental concentrations indicated little similarity in spatial patterns, the habitat generalizations reported in this study and that of Chittaro et al. (2005) are species specific.

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