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The Effects of Spatial Scale on Assigning Nursery Habitats in Atlantic Goliath Groupers (*Epinephelus itajara*) Using Non-lethal Analyses of Fin Rays

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Abstract We evaluated Atlantic Goliath Groupers, *Epinephelus itajara*, in their nursery habitats via microchemical analyses of fin rays. Juveniles were sampled from known nursery habitats off southwest Florida, and adults were primarily sampled from a spawning aggregation off southeast, Florida. We collected fin rays using a non-lethal technique that is minimally invasive with no known negative effects on growth or survival. Trace metal constituents in the fin rays were quantified with an inductively coupled plasma mass spectrometer via laser ablation (LA-ICP-MS). Two spatial scales were quantified to test the limitations of grouping individuals based on elemental compositions. On a small spatial scale (i.e., 100s of m), individuals were correctly classified within individual watersheds 64% of the time. On a larger spatial scale (i.e., 10s–100s of km), juveniles were classified with 100% accuracy. Trace metals in adults were analyzed by back-tracking across fin ray annuli to a year in which our previous studies have shown these adults occupied their juvenile habitats (i.e., 2006). These fish were grouped using a measure of dissimilarity and then analyzed to test whether we could reclassify them into these same groupings based solely on the chemical components in their fin rays, which was done with over 84% accuracy. Although juvenile habitats of the adults could not be determined due to the lack of baseline data, classifications were driven by similar elements to those that drove the classification of juveniles, suggesting similar physiological mechanisms. The results highlight the importance of spatial scale for interpreting microchemical analyses on calcified structures in fishes.

Keywords Fin ray chemistry · Ten Thousand Islands · Natural tags · Nursery of origin · Trace element analysis · Chemical fingerprints

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Introduction

Estuarine habitats have long been assumed to be important nurseries for many fishes and invertebrates based on the observed high abundances of juveniles associated with them (Beck et al. 2003). However, the relative contribution of juveniles from a particular habitat to the adult population, by way of ontogenetic migrations, is a more meaningful criterion for "essential nursery habitats (ENH)" than abundance alone (sensu Beck et al. 2001; Stoner 2003; Dahlgren et al. 2006). ENHs are particularly relevant to recovering stocks of depleted species, as recruitment success can be paramount to the persistence of their populations (Sheaves et al. 2006).

The ability to assign members of the adult population to their juvenile habitats offers a quantifiable metric to assess ENH and can direct the management of endangered species by suggesting preservation sites at nursery grounds. Until recently, the research on ENHs has been largely theoretical due to the difficulties associated with tracking individuals throughout the course of their ontogenetic migrations (i.e., measuring movement between juvenile and adult habitat). Tagging studies that aim to quantify the contributions of juvenile habitats are costly and often suffer from low return rates (Pine et al. 2003). Studies that use natural tags offer a viable alternative, but still require the characterization of individual

nurseries on multiple spatial scales so that adults can be traced back to their nursery origin (Gillanders et al. 2003). Spatial heterogeneity confounds background levels of trace elements in the marine environment and may result in unique background signatures, in time and space, at very small spatial scales that cannot then be characterized on larger scales (e.g., Gao et al. 2009). Chemical heterogeneity in the marine environment may limit the quantification and characterization of habitats on different spatial scales, depending on the ecosystem. Traditionally, the study of ontogenetic movements of marine fishes has relied on otolith microchemistry (Gillanders and Kingsford 2000; Hobbs et al. 2010; Mercier et al. 2011), requiring sacrifice of the study organisms.

The Atlantic Goliath Grouper (*Epinephelus itajara*) is critically endangered throughout its range (Pusack and Graham 2009) and is extirpated in waters off western Africa (Craig et al. 2009). As a result of their exceptionally low abundances, a federal moratorium in the USA has prohibited landings of the species since 1990 in US continental waters (primarily off Florida). In the early 2000s, the *E. itajara* population in Florida waters began showing early signs of recovery, initially off the southwest coast, and more recently throughout the state (Koenig et al. 2011).

The ongoing recovery of *E. itajara* in Florida highlights the role that ENHs can play in the restoration of a depleted population (Koenig et al. 2007). Postlarval juveniles of the species settle into leaf litter in mangrove lagoons (Lara et al. 2009). They remain in the mangrove ecosystem for the initial 4–7 years of their lives, where they typically inhabit deep undercuts and submerged structure such as mangrove roots (Koenig et al. 2007). Indeed, the extensive and intact mangrove habitat off the southwest coast of Florida in the Ten Thousand Islands (TTI) region is the presumed ENH for the species and is thought to be largely responsible for its recovery (Koenig et al. 2007). The TTI borders the Big Cypress National Preserve, which prohibits development and limits anthropogenic influences. As a result, the mangrove habitat in the TTI has relatively high water quality (Fourqurean et al. 2003), which may produce ideal conditions for the ENH of *E. itajara*. However, the information currently available regarding nursery use is based on tagging studies with tag returns of less than 5% for juveniles that were tagged and then recaptured as adults (Eklund and Schull 2001; Koenig et al. 2011).

Our objectives were to characterize the *E. itajara* juvenile habitats at multiple spatial scales within the TTI region and the surrounding areas in order to measure future contributions to the adult population, by creating habitat-specific profiles that can be matched to adult samples in future studies. Specifically, we identified chemical indicators, or "fingerprints," of juvenile habitats by sampling multiple individuals within each location. Due to the endangered status of *E. itajara*, we employed a non-lethal and minimally invasive technique to study microchemical trends among individuals as an alternative method to examine nursery habitats at multiple spatial scales. Specifically, we identified the chemical fingerprints of juvenile habitats embedded in fin rays of both juvenile and adult *E. itajara*. Our approach was possible because the annuli within the fin rays of *E. itajara* correspond to yearly depositions (Clarke et al. 2007; Murie et al. 2009), which retain chemical properties over time (Tzadik et al. 2015).

Methods

Sample Collection and Study Area

We collected fin rays from 40 juveniles in southern Florida. Sampling occurred during June through August 2014 in the TTI region, Pine Island Sound, and the Lower Florida Keys, which are all areas where *E. itajara* juveniles are abundant (Fig. 1). Due to the ENHs' role previously suggested in the TTI region for *E. itajara* (Koenig et al. 2007; Lara et al. 2009), we focused more effort there than Pine Island Sound and the Lower Florida Keys. Sampling sites within TTI were

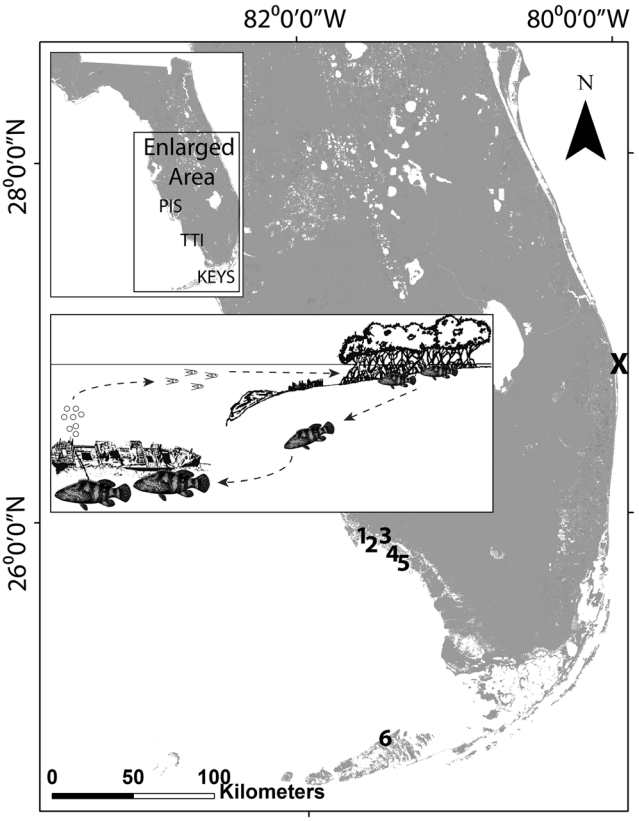

Fig. 1 Map of southern Florida showing sampling locations for juvenile *E. itajara*, with a diagram of its life history. The general sampling locations, Pine Island Sound (PIS), Ten Thousand Islands (TTI), and the Florida Keys (Keys) are labeled on the *large-scale inset*. *Numbers* on the *map* correspond to sample sites for Fig. 2. The large "X" represents the sampling location of adults in southeastern Florida

categorized by watershed and drainage basin (Fig. 1). All juvenile sites had high-relief, subtidal structure. Most sites contained natural habitat such as mangrove prop roots or rock undercuts, but three sites were artificial structures such as ship wreckage and concrete pilings. We also sampled 54 adults from known spawning aggregations (Koenig et al. 2016a, b) at offshore locations in southeastern Florida during August through September 2013, and an additional 11 adult samples from TTI were donated by collaborating fishermen. All adult sites had high structural relief, either natural or artificial reefs.

Juvenile Sampling

Juveniles were captured using traps, set lines, and hand lines. Blue-crab traps (61 cm × 61 cm × 46 cm) were used based on previously documented effectiveness (Koenig et al. 2007). These traps were constructed of coated wire mesh with two funnels (proximal openings of 19 cm × 12.5 cm and distal openings of 18 cm × 7.5 cm) leading into the lower chamber and another two funnels (both proximal and distal openings of 18 cm × 7.5 cm) leading into the upper chamber. Traps were placed next to mangrove roots, primarily in low-current canals, and weighted using 1-kg lead weights. Roughly two thirds of all traps were baited (using dead baitfish, e.g., *Ariopsis felis*, *Bagre marinus*, *Lagodon rhomboides*, *Orthopristis chrysoptera*), while the remaining traps were un-baited.

Set lines were made using 14/0 or 15/0 circle hooks that were attached to 50 cm of a 400 kg test monofilament. The monofilament sections were attached to 3–4 m of 0.16 cm stainless steel cable with a 170 g weight to keep the line taught. The end of the cables was attached to an 8/0 gangion clip. Lines were baited with either live or dead fish (i.e., *A. felis*, *B. marinus*, *L. rhomboides*, or *O. chrysoptera*). We attached all lines to mangrove prop roots in areas with deep undercuts and high currents.

We used hand lines opportunistically in locations where set lines were not practical, such as areas of exceptionally high currents or where water clarity allowed snorkelers to place the bait directly in front of the fish. Hand lines comprised a 15/0 circle hook and two 170 g weights attached to a 135 kg test monofilament that was wrapped around a hand reel. As described above, hooks were baited with either live or dead fish.

Juveniles were tagged ventrally with individually numbered stainless-steel-core internal anchor tags (Floy Tag Company) and measured for total length. We excised soft dorsal fin rays 6 and 7 to maintain consistency with a companion study (Koenig et al. 2016a, b). Fin membranes on either side of the two rays were cut with a knife to the base of the fin, and the fin ray was excised as close to the base as possible, using 15 cm cutting pliers. Juveniles were never held out of the water for more than 3 min.

Adult Sampling

Adults were captured using hand lines in collaboration with a companion study to determine the age structure of *E. itajara* in Florida (Koenig et al. 2016a, b). Adults were measured (total length) and tagged both externally (livestock tag) and internally (Passive Integrated Transponder). We removed the soft dorsal fin rays 6 and 7 in the same manner as described for juveniles. Sampling adults typically took 5–10 min, so we flushed ambient water over the gills and placed a damp towel over the eyes while the fish were being processed on deck. Individuals were released immediately following sampling.

Fin Ray Analysis

Immediately after excision, fin rays were bagged, labeled, and stored on ice. Samples were stored in a freezer at −20 °C until further processing. Fin rays were thawed by removing them from the freezer and were immediately placed in a drying oven for 3 h at 55 °C. Once thawed, the fat, membrane, and muscle tissues were removed using rubber-tipped forceps. We then soaked the rays in trace metal grade 30% hydrogen peroxide (H_2O_2) for 5 min to loosen any remaining tissues, which were removed using rubber-tipped forceps and paper towels.

Each cleaned ray was attached to a petrographic microscope slide using Crystalbond™ adhesive (SPI Supplies, Westchester, PA, USA). Two cross sections, each 0.5 mm thick, were cut from the ray as close to the base as possible using a Buehler IsoMet™ slow-speed saw (Buehler, Lake Bluff, IL, USA). We used one cross section for aging and the other for chemical analysis. Cross sections did not typically require polishing to expose the annuli, but when necessary, we polished the section using 800-grit wet sandpaper. Cross sections were independently aged by two readers. If there was disagreement between age estimates, a third reader was used. All adult samples were also sent to the age and growth lab at the University of Florida for verification.

The second cross section from each fish was mounted on petrographic slides using Crystalbond™ and sonicated in ultrapure Milli-Q™ water for 5 min. After sonication, samples were air dried for 24 h in a class-100 laminar flow clean hood. The second sections were attached to acid-washed petrographic slides so that roughly 20 samples were attached to a single slide. All samples were assayed using an Agilent Technologies 7500 ICP-MS coupled with a Photon Machines Analyte 193 excimer UV laser ablation system (LA-ICP-MS).

We used a sequence of replicate spot samples (n = 3) of 64 μm diameter at the outermost annulus for juvenile samples and the annulus corresponding to the year 2006 for the adult samples. The year 2006 was chosen for analysis as the majority (>80%) of the adults were believed to still be in their nursery habitats at that time, based on their ages (≤5 years

old to ensure a conservative estimate). The laser system operated at a wavelength of 193 nm and a set point of 7.0 mJ. Fin ray ablations were conducted with 86% power and a 5 Hz frequency. Background levels were collected for 60 s between each spot scan. We used a single glass standard (NIST 612) with known isotopic compositions to calibrate the instrument. The NIST 612 standard was analyzed prior to and after each sample slide. We also analyzed the standard after every two samples to account for instrument drift. Measurements were made for 26 unique isotopes[1] to quantify the trace elemental compositions within the structure. An internal standard is essential to these measurements due to biases in yield that are apparent during the ablation process over an irregular surface such as fin ray sections. Calcium (Ca) was used as the internal standard due to its abundance and stoichiometric consistency in hydroxyapatite (Wopenka and Pasteris 2005). During a prior analysis using solution-based methods (SB-ICP-MS), Ca concentrations in fin rays were measured via digestion in 16 N HNO_3 within polypropylene vials at 180 °C for 2 h. Samples were diluted with 2% HNO_3. These solutions were then quantitatively analyzed in the ICP-MS to obtain Ca concentrations. Drift of the SB-ICP-MS was monitored and corrected using scandium (Sc) added as an internal standard. The calibration line measured from 5 to 50 ppm for Ca. Based on our previous analysis, Ca concentration was measured as 27.5% of the molecular weight of fin rays.

The Agilent Technologies Instrument control software was used for data collection. Isotopic values of each element of interest were recorded as counts per second. These counts were then converted to concentration (ppm) using Matlab version R2015a, with functions created in the Fathom Toolbox for Matlab (Jones 2014). We used parts per million values in all subsequent analyses.

Statistical Analyses

We classified juvenile samples according to the location where they were captured to test whether we could reassign them based on their chemical properties. Two separate groupings were created based on relevant spatial scales: (1) sites separated by hundreds of meters (hereafter "small scale") and (2) sites separated by tens of kilometers (hereafter "large scale"). By analyzing groups of juveniles on these spatial scales, we assumed that the chemical constituents of the rays were discernable by the influence of ambient water over the variability among individuals. Sites with less than three individuals were not considered due to the small sample size. Given that absolute concentrations of elements naturally varied by up to three orders of magnitude, we standardized them to z-scores to equally weight them (Legendre and Legendre 2012). All variables with measurements that were below the limits of detection were removed prior to further analysis. In order to test and visualize differences among groups at each spatial scale, we used a canonical analysis of principal coordinates (CAP) based on a Euclidean distance matrix (Anderson and Willis 2003). The CAP generated a leave-one-out (LOO) cross-validation matrix, and we used a proportional chance criterion (PCC) to assess the performance of the CAP model and the probability that it performed better than a null model generated by random chance (Morrison 1969). Indicator values were calculated for elements with significant influences on the groupings (at $\alpha < 0.05$) via the indicator value method (IndVal, Dufrene and Legendre 1997).

For adults, we calculated a dissimilarity matrix for all samples because their nursery locations were unknown. A similarity profile analysis (SIMPROF) based on Ward's minimum variance method (Ward's cluster analysis) and a Euclidean distance matrix were implemented via the dissimilarity profile analysis (DISPROF) function in the Fathom Toolbox (Jones 2014). The DISPROF identified groups that were formed based on the dissimilarities of elemental compositions among individuals (Clarke et al. 2008). The IndVal method was used to identify indicator elements for each group. For Sr, a natural break existed at one standard deviation above the mean, between the 12 highest values and the remaining samples. High Sr values are representative of high salinity water, due to the influence from marine limestone and other sediments. These 12 individuals with the highest Sr values were presumed to have moved out of their nursery habitat, or still occupying upriver locations, by the year 2006, possibly due to size-driven egress (Koenig et al. 2007), and were therefore not representative of the juvenile habitats of interest (Elsdon and Gillanders 2003). We excluded these 12 individuals from further analysis.

Last, we used a random forest analysis on the remaining adult samples to model the relationship among elemental concentrations in fin rays and the DISPROF groups, while also reclassifying unknowns to assess the accuracy of the model (Breiman 2001; Cutler et al. 2007; Mercier et al. 2011). The forest was a collection of unique classification trees, each originating from a root node of a bootstrapped training dataset derived from the elemental concentration data. Data from each root node were successively divided into progressively smaller and more homogenous nodes (i.e., branches). At each node, a random set of predictor variables was analyzed to find the one that minimized the sum-of-squared errors among the remaining observations, which was then used to split the data. Trees were grown until the data at the terminal nodes could not be split into more homogenous groups. Once the trees were grown, fitted values of the categorical variable (i.e., the grouping vector) were assembled from their terminal nodes and weighted to produce the final predicted response of the

[1] Li^7, Na^{23}, Mg^{24}, P^{31}, Ca^{43}, Sc^{45}, V^{51}, Cr^{53}, Mn^{55}, Fe^{57}, Co^{59}, Ni^{60}, Cu^{63}, Zn^{64}, Cu^{65}, Ge^{72}, Rb^{85}, Sr^{88}, Y^{89}, Cd^{114}, Sn^{118}, Ba^{137}, Au^{197}, Pb^{208}, Th^{232}, and U^{238}

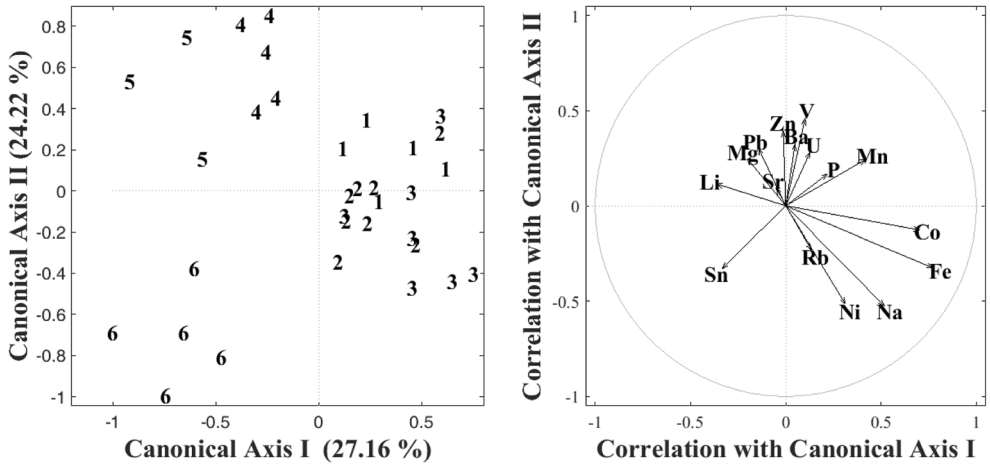

Fig. 2 The canonical analysis of principal coordinates (CAP) for juveniles that were analyzed on a small spatial scale. The length of each vector corresponds to its relative importance in grouping individuals in the direction in which it is pointing. Correlation vectors directly correlate and are proportional to the ordination plot. Site numbers correspond to those presented in Fig. 1

forest. For the adult samples, we used a non-linear random forest model instead of a linear CAP model because it fit the data better.

Results

Age estimates based on the cross sections of fin rays ranged from 2.0 to 6.2 years for juveniles (median = 4.2) and from 5.0 to 14.0 years for adults (median = 10.0). Total lengths ranged from 33.2 to 124.0 cm for juveniles (median = 62.0 cm) and from 122.0 to 222.0 cm for adults (median = 171.0). In the present study, we classified fish by habitat (juveniles in mangroves and adults on offshore reefs) instead of by age or size.

Chemical Fingerprints in Juvenile Habitats

When juveniles were evaluated on a small spatial scale, six areas were classified into a grouping vector based on location. The chemical data from the fin rays were used to correctly classify juveniles 64% of the time with the output model created by the CAP (as compared to 18% by the PCC null, $p = 0.001$). Locations as close as 200 m apart were distinguished to be different by the CAP and were largely influenced by the relative concentrations of cobalt (Co) and barium (Ba) (Fig. 2 and Table 1).

When we categorized the juveniles into groups at the larger spatial scale, three areas were identified. The three groups comprised two sites within TTI and a third from the Lower Florida Keys. The classification success rate for the output model produced by the CAP was 100% (as compared to 42% by the PCC null, $p = 0.001$). Groupings at this spatial scale were precise with no apparent among-group overlap (Fig. 3). The primary drivers of these classifications were Co and manganese (Mn) (Table 2). Most sites in the TTI region grouped together (largely driven by iron (Fe)), while those from two southern TTI sites in Pumpkin Bay grouped on their own (driven by zinc (Zn), Ba, and magnesium (Mg)). A third group, characterized by elevated levels of tin (Sn), was identified as samples from the Lower Florida Keys.

Adult Classification

The DISPROF clustering method identified four groups ($p < 0.05$) from the adult samples (Fig. 4). These groups varied in size (i.e., $n = 25$, $n = 23$, $n = 13$, $n = 4$). Note that all individuals that were subsequently removed from further analyses due to high Sr values came from a single group (Fig. 4, group B). The output model produced by the random forest clustered samples with a classification rate of 85% (as compared to 32% by the PCC null, $p = 0.001$) and was significantly driven by six elements: Mn, Fe, Sr, Sn, Ba, and lead (Pb) (Fig. 5 and Table 3).

Table 1 Indicator values for the significant elements when juvenile samples were grouped on a small spatial scale

Element	Atomic weight	IndVal	*p* value
Li	7	22.59	0.001
Na	23	20.40	0.001
Mg	24	19.09	0.001
V	51	31.00	0.013
Fe	57	19.65	0.001
Co	59	31.73	0.001
Zn	64	22.49	0.004
Rb	85	22.41	0.009
Sr	88	18.13	0.026
Ba	137	26.98	0.005

IndVal indicator value, *p value* significance based on 1000 permutations

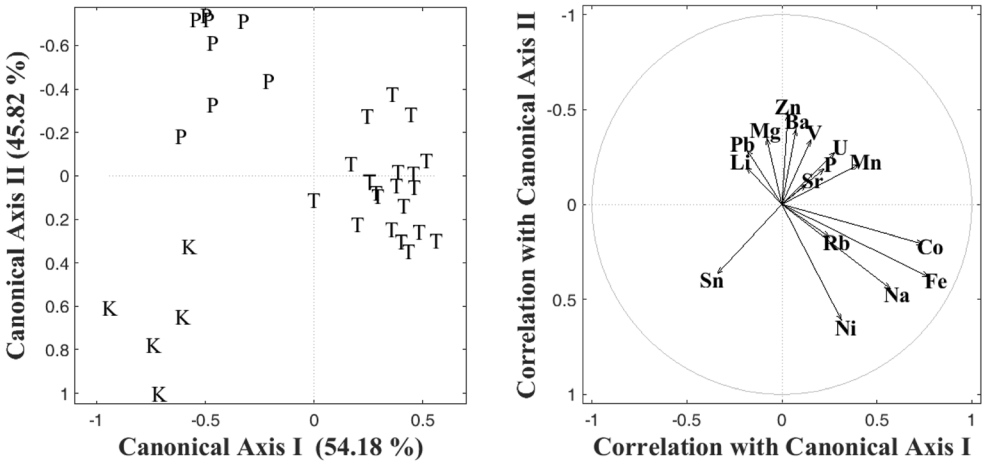

Fig. 3 The canonical analysis of principal coordinates (CAP) for juveniles that were analyzed on a large spatial scale. The length of each vector corresponds to its relative importance in grouping individuals in the direction in which it is pointing. Correlation vectors directly correlate and are proportional to the ordination plot. Site labels run from north to south (i.e., *T* = TTI northern bay system, sites 1, 2, and 3; *P* = Pumpkin Bay system, sites 4 and 5; *K* = Lower Florida Keys, site 6)

Discussion

Juvenile habitats used by *E. itajara* can be accurately distinguished at varying spatial scales in the state of Florida using the microchemical analyses of fin rays. The chemical fingerprints that were incorporated into the fin rays of *E. itajara* acted as natural tags that allowed us to classify specific locations where individuals were sampled. The use of these natural tags may be used to augment tagging studies that commonly experience low return rates. Using a baseline of chemical fingerprints (composed of juvenile fin rays, sampled yearly via a random-stratified sampling design), individuals of unknown origins can be classified by nursery location.

The current application of our methodology was used to identify and characterize juvenile habitats for *E. itajara*. The methods, however, also are applicable to studying movements and ontogenetic migrations in other fishes. Indeed, our methods were largely derived from studies that tracked movements in diadromous fishes over long periods, in some cases over 30 years (Allen et al. 2009; Jaric et al. 2012). Due to the preservation of chemical properties, as previously documented in fin rays (Tzadik et al. 2015; Tzadik et al. 2017), we suggest that juvenile habitats can be assigned to species of interest over long time periods.

Table 2 Indicator values for the significant elements when juvenile samples were grouped on a large spatial scale

Element	Atomic weight	IndVal	*p* value
Na	23	38.03	0.003
Mg	24	35.50	0.016
Mn	55	44.99	0.014
Fe	57	39.20	0.001
Co	59	69.32	0.001
Zn	64	40.72	0.007
Ba	137	43.27	0.029

IndVal indicator value, *p value* significance based on 1000 permutations

Chemical Fingerprints of Juvenile Habitats

Chemical fingerprints of juvenile habitats were distinguishable at two spatial scales. At the small spatial scale, the relatively high correct classification rate demonstrated that the chemical fingerprints in our study system were distinctive even for closely located sites. Indeed, individuals from two sites (groups 1 and 2 in Fig. 2) that were separated by only 157 m (Fig. 1) were distinguishable based on the concentrations of trace elements in their fin rays. Most trace element concentrations in the body parts of fishes are thought to derive primarily from ambient water chemistry (Kerr and Campana 2014), and previous tagging studies have clearly demonstrated high site fidelity for juvenile *E. itajara* at similarly small spatial scales (Eklund and Schull 2001; Koenig et al. 2007; Koenig et al. 2011). Thus, the differences in fin ray chemistry may have derived directly from differences in ambient water chemistry at these two sites. The high indicator value for Ba in separating the two sites may suggest that it was driven in some part by haloclines in the mangrove lagoons, particularly as Ba is derived almost exclusively from ambient water as opposed to diet (Walther and Thorrold 2006). Regardless of the mechanism, the presence of small-scale microhabitats may have some utility for informing management (e.g., determining boundaries of nursery reserves) and the life history of *E. itajara*. Sequential microhabitat use and strong site fidelity

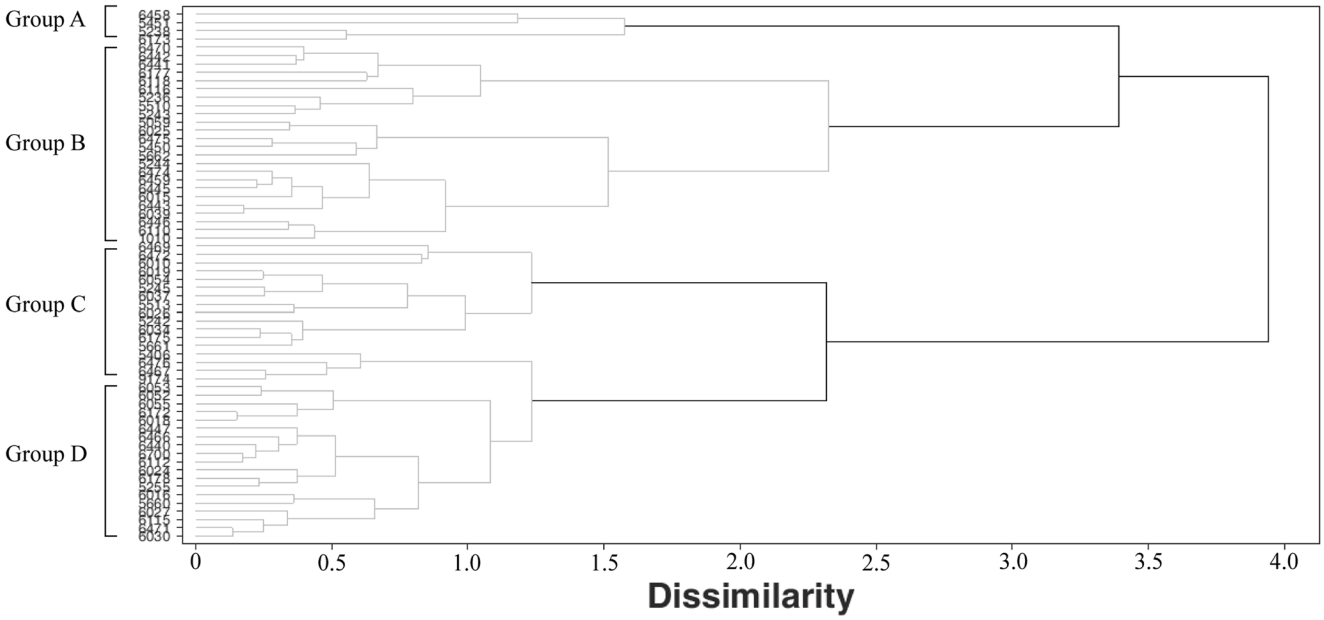

Fig. 4 A DISPROF-based cluster analysis for the adult samples in the study. *Solid lines* indicate significant divisions of classification. These groupings were used in the subsequent random forest

occur in the juvenile phases of other estuarine fishes (e.g., Brame et al. 2014) and have been suggested for *E. itajara* based on observational and tagging studies (Koenig et al. 2007; Lara et al. 2009). Using natural tags in fin rays, future studies can expand upon current knowledge of microhabitat use by *E. itajara* in its juvenile phase. However, the unique chemical fingerprints among microhabitats may confound results of future studies, as comprehensive sampling across all locations may not be feasible.

The groups that formed at the large spatial scale, which had a reclassification accuracy of 100%, are likely more relevant for management and conservation purposes under most circumstances. The mechanisms that drive different chemical fingerprints at this scale are more interpretable than at a small scale (due to a stronger signal-to-noise ratio), and may be directly influenced by both natural and anthropogenic processes in the vicinity.

The main TTI group was largely driven by Fe concentrations (Fig. 3), which are physiologically regulated (Gauldie and Nathan 1977). Importantly, the TTI group was not characterized by elements with anthropogenic sources (e.g., Zn, Cu), which suggests the juvenile habitats had minimal anthropogenic influence. In contrast, the combination of elements from the samples collected at Pumpkin Bay may have resulted from the upstream water source, the Faka Union Canal, which is dredged and has more boat traffic (Browder et al. 1986). Indeed, the downstream water of the two bays that neighbor Pumpkin Bay (i.e., Faka Union Bay and Fakahatchee Bay), as

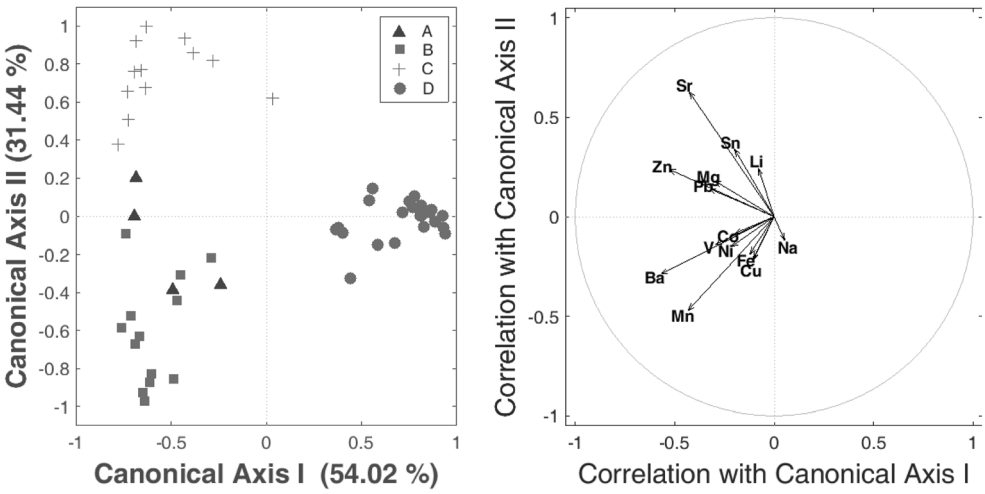

Fig. 5 Adults analyzed via a random forest from Fig. 4. The length of each vector corresponds to its relative importance in grouping individuals in the direction in which it is pointing. Correlation vectors directly correlate and are proportional to the ordination plot

Table 3 Indicator values for the significant elements when the adult samples were grouped

Element	Atomic weight	IndVal	*p* value
Mn	55	30.05	0.033
Fe	57	18.14	0.029
Sr	88	17.68	0.006
Sn	118	26.64	0.010
Ba	137	30.62	0.006
Pb	208	27.36	0.045

IndVal indicator value, *p value* significance based on 1000 permutations

well as that of Pumpkin Bay, is influenced by the outflow of the Faka Union Canal (Browder et al. 1986). The Faka Union Canal effectively begins at a dam location that traps freshwater from the Everglades and periodically flushes it into the canal. The dam location also houses a marina and a frequently used boat ramp. The freshwater input over the dam could contribute to the high concentrations of Ba, while the heavy boat traffic could contribute to the elevated levels of Zn and Mg, which are commonly used as sacrificial anodes on boat engines (Shanmugam et al. 2007).

The elements that were characteristic of the group from the Lower Florida Keys may be reflective of heavy boat traffic in the area, as Sn is a common alloy used in the forging of industrial metals, particularly aluminum, a common material used in the marine industry (Li and Feng 2003; Yan et al. 2013; Naeem et al. 2014). High levels of Sn may also result from regional use of illegal anti-fouling agents containing the element, even though such agents (primarily tributyltin) have been banned in the USA for several decades (Yebra et al. 2004). Due to the influence of several anthropogenic-derived elements, future considerations and studies should include long-term water sampling to test uptake patterns.

Adult Stock Origins

Five of the six significant indicator elements that were the most influential in clustering the adult samples (i.e., $n = 53$) were also drivers in the juvenile analyses, suggesting a similar mechanism of elemental substitution and retention (Tables 1, 2, and 3). Pb, which is often associated with fuel docks (Duarte et al. 2012), was the only element unique to the adult analysis, possibly as a result of individuals living near fueling stations. Three other ordinated groups were evident from the random-forest classification of the adults. One was characterized by the abundance of Sr and Sn, a second by Ba and Mn, and a third group by lower abundances of most of the elements measured. The lack of a baseline from 2006 (i.e., fin rays from juveniles sampled in all possible nursery habitats from that year) precludes the possibility of reclassifying adults into their nursery habitats. However, the grouping of adults, as influenced by nearly the same elements as the juveniles in 2014, suggests that similar mechanisms may be driving the groupings found in both adults and juveniles.

Conclusions and Implications

The technique described in our study can be used to study ENHs of endangered fishes and others of management concern. Our study is the first of which we are aware to use fin rays to establish chemical fingerprints with the objective of discerning ENH in a marine fish. Future applications include long-term monitoring projects that could be used to reclassify members of adult stocks into their nursery habitats. Chemical fingerprints can act as natural tags and are imprinted onto every individual in a population, thereby increasing inference to the entire population, rather than only the ones with implanted tags. However, the microchemical variability on exceptionally small scales can present challenges to future work on essential nursery habitats in marine ecosystems. Specific to our study, the ability to assign individuals to nursery habitats among the northern TTI bay system, the Faka Union Bay system (i.e., Pumpkin Bay, Faka Union Bay, and Fakahatchee Bay), and the Lower Florida Keys suggests that the role of spatial scale in habitat classification is paramount to studies that aim to quantify nursery habitats. Future research for *E. itajara* should aim to classify additional habitats, possibly via an annual random-stratified sampling design to minimize the possibility of type I errors (i.e., a false positive) in the reclassification of adults.

Similar techniques, using otoliths, have been used for the same purposes, but require lethal sampling. The use of fin rays allows non-lethal sampling and can be used in a manner similar to otoliths to differentiate among nursery habitats. The process outlined in our study is particularly relevant for recovering stocks, such as *E. itajara*, that must depend on their ENHs to help rebuild their population.

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