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The role of extrinsic variation — cohabiting juvenile fish species exhibit similar otolith elemental signatures

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ABSTRACT: The effect of extrinsic (environmentally based) and intrinsic (physiologically based) controls on otolith elemental signatures remains poorly understood. We evaluated the relative importance of both extrinsic and intrinsic factors using juvenile fish in Eastern Tropical Pacific (ETP) mangroves. To assess extrinsic influences, we compared the cohabiting yellow snapper *Lutjanus argentiventris* and sailfin grouper *Mycteroperca olfax* from the Galápagos Archipelago. To evaluate intrinsic influences, we compared yellow snapper from the Gulf of California (Mexico) and the Galápagos Archipelago (Ecuador). The 2 cohabiting species in the Galápagos exhibited very similar otolith elemental signatures, with no significant differences observed for Li, Cu, Mg, Mn, Rb, and Sr (univariate ANOVAs, $p > 0.05$), and a small separation achieved between these species (ANOSIM test, $R = 0.01$, $p = 0.038$). The yellow snappers from Galápagos and the Gulf of California exhibited distinct elemental signatures increasing from Rb, Cu, Mn, Sr, Li to Ba (univariate ANOVAs, $p < 0.05$), with a large separation between them (ANOSIM test, $R = 0.55$, $p = 0.001$). The present study suggests that extrinsic factors (e.g. water chemistry, temperature, salinity) can be more important than intrinsic factors (e.g. physiology, growth rates, genetics) for influencing elemental uptake in the otoliths of juveniles from mangrove waters. However, improved understanding of factors influencing elemental incorporation is still needed to ensure accurate interpretation of field data, especially in dynamic oceanographic systems, which is the case for both the Gulf of California and the Galápagos Archipelago.

KEY WORDS: Environmental effect · Otolith microchemistry · Mangrove · *Lutjanus argentiventris* · *Mycteroperca olfax* · Vital effect · Eastern Tropical Pacific · ETP

1. INTRODUCTION

Otoliths — calcium carbonate structures in the inner ear of teleost fishes — present unique chemical and chronological properties as they can record aspects of both the environment and fish life history strategy (Campana & Thorrold 2001). The chemistry of otoliths can be influenced either directly by variation in environmental conditions (e.g. ambient water chemistry, salinity, temperature) or indirectly through the environmental effects on fish physiology (e.g. growth, metabolism, stress). Laboratory experiments demon-

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strating how elemental signatures in otoliths reflect environmental conditions have allowed insight to be gained on various aspects of the life history dynamics of fish including freshwater–marine transitions in anadromous and catadromous species (Kalish 1990, Secor 1992), population connectivity (Chittaro et al. 2004), population structure (Campana et al. 1994, Ashford et al. 2006, Clarke et al. 2011), and utilization of nursery habitats and natal homing (de Pontual et al. 2000, Thorrold et al. 2001, Gillanders et al. 2003, Mateo et al. 2010). Otolith microchemistry has also been useful in developing environmental proxies for hypoxia events (Limburg et al. 2015) and pollution exposure (Geffen et al. 1998, Halden & Friedrich 2008).

Although otolith chemical analyses have become a common research tool in fish ecology and fisheries management, the precise mechanisms governing elemental incorporation into the otoliths are not fully understood (Campana 1999). This is due to the complex interaction of multiple intrinsic (e.g. physiology, growth rates, metabolism, genetics) and extrinsic (e.g. temperature, salinity, dissolved oxygen, water chemistry) factors that can disrupt the simple linear relationships between an element and a single environmental parameter (Grønkjær 2016, Walther 2019). The current discussion on how intrinsic or species-specific 'vital effects' affect the use of otolith chemistry as a natural tag has been addressed in several recent studies (Chang & Geffen 2013, Sturrock et al. 2015, Walther 2019), which suggest that the influence of physiological controls may play a key — usually underestimated — role in elemental incorporation (Sturrock et al. 2014, 2015, Thomas et al. 2017, Izzo et al. 2018). Moreover, the degree to which the relationship between environment and otolith chemistry can be generalized across species with physiological differences remains poorly understood. Comparison of different species experiencing the same environmental variation could provide insights into whether the influence of environmental variation on otolith chemistry remains consistent across species.

In addition to the lack of knowledge of the underlying mechanisms affecting otolith chemical composition, otolith microchemistry work is still not broadly available in developing countries or tropical and subtropical regions (Avigliano & Volpedo 2016) due to the high costs and qualified technical knowledge required. This is the case in the Gulf of California (Mexico) and the Galápagos Archipelago (Ecuador), an area for which just 1 microchemistry paper is currently available (Ruttenberg & Warner 2006). Nonetheless, these subtropical regions are particularly interesting from an ecological perspective because they experience high seasonal variation in sea surface temperature (SST) and primary productivity. For example, the intra-annual SST and chlorophyll *a* vary up to 8°C and 10-fold among bioregions in Galápagos, an archipelago located at the equator (Wellington et al. 2001), and the intra-annual SST varies up to 17°C in the Gulf of California, which is one of the most productive marginal seas in the world (Álvarez-Borrego 2012). In addition, both regions have high percentages of endemic fish — 13.6% for Galápagos (McCosker & Rosenblatt 2010) and 10% for the Gulf of California (Lluch-Cota et al. 2007) — that are subject to fisheries and whose management could use tools such as otolith microchemistry analysis. For example, otolith microchemistry could help to identify and address the contribution of nursery sites for the adult populations (Thorrold et al. 2001, Chittaro et al. 2004, Mateo et al. 2010), and/or to assess stock structure and connectivity patterns (Thorrold et al. 2007).

The present study is the first attempt to evaluate extrinsic and intrinsic influences on the elemental composition of otoliths of juvenile fishes inhabiting mangrove forests in the Eastern Tropical Pacific Ocean (ETP). To evaluate the relative importance of extrinsic drivers, we compared different species in the same environment: yellow snapper *Lutjanus argentiventris* and sailfin grouper *Mycteroperca olfax* in Galápagos. We used snappers and groupers as model species because they have different life histories, growth rates and diets (Aburto-Oropeza et al. 2009, Usseglio et al. 2015) but both inhabit mangroves during their juvenile stages. If the 2 species exhibit similar elemental fingerprints in Galápagos, then these would likely reflect the characteristics of the environment, such as water chemistry, temperature and salinity. Conversely, if they exhibit different elemental fingerprints, that would indicate an effect of species-specific physiologies, taxonomic differences and/or microhabitat preferences. Furthermore, to improve our understanding of the role that intrinsic variation can play in the elemental composition of the otoliths, we also compared the elemental signatures of yellow snapper *L. argentiventris* occupying 2 different dynamic ecosystems: the Galápagos Archipelago and the Gulf of California. Although this interspecies comparison has a temporal component we cannot control for (snappers were collected in each location ~12 yr apart), this comparison provides an initial evaluation of the magnitude of variation in otolith chemistry between yellow snappers at their northern (Mexico) and southern (Ecuador) limits of distribution.

We hypothesized that (1) the water environmental signal in the Galápagos Archipelago — driven by the convergence of 3 major oceanographic currents — is stronger than the taxonomic or physiological factors regulating the incorporation of trace elements into fish otoliths, and (2) physiological factors regulating the incorporation of trace elements into fish otoliths may be minor when comparing the same species across completely distinct ecosystems.

2. MATERIALS AND METHODS

2.1. Model species and data selection

The yellow snapper *Lutjanus argentiventris* occurs throughout the ETP, from southern California to Peru (Allen 1985). In the Gulf of California, adults spawn on the continental shelf, and their larvae are transported to mangroves where they metamorphose and settle at around 19 to 26 d after hatching. The juveniles remain close to the substrate and to the mangrove roots until they are approximately 10 cm in total length (TL) (approx. 150 to 200 d old), when they begin to migrate offshore to join adults on rocky reefs (Aburto-Oropeza et al. 2009). In Galápagos, juvenile yellow snappers are also present in mangroves, but up to a larger size (~20 cm TL), suggesting that they might migrate at larger sizes than the juveniles from the Gulf of California (J. Marin pers. comm. 2017). This species sustains an important artisanal fishery in both the Gulf of California and Galápagos. There are signs of overexploitation, such as a decline in the size-at-capture in the former (Piñón et al. 2009) and reduced abundance and biomass in the latter region (Ruttenberg 2001).

The Galápagos sailfin grouper *Mycteroperca olfax* is endemic to several islands of the ETP and in Galápagos it has a high economic and cultural value (Reck 1983). Despite its importance, the Galápagos sailfin grouper faces severe overexploitation (Usseglio et al. 2016) due to the combination of a strong fishery pressure, the direct targeting of spawning aggregations (Salinas-de-León et al. 2015) and its K-selected life-history strategy of slow growth and high longevity (Usseglio et al. 2015, 2016, Eddy et al. 2019).

We examined 70 grouper juveniles and 88 yellow snapper juveniles from the Galápagos Archipelago, and 174 yellow snapper juveniles from the Gulf of California, for a total of 332 fishes (Fig. 1). We selected the juvenile stage of these species because they all come from mangrove sites and do not have physiological changes induced by reproduction, which is known to affect the chemistry of otoliths (Kalish 1989). Snappers and groupers from Galápagos were collected in 2 sampling events, in April 2015 and April 2016, while snappers from the Gulf of California were sampled in 4 collection events, in June and October 2003 and June and October 2004 (Table 1). Most of the Galápagos samples (93% of the fishes) were collected in April 2015, while 60% of the Gulf of California samples were collected in June and October 2003, and 40% in June and October 2004.

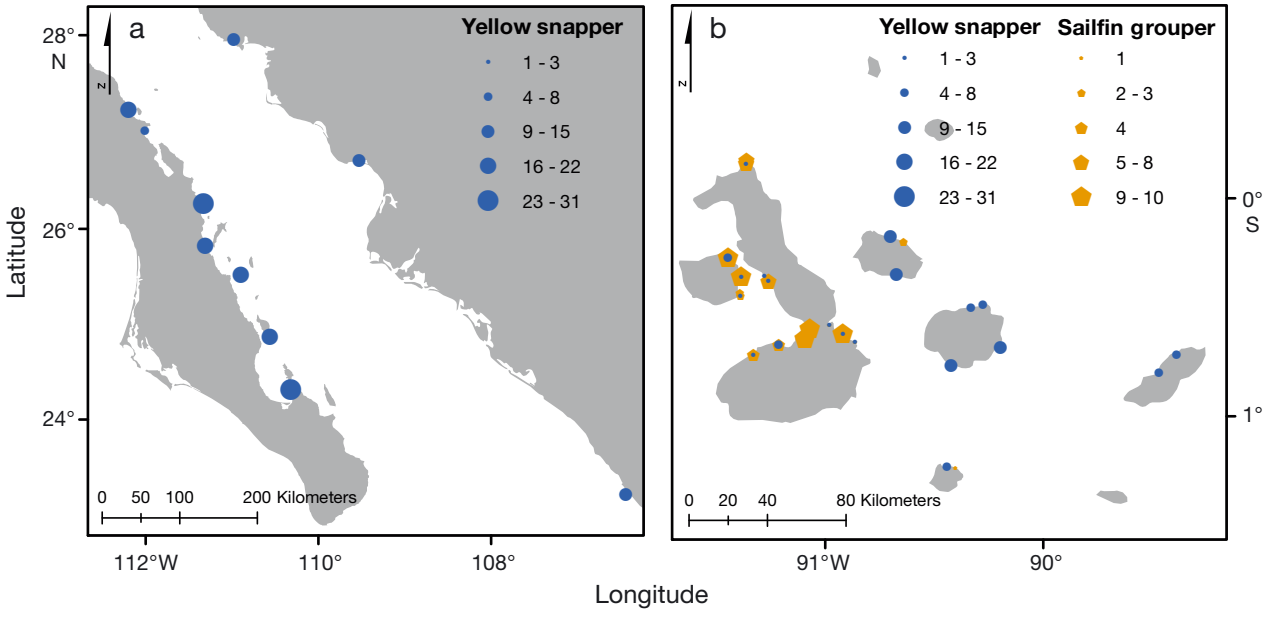

Fig. 1. Fish collection area in (a) the Gulf of California (n = 174) and (b) the Galápagos Archipelago (n = 158) mangroves

Table 1. Date of collection, sample size (n) and size range of yellow snapper *Lutjanus argentiventris* and sailfin grouper *Mycteroperca olfax* from the Galápagos and the Gulf of California. TL: total length

Ecosystem	Date of collection	Species	n	Age (yr)	Min. TL (mm)	Max. TL (mm)
Gulf of California	June 03	Yellow snapper	24	0	5.45	15.19
Gulf of California	October 03	Yellow snapper	80	0	2.7	12.41
Gulf of California	June 04	Yellow snapper	20	0	2.21	11.15
Gulf of California	October 04	Yellow snapper	50	0	2.44	12.02
Galápagos	April 15	Yellow snapper	83	0	2.8	19.5
Galápagos	April 16	Yellow snapper	5	0	10.3	24
Galápagos	April 15	Sailfin grouper	64	0	7.2	25.7
Galápagos	April 16	Sailfin grouper	6	0	11.5	24

For the present study, we included only sailfin grouper juveniles under 26 cm TL (Table 1), which had not yet formed the first annual ring in their otolith. Since this species begins to reproduce at ~6.5 yr and 65 cm TL (Usseglio et al. 2016), all groupers collected in this study were immature. All snappers were age-0 (Fig. 2, Table 1), estimated from counts of daily growth rings validated by Zapata & Herrón (2002). The snappers from the Gulf of California represented 4 different cohorts — the winter and summer of 2003, and the winter and summer of 2004, while the snappers from Galápagos represented 2 cohorts — the winter of 2014 and summer of 2015 (Fig. 3).

2.2. Otolith preparation and elemental analysis

Otolith sections from all samples were mounted in random order on microscope slides using thermoplastic adhesive (Crystalbond™), cleaned in ultrapure water and dried in a Class 100 clean bench (for details of otolith preparation see Text S1 in the Supplement at www.int-res.com/articles/suppl/m646p109_supp.pdf). The elemental composition of *L. argentiventris* and *M. olfax* otoliths was quantified using a Thermo Scientific X-series II quadrupole inductively coupled plasma mass spectrometer with a Photon Machines Analyte G2 laser system (LA-ICPMS) at the Oregon State University WM Keck Collaboratory for Plasma Spectrometry in Corvallis, Oregon (see Text S1 for details of elemental acquisition). Laser transects were positioned along the longest axis of the otoliths, from the outer edge of the core, passing through the core to the opposite edge of the otolith, in order to collect a time series of elemental composition (Fig. 4a). We collected data on lithium (^{7}Li), magnesium (^{24}Mg), calcium (^{43}Ca), manganese (^{55}Mn), copper (^{65}Cu), rubidium (^{85}Rb), strontium (^{86}Sr), barium (^{138}Ba), zinc (^{66}Zn) and lead (^{208}Pb). The trace elements were divided by Ca (Me/Ca, where the Me represents a metallic element) and data were converted to molar ratios based on repeated measurements of the NIST 612 standard. Elemental ratios are presented as mmol mol^{-1} (Mg, Mn and Sr) or µmol mol^{-1} (Li, Cu, Zn, Rb, Ba and Pb). The mean percent relative standard deviations (%RSD) of multiple NIST 612 standards (n = 58) were used to evaluate precision (see Table S1 in the Supplement). Accuracy was estimated with a calcium carbonate standard of known composition (USGS MACS-1, n = 45) and measured values were within 10% of known values for all elements, except for Mg:Ca and Pb:Ca (Table S1).

The otolith microchemistry data along each transect were separated into different life stages (i.e. larva, settler, post-settler and non-migratory imma-

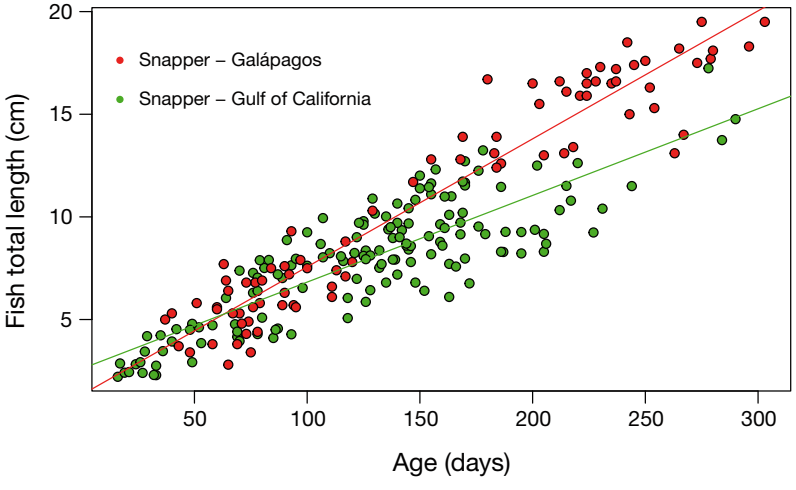

Fig. 2. Linear regression between the fish total length and age estimated from the otolith sagittae of yellow snapper *Lutjanus argentiventris* juveniles collected in the Gulf of California (n = 148) and Galápagos (n = 82)

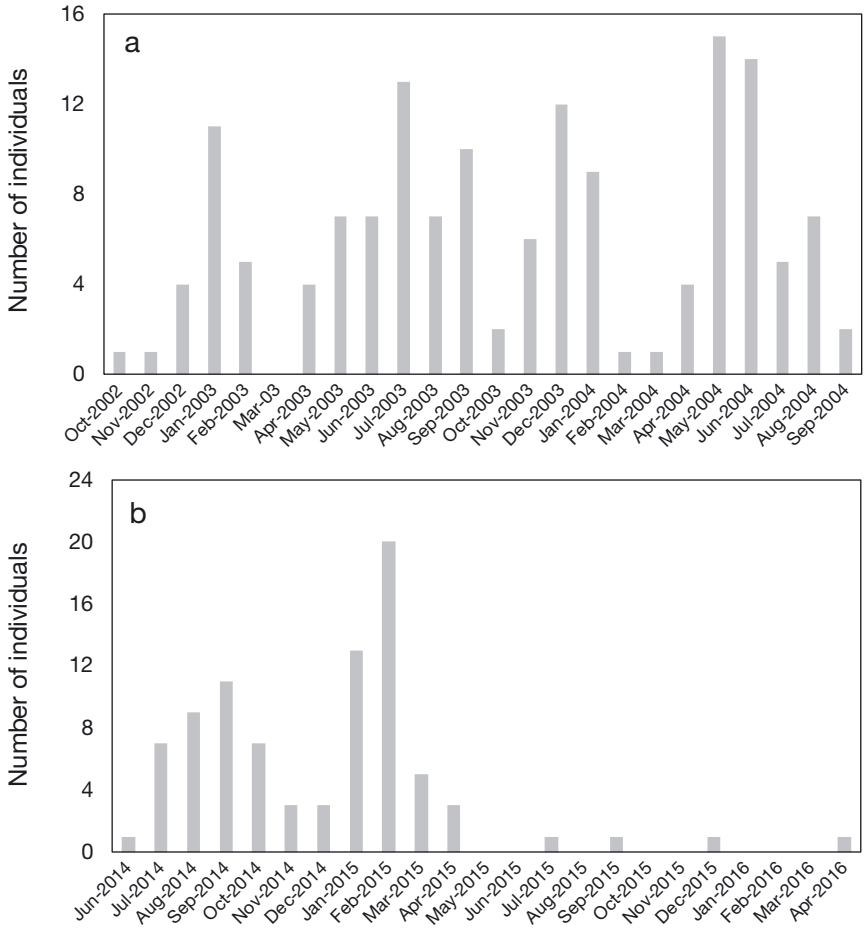

Fig. 3. Monthly distribution of back-calculated hatch date for the juvenile yellow snapper *Lutjanus argentiventris* collected in (a) the Gulf of California (n = 148) and (b) Galápagos (n = 82). Please note that in the Galápagos Archipelago, the cold season is between July and November and the warm season is between January and May, with June and December being transition months

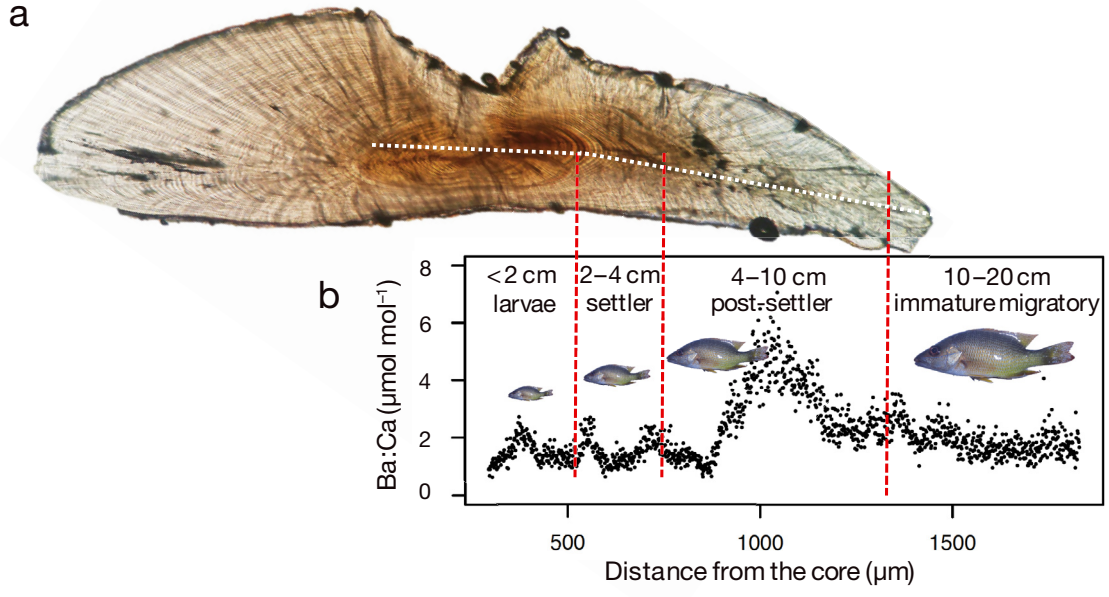

Fig. 4. (a) Ablated laser transect across a juvenile yellow snapper otolith (white dashed line) and (b) an example of the mean Ba:Ca trace elemental ratio (µmol mol^{-1}) estimated at each life stage

ture) to examine stage- and habitat-specific elemental fingerprints. For example, larval snappers and groupers utilize open water habitats, while settlers, post-settlers and other non-migratory immature fish (hereafter referred to as 'juveniles') reside in mangroves. In order to calculate the average elemental ratio (Me:Ca in µmol mol^{-1} or mmol mol^{-1}) at each life stage of the juvenile fish (i.e. larva, settler, post-settler and immature), we used the relationship between the total length of the fish (TL, cm) and the ablated length in each otolith (AL, µm) (see Fig. S1 in the Supplement).

2.2.1. *Lutjanus argentiventris*

The yellow snapper juveniles from the Gulf of California ranged from 2.21 to 15.19 cm TL and those from Galápagos were from 2.8 to 24 cm TL. The relationship between the TL of yellow snappers and the AL for each of their otoliths was linear (TL = 0.0088 AL, $R^2 = 0.92$, $p < 0.05$ for the Gulf of California, and TL = 0.0097 AL, $R^2 = 0.90$, $p < 0.05$ for Galápagos) (Fig. S1). The regression slopes were significantly different between fish from the Gulf of California and the Galápagos (multiple linear regression, $p < 0.0001$), partially because Galápagos juveniles were larger than those from the Gulf of California. Using these 2 relationships between fish TL and otolith AL, we calculated the average Me:Ca (µmol mol^{-1} or mmol mol^{-1}) for each section of AL along the otolith that corresponded to a specific range of TL in the fish (Fig. 4b). These specific ranges of TL were based on a previously established classification of juvenile size classes (Aburto-Oropeza et al. 2009), in which larvae were <2 cm TL, settlers between 2 and 4 cm TL, post-settlers between 4 and 10 cm TL, and immatures between 10 and 20 cm TL. The average number of days and growth rates corresponding to each of these life stages were also calculated (Table 2).

2.2.2. *Mycteroperca olfax*

For sailfin groupers, we included unpublished data from adult samples to improve the relationship between TL and the AL for each of their otoliths. By including adults, we were able to better adjust the intercept for our regression to improve the estimate of the range in lengths and average elemental ratios for the youngest life stages considered (e.g. larvae and settlers). The relationship between the TL of 189 adult and juvenile sailfin groupers from 7.20 to 89.25 cm TL and their otolith AL was linear (TL = 0.0239 AL, $R^2 = 0.7426$, $p < 0.05$) (Fig. S1). The differences in the fit of the linear relationships between TL and AL for the sailfin groupers and yellow snappers indicate differences in growth rates and otolith accretion rates between these species, since the groupers were larger than the snappers. In the microchemistry runs, we only included juveniles (n = 70) between 7.2 and 25.7 cm of TL that were age-0 (Table 1). Since there was no available literature for the size ranges of *M. olfax* from the larval to the post-settler stages or for the genus *Mycteroperca*, we used size-class range values from a species with similar growth parameters, the Nassau grouper *Epinephelus striatus* (Eggleston 1995). Using those reference values and the linear relationship between TL and AL, we assigned individuals to different life stages, defining larvae as individuals <2.5 cm TL, settlers between 2.5 and 3.5 cm TL, post-settlers between 3.5 and 15 cm TL, and immatures between 15 and 65 cm TL. The average Me:Ca (µmol mol^{-1} or mmol mol^{-1}) for each of these life stages was calculated in the same way as for yellow snappers (Fig. 4, Fig. S1).

To compare only those juveniles that experienced the same temporal variability of the elements in the environment, we subsampled 97 juveniles of similar sizes, ages and hatch dates within each ecosystem. These juveniles included yellow snappers from 5 to 10 cm TL collected in October 2003 in the Gulf of California (n = 40) and yellow snappers and sailfin

Table 2. Average (SD in parentheses) of the total length (TL) and age, and mean growth rate of yellow snapper *Lutjanus argentiventris* in the Gulf of California and Galápagos ecosystems

Ecosystem	Species	Life stage	TL (cm)	Age (d)	Mean growth (mm d^{-1})
Gulf of California	Yellow snapper	Settler	2.93 (0.61)	33 (15)	0.089
		Post-settler	7.46 (1.67)	124 (43)	0.060
		Immature	11.75 (1.53)	182 (44)	0.065
Galápagos	Yellow snapper	Settler	3.41 (0.39)	60 (12)	0.057
		Post-settler	6.36 (1.20)	82 (21)	0.078
		Immature	15.68 (2.25)	224 (41)	0.070

groupers of 15 to 20 cm TL collected in April 2015 in the Galápagos (n = 57).

2.3. Sea surface temperature and chlorophyll *a* adjacent to mangrove sites — Gulf of California and Galápagos

We downloaded daily SST and chl *a* in 64 km^2 polygons adjacent to our mangrove sites during the lifetimes of the juveniles at each ecosystem. For the Gulf of California, the full-resolution data products were merged from data from multiple sensors: SeaWiFS (1997 to 2010), MODIS-Terra (MODIST, 2000 to present), MODIS-Aqua (MODISA, 2002 to present), MERIS (2003 to 7 April 2012). For the overlapping periods, datasets from all available sensors were merged. Corresponding SST products were created from MODIST (2000 to present) and MODISA (2002 to present). For Galápagos, the data products were from VIIRS-SNPP sensors on NASA satellites (https://oceancolor.gsfc.nasa.gov).

2.4. Statistical analysis

To determine how the overall elemental composition of otoliths differed between species or ecosystem of origin, we used a permutational multivariate analysis of variance (PERMANOVA) (see Text S2 in the Supplement for PERMANOVA assumptions). Two PERMANOVA models were run with the average Me:Ca across the entire life of the juveniles (i.e. elemental ratios) as the dissimilarity matrix and species or region as the independent variables (Anderson 2001, 2014, McArdle & Anderson 2001). For the 2 species in the same region (*L. argentiventris* from Galápagos vs. *M. olfax* from Galápagos), we used a model where the species was the independent variable. For the same species in different regions (*L. argentiventris* from Galápagos vs. *L. argentiventris* from Gulf of California), we used a model where the region was the independent variable. In addition, univariate ANOVA comparisons and Tukey tests were used to test for significant differences in single trace elemental ratios (see Text S2 for ANOVA assumptions).

In order to complement the PERMANOVA analysis, an analysis of similarity (ANOSIM) was used to test whether there was significant separation based on species (*L. argentiventris* from Galápagos vs. *M. olfax* from Galápagos) or their respective ecosystem of origin (*L. argentiventris* from Galápagos vs. *L. argentiventris* from Gulf of California) using the average Me:Ca across the entire life of the juveniles (see Text S2 for ANOSIM model interpretation).

To visualize the level of similarity or dissimilarity in the elemental composition of otoliths by species (*L. argentiventris* vs. *M. olfax* from Galápagos) or ecosystem of origin (*L. argentiventris* from Gulf of California vs. *L. argentiventris* from Galápagos), we used Principal Coordinate Analysis (PCoA), also known as Classical Multidimensional Scaling (MDS) (see Text S2 for PCoA model details). We used the average Me:Ca of larval stages to reflect open ocean residence and the average Me:Ca from settlers to immature stages (i.e. 'juveniles') to reflect residence inside the mangrove sites. Confidence intervals (CI) of 95% were used to assess the overlap between sampling areas or species and to better visualize the group separation or overlap achieved between the different species–region combinations (*L. argentiventris* from Gulf of California, *L. argentiventris* from Galápagos and *M. olfax* from Galápagos) of both larval and juvenile stages.

To investigate temporal variability in elemental composition of otoliths for the yellow snappers and sailfin groupers from different cohorts, we used PCoAs of the elemental ratios of juveniles (i.e. post-settlers to immature) based on their species (*L. argentiventris* vs. *M. olfax*), ecosystem of origin (Gulf of California vs. Galápagos), and month and year of collections (April 2015 and 2016 for Galápagos, and June and October 2003 and 2004 for the Gulf of California) (see Text S2, Figs. S2 & S3 in the Supplement).

To examine the role of growth on the elemental composition of yellow snappers from different ecosystem of origin (Gulf of California vs. Galápagos), we performed a PCoA only using fish with similar ages, sizes and growth rates. For this analysis, we included fish less than 10 cm TL and ~150 d old because their growth rates were similar at these sizes and ages (Fig. 2). All analyses were performed in the program R version 3.6.1 (R Core Team 2019). We used the package 'vegan' (Oksanen et al. 2019) to perform the PERMANOVA, ANOSIM and PCoAs.

3. RESULTS

The elemental ratios at each life stage of the subset of juveniles (n = 97) (see Fig. S4 in the Supplement) were very similar with the elemental ratios at each life stage including all samples (n = 332) (see Fig. 7), with differences being within 1 SD, with the exception of Li:Ca and Cu:Ca, which were within 2 SD.

Therefore, we decided to include all juveniles in our statistical analysis, with the main assumption that the environment in Galápagos as a whole would be important for imparting local chemical signals to 'larvae' and 'juveniles' inhabiting it, despite likely seasonal variations.

3.1. Interspecific differences within the same ecosystem

Within Galápagos, the multi-elemental otolith signatures of the yellow snappers were significantly different from those of the sailfin groupers, but the difference between species explained only 2.1% of the variance (PERMANOVA, F = 3.59, R^2 = 0.021, p < 0.02) (Table 3). Univariate results indicated that the differences were primarily due to 2 elemental ratios (Ba:Ca and Pb:Ca) (Table 4, Fig. S5 in the Supplement). The elemental ratios of Sr:Ca, Cu:Ca, Li:Ca, Rb:Ca, Mn:Ca and Mg:Ca were very similar in the 2 cohabiting species. The ANOSIM test showed little separation between these 2 species from Galápagos (ANOSIM test, R = 0.016, p = 0.038) (Fig. 5a). The PCoA showed a high degree of overlap among the elemental signatures, irrespective of the life stage (Fig. 6). Moreover, the pattern of elemental composition and range of elemental ratio values across all life stages (larvae, settlers, post-settlers and immatures) was similar, with the exception of Pb (Fig. 7).

3.2. Spatial differences within the same species

The multi-elemental otolith signatures of the yellow snappers from Galápagos and the Gulf of California were significantly different and the difference between regions explained 32.4% of the variance (PERMANOVA, F = 126.58, R^2 = 0.324, p < 0.001) (Table 3). The majority of elements were significantly different between regions, except for Mg:Ca, Zn:Ca and Pb:Ca (Table 4, Fig. S5). There was a larger separation between the snappers from Galápagos and snappers from the Gulf (ANOSIM test, R = 0.558, p = 0.001) (Fig. 5b) than between the snappers and groupers from Galápagos. The PCoA partially separated the yellow snappers of Galápagos from those of the Gulf of California (Fig. 6). In addition, the pattern of elemental composition across life stages was different for yellow snappers from the Galápagos and the Gulf of California. For example, Ba:Ca was around 3 times higher in the snappers from the Gulf of California than in those from Galápagos and Li:Ca was almost 9 times lower in the snappers from the Gulf than in those from Galápagos (Fig. 7).

3.3 Temporal variation

The PCoA partially separated the yellow snappers and sailfin groupers in the Galápagos from the yellow snappers in the Gulf of California, irrespective of the distinct cohorts from different sampling events (Fig. 8). While it was not possible to test for temporal variation in the otolith elemental fingerprint for Galápagos fishes due to the low number of samples collected in April 2016 (n = 12), their elemental fingerprint overlapped well with the remaining fishes collected the previous year (n = 160) (Fig. 8). However, the multi-elemental otolith signatures of yellow snapper juveniles from the Gulf of California were significantly different between the years of collection, 2003 and 2004 (PERMANOVA, F = 46.25, p < 0.001), as well as for some of the elemental ratios averaged for different life stages (Fig. 9). For example, Cu:Ca, Rb:Ca and Zn:Ca were approximately 2 times higher in the snappers from 2004 than in those from 2003, while other elemental ratios were within the same range of values (Fig. 9, Table S2 in the Supplement). There were no significant differences for Ba:Ca, Mg:Ca, Sr:Ca, Pb:Ca and Zn:Ca among the life stages compared across years for the snappers in the Gulf (Fig. 9, Table S2).

3.4. Growth rate effects

The PCoA still partially separated the yellow snappers from Galápagos from those from the Gulf of Cal-

Table 3. Results of PERMANOVA comparing trace element signatures in yellow snapper otoliths collected from the Gulf of California (GOC) and Galápagos (GAL) and comparing yellow snapper and sailfin grouper otoliths collected from Galápagos (GAL). Pr: p-values based on 999 permutations. *p = 0.05, **p = 0.01, ***p = 0.001

Species and region	Factor	df	SS	R^2	F	Pr > F
Yellow snapper GOC vs. yellow snapper GAL	Region	1	1.868	0.324	126.580	0.001***
Yellow snapper GAL vs. sailfin grouper GAL	Species	1	0.084	0.021	3.586	0.027*

ifornia (Fig. S6 in the Supplement) for those individuals with similar growth rates, suggesting that factors beyond growth are driving this separation pattern.

4. DISCUSSION

Unraveling the effect of extrinsic and intrinsic factors on elemental composition of otoliths is essential for interpreting connectivity patterns, life history exposure, and the role of environment and vital effects in fish ecology (Thorrold et al. 2007, Sturrock et al. 2014, 2015). This study demonstrated consistent otolith microchemistry between 2 species sampled in various locations across the Galápagos. Furthermore, our results indicate that the same species under the influence of dynamic ecosystems had different elemental compositions in their otoliths. To our knowledge, this is the first time that otolith microchemistry has been used to assess the relative role of environment and physiology in juvenile fish at their northern and southern distributional limits in the ETP.

4.1. Extrinsic vs. intrinsic influences on otolith elemental composition

The factors that affect the quantification and the elemental composition of otoliths include (1) methodology (Ruttenberg & Warner 2006), (2) temperature (affects fish metabolism and growth rate, and can influence how the elements are incorporated into the crystal matrix) (Radtke & Shafer 1992), (3) ontogeny (developmental changes can lead to changes in otolith deposition) (Ruttenberg et al. 2005), (4) phylogeny (species differences may be due to taxonomic changes in otolith composition) (Chang & Geffen 2013), (5) water chemistry (Thorrold et al. 1997) and (6) dietary sources (Buckel et al. 2004, Mathews & Fisher 2009).

In our study, methodological influence was discarded as an important factor because data collection occurred using calibration controls during the 1 wk LA-ICPMS analysis. The potential for growth variation to generate the observed variation in elemental composition appears to be limited, because juveniles younger than 150 d old were growing at similar rates during these periods (Fig. 2) and still exhibited marked differences in their elemental signatures (Fig. S6). Potential ontogenetic effects were reduced by including only immature fishes prior to reproductive investment (Kalish 1989, Sturrock et al. 2015), and by comparing elemental signatures across discrete stages. Phylogenetic effects were also less evident than the region of origin, as the elemental signatures of the 2 Galápagos species were more similar than those of yellow snapper from Galápagos and Gulf of California, even when separated by life stage.

Most of the snapper juveniles from Galápagos came from the eastern side of the archipelago, whilst most of the sailfin grouper juveniles came from the western side. Nonetheless, both species exhibited more similar elemental signatures compared to snappers from the Galápagos and the Gulf of California. This pattern of similar trace elemental composition in the 2 species might be due to the unique geographic position of the archipelago, being the only subtropical archipelago located at the confluence of major warm- and cool-water current systems, including the: (1) warm south-westerly flowing Panama Current; (2) cool north-westerly flowing Peru Current; and (3) cold eastward-flowing subsurface equatorial undercurrent (EUC). In Galápagos, the EUC divides into a northern and southern branch, leading to local upwelling all around the archipelago and a

Table 4. Results of univariate ANOVA comparing elemental ratios between species (yellow snapper GAL vs. sailfin grouper GAL) and between regions (yellow snapper GOC vs. Yellow snapper GAL). p-bonf: p-values adjusted for multiple comparisons using the 'Bonferroni' procedure. *p = 0.05, **p = 0.01, ***p = 0.001; ns: non-significant

Element	Response	df	SS	MS	*F*	p-bonf
Ba	Species	1	1.94	1.94	20.18	<0.001***
	Region	1	50.91	50.91	342.91	<0.001***
Cu	Species	1	0.44	0.44	0.82	ns
	Region	1	21.32	21.32	58.90	<0.001***
Li	Species	1	0.99	0.99	1.09	ns
	Region	1	115.77	115.77	273.62	<0.001***
Mg	Species	1	0.77	0.77	5.85	ns
	Region	1	0.41	0.41	5.12	ns
Mn	Species	1	0.21	0.21	5.24	ns
	Region	1	4.67	4.67	68.68	<0.001***
Pb	Species	1	33.81	33.81	29.88	<0.001***
	Region	1	0.53	0.52	0.49	ns
Rb	Species	1	0.14	0.14	0.46	ns
	Region	1	4.70	4.70	20.73	<0.001***
Sr	Species	1	0.01	0.01	2.22	ns
	Region	1	0.47	0.47	94.72	<0.001***
Zn	Species	1	1.94	1.94	3.99	ns
	Region	1	1.52	1.52	2.63	ns

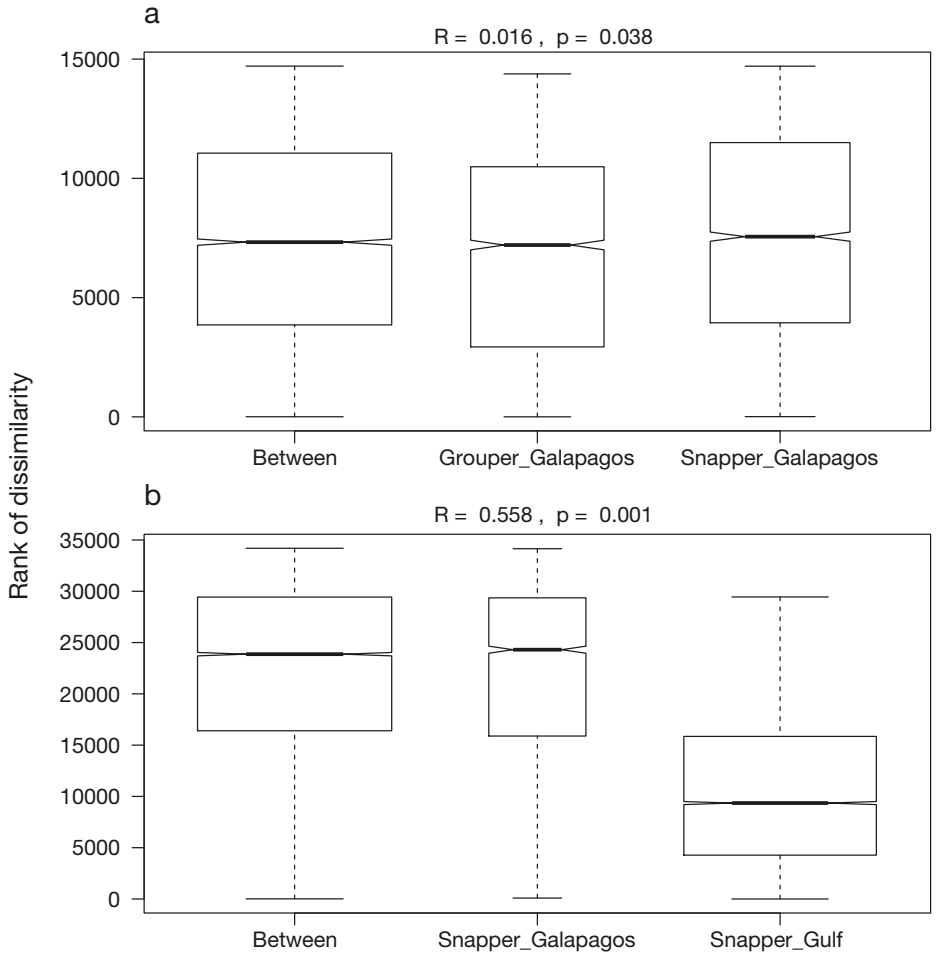

Fig. 5. ANOSIM test between (a) the sailfin grouper and yellow snapper from the Galápagos Archipelago, and (b) yellow snapper from Galápagos and yellow snapper from the Gulf of California. Notched boxplots indicate the dissimilarity rank distributions for between and within species presented in plots

complicated pattern of internal eddies that allows for horizontal interchange and mixing of water masses (Houvenaghel 1978), likely homogenizing the water across the entire archipelago. This homogenous environment would further support the observations made by Ruttenberg & Warner (2006), who found that otolith chemical signatures did not vary over larger spatial scales (~100 km) across the Galápagos Archipelago but observed some spatial differences at small spatial scales of 10s of km, which they attributed to localized upwelling events and their variation in intensity among the islands.

The similarity of elemental ratios between species (snappers and groupers) observed in Galápagos does not agree with previous studies where interspecific differences were observed for juvenile fishes living in the same environment (Swearer et al. 2003, Hamer & Jenkins 2007, Reis-Santos et al. 2008). The interspecific similarity of elemental ratios for Galápagos fishes may, however, support interspecific classification of natal sources, where otolith microchemistry signatures obtained for one species may be used to predict those of co-occurring species for which natal source otolith microchemistry information is unavailable (Prichard et al. 2018). These similarities also hold promise for using one species as a proxy for a congener (Patterson et al. 2014).

The differences in elemental concentration observed between snappers from Galápagos and the Gulf might be due to both environmentally and physiologically mediated mechanisms, as temperature and the amount of productivity within the mangrove lagoons can also affect metabolic rates and the rates of growth of somatic tissue and otoliths. For example, the seasonal SST was more variable in the Gulf (~16°C) than in Galápagos (~7°C) (Table S3 in the Supplement), for the juvenile lifetime examined herein (i.e. <1 yr old). The strong SST seasonality in the Gulf

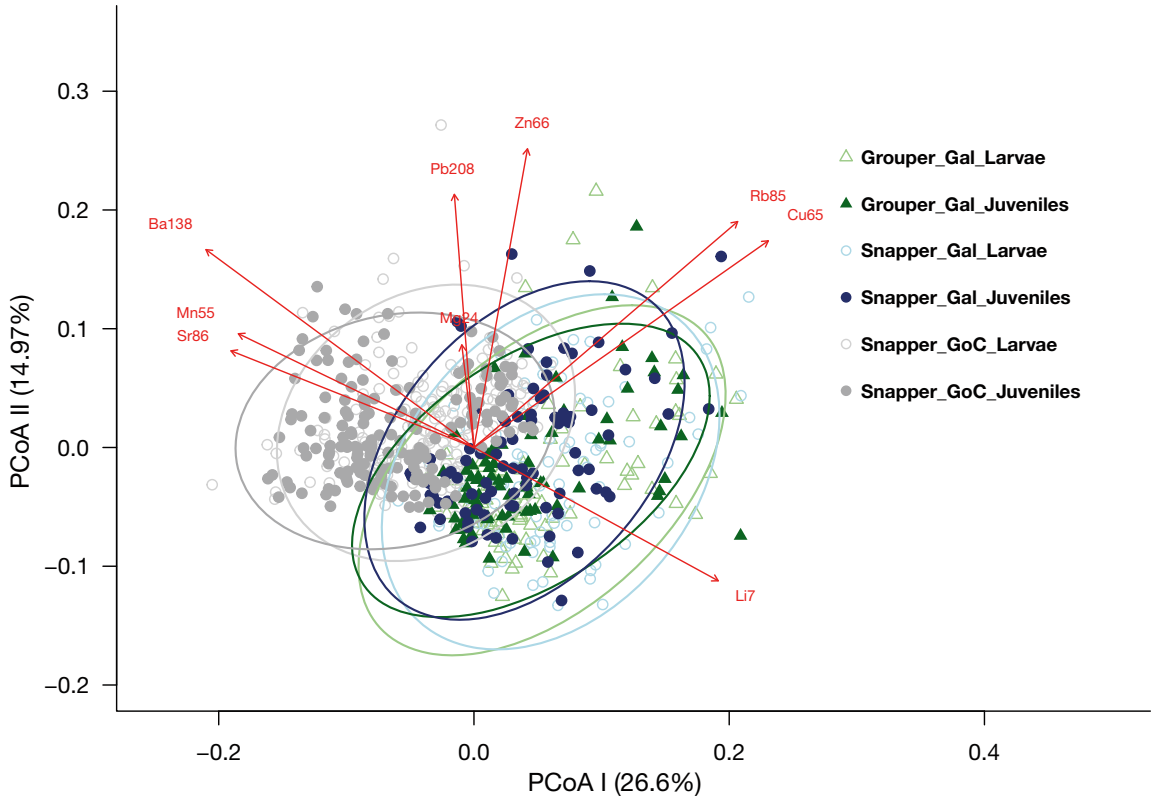

Fig. 6. Principal Coordinate Analysis (PCoA) of fish otoliths from Galápagos (Gal) and the Gulf of California (GoC). Each symbol represents the elemental ratios (Me:Ca) of a single otolith during the larval stage (open ocean) or the juvenile stage (mangrove sites). Environmental vector correlations are included to indicate relationships between trace element ratios (Me:Ca) and PCoA axes

probably led to a higher variability in growth rates of its snapper juveniles compared with those from Galápagos (Fig. 2).

4.2. Trace elements as proxies for large-scale environmental processes

Recent experimental and field observations found that fish physiology affects softer elements (Mn, Cu, Zn and Pb) and quasi-conservative elements (Sr and Ca) more than hard acid metal ions (Li, Mg, Rb and Ba) in otoliths (Sturrock et al. 2012, 2014, Grammer et al. 2017). Partially aligned with these studies, Thomas et al. (2017) observed the occurrence of Li, Mn and Rb only in the salt fraction of otoliths, which likely reflects changes in the physicochemical environment; Ba and Sr in both the salt and proteinaceous fractions, which likely reflects both endogenous and exogenous processes; and Cu, Zn and Pb only in the proteinaceous fraction, which likely reflects physiologically mediated mechanisms. In the present study, most hard acid metal ions (e.g. Li, Rb, Ba) and elements occurring only in the salt fraction (e.g. Mn) of otoliths were significantly different between Galápagos and the Gulf (Table 4), supporting the hypothesis that those elements are less affected by physiology and more influenced by the environment.

Ba:Ca and Sr:Ca were higher for the Gulf snappers than the Galápagos snappers, while Li:Ca was higher for the Galápagos fishes than the Gulf snappers (Fig. 7). In marine systems, these hard acid cations tend to be less physiologically influenced and accepted more readily into the otolith crystal lattice, but are relatively homogeneous in seawater (Sturrock et al. 2012). Ba is often used to identify freshwater occupancy due to the commonly observed relationship of increasing ambient and otolith Ba:Ca with decreasing salinity (Walther & Thorrold 2006), and greater Ba concentrations are also associated with upwelled waters and primary productivity (Kingsford et al. 2009). In our study, Ba:Ca was the most important element defining the spatial pattern observed for the Gulf of California juvenile snappers (Fig. 6), probably associated with the presence of the rivers Sonora, Yaqui and Fuente (mainland side of

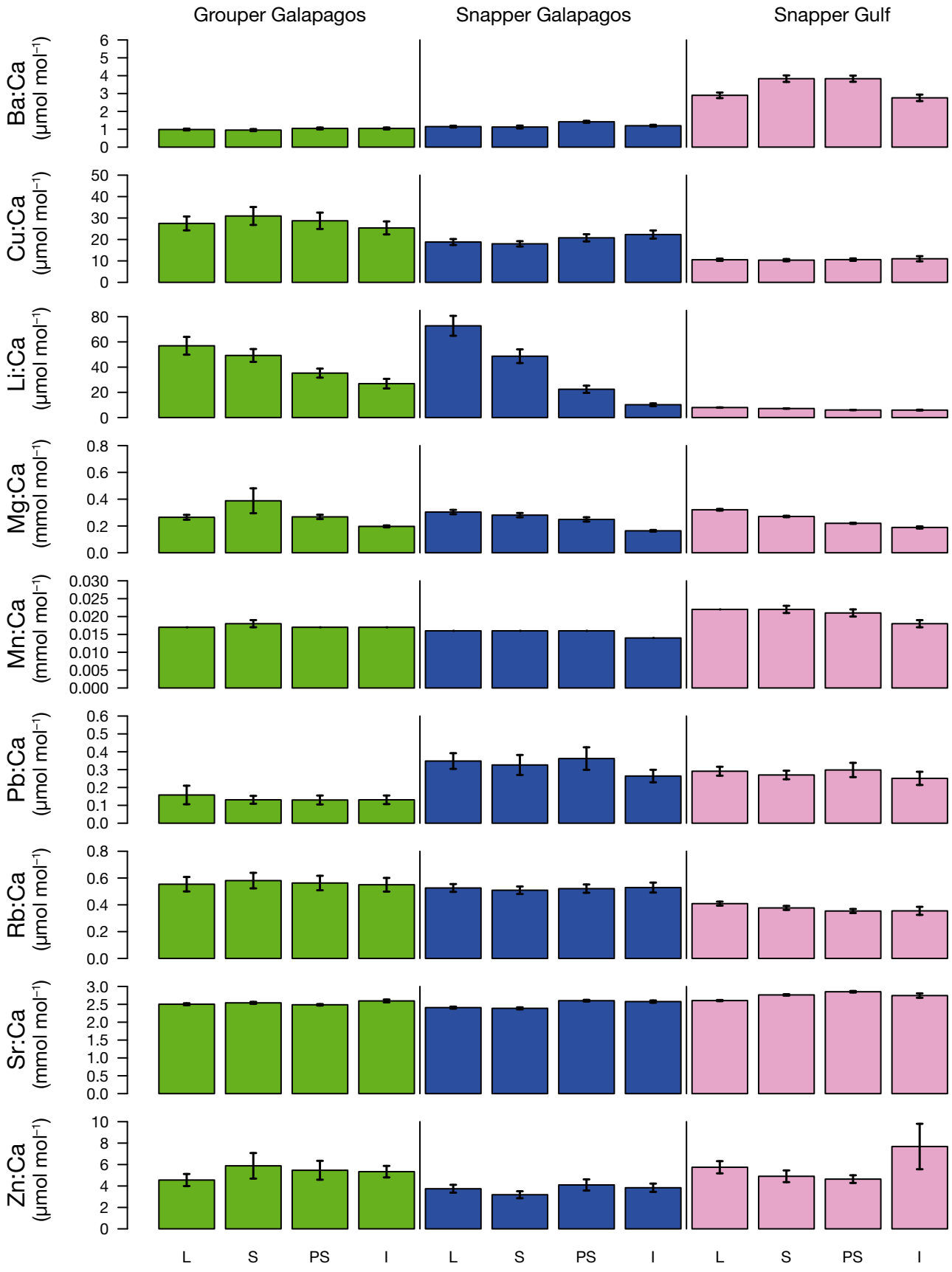

Fig. 7. Average ± SE of element to calcium ratios per juvenile size class for sailfin grouper from Galápagos, yellow snapper from Galápagos and yellow snappers from the Gulf of California. L: larvae; S: settlers; PS: post-settlers; I: immatures

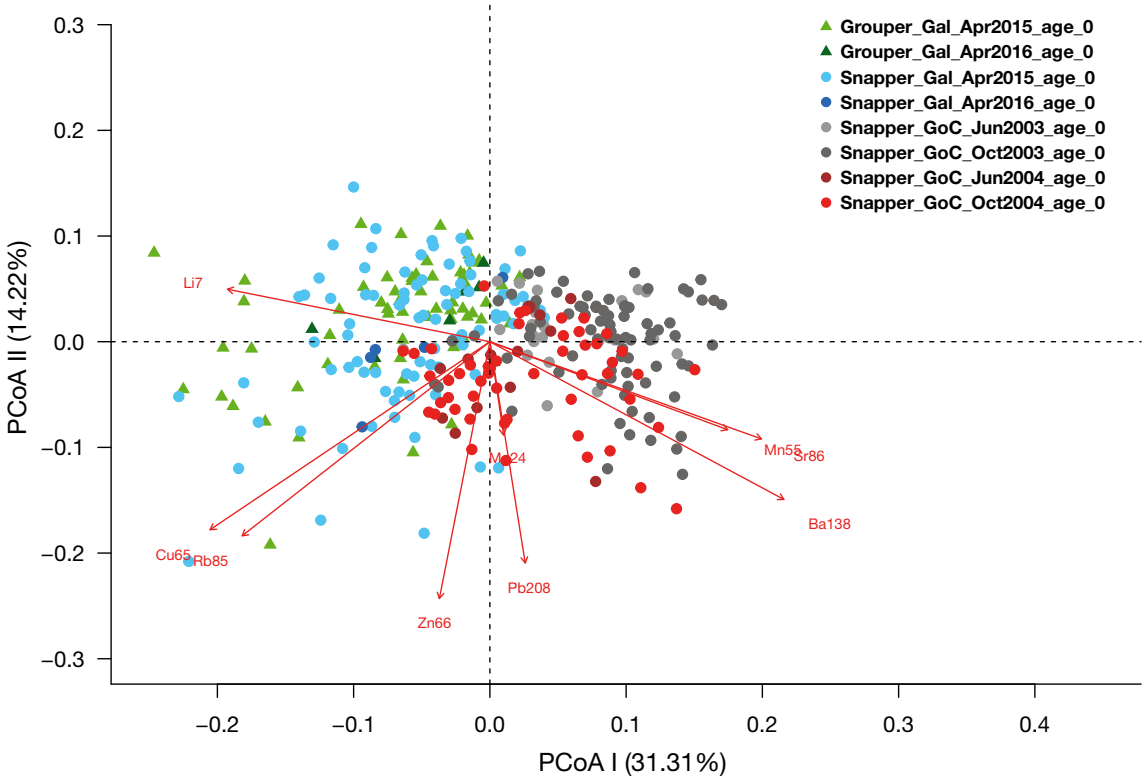

Fig. 8. Principal Coordinate Analysis (PCoA) of fish otoliths from Galápagos (Gal) and the Gulf of California (GoC). Each symbol represents the elemental ratios (Me:Ca) of the juvenile stage of a single otolith (i.e. representing mangrove waters) across different cohorts. Environmental vector correlations are included to indicate relationships between trace element ratios (Me:Ca) and PCoA axes

the Gulf), and also with the high primary productivity in this marginal sea due to upwelling events (Álvarez-Borrego & Lara-Lara 1991) (Table S3).

The Sr:Ca ratios for the Gulf of California fishes were significantly higher than Galápagos fishes (e.g. Sr:Ca was ~2.71 mmol mol^{-1} in the Gulf and ~2.47 mmol mol^{-1} in Galápagos). The Gulf of California is characterized by a positive salinity anomaly due to higher evaporation rates compared to precipitation rates and the current lack of freshwater inflow from the Colorado River. The annual mean salinity in the Gulf of California decreases from 35.26 ± 0.01 at the head to 34.75 ± 0.01 at the mouth (Beron-Vera & Ripa 2002), which is slightly higher than the Galápagos Archipelago, where our *in situ* salinity measurements at the time of fish collection were ~34.44. However, because these salinities were within a very small range, the significant differences observed for Sr:Ca ratios are probably related to regional variations in the bedrock geology between these ecosystems or due to differences in the water temperature, as temperature can also significantly affect Sr incorporation (Bath et al. 2000).

For Galápagos, Li:Ca was the most important element defining the spatial pattern observed for its juvenile fishes and presented ratios up to 10 times higher than those reported in the literature for otoliths (Chang & Geffen 2013). The major sources of Li in the ocean are primarily from river input and hydrothermal activity (Edmond et al. 1979). Sailfin groupers and yellow snappers are found among rock walls, underwater lava ridges and all kinds of vertical rock formations. Juveniles can also be found in shallow lava reefs and inland lava ponds. It is possible that the substrates of these habitats are important sources of lithium, since there are no rivers across the archipelago. In addition, Swan et al. (2003) suggest that the higher concentrations of Li, Rb, Cu and Pb in areas with hydrothermal activity can lead to detectable concentrations of those trace elements in otoliths. In agreement with this study, Rb:Ca and Cu:Ca were consistently higher in fishes from the Galápagos and were also important in defining the spatial pattern of elemental ratios observed for this region (Fig. 6). Pb:Ca and Zn:Ca explained less variance on the first axis of our PCoA and were not significantly different between the Gulf and Galápagos snappers. Pb:Ca is highly toxic at higher concentrations, so fish can present specific mechanisms to control its uptake. For instance, Geffen et al. (1998)

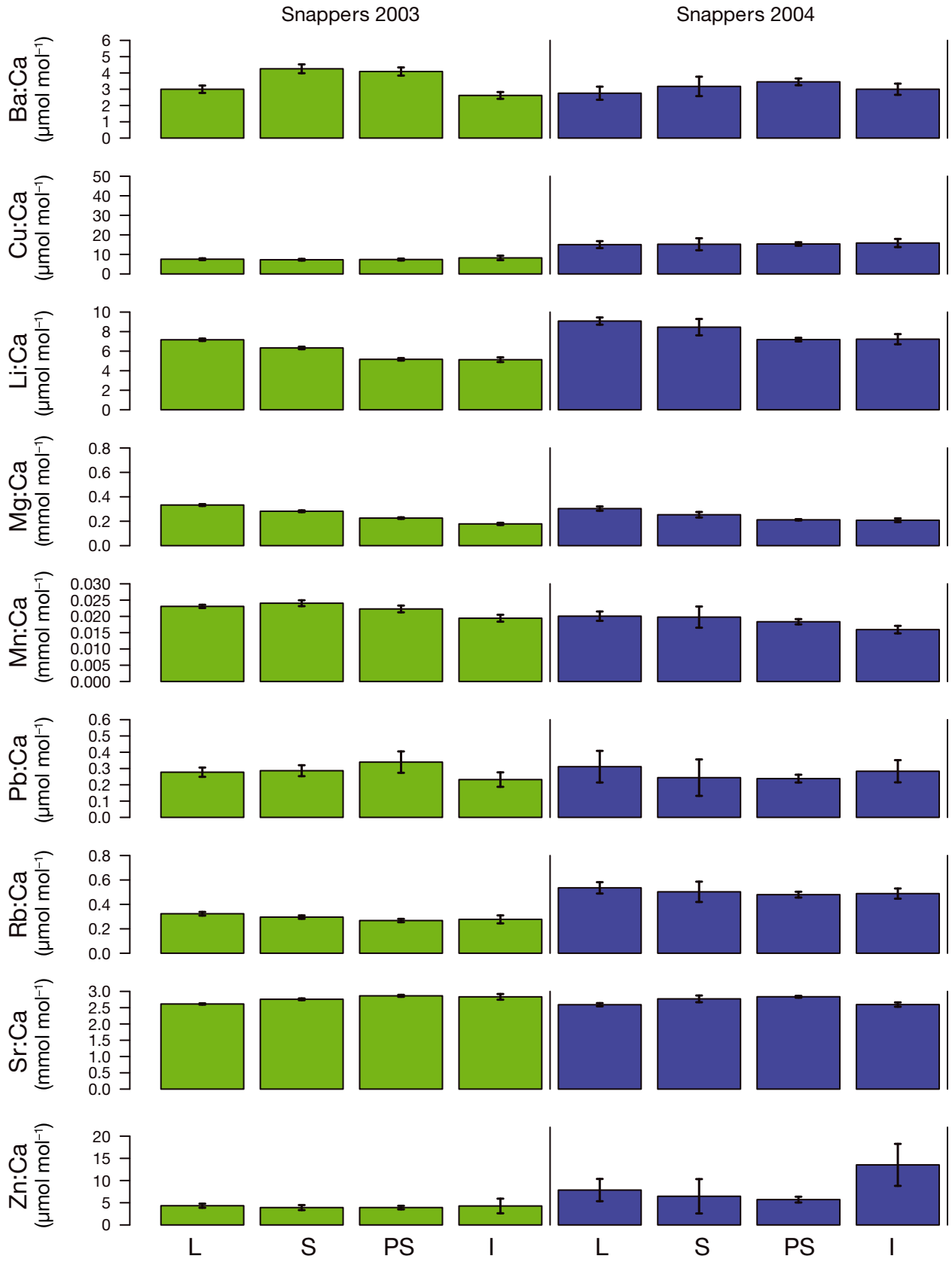

Fig. 9. Average ± SE of element to calcium ratios per juvenile size classes for yellow snapper collected in 2003 and 2004 from the Gulf of California. See Fig. 7 and Section 2.2.1 for abbreviations and size ranges

observed that the relationship between exposure and metal incorporation in otoliths was not always direct for juvenile sand gobies *Pomatoschistus minutus*, plaice *Pleuronectes platessa* and sole *Solea solea*, suggesting that physiological mechanisms operate to regulate lead and that at higher concentrations, lead is sequestered or removed from circulation so that it does not reach the growing otolith.

Diet is the primary source of intake for Zn in teleost and elasmobranch fishes (Mathews & Fisher 2009). It is therefore suspected to be an unreliable proxy for ambient environmental conditions (Miller et al. 2006). In the present study, the lack of difference in Zn ratios between species and ecosystems suggest that these juveniles were feeding on similar prey items. Indeed, snappers feed primarily on decapod crustaceans *Upogebia* sp. in the Gulf of California (Vázquez et al. 2008), a food group also found in the Galápagos mangroves.

4.3. Temporal variation

Trace element signatures in otoliths can vary between years in the same system, as demonstrated by the differences we measured for snappers captured in consecutive years in the Gulf of California (Fig. 9). Inter-annual variability in otolith signatures has been previously reported for fish inhabiting dynamic environments such as estuaries (Thorrold et al. 1997, Gillanders & Kingsford 2000, Swearer et al. 2003) and mangroves (Chittaro et al. 2004). Mateo et al. (2010), however, reported consistent elemental signatures between consecutive years in otoliths of 2 species inhabiting seagrass and mangrove habitats on Caribbean Islands. Thus, the degree of inter-annual variation appears to be region- and habitat-specific and must be assessed before multi-elemental fingerprints are considered 'permanent' markers of any specific nursery ground.

5. CONCLUSION

The present study suggests that extrinsic factors (e.g. water chemistry, temperature, salinity) can be more important than intrinsic factors (e.g. physiology, growth rates, genetics) for influencing elemental uptake in the otoliths of juveniles from the Gulf of California and Galápagos. In the future, these elements present the potential to be used as proxies for environmental processes that occur within and adjacent to mangroves, such as for the quantification of hydrothermal activity, pollution, hypoxia and primary productivity levels. We postulate that the combination of terrestrial and submarine volcanos in the Galápagos and the convergence of different oceanographic currents act to create a homogenous and distinctive water chemistry across the entire archipelago that is imparted into calcareous structures of fishes and potentially other marine organisms such as corals and mollusk shells. For the Gulf of California, the trace element ratios found in this study (especially Li:Ca, Cu:Ca, Rb:Ca, Zn:Ca, Mn:Ca and Pb:Ca) can serve as a benchmark for future comparison, in light of the potential changes in water chemistry in sediment plumes from planned mining operations. For example, the Clarion-Clipperton Zone boasts one of the world's largest untapped collections of rare-earth elements, stretches from Hawaii to the Baja California Peninsula, and is projected to be explored within the next 10 yr with 16 licenses already granted for contractors (Heffernan 2019). Finally, we also hope this simple comparison will set the scene for future interspecific comparisons of fish inhabiting ETP mangroves.

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