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Stable isotope analysis of eye lenses from invasive lionfish yields record of resource use

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ABSTRACT: Patterns of stable isotopes recorded in metabolically stable, serially synthesized, structures such as eye lenses can yield robust descriptions of resource use across the life histories of individual fish. We performed stable isotope analysis of eye lenses sampled from invasive lionfishes *Pterois* spp. and a potentially competitive native mesopredator, the graysby *Cephalopholis cruentata*, to compare lifelong patterns of trophic resource use on a coral reef ledge in Biscayne National Park, Florida, USA. In both lionfishes and graysby, stable isotope values increased logarithmically with eye-lens radius, likely reflecting increases in trophic position with growth. Tissue samples toward the interior of the lens were the most isotopically similar between lionfish and graysby, suggesting interspecific resource use overlap may be strongest in smaller fish. We observed substantial variation in isotopic chronologies around the underlying logarithmic trend within individual fish, potentially driven by patterns of movement across measured environmental isotopic gradients, intraspecific variation in resource use specificity, or other ecological variables of interest. These results are the first to describe patterns of size-structured resource use across the life of individual lionfish, an important objective for researchers studying the interactions of this highly invasive species with the surrounding ecological communities. Additionally, through this example, we illustrate analytical approaches and considerations for the application of eye-lens stable isotope analysis to the study of vertebrate ecology.

KEY WORDS: Isoscape · Competition · Habitat use · Trophic growth · Ontogeny · Reef ecology

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1. INTRODUCTION

Stable isotope analysis (SIA) has emerged as a prominent and powerful tool for ecological research, and has been applied to the study of diet, movement, and species interactions in many taxa including teleost fishes (Fry 2006). The 2 most commonly sampled isotopes are ^{13}C, which reflects basal-resource dependence (DeNiro & Epstein 1978, Peterson & Fry 1987, Grippo et al. 2011), and ^{15}N, which can serve as a proxy for trophic position (DeNiro & Epstein 1981). Stable isotope values integrate dietary input over the metabolic turnover period of the sampled tissue and can thus be applied to describe spatiotemporal patterns in consumer resource use, providing information about both foraging (Peterson & Fry 1987, Araújo et al. 2007) and movement among isotopically diverse habitats (Hobson 1999, Davis et al. 2015). Sequentially synthesized metabolically stable tissues are particularly useful for SIA-based studies of animal ecology (see review by Tzadik et al. 2017), as these can record stable isotope values across the entire lifespan of individual organisms (Campana 1999, McMahon et al. 2011). Such isotopic data series provide information about ontogenetic shifts in diet and habitat, individual resource use specificity, and other aspects of consumer life history (Kim et al. 2012, Hanson et al. 2013, Tzadik et al. 2015).

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Mineral-rich structures such as otoliths, scales, and bones have been previously used to construct isotopic chronologies (Campana 1999, Clarke et al. 2007). However, otoliths and other 'hard parts' may contain small proportions of nitrogen (N)-bearing organic material which can complicate measurement of δ^{15}N, which is frequently quantified to provide information about consumer diet and movement (Pinnegar & Polunin 1999). The eye lens has recently been proposed as an alternative, N-rich isotopic recorder that may be used to describe animal life histories (Hunsicker et al. 2010, Wallace et al. 2014, Quaeck-Davies et al. 2018). Eye lenses are structured in concentric layers (laminae) composed of cells that undergo a modified version of apoptosis shortly after formation (Nicol 1989, Lynnerup et al. 2008). Following the cessation of metabolic activity, laminae retain the chemical composition associated with their period of synthesis (Vihtelic 2008) and therefore have the potential to be highly valuable for the reconstruction of isotopic chronologies. Despite a demonstrated capacity for recording age and chemical histories via radiocarbon (Askaner & Hansson 1967, Lynnerup et al. 2008, Nielsen et al. 2016), the study of stable isotopes in eye lenses in marine vertebrates has been limited to a small number of research efforts in teleost and elasmobranch fishes (Wallace et al. 2014, Quaeck-Davies et al. 2018, Kurth et al. 2019, Simpson et al. 2019).

We aimed to further explore the utility of eye-lens SIA within a novel ecological context, specifically by studying how resulting chemical chronologies could help describe resource use throughout the life history of an invasive species. We applied this technique to a well known marine invader, Indo-Pacific lionfishes *Pterois* spp. (hereafter lionfish), which have become a pervasive threat to native species throughout the western Atlantic (Côté et al. 2013, Albins & Hixon 2013). Although lionfish diet (Morris & Akins 2009, Muñoz et al. 2011, Peake et al. 2018) and movement (Akins et al. 2014, Bacheler et al. 2015, Tamburello & Côté 2015) have been characterized at the population level, similar measurements throughout the lifespan of individual fish could yield additional insights that could be useful to conservation scientists and resource managers. Comparative analyses of isotopic chronologies in lionfish and native mesopredators could also refine our understanding of how diet and habitat overlap among potential competitors at different sizes and ages; this is an important aspect of lionfish ecology in the western Atlantic that remains to be thoroughly quantified (Layman & Allgeier 2012, O'Farrell et al. 2014, Ellis & Faletti 2016, Curtis et al. 2017).

One of the features expected to chiefly characterize isotopic chronologies from eye lenses of generalist piscivores such as lionfish is an increase in trophic position with size, or 'trophic growth' (Wallace et al. 2014, Tzadik et al. 2015, Simpson et al. 2019). Such ontogenetic shifts are prevalent in predatory fishes (Romanuk et al. 2011), primarily due to a widening of gape size that enables consumption of larger prey (Arim et al. 2010). However, evidence of a relationship between lionfish size and muscle stable isotope values has been equivocal, varying geographically and by habitat (Morris & Akins 2009, Muñoz et al. 2011, Layman & Allgeier 2012). Thus, descriptions of trophic growth based on eye-lens stable isotopes may clarify the nature of size-based diet shifts at both the individual and population levels (Kim et al. 2012). Importantly, comparisons of these attributes with those of ecologically similar native predators could also help predict relative trophic positions and the amount of dietary overlap across multiple life stages of potentially competitive species, providing insight into which size classes of both species are most likely to engage in competition in a resource-limited environment (Simpson et al. 2019).

Additionally, careful examination of deviations from trophic growth in individual eye-lens isotopic records could help to better describe other important aspects of lionfish ecology. Sharp or persistent fluctuations within individual chronologies that cannot be reasonably explained by corresponding substantive changes in diet or the prey community could be indicative of movement or shifts in habitat use in individual lionfish (McMahon et al. 2011, Kurth et al. 2019), especially in an environment with spatially structured isotopic baselines (Radabaugh et al. 2013, 2014). Lionfish movement is still relatively unquantified at the individual level, yet is critical to understand for accurately predicting the scale of foraging activities, patterns of dispersal throughout invaded ecosystems, and the potential for habitat overlap and interactions with native mesopredators (Akins et al. 2014, Bacheler et al. 2015, Tamburello & Côté 2015). Besides indicating movement, variation in individual resource use chronologies can be measured to study traits such as resource use specificity (Kim et al. 2012), which has previously been suggested is an underappreciated aspect of lionfish foraging ecology (Layman & Allgeier 2012).

We applied isotopic analysis of eye-lens laminae to compare lifelong patterns of resource use in lionfish with that of a native mesopredator, the graysby *Cephalopholis cruentata*, on a south Florida coral reef tract. The graysby is a small grouper that is a plausible competitor with lionfish due to similarities in maximum size, diet, and habitat use (Nagelkerken 1979, Popple & Hunte 2005). Previous SIA-based measurements indicate a high likelihood of resource use overlap between these species, mostly based on the similarity of their isotopic niches and parallel increases in trophic position (from δ^{15}N values) with increases in fish length (Curtis et al. 2017).

In this study, we intended to further compare patterns of resource use between lionfish and graysby, especially at the individual level and across multiple life stages. First, we estimated population-level trophic growth curves for the 2 species. Then we examined deviations from trophic growth within individuals in order to detect possible influences of movement among isotopically distinct habitats or individuality in resource use ontogeny. In order to provide important context for interpretations of isotopic chronologies, we first had to create spatial and temporal frames of reference in our study system. First, we described regional spatial variation in isotopic values of muscle from both species, which facilitated interpretation of features in isotopic chronologies as possible evidence of movement across environmental baselines. Additionally, we derived the first relationship between lens size and fish length for lionfish and graysby, which allowed ascription of isotopic patterns within eye-lens chronologies to specific size and age classes in these species.

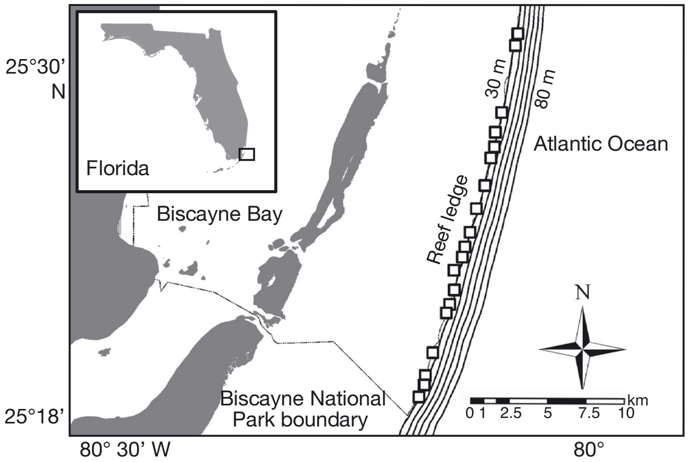

Fig. 1. Study area and sites (squares) along the reef ledge of Biscayne National Park in south Florida, USA. Solid gray lines: isobaths at 10 m increments from 30–80 m depth

2. MATERIALS AND METHODS

2.1. Study region and sample collection

This study was conducted in Biscayne National Park (BNP) in south Florida (Fig. 1), where complex contiguous reefs support resident populations of both lionfish and graysby. Fish were collected using pole spears in association with a lionfish removal experiment between January 2014 and September 2015. Specimens were collected from 20 sites, each 1000 m^2, distributed along 25 km of reef-ledge habitat (Stallings & Albins 2016, see Text S1 in the Supplement at www.int-res.com/articles/suppl/m637p181_supp.pdf for details). Sampled lionfish were frozen and transported to the University of South Florida (USF) for dissection, whereas graysby were stored on ice after each dive and dissected on the same day. Occasionally, graysby collections were facilitated with the use of a 20 % quinaldine solution ($C_{10}H_9N$), a common fish anesthetic. Collections were made under the auspices of the USF Institutional Animal Care and Use Committee (protocol W4193) with approval from the US National Park Service (permit BISC-2014-SCI-0025).

2.2. Dissection and eye-lens delamination

Before dissection, all frozen lionfish were thawed at room temperature. Thawing was not required for graysby, which were dissected on the day of capture. The standard length (cm) of each individual was measured, and approximately 1 cm^3 of white muscle was removed posterior to the dorsal fin and frozen. Intact eyes were removed by severing the optic nerve and connective tissue between the eye and surrounding skeletomusculature, then wrapped in aluminum foil and frozen. Freezing was chosen as the preferred method for fish tissue storage, as it does not impart preservation-driven offsets in stable isotope values (Stallings et al. 2015).

We dissected eye lenses from a subsample of lionfish (n = 13) and graysby (n = 14) collected during July or September 2015 through a process called 'delamination'. Individuals were selected from a standard-length range which offered the largest number of similarly sized fishes (lionfish: 16.0–22.0 cm; graysby: 15.8–22.1 cm). Although stable isotope records do not appear to vary between left and right eyes (Wallace et al. 2014), the left eye lens was preferentially used for analysis unless it had been damaged during fish collection. Before delamination, each eye was thawed at room temperature. Lenses were then extracted, the lens epithelium was removed, and the remainder of the lens was rinsed in de-ionized water to remove semi-fluid cortical material. Lens laminae were sequentially peeled under a dissecting microscope using a pair of fine-tipped forceps, following the methods described in Wallace et al. (2014); this technique has been described as the first-choice methodology for delaminating eye lenses (Quaeck-Davies et al. 2018). Before and after the removal of each lamina, the diameter of the lens was measured at its widest point (rounded to the nearest 0.05 mm) using an ocular micrometer. The distance of each lamina relative to the center of the lens was defined as the 'radial midpoint', which is the average of the lens diameter before and after each delamination. Between laminar removals, tools were rinsed and dried with a lint-free cloth, and a small amount of deionized water was applied to the lens to facilitate subsequent delamination. This process was repeated until the lens diameter was ≤1 mm, the size at which each individual lamina no longer provided enough material for independent isotopic analysis with our available instrumentation.

2.3. SIA

Muscle samples were freeze-dried at −40°C for 36–72 h and were mechanically homogenized using a mortar and pestle, while eye-lens laminae were air-dried at room temperature for 1 h. For muscle, 400–1000 µg of muscle was weighed on a Mettler-Toledo precision microbalance, while 300–600 µg of material was sectioned and weighed from each lens lamina when enough material was available. If laminar mass was <300 µg, equal material from the adjacent interior lamina was included in the sample, and radial midpoints were averaged to describe location within the lens.

Tissue samples were wrapped in a tin capsule and loaded into a Costech Technologies Zero-Blank Autosampler. Samples were combusted at 1050°C in a Carlo-Erba NA2500 Series-II elemental analyzer coupled in continuous-flow mode to a Finnigan Delta Plus XL isotope ratio mass spectrometer. Measurements of C:N ratios were calibrated and isotopic measurements were normalized to the AT-Air and VPDB scales, respectively, using NIST 8573 and NIST 8574 L-glutamic acid Standard Reference Materials. Measurements were expressed in units per mil (‰) using δ notation, where $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, and R is the isotopic ratio of interest (e.g. ^{13}C:^{12}C). Analytical precision, estimated from repeated measurements of a working standard (NIST 1577b, bovine liver, n = 253) was ±0.17‰ (δ^{15}N) and ±0.13‰ (δ^{13}C). Two replicate samples were analyzed for each fish, and the results were averaged for further statistical comparison. The mean difference (±SD) between replicate samples was 0.00 ± 0.15‰ for both δ^{13}C and δ^{15}N, indicating a high degree of consistency in isotopic measurements.

2.4. Environmental isotopic baseline

The establishment of a baseline map of environmental isotopic values, which at its most refined is called an 'isoscape' (Barnes et al. 2009, Radabaugh et al. 2013), is generally a prerequisite to determining how patterns of movement may become manifest in isotopic chronologies within a geographic setting (Wallace et al. 2014, Kurth et al. 2019). These reference frames can be derived from spatially structured measurements of multiple materials, including fish muscle (Radabaugh et al. 2013). To create an environmental baseline for BNP, we examined spatial trends along the reef ledge by measuring site-averaged stable isotope values of both lionfish and graysby muscle across gradients in depth (18–25 m) and latitude (25° 18′ 10″ N to 25° 31′ 26″ N). In lionfish, muscle samples (n = 276) were analyzed from fish collected across 12 of our 20 study sites, as logistical constraints at the other 8 locations restricted sampling to either very few individuals or a highly skewed size distribution, and therefore could not be included in a generalizable assessment of local stable isotope baselines (see Text S1 & Table S1 for context). Muscle samples from graysby included in the analysis (n = 172) were collected across all 20 study sites. To represent depth, we used the maximum value recorded during the establishment of each site. For latitude, we used GPS coordinates measured with a Garmin GPSMAP 4210 at the center of each site. Because sites were oriented in a line running approximately north to south, latitude provided an appropriate descriptor of relative site position.

Isotopic trends with depth and latitude were analyzed using a multiple linear regression model. Site depth and latitude were included as main effects, as well as site averages of fish size (standard length) to account for variation driven by length–isotopic relationships in these species (Curtis et al. 2017). We initially included all possible 2-way interaction terms between depth, latitude, and size in the regression, but these were excluded after none were found to significantly improve the overall fit.

2.5. Lens–length relationships

Estimates of the relationship between lens size and fish length provide another important reference frame for attributing isotopic trends observed in interior lens material to specific life stages or age classes. Though lens–length relationships are generally linear, parameters may vary on a species-by-species basis; it is therefore important to establish this relationship in taxa that are undergoing eye-lens isotopic analysis for the first time (Quaeck-Davies et al. 2018, Simpson et al. 2019). We compared length and lens measurements across a size distribution that was representative of the broader population within each species (n = 58 graysby, 10.2–26.8 cm total length [TL]; n = 48 lionfish, 9.8–41.3 cm TL). Analyzed lenses were extracted from thawed, intact eyes and measured at their widest point using an ocular micrometer, while TL was measured during initial fish dissection. Sample sizes were determined to be appropriate based on previous analyses comparing lens diameter and fish length, as was the use of linear regression to describe the lens–length relationship (Quaeck-Davies et al. 2018, Simpson et al. 2019).

2.6. Eye-lens chronologies

Analyses of eye-lens chronologies yielded by SIA were performed using both quantitative tests and qualitative observations. To describe general trends in lionfish and graysby eye-lens chronologies, we examined the relationship between isotope values and lens radius using nonlinear least-squares regression on data aggregated from multiple eye lenses for both lionfish (n = 139 lamina from 12 ind.) and graysby (n = 220 lamina from 14 ind.). Specifically, we measured the fit of a logarithmic curve with the equation $y = a + b \times \log(x)$, where y is the isotopic value of interest, x is radial midpoint, a is the parameter controlling curve location on the y-axis, and b is the parameter controlling curve shape. This model was chosen as a simplified version of growth equations commonly used in fish, such as von Bertalanffy and logistic curves (von Bertalanffy 1938, Ricker 1975). The resulting regression trends could theoretically be attributable to changes in trophic position with somatic growth. Although isotopic fractionation with trophic position is smaller in magnitude and less consistent in $\delta^{13}C$ values than in $\delta^{15}N$ values (~1 vs. 3‰ enrichment per trophic step), a trend is still expected in both isotopes if lionfish and graysby trophic positions are related to size (DeNiro & Epstein 1978, 1981, Fry 2006). To determine whether model parameters varied between lionfish and graysby, we calculated 95% confidence intervals (CI) for the differences between parameters derived from bootstrapped iterations of the regression (n = 1000) and assigned significance if the 95% CI did not include 0. One lionfish was excluded as an outlier from the regression analysis based on measurements of $\delta^{15}N$ values that were >10 standard deviations higher than the mean of other sampled individuals.

To describe deviation from modeled trophic-growth curves within individual eye-lens chronologies, we fit a nonlinear mixed effects model using the same equation as the nonlinear regression described above, with lens radius as a fixed effect and fish identity as a random effect. We then calculated the root mean square error (RMSE) value for each species–isotope pairing to assess the degree of intra-individual variation, with higher values indicating higher deviations from logarithmic trends within chronologies. Data were standardized as *z*-scores before analysis to increase comparability of RMSE values, which can be scale-dependent.

To qualitatively examine whether any consistent life history events explained specific patterns of variation in isotopic chronologies, we plotted stable isotope values against laminar distance from the center of the lens (radial midpoint) as a proxy for age. Similar fluctuations at consistent radial midpoints could theoretically be attributable to shifts in diet or habitat during specific developmental stages (Wallace et al. 2014, Quaeck-Davies et al. 2018, Kurth et al. 2019). Hereafter we refer to this arrangement of the data as 'age synchronized'. Similarly, by plotting stable isotope values in reverse chronology (i.e. radial distance from the outer lens margin instead of from the lens center), we examined whether any consistent environmental influence, such as seasonal variation in abiotic conditions or the prey community, may have influenced patterns in recently formed laminae (toward the outermost edge of the lens). Only individuals collected during September 2015 (n = 6 lionfish, n = 10 graysby) were used in this 'time synchronized' analysis to avoid inclusion of potentially confounding temporal variation.

2.7. Statistical analyses

For all quantitative analyses in this study, statistics were calculated with distribution-free, non-parametric tests modified from the Fathom Toolbox for MATLAB (Jones 2017). Significance was assessed using p-values derived by comparing the position of test statistics calculated from the original data relative to a distribution of the same statistic calculated from n = 1000 randomized permutations of the data set (Moore et al. 2014). Statistics that were more extreme than 95% of permuted values (2-tailed) were considered to be significant (α = 0.05). Although these methods allowed us to relax some of the strict assumptions of parametric statistical tests, particularly that of normally distributed errors, they can be sensitive to the inclusion of dependent or spatially structured data (Anderson 2001). Therefore, for tests involving multiple individuals collected from the same study site or multiple samples collected from the same individual, which represent subsamples rather than independent replicates, randomization was constrained within study sites or individual specimens, respectively (Legendre & Legendre 1998)

3. RESULTS

3.1. Environmental isotopic baseline

In both graysby and lionfish muscle tissue, δ^{13}C values were lower at deeper sites, whereas δ^{15}N values were not related to depth (Table 1, Fig. 2a–d). Conversely, although δ^{13}C values did not vary with latitude, δ^{15}N values were higher on northern sites in both graysby and lionfish (Table 1, Fig. 2e–h). All spatial trends were detected after accounting for effects of fish size on stable isotope values (via inclusion of size as a main effect in the regression model), which were consistent with relationships reported in our previous work along the BNP reef ledge (Curtis et al. 2017).

Table 1. Statistics for multiple linear regressions of stable isotope values (δ^{13}C and δ^{15}N) measured in muscle of graysby and lionfish with spatial variables (latitude and depth) and average fish size (standard length) among study sites. For graysby, all sites were initially included for analysis (n = 20); a smaller subset of sites were included for lionfish (n = 12; see Section 2.4 and Text S1). Regressions were randomization-based (n = 1000 permutations); *p ≤ 0.05

Analysis	Variable	Coefficient	Coeff SE	*t*	p
Graysby δ^{13}C	Intercept	−15.03	0.05	−292.34	0.001*
	Depth	−0.06	0.03	−2.18	0.038*
	Latitude	−1.32	0.81	−1.63	0.146
	Size	−0.05	0.03	1.61	0.128
Graysby δ^{15}N	Intercept	9.17	0.02	542.15	0.001*
	Depth	0.01	0.01	0.56	0.745
	Latitude	1.18	0.27	4.45	0.001*
	Size	0.04	0.01	4.41	0.001*
Lionfish δ^{13}C	Intercept	−14.66	0.04	−373.15	0.04*
	Depth	−0.11	0.03	−3.21	0.013*
	Latitude	0.51	0.69	0.74	0.458
	Size	−0.04	0.02	−1.96	0.076
Lionfish δ^{15}N	Intercept	9.13	0.02	538.17	0.001*
	Depth	−0.02	0.02	−1.17	0.291
	Latitude	1.67	0.30	5.62	0.001*
	Size	−0.04	0.01	4.06	0.003*

3.2. Eye lenses

In both graysby and lionfish, lens diameter was positively linearly related to TL (graysby: R^2 = 0.64, F_{56} = 99.8, $p < 0.001$; lionfish: R^2 = 0.77, F_{44} = 147.3, $p < 0.001$; Fig. 3). Nonlinear regression revealed a logarithmic trend of δ^{13}C and δ^{15}N in the aggregated age-synchronized chronologies in both graysby and lionfish (Table 2, Fig. 4). All regression parameters were different from one another except for the location parameter (*a*) in graysby and lionfish δ^{15}N chronologies (Table 3). Variation around logarithmic curves within individuals was higher in δ^{15}N chronologies than in δ^{13}C chronologies in both species (RMSE values: graysby δ^{13}C = 0.41, δ^{15}N = 0.27; lionfish δ^{13}C = 0.49, δ^{15}N = 0.16). However, the amount of within-individual variation was higher in graysby δ^{15}N than lionfish δ^{15}N chronologies, but lower in graysby δ^{13}C than lionfish δ^{13}C chronologies. The shapes, patterns, and features of individual data series varied substantially among individuals (Figs. S1–S4). Many of the chronologies were characterized by sharp deviations, peaks, and valleys of different magnitude and periodicity, with no obvious, consistent trends that could be attributed to environmental or temporal covariates. Whether plotted forwards (age synchronized) or in reverse (time synchronized), fluctuations in the curves did not appear to coincide with any common position within the eye lens (Fig. 5).

4. DISCUSSION

To our knowledge, this study provides the first descriptions of resource use patterns across the lifespan of individual invasive lionfish, and it is the first to apply SIA of metabolically stable, sequentially synthesized, tissues for the comparison of life histories of invasive and native fishes. These results may prove useful to those studying marine fishes or invasive spe-

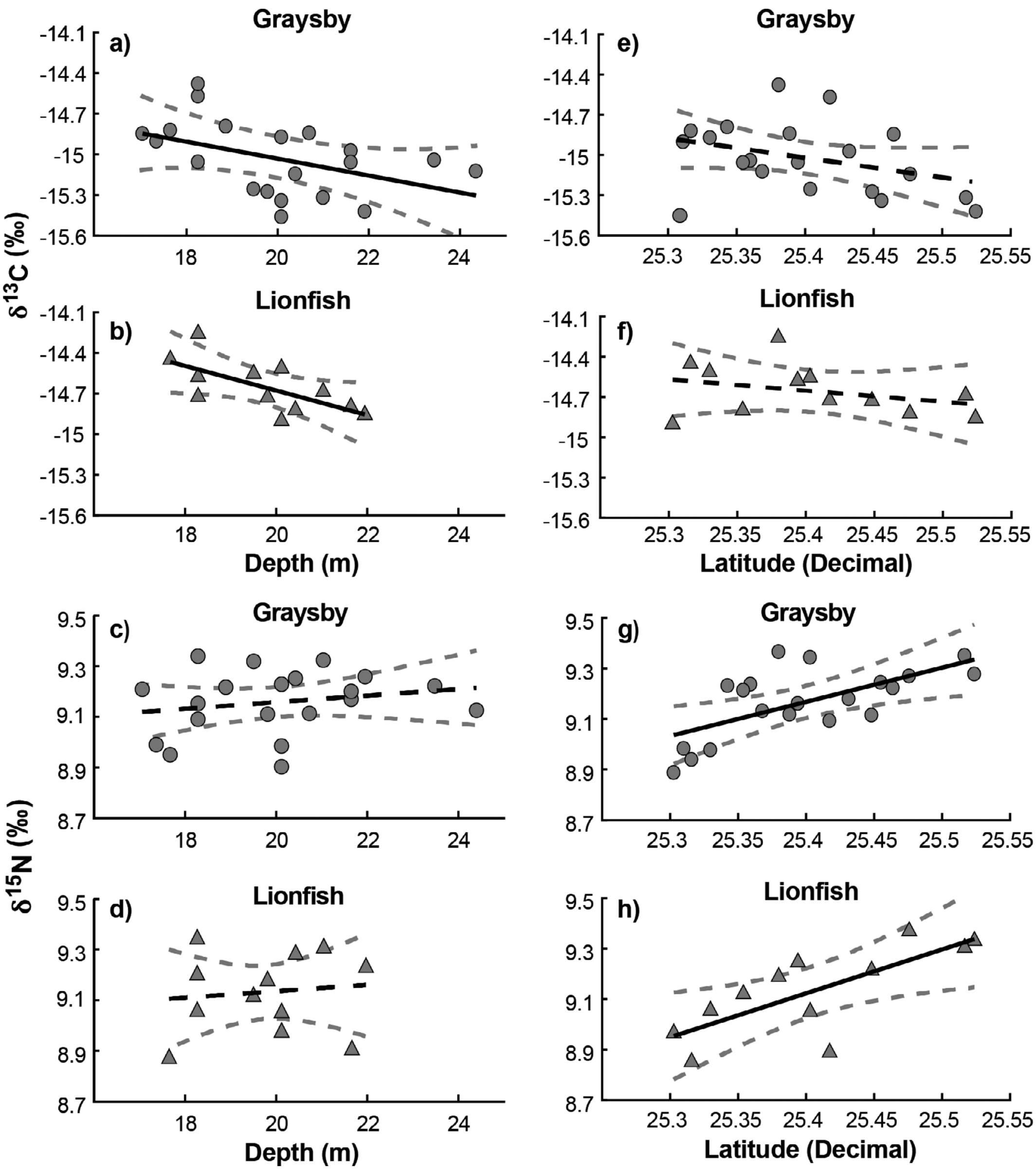

Fig. 2. Linear regressions of average $\delta^{13}C$ and $\delta^{15}N$ values from muscle samples of graysby (circles) and lionfish (triangles) with maximum site (a–d) depth and (e–h) latitude. Graysby samples were collected from all sites (n = 20); lionfish samples were collected from a subset of sites where appropriate samples were available (n = 12; see Section 2.4 and Text S1). Statistically significant regressions (see Table 1) are depicted as solid black lines ($p < 0.05$). Grey dashed lines: bootstrapped 95% confidence bands for the regression

cies, and more broadly, could suggest analytical approaches for ecological investigations in taxa featuring similar eye-lens physiology (including most vertebrates and several cephalopods). Our study corroborates the growing understanding that the chemical archives of eye lenses can be consulted to uncover novel insight, especially in systems where variation in stable isotopes can readily be related to geography or available resources.

We detected spatial isotopic gradients along the reef ledge of BNP that provide important environmental context for interpretation of eye-lens chronologies. We measured a consistent decreasing trend in $\delta^{15}N$ values with latitude, which can likely be attributed to the influence of anthropogenic wastewater and urban runoff on northern sites closer to the heavily developed coastline of Miami (McClelland et al. 1997). The negative depth–$\delta^{13}C$ relationship measured in both lionfish and graysby was consistent with patterns found in multiple marine taxa, including other teleost fishes (Barnes et al. 2009, Radabaugh et al. 2013, Kurth et al. 2019).

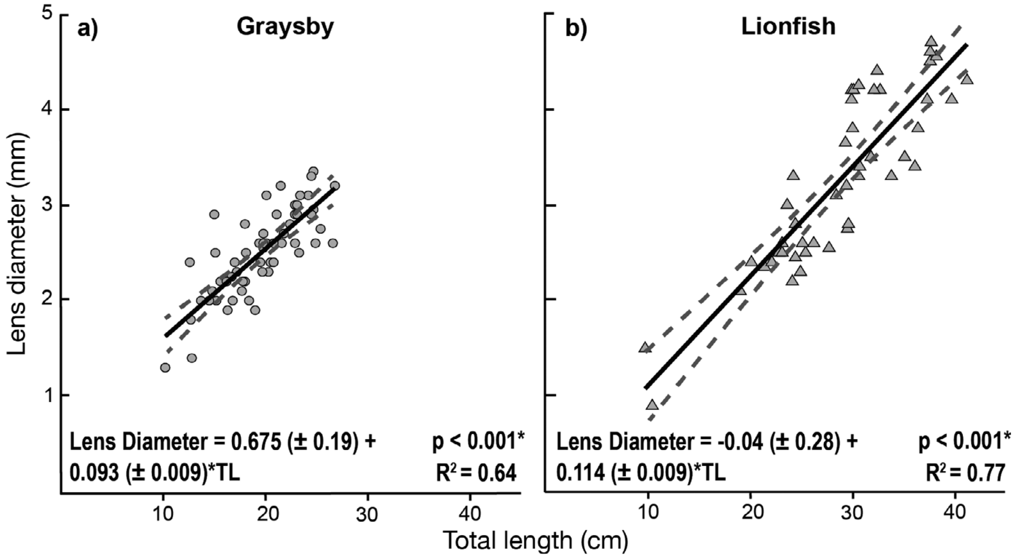

Fig. 3. Linear regressions of total length and lens diameter in graysby (n = 58) and lionfish (n = 45), including regression lines (solid) and associated 95% confidence bands (dashed). Regression parameters (±SE) and goodness-of-fit estimates (R^2) are provided. $^*p < 0.05$.

Generally, this trend can be explained by both reduced negative photosynthetic fractionation in primary producers experiencing lower light availability (Wefer & Killingley 1986) and less trophic input from benthic primary producers with increasing depth (Sierszen et al. 2014, Radabaugh et al. 2014). Due to the small magnitude of variation of both isotopes across the study area (~0.4‰ in $\delta^{15}N$, ~0.7‰ in $\delta^{13}C$), deterministic tracking of regional movement and foraging using data collected for this study alone may not be possible (Authier et al. 2012). However, the manifestation of spatial isotopic trends in both lionfish and graysby suggests the presence of consistent environmental relationships that may be more geographically expansive (e.g. as described in the eastern Gulf of Mexico by Radabaugh et al. 2013), although determining the scale of these patterns would require further sampling. Still, the detected gradients suggest that broad movement of fish along the BNP reef ledge and across depths would likely materialize in chemical chronologies of long-term resource use. The most likely pattern in isotopic values caused by movement would be features that cannot reasonably be attributed to changes in trophic position or diet alone (i.e. dramatic variation around the trophic growth curve or large persistent offsets in isotopic baselines).

Table 2. Statistics for nonlinear least squares regressions of stable isotope measurements ($\delta^{13}C$ and $\delta^{15}N$) from eye lenses of lionfish and graysby with laminar radial midpoint (mm). Regressions were made to test the fit of a logarithmic growth equation, $y = a + b \times \log(x)$ (see Section 2.6), to aggregated measurements from all sampled eye lenses. Regressions were conducted using a randomization-based approach (n = 1000 permutations); randomization was constrained to within individual fish to account for a lack of independence among sequentially collected data points from a single individual. Parameters are presented with bootstrapped 95% confidence intervals (CI) (n = 1000 iterations); $^*p \leq 0.05$

Regressed with radial midpoint	n	*a* (95% CI)	*b* (95% CI)	*F*	p
Graysby $\delta^{13}C$	220	−15.55 (−15.69, −15.40)	1.36 (1.16, 1.54)	78.4	<0.001*
Lionfish $\delta^{13}C$	139	−15.05 (−15.18, −14.91)	0.44 (0.23, 0.62)	9.76	<0.001*
Graysby $\delta^{15}N$	220	8.22 (8.13, 8.32)	1.97 (1.84, 2.09)	501	<0.001*
Lionfish $\delta^{15}N$	139	8.27 (8.15, 8.36)	0.84 (0.71, 1.00)	70.2	<0.001*

The strong correlations measured between fish length and lens size in both lionfish and graysby corroborates a predictable linear relationship that allows attribution of characteristics in lens-derived isotope chronologies to specific size- and age-classes in these species. Although the absence of measurements in very small (larval and newly settled) individuals precludes concrete determination of the length or age at which graysby and lionfish have developed lenses smaller than ~1.5 mm in radius, the high goodness-of-fit estimates of our regression models justify cautious interpretations of trophic resource use trends at life stages outside of our included size range. In lionfish in particular, the intercept of the linear model was estimated at almost exactly 0, suggesting that the lens grows proportionally with length throughout develop-

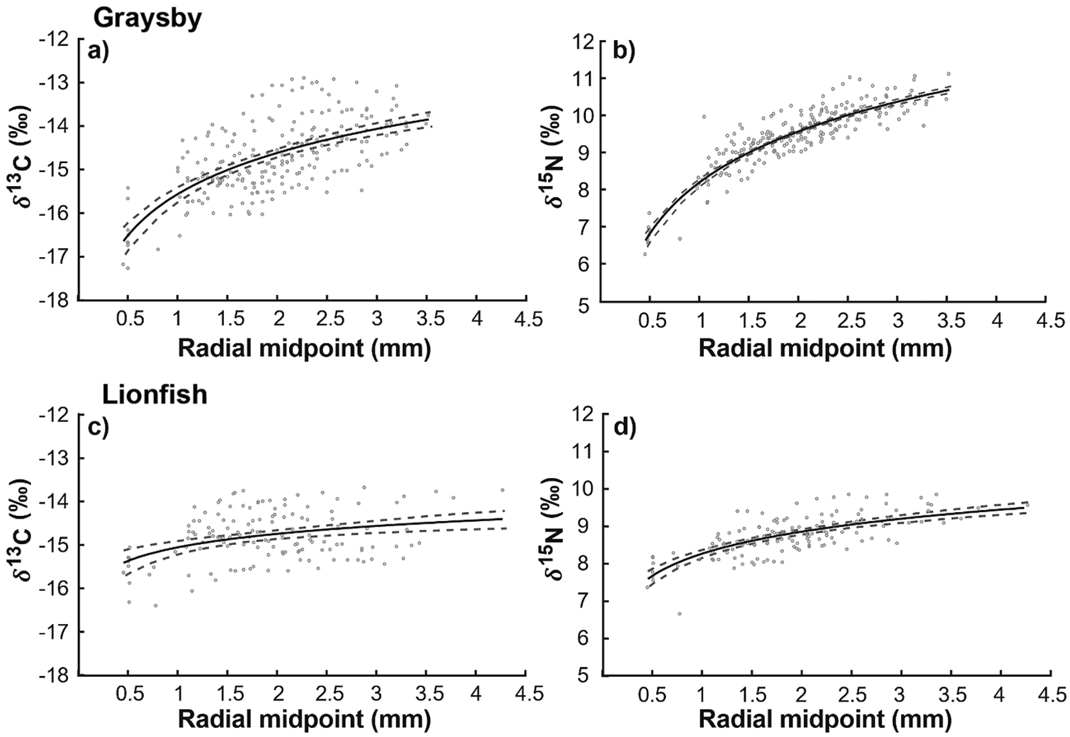

Fig. 4. Nonlinear regressions (solid lines) of (a,c) δ^{13}C and (b,d) δ^{15}N values with laminar radial midpoint in graysby (a,b) and lionfish (c,d) using a logarithmic model. Dashed lines: 95% CI bands calculated using error propagation via a Monte Carlo simulation (n = 1000 iterations). Regression statistics are provided in Table 2; regression parameters are provided in Table 3

ment. The measurement of a slightly positive intercept in graysby indicates that there may be some non-linearity in lens–length relationships at very small sizes in this species, which previous works indicate could be a common feature in fishes (Quaeck-Davies et al. 2018, Simpson et al. 2019). Generalities regarding these relationships will only become clearer with further study in diverse taxa and environmental contexts, which will allow refined projections from lens radius to the size and age at which an individual lamina was synthesized in a variety of systems.

Table 3. Bootstrapped 95% CI (n = 1000 iterations) for interspecific differences in parameters *a* and *b* calculated via nonlinear regression of stable isotope values with eye-lens radius in lionfish and graysby (see Table 2 for regression results). The difference in parameters was considered to be statistically significant if the 95% CI did not contain 0

Regression	Parameter	95% CI of difference (graysby–lionfish)	Contains 0
δ^{13}C vs. lens radius	*a*	−0.70, −0.32	No
	b	0.64, 1.18	No
δ^{15}N vs. lens radius	*a*	−0.19, 0.11	Yes
	b	0.92, 1.31	No

Alongside our spatial and temporal reference frames, measurements of stable isotopes in eye-lens laminae yielded information about the life histories of lionfish and graysby. The logarithmic increase in the δ^{13}C and δ^{15}N values of both lionfish and graysby with lens radius provides a novel characterization of how trophic position might change with age and size in these generalist mesopredators. Indeed, these are the first data to examine ontogenetic shifts in resource use across the life history of individual wild-caught specimen of either species. The observed patterns broadly match trends between diet and size measured in lionfish and graysby at the population level (Nagelkerken 1979, Morris & Akins 2009, Peake et al. 2018), and are similar in shape to curves derived from serially sampled isotopic records in other fishes known to switch diet and increase trophic position as they grow (Kim et al. 2012, Wallace et al. 2014, Tzadik et al. 2015). The logarithmic trend resembles traditional models of fish growth, except that our chronologies generally lack a horizontal asymptote (von Bertalanffy 1938, Ricker 1975). That isotopic values did not usually level out in the outermost laminae may be partly due to the size range of sampled fish, which was smaller than the maximum potential length of

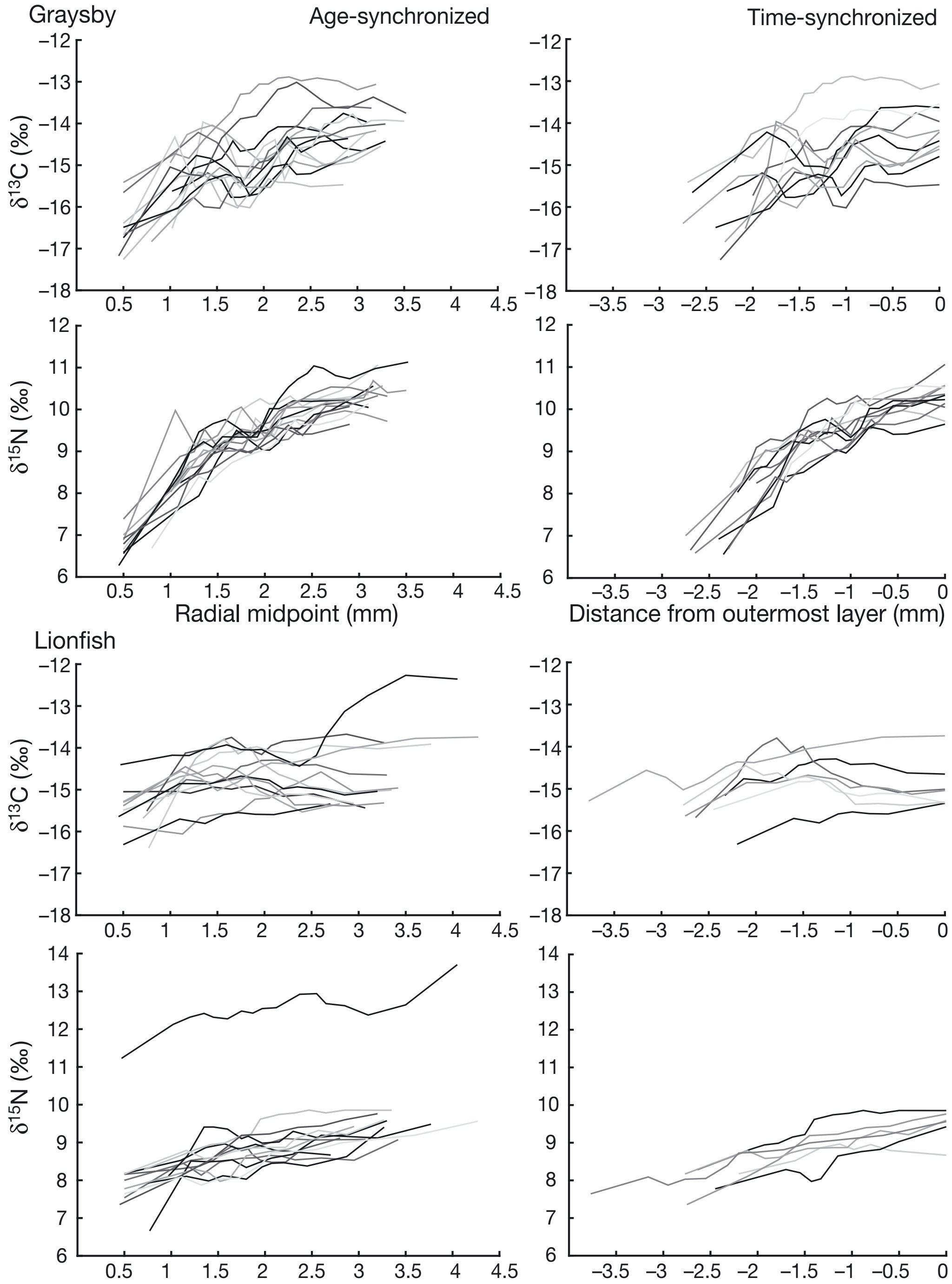

Fig. 5. Chronologies of δ^{13}C and δ^{15}N measured in eye lenses of graysby and lionfish. Data are plotted both against distance of laminar radial midpoint from the lens nucleus (age-synchronized) and against distance from the outermost lamina (time-synchronized). In age-synchronized plots, time is represented as ontogeny; differential somatic growth rates cause ontogenetic synchrony to be higher at smaller radial midpoints. In time-synchronized plots, time is synchronized with date of capture in this figure (September 2015); differential growth rates cause synchrony to be lower at smaller radial midpoints

both lionfish and graysby. Similar measurements in larger fish may demonstrate whether eye-lens isotopic values eventually reach a horizontal asymptote in these species.

Comparing modeled trophic growth curves between species helps identify the life stages during which lionfish and graysby are most likely to overlap in diet, offering a more refined approach to assessing interspecific patterns of size-structured resource use than provided by previous population-level estimates for lionfish (e.g. Layman & Allgeier 2012, O'Farrell et al. 2014, Curtis et al. 2017). Graysby isotopic values were lower than those of lionfish at the interior of the eye lens, but increased more rapidly with distance from the nucleus, resulting in higher values in outer laminae (Fig. 4). The fitted logarithmic trendlines intersect at a radial midpoint of 1.0 mm (δ^{15}N) and 1.75 mm (δ^{13}C) within a ~4 mm lens. Consulting our lens size–length relationships, graysby measuring ~4–12 cm TL are therefore most likely to overlap in resource use with lionfish measuring ~9–16 cm TL. These size ranges would be characteristic of graysby younger than 2 yr (Potts & Manooch 1999) and lionfish younger than 1 yr (Edwards et al. 2014, Johnson & Swenarton 2016). It is therefore possible that juvenile graysby may experience heightened resource use overlap with lionfish for an additional year of their development due to their slower somatic growth, which could lend a competitive 'edge' to lionfish in resource-limited environments. Conversely, isotopic measurements in the exterior-most lens layers of graysby suggest both slightly higher long-term trophic level than cohabitant lionfish and greater differentiation of interspecific resource use among adult fish.

In addition to comparing the logarithmic trends which generally characterized eye-lens isotopic records in lionfish and graysby, examining deviations from modeled trophic growth can yield further information about the ecology of these species. There was more apparent variation around the fitted curves in eye-lens δ^{13}C values compared to δ^{15}N. The finding that δ^{15}N values corresponded better with modeled trophic growth was not surprising, because δ^{15}N relates more predictably to trophic position than δ^{13}C (DeNiro & Epstein 1978, 1981, Pinnegar & Polunin 1999). We also observed higher variation around logarithmic models in lionfish δ^{15}N chronologies compared to those of graysby, while δ^{13}C chronologies varied more relative to trophic growth curves in the invasive species. One possible interpretation given the opposing orientation of measured spatial gradients in these isotopes is differences in the directionality of fish movement. If lionfish traveled more persistently across depth–δ^{13}C isoclines while graysby moved more broadly across latitudinal δ^{15}N gradients (i.e. along the reef ledge), it could explain the disparity in relative magnitudes of variation measured in the chronologies of each isotopic type. Furthermore, the lower amount of deviation from trophic growth within lionfish δ^{15}N chronologies might suggest that individual variation in patterns of ontogenetic diet shifts is smaller than in graysby (Kim et al. 2012). The comparatively high variation within δ^{13}C records could also indicate that individual specificity in trophic ontogeny, or resource partitioning among individuals, is driven more by differences in productivity source than trophic level in both species (Simpson et al. 2019). Additional sampling in a variety of environmental and geographic settings would be necessary to distinguish the relative influence of these possible interpretations, and to more broadly discern how movement and individual resource use specificity are recorded in eye-lens chronologies within these and other species of interest.

The lack of obvious consistency in the shape or direction of isotopic fluctuations at a particular lens radius make it difficult to conclusively or generally attribute patterns of variation within measured chronologies to particular aspects of lionfish or graysby ecology. Indeed, no 2 data series appeared completely alike in either species when visualized in either an age synchronized (i.e. relative to life history events) or time synchronized (i.e. relative to environmental fluctuations) manner. Though such asynchrony could itself be important evidence of high amounts of individual resource use specificity in these species, larger sample sizes would be necessary to draw this interpretation from our results. Still, specific marked features in individual chemical chronologies point toward the capacity to glean ecological insight from eye-lens isotopic records. In the most extreme example provided by our data, we recorded a distinct elevation of δ^{13}C values in the outermost lens laminae of a sampled lionfish that was also found to have strongly enriched δ^{15}N values in both eye-lens and muscle tissues (>3‰ higher than the average; Figs. S1a & S2a). Based on their magnitude and coincidence, these exceptional deviations infer movement across environmental isotopic baselines rather than persistent changes in diet (Grippo et al. 2011), though more extensive estimates of regional isotopic values would allow refined estimates of the relative influences of migration and any concurrent changes in foraging habits. Although we cannot draw general inference from a single fish, this chronology could provide the first description of broad movement across the entire lifespan of a wild-caught lionfish if an elaborated isoscape of the BNP reef ledge were made available for consultation.

Variation within isotopic ontogenies could be studied to characterize ecological events of interest besides trophic growth and movement, such as seasonal variation in the local prey community, transitions into and out of spawning cycles, or variation in growth rates, all of which would be likely to manifest as chemical fluctuations in eye lenses. To study such questions using lens chronologies would require greater numbers of fishes, preferably sampled using a rigorously spatiotemporally structured scheme that would control for influential environmental variables. Still, the apparent amount of variation in our data series suggests that performing eye lens analyses in natural or experimental settings where diet, movement, and environmental context are manipulated or more strictly accounted for has the potential to yield insight regarding a wide array of ecological patterns and processes in marine fishes.

5. CONCLUSIONS

Our study demonstrates that chemical chronologies recorded in eye lenses can be consulted to yield novel insights about trophic resource-use ontogeny in invasive marine fishes. Providing useful context for further investigations, we measured rudimentary spatial trends in δ^{15}N (with latitude) and δ^{13}C (with depth) around BNP's reef ledge that may be reflective of broader geographic patterns (Fig. 2). Additionally, we provided the first measurements of predictable, linear lens–length relationships in lionfish and graysby. In combination, these reference frames allowed interpretation of isotopic chronologies within eye lenses that could be consulted to describe aspects of individual and population-level life histories of graysby and lionfish.

The observed logarithmic increase in lifetime δ^{13}C and δ^{15}N values of both lionfish and graysby (Fig. 4) may reflect trophic position changes with age and size (trophic growth). These patterns broadly match previously reported diet trends for these fishes, and are similar to lifetime trends in other fishes that increase their trophic position with size. Graysby isotopic values were generally lower than those of lionfish at the interior of the eye lens, but increased more rapidly later in life, suggesting that graysby could eventually reach a higher trophic position than lionfish at a smaller relative length (Fig. 4). However, based on their comparatively low trophic position at small sizes and prolonged exposure to apparent resource use overlap during early development, our data imply that small, young graysby may be particularly susceptible to potential competition from cohabitant invasive lionfish.

We suggest that the short-term isotopic variations superimposed on broader lifelong trends are likely caused by fluctuations in habitat use (movement) or differences in foraging selectivity/specificity. The sum of these patterns appears to produce distinctive isotopic histories (Figs. 5 & S1–S4) that could feasibly be consulted to assess trends in resource use throughout the life history of individual fish. Though sample sizes available for this study were too small to support concrete interpretation of specific features in lens chronologies, the combined existence of both underlying population-level trends and high amounts of individual variation imply that similar analyses could uncover useful ecological information, especially in settings where factors that influence isotopic values (diet, movement, and environmental context) are well known or experimentally controlled. Creating similar isotopic histories in populations of marine teleosts and other vertebrates could help uncover information about short- and long-term trends in foraging and habitat use that would be useful to ecologists and resource managers, particularly in species that are difficult to monitor or when more traditional *in situ* sampling techniques are infeasible. We encourage exploratory applications of this technique in a broad variety of taxonomic and environmental contexts to determine in which systems the use of serially synthesized eye-lens stable isotopes may be most insightful and appropriate.

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