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Life history through the eyes of a hogfish: trophic growth and differential juvenile habitat use from stable isotope analysis

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ABSTRACT: Understanding ontogenetic linkages among fish habitats is critical for conservation of fish populations and the ecosystems on which they rely. Natural tags such as stable isotopes are effective for investigating ecological questions regarding fish movement and habitat use. We analyzed stable isotopes from sequentially deposited laminae of hogfish *Lachnolaimus maximus* eye lenses from the eastern Gulf of Mexico (eGOM) to investigate trophic and geographic changes across individual life histories. We documented evidence of 1 to 2 step trophic level increases through $\delta^{15}N$ increases. We also observed depth separation at the juvenile stage, evidenced by $\delta^{13}C$ variation early in life. These results suggest that adult hogfish in deeper habitats likely inhabited deeper juvenile habitats (i.e. nearshore reefs), while adult hogfish inhabiting shallower adult habitats likely used shallower juvenile habitats (i.e. estuaries). This novel finding for eGOM hogfish contradicts prior literature that solely discusses seagrass as juvenile habitat. We used muscle tissue isotopes to characterize juvenile hogfish habitats and linear discriminant function analysis (LDA) to determine the habitats previously inhabited by adults in this study. The LDA revealed Cedar Key as the most used juvenile hogfish habitat in this study, but more evidence is needed to determine its status as a nursery. This study provides the first evidence for ontogenetic migration of individual hogfish using natural tags as tracers and demonstrates a mechanism for identifying juvenile habitats based on eye lens stable isotope analysis. Identifying ontogenetic patterns and habitat use can help to better conserve stocks and essential fish habitats.

KEY WORDS: Ontogeny · Sclerochronology · Connectivity · Carbon · Nitrogen · Reef fish

1. INTRODUCTION

Ontogenetic movement and differential habitat use of fishes can greatly affect population demographics and distributions of species (Searcy & Sponaugle 2001). However, there are myriad factors that can also influence population distribution and demographics, such as differences in settlement patterns or selective mortality. For example, differences in habitat selection of recruits can directly influence the abundance and distribution of adult coral reef fishes (Victor 1986, Gutiérrez 1998). During the juvenile stage, inhabiting complex habitats such as seagrass beds can increase fish survival rates by reducing predation efficiency, therefore increasing densities of juvenile fish (Searcy & Sponaugle 2001, Chacin & Stallings 2016). Furthermore, adult fish density, size, and spatial distribution can be significantly affected when portions of the population inhabit areas that are susceptible to higher fishing intensity (McBride & Richardson 2007, Heppell et al. 2012, Frank et al. 2018). These processes can all affect the distribution and demographic structure of fish populations. It is therefore important to gather information on life history, such as settlement patterns and ontogenetic habitat use, to disentangle these influences.

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Animal movement has been studied with a wide variety of marks or tags applied to individuals or groups of animals. These studies are often conducted with limited spatial and temporal resolution and often depend on recapture or detection of tagged individuals (Lindholm et al. 2006, Hazen et al. 2012). Tagging studies provide valuable snapshots of information post-tagging, but the use of natural chemical tags can be used to build upon these data. Natural tags reduce the logistical challenges associated with tagging since they only require one capture occurrence, and they can provide retrospective data on individual movement. Trace elements and stable isotopes have been used to investigate many ecological questions regarding fish movement and habitat use (see reviews by Trueman et al. 2012, Tzadik et al. 2017). For example, otolith microchemistry has been used to identify differences in trace element composition among otoliths, signifying different habitats (Gillanders 2002), and revealed changes in habitat use by individuals (Gillanders & Kingsford 1996, Vasconcelos et al. 2007). This method has been used to trace movement for many marine species. For instance, stable isotopes in Atlantic bluefin tuna *Thunnus thynnus* otoliths have been used to identify trans-oceanic migration patterns and natal origin for individual fish from several locations in the North Atlantic (Rooker et al. 2008, Schloesser et al. 2010). Stable isotopes from soft tissues have also been used to examine coastal shelf movements (e.g. Herzka 2005). Fry et al. (1999) compared muscle tissue isotope values between juvenile and adult pink shrimp *Farfantepenaeus duorarum* to reveal migration patterns from seagrass beds to offshore habitats in the Gulf of Mexico. However, muscle, liver, and blood samples can only be used to examine recent time periods due to the metabolic activity of these tissues, resulting in relatively short turnover rates on the scale of weeks to months (Trueman et al. 2012).

Isotopic analyses over longer time periods (e.g. whole-life studies) require the use of incrementally grown tissues that retain their chemical composition through time. Sclerochronology, the study of chemical variability in incrementally grown tissues, can be used to retrospectively analyze entire life histories of individuals by using isotopic or elemental information (Tzadik et al. 2017). Similar to incrementally grown otolith annuli, the sequentially deposited laminae of fish eye lenses serve as chronological isotope recorders and can also be used for retrospective life history analyses (Wallace et al. 2014). Stable isotope analysis (SIA) of eye lenses has been used with increasing frequency to examine trophic and geographic shifts across individual life histories for a variety of species, including elasmobranchs (Nielsen et al. 2016, Quaeck-Davies et al. 2018, Simpson et al. 2019), teleosts (Wallace et al. 2014, Kurth et al. 2019, Curtis et al. 2020, Vecchio et al. 2021), and cephalopods (Meath et al. 2019). The eye lens nucleus is formed during the gastrula stage (Vihtelic 2008), and layers of lens fiber cells are accreted at a linear proportion to allometric growth (Quaeck-Davies et al. 2018). Lens fiber cells undergo attenuated apoptosis, a process by which organelles are removed from lens cells, leaving only structural crystallin proteins that retain carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios and preserve them within the lens. These fiber cells are then layered on top of one another parallel to the lens surface (Vihtelic 2008). The inner core of the lens is not vascularized and is therefore metabolically inert once formed (Lynnerup et al. 2008). Thus, the lens allows for retrospective investigation of isotopic ontogeny, which can be particularly useful for providing data on early and cryptic life history stages of fishes that are difficult to sample, tag, or observe directly.

Stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ along the West Florida Shelf in the eastern Gulf of Mexico (eGOM) are geographically predictable and vary orthogonally to one another, thus making it an ideal study region for assessing the connectivity among habitats for marine species (Radabaugh et al. 2013, Radabaugh & Peebles 2014). Higher $\delta^{13}C$ values are found nearshore on the shelf, and lower $\delta^{13}C$ are found offshore, due to gradients in photosynthetic fractionation (McConnaughey & McRoy 1979). Photosynthetic fractionation occurs in primary producers that selectively fix ^{12}C over ^{13}C, resulting in lower $\delta^{13}C$ values (France 1995). Stable isotope values tend to differ between primary producer types. For example, Moncreiff & Sullivan (2001) found that isotope values were distinct between seagrass, epiphytic algae, and phytoplankton within seagrass habitats of the northern Gulf of Mexico. Gradients in $\delta^{13}C$ across depth may also be influenced by light attenuation, which leads to differences in growth rates and fractionation (Radabaugh & Peebles 2014). Thus, $\delta^{13}C$ in marine systems can be dependent on differences in the types of dominant primary producers, light attenuation, or a combination of the two. Differences in $\delta^{13}C$ values can therefore help to infer whether basal resources are dominated by benthic (higher $\delta^{13}C$) or pelagic (lower $\delta^{13}C$) primary production (Kurth et al. 2019). In addition, $\delta^{13}C$ is generally conserved with increasing trophic level, with enrichment of only ~1‰ per trophic step (Deniro & Epstein 1978, Peterson & Fry 1987).

Nitrogen isotope values depend on biogeochemical processes such as nitrogen fixation and remineralization as well as nutrient inputs from anthropogenic activity. In the eGOM, $\delta^{15}N$ values are likely linked to gradients in nitrogen fixation and nutrient input from the Mississippi River (Radabaugh & Peebles 2014). These influences lead to higher background $\delta^{15}N$ levels found close to this large nutrient source in the north, and lower values found further south in more oligotrophic waters. However, interpreting isotopic data for $\delta^{15}N$ is somewhat more complex due to stronger trophic influences. In consumers, light ^{14}N is excreted preferentially over heavier ^{15}N, leading to enrichment of ^{15}N with increasing trophic level of ~3‰ per trophic step (Deniro & Epstein 1978, Peterson & Fry 1987). Here, we used variation in $\delta^{13}C$ sampled from marine fishes in the eGOM to infer on-to-offshore geographic movement and variation in $\delta^{15}N$ as a proxy for trophic level. The geographic patterns and predictability of isotopes in the eGOM provide an opportunity to interpret ontogenetic migration of individual marine organisms using stable isotopes archived within the eye lens.

Hogfish *Lachnolaimus maximus* are harem-forming protogynous hermaphrodites (Collins & McBride 2015) that exhibit high site fidelity on short temporal scales (Colin 1982, Lindholm et al. 2006, Cooper et al. 2013). However, movement patterns on longer, lifelong (1−2 decades) time scales are still unclear. Hogfish are an important component of Florida's commercial and recreational fisheries (GMFMC 2018), with landings being highest along the West Florida Shelf and historically dominated by spearfishing gears (McBride & Murphy 2003). Such fishing practices limit most harvest activity of hogfish in the eGOM to depths shallower than 30 m due to distance from shore and recreational diving limitations (Collins & McBride 2011, McBride & Richardson 2007), which could result in deepwater refugia (Tupper & Rudd 2002). In fact, hogfish residing offshore (>30 m depths) are significantly larger than those captured nearshore (<30 m depths) in the eGOM, even within the same age class (Collins & McBride 2011). This suggests that a mechanism other than ontogenetic movement (e.g. selective mortality) may be influencing the observed depth-specific size distributions.

Ontogenetic movement is commonly inferred from observed differences in size and abundance across depth and habitat, without evidence from movement studies (Lindeman et al. 2000, Tupper & Rudd 2002), which has been the case for hogfish (Lindholm et al. 2006, Cooper et al. 2013, Switzer et al. 2013). However, selective exploitation has been shown to result in an ontogenetic-like deepening of marine fishes as well (Frank et al. 2018). Since both of these processes can lead to geographically specific size distributions of fishes (Lindeman et al. 2000, McBride & Richardson 2007), the observed size distribution of hogfish could be attributed to offshore movement with increasing size or age, selective fishing mortality, or a combination of these factors. Current research on hogfish movement is limited to small-scale studies using acoustic tags and diver observations (Lindholm et al. 2006, Munoz et al. 2010). While small-scale movement studies are valuable for understanding spawning and social dynamics, much less is known about long-term, large-scale movement. Further information on hogfish movement is needed to disentangle the relative influences of selective fishing mortality and movement on the eGOM hogfish population.

While adult hogfish in the eGOM reside primarily near hardbottom or reef habitats, juveniles are commonly found in shallow seagrass habitats (Switzer et al. 2013, Tabb & Manning 1961). Juvenile nursery habitats have yet to be defined for hogfish, although seagrass beds of Florida's Big Bend region in the eGOM have been hypothesized to serve this role based on high densities of juveniles (Switzer et al. 2013). Estuaries and seagrass beds provide numerous benefits to juvenile fishes, including protection from predators (Heck et al. 2003) and high food availability (Orth et al. 1984, Shulman 1985). The identification and protection of habitats used by juvenile fishes is therefore critical for management and conservation of marine species and the habitats on which they rely. Beck et al. (2001) described a nursery specifically as a habitat that contributes the greatest number to the adult population per unit area relative to other habitats. The designation of a nursery habitat therefore requires empirical study that traces species movements from habitats of juveniles to those of adults (e.g. Gillanders et al. 2003). Tracing hogfish habitat use back to early life stages can help inform management on the presence and importance of a potential nursery habitat for the eGOM hogfish population.

Estuaries in the eGOM are defined by several characteristics that influence ambient isotopes. Estuaries in the northern eGOM (e.g. Cedar Key, Big Bend) receive large amounts of river discharge from vast watersheds which lead to higher nutrient inputs, while more southerly estuaries (e.g. Tampa Bay, Charlotte Harbor) receive much lower fluvial discharge (US Geological Survey 2020). This difference in riverine input, and therefore nutrient load, influences the dominant basal resources present in each region. Phytoplankton tend to outcompete benthic growth with high sediment and nutrient flux, while lower nutrient inputs and less light attenuation allow benthic microalgae to outcompete phytoplankton (Sand-Jensen & Borum 1991). Regional variation in dominant macrophytes has also been observed in the eGOM, which can influence the biomass and production of epiphytic algae (Zhang & Huang 2008). Higher coverage of the seagrass *Thalassia testudinium* is found in southerly estuaries, which are characterized by wide, flat grass blades that can support higher biomass of epiphytic algae, while more northerly estuaries are dominated by primarily *Syringodium filliforme* with thinner, cylindrical leaves (Stallings et al. 2015a). The relative abundance of these primary producers across different habitats can influence the ambient δ^{13}C values (Moncreiff & Sullivan 2001) and allow for isotopic distinctions among habitats. Gradients in basal resources are also reflected in geographic isoscapes for the eGOM (Radabaugh et al. 2013). Given these distinctions among potential habitats of juvenile hogfish, and known isoscapes, we can infer past habitat use by using methods in retrospective isotope analysis.

More specific information about hogfish movement patterns and habitat use is needed to determine the influences of life history and fishing intensity on the observed depth-specific size distributions and identify a potential nursery area for eGOM hogfish. Here, we addressed the following questions: (1) how do δ^{13}C and δ^{15}N values change throughout the life of individual hogfish in the eGOM; (2) at what life stage do specific changes in trophic level or habitat use occur; and (3) which habitats for juvenile hogfish contribute to the adult eGOM hogfish population? These data will provide the first empirical evidence of ontogenetic changes in hogfish habitat and diet using techniques in stable isotope ecology.

2. MATERIALS AND METHODS

2.1. Sample collection

Hogfish were collected at depths ranging from 1.3–60.9 m between June 2016 and December 2018. Most adult specimens were donated by spearfishers, resulting in most sizes being above the legal harvest size (30.5 cm fork length [FL] in 2016–2017; 35.6 cm FL in 2018). Divers provided information on harvest depth and distance from shore, which allowed for estimation of capture location coordinates. Hogfish smaller than the recreational size limit were collected via SCUBA divers with permission from Florida Fish and Wildlife Conservation Commission (Special Activities License No. SAL-15-1673A-SR) in order to obtain specimens across a greater size range. Additional hogfish samples were provided by the Florida Fish and Wildlife Research Institute (FWRI) Fisheries Independent Monitoring Program and the Southeast Monitoring and Assessment Program (SEAMAP). Estuarine samples from FWRI were collected using a 21.3 m center-bag seine with 3.2 mm mesh netting or with 6.1 m otter trawls. Offshore SEAMAP samples were collected with a 12.8 m trawl. Additional survey details for FWRI and SEAMAP sampling can be found in Matheson et al. (2017) and Rester (2017), respectively.

Collections for this study were confined to the West Florida Shelf within the eGOM, and the study area was divided into 4 latitudinal regions: the Florida Keys (KE; 24.5–26° N), Charlotte Harbor (CH; 26–27° N), Tampa Bay (TB; 27–28° N), and the Big Bend (BB; north of latitude 28° N; Fig. 1a). The western boundary of each deep region was confined to 85° W based on the boundaries of the National Marine Fisheries Service statistical zones. These regions were further sub-divided based on depth into nearshore (<30 m) and offshore (≥30 m).

For juvenile hogfish, 8 potential habitats were identified within the eGOM study area based on previous observations of juvenile hogfish presence (<15 cm; Switzer et al. 2013, FWRI 2018, M. E. Faletti pers. obs.). Four estuarine habitats were selected (either semi-enclosed bays or in estuarine areas <5 m depth): Big Bend (EBB), Cedar Key (ECK), Tampa Bay (ETB), and Charlotte Harbor (ECH); and 4 nearshore areas (we sampled both adult and juvenile hogfish from nearshore areas) were also selected as potential habitats between 5 and 30 m depth: Big Bend (NBB), Tampa Bay (NTB), Charlotte Harbor (NCH), and the Keys (NKE; Fig. 1b).

2.2. Sample processing

For each fish, FL was measured to the nearest 0.1 cm. Whole eyes and muscle tissue samples were extracted from each fish. Whole eyes were wrapped in aluminum foil and frozen at −18°C until processed. White muscle tissue samples were collected anteriorly to the first dorsal fin ray and frozen at −18°C until processed. We froze tissue samples because the method does not impart preservation-based offsets on measured isotope values (Stallings et al. 2015b). Muscle tissue samples were freeze-dried at −40°C in a vacuum of 50–100 microbar for 48 h, then pulverized with mortar and pestle for SIA preparation.

2.3. Lens delamination

Whole eyes were thawed prior to lens dissection. An incision was made in the cornea with a scalpel to extract the lens. The whole lens, including the outer epithelial layer, was measured using a caliper to the nearest 0.01 mm. Eye lens delamination was performed by first placing the lens in a Petri dish of deionized water under a stereomicroscope, then the epithelium was removed using fine-tip forceps until the outer lens layer was revealed and cleared of all epithelial material. Epithelial material was stored in a separate microcentrifuge tube and retained. With the lens pole facing up, each lens lamina was then peeled from the eye with fine-tip forceps, ensuring that the same amount of material was removed from the entire surface of the lens. Layers were peeled until the core was reached (~0.5 mm diameter), which is the smallest unit of the lens at which it maintains its structural integrity. Each lens layer was stored in a separate microcentrifuge tube and labeled in reverse order from which they were peeled (core labeled as zero, with subsequent layers labeled in increasing order). Between each layer, lens diameter was meas-

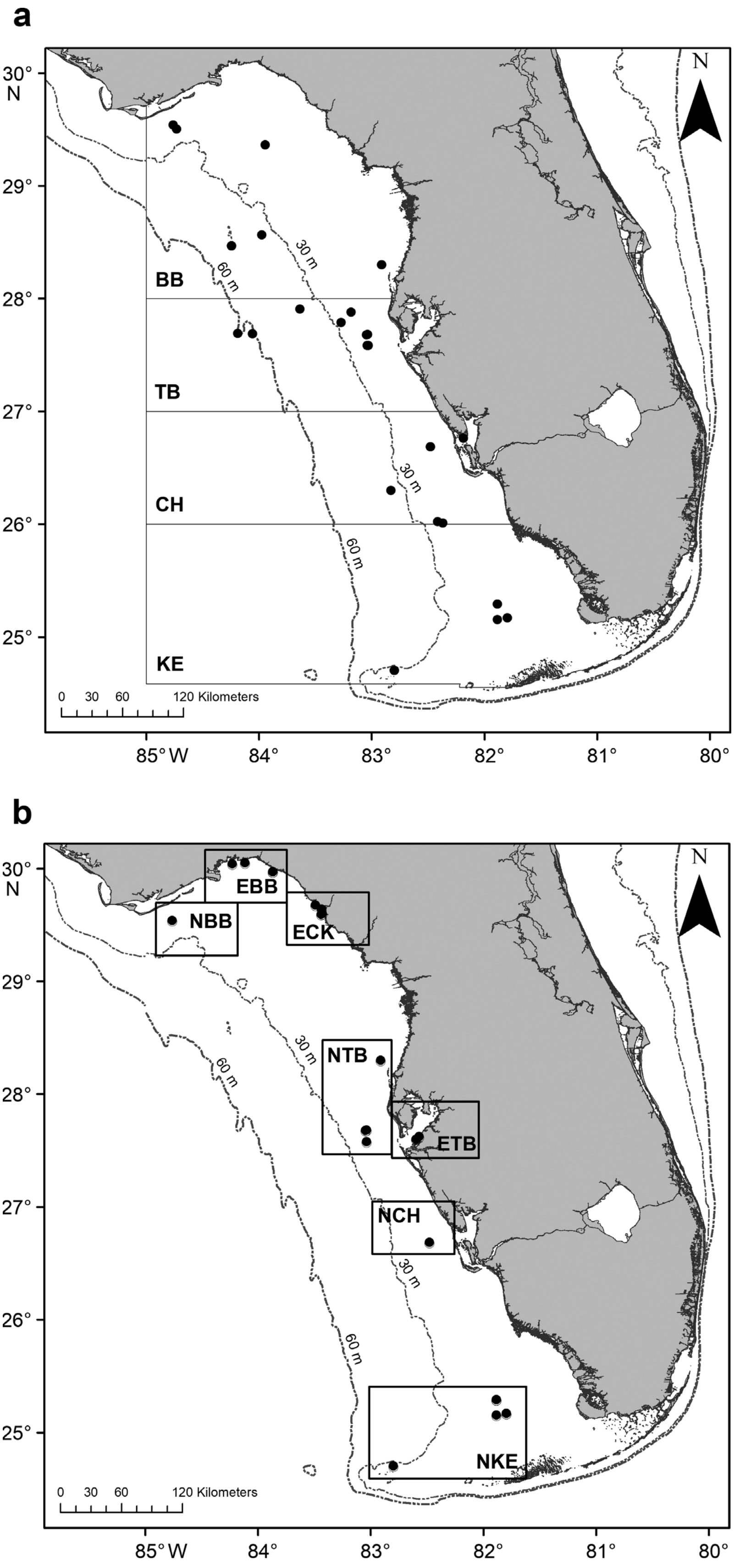

Fig. 1. (a) Capture locations for hogfish used in the stable isotope analysis, latitudinal regions, and depth contours (30 and 60 m). West Florida Shelf was divided into 4 latitudinal regions: BB: Big Bend; TB: Tampa Bay; CH: Charlotte Harbor; and KE: Keys. (b) All hogfish capture locations used in juvenile analysis. Polygons encompass potential habitats of juveniles used in linear discriminant analysis for retrospective habitat usage predictions. EBB: estuarine Big Bend; NBB: nearshore Big Bend; ECK: estuarine Cedar Key; NCK: nearshore Cedar Key; ETB: estuarine Tampa Bay; NTB: nearshore Tampa Bay; NCH: nearshore Charlotte Harbor; NKE: nearshore Keys

ured (to the nearest 0.01 mm) and used to calculate the radial midpoint (RM), or distance from the nucleus:

$$RM = (d_o - d_i) / 2 \tag{1}$$

where d_o is the outer lens diameter (prior to peeling) and d_i is the inner lens diameter (after peeling). Following delamination, each layer was dried at 70°C for 18 h.

2.4. SIA

Subsamples of dried muscle and eye lens laminae were weighed on a Mettler-Toledo precision microbalance to ensure adequate sample weight for analysis, then wrapped in tin capsules in preparation for SIA. Samples were analyzed for bulk stable isotope ratios (δ^{13}C, δ^{15}N) and carbon and nitrogen (C, N, C:N ratios). Measurements are expressed in per mil (‰) using δ notation, where R is the isotopic ratio of interest (e.g. ^{13}C:^{12}C), and X is the isotope of interest:

$$X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000 \tag{2}$$

Samples were combusted in a Carlo-Erba NA2500 Series-II Elemental Analyzer coupled to a Thermo-Finnigan Delta+XL isotope ratio mass spectrometer, located at the University of South Florida College of Marine Science. Analytical precision was obtained by replicate measurements of Bovine Liver standard NIST1577B. For eye lens cores that did not have adequate mass for analysis (<100 µg), samples were combined with the other eye lens core of its respective pair. Left and right eye lens isotope values from the same fish do not vary from one another (Wallace et al. 2014), so combining the cores provided sufficient mass for analysis without affecting the resulting isotopic values. The δ^{13}C values were measured relative to a PeeDee Belemnite (PDB) standard, and δ^{15}N was measured relative to air. Samples were calibrated to NIST8573, NIST8574 L-glutamic acid Standard Reference Materials.

2.5. Statistical analyses

A total of 27 adult hogfish were analyzed for eye lens SIA, chosen based on geographic distribution and across a wide range of sizes to best represent the eGOM hogfish population. This subsample included 8 fish from the BB region (5 offshore, 3 nearshore), 7 from the TB region (4 offshore, 3 nearshore), 7 from the CH region (1 offshore, 6 nearshore), and 5 from the KE region (5 nearshore). Not every region was equally represented due to sampling constraints. Linear regression of lens radius and FL at time of capture was used to back-calculate estimated FL at each measured RM from the eye lens:

$$FL_{est} = FL_{capture} (RM_{layer} / r_{lens}) \tag{3}$$

where RM_{layer} is the radial midpoint of the layer of interest, and r_{lens} is the total lens radius for the individual fish. This assumes that lens growth was proportionate to somatic growth, since lens growth rate decreases through life (Bron et al. 2000), leading to near-isometric growth of lens diameter compared to total body size (Quaeck-Davies et al. 2018). The lens epithelium was excluded from these calculations due to its disproportionate thickness and its source of metabolic activity for lens formation (Andley 2008).

Stable isotope values of δ^{13}C and δ^{15}N were each plotted against estimated FL for each individual to analyze changes in habitat and basal resource dependency (δ^{13}C) and trophic level (δ^{15}N). To analyze overall changes in isotope ratios across life, linear mixed models were fit to measure the relationship between isotope values and RM, with individual fish included as a random variable. The data were assumed to be Gaussian distrusted as indicated by visual inspection of histograms. The log of δ^{15}N was used to account for the nonlinear relationship it had with RM. These models were chosen based on the most parsimonious fit. Linear mixed models were conducted in R (R Core Team 2018).

Assuming diet does not shift significantly from benthic to pelagic based food webs, at least 2 patterns of isotope values may be observed (Fig. 2). If hogfish exhibit gradual ontogenetic movement offshore, the corresponding δ^{13}C values will show a steady decrease through life, exhibited from the nucleus to the edge of the eye lens (Fig. 2a). In contrast, if hogfish move either to nearshore or offshore reefs, and subsequently become permanent residents there, δ^{13}C will change abruptly, then remain constant (Fig. 2b). The latter scenario would suggest that the distinct size distributions observed on shallow versus deep reefs are driven by external factors limiting hogfish growth and size in shallow water (e.g. resource availability, fishing intensity).

Raw eye lens isotope data were interpolated using a cubic spline function (SRS1 cubic spline for Microsoft Excel add-on; SRS1 Software 2015). This allowed for direct comparison of isotope values across estimated sizes between depths and among regions using linear mixed models to determine how δ^{13}C or δ^{15}N changed with ontogeny and space. Since we were interested in the effects of both depth and size, we included the in-

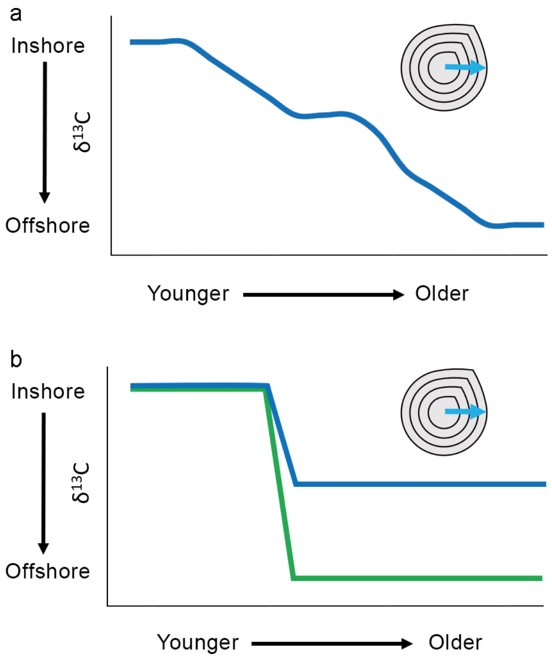

Fig. 2. Conceptual model of $\delta^{13}C$ in hogfish eye lens associated with hypothesized ontogenetic migrations. Plots display 2 potential patterns: (a) gradual ontogenetic movement offshore and (b) depth-specific habitat use (i.e. hogfish move either to nearshore [blue] or offshore [green] reefs, and subsequently become permanent residents)

teraction for those models. We ran additive models for the effects of zone and size on isotope values.

To characterize the isotope values of juvenile habitat, muscle tissue from 37 small (<25 cm) hogfish were sampled. Muscle tissue samples were taken from these fish to estimate the average ambient isotope values for each habitat, in order to allow isotope values from adult eye lenses to be mapped back to one of these regions. Muscle tissue samples were analyzed using permutational analysis of multivariate homogeneity of group dispersions. These data failed to meet the assumption of homogeneous dispersions for perMANOVA, despite data transformations. Juvenile muscle tissue isotope values were therefore analyzed for overlap using the stable isotope Bayesian ellipses (SIBER; Jackson & Parnell 2019) package in R (R Core Team 2018) to identify isotopically distinct juvenile habitats. Ellipses were generated to display 95% confidence intervals for 5 juvenile regions with adequate sample sizes ($n \geq 5$), and proportion of overlap was calculated for each pair. Biologically significant overlap for ellipses was defined as overlap of 60% (Smith 1985) or higher. For regions that did not have adequate sample size ($n < 5$) for ellipse generation, points were included on the SIBER plot for visual comparison.

Linear discriminant analysis (LDA) was used to predict the habitat that each adult individual previously inhabited in the juvenile stage. The function was tested with muscle tissue isotope data from juvenile hogfish (for which capture region was known) to see how accurately it classified each individual. The function was then applied to adult eye lens isotope data corresponding to the time at which each fish was in the juvenile stage (10 cm FL) to predict the region from which each fish originated. The LDA was conducted using the 'MASS' (Ripley et al. 2018) package in R v.3.5.1 (R Core Team 2018).

Eye lens cores are representative of the earliest stages of life, or may reflect maternal provisioning of the egg, and can therefore be used to infer spawning location or natal origin (Vecchio & Peebles 2020). To determine if area of natal origin differed by region of capture, eye lens cores from 27 adult individuals were analyzed for differences using perMANOVA, with latitudinal regions (BB, TB, CH, KE) and depth strata (nearshore: <30 m, offshore: ≥30 m) as predictors and isotope values ($\delta^{13}C$, $\delta^{15}N$) as the responses. To identify isotopic distinction among spawning areas, eye lens core isotope values were analyzed for overlap using the SIBER package and analyzed via linear regression to determine any potential relationship with latitude (i.e. $\delta^{13}C$ ~ latitude, $\delta^{15}N$ ~ latitude).

3. RESULTS

A total of 27 adult hogfish were sampled for eye lens SIA, with length ranging from 27.2–74.6 cm (mean ± SE: 50.4 ± 2.4 cm). The number of layers peeled from each lens varied and ranged from 7–16 (median: 10) fish^{-1}, with a grand total of 277 eye lens laminae analyzed. Lamina thickness ranged from 0.05–0.85 mm (0.25 ± 0.006 mm). Values of $\delta^{13}C$ and $\delta^{15}N$ were plotted against RM for each eye lens layer sampled to illustrate individual isotope chronologies (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m666p183_supp.pdf). The $\delta^{13}C$ of adult hogfish eye lens layers ranged from −21.27 to −12.67‰. There was no significant relationship between eye lens $\delta^{13}C$ and RM ($F_{1,270} = 2.08$, $p = 0.15$; Fig. 3, Table S1 in the Supplement). The $\delta^{15}N$ values of adult hogfish eye lens layers ranged from 4.74–12.57‰. There was a positive logarithmic relationship between eye lens $\delta^{15}N$ and RM ($F_{1,270} = 189.60$, $p < 0.001$; Fig. 4, Table S2). There was a negative relationship between $\delta^{15}N$ and $\delta^{13}C$ across all measured adult eye lens data ($F_{1,270} = 48.61$, $p < 0.001$; Fig. 5, Table S3).

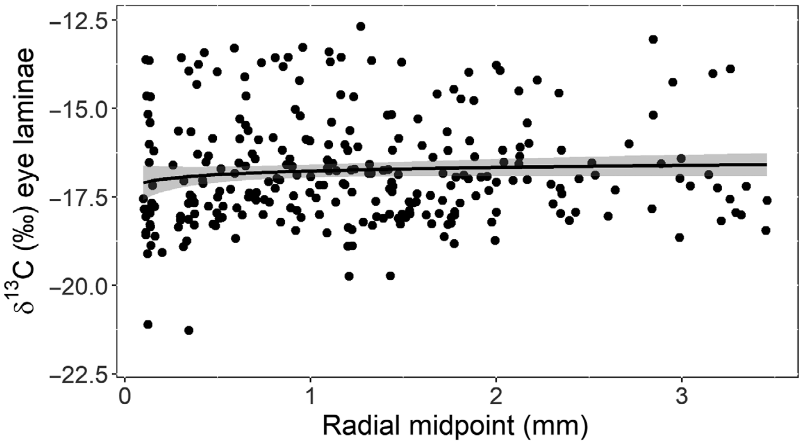

Fig. 3. Observed δ^{13}C values in hogfish eye lens with fitted generalized linear mixed model trendline (gray shading = 95% CI) by radial midpoint for all individuals sampled (n = 27 fish; n = 277 layers)

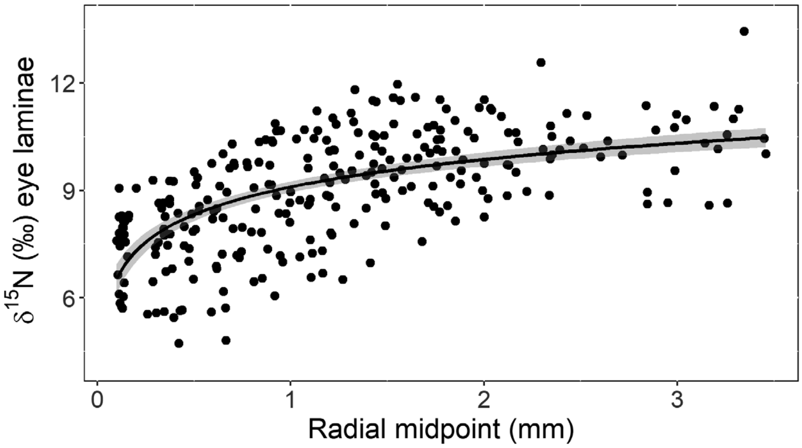

Fig. 4. Observed δ^{15}N values in hogfish eye lens with fitted generalized linear mixed model trendline (gray shading = 95% CI) by radial midpoint for all individuals sampled (n = 27 fish; n = 277 layers)

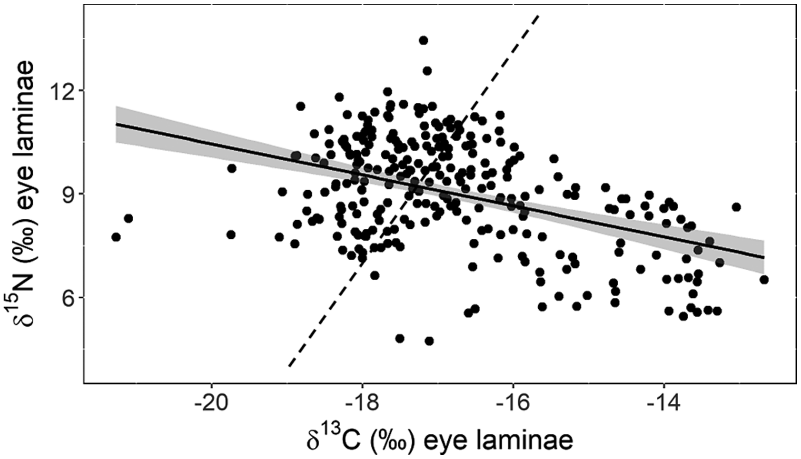

Fig. 5. δ^{15}N plotted against δ^{13}C for each hogfish eye lens layer sampled, with fitted generalized linear mixed model trendline (gray shading = 95% CI). Dashed line: 3:1 relationship expected due to trophic increase

After accounting for size, δ^{13}C values in eye lenses were higher in nearshore depths compared to offshore ones ($F_{1,1368}$ = 120.35, $p < 0.001$; Fig. 6a, Table S4). Although suggestive of an interaction between depth and size ($F_{1,1368}$ = 3.79, p = 0.052), inspection of the 2 slopes across the sizes of hogfish studied indicated it was not ecologically important. Values of δ^{13}C were also different across regions ($F_{3,1367}$ = 226.12, $p < 0.001$) with higher values in the KE region, and moderately higher values in both the CH and TB regions compared to BB (Fig. 7a, Table S5). For δ^{15}N values, we observed an interaction between depth and size ($F_{1,1368}$ = 47.56, $p < 0.001$; Table S6), so we separated the analysis between the depth zones. There were positive relationships between size and δ^{15}N values in both nearshore ($F_{1,1368}$ = 47.56, $p < 0.001$) and offshore depths ($F_{1,1368}$ = 47.56, $p < 0.001$; Fig. 6b, Tables S7 & S8). Values of δ^{15}N differed by region ($F_{3,1367}$ = 84.7, $p < 0.001$; Fig. 7b, Table S9), with notably lower values in the KE compared to the other locations.

Muscle tissue isotope values were analyzed from 37 small hogfish (<25 cm FL) from 8 different habitats of juveniles (Fig. 1b): EBB (n = 5), ECK (n = 5), NBB (n = 5), ETB (n = 3), NTB (n = 3), ECH (n = 1), NCH (n = 5), and NKE (n = 10). The 95% CI ellipses generated in the SIBER analysis (Fig. 8) revealed very little overlap among hogfish muscle tissue isotopes from these habitats. The highest levels of overlap were observed between fish in the NBB and NCH regions (29.82%), NBB and NKE (11.58%), and EBB and ECK (11.28%; Table 1). Based on the significance overlap threshold of 60% (Smith 1985), all juvenile hogfish habitats were significantly different from one another (Fig. 8).

Since habitats used by juveniles were isotopically distinct from one another, the LDA was suitable for estimating the habitat from which each adult originated. The LDA function accurately predicted 44.9% of individ-

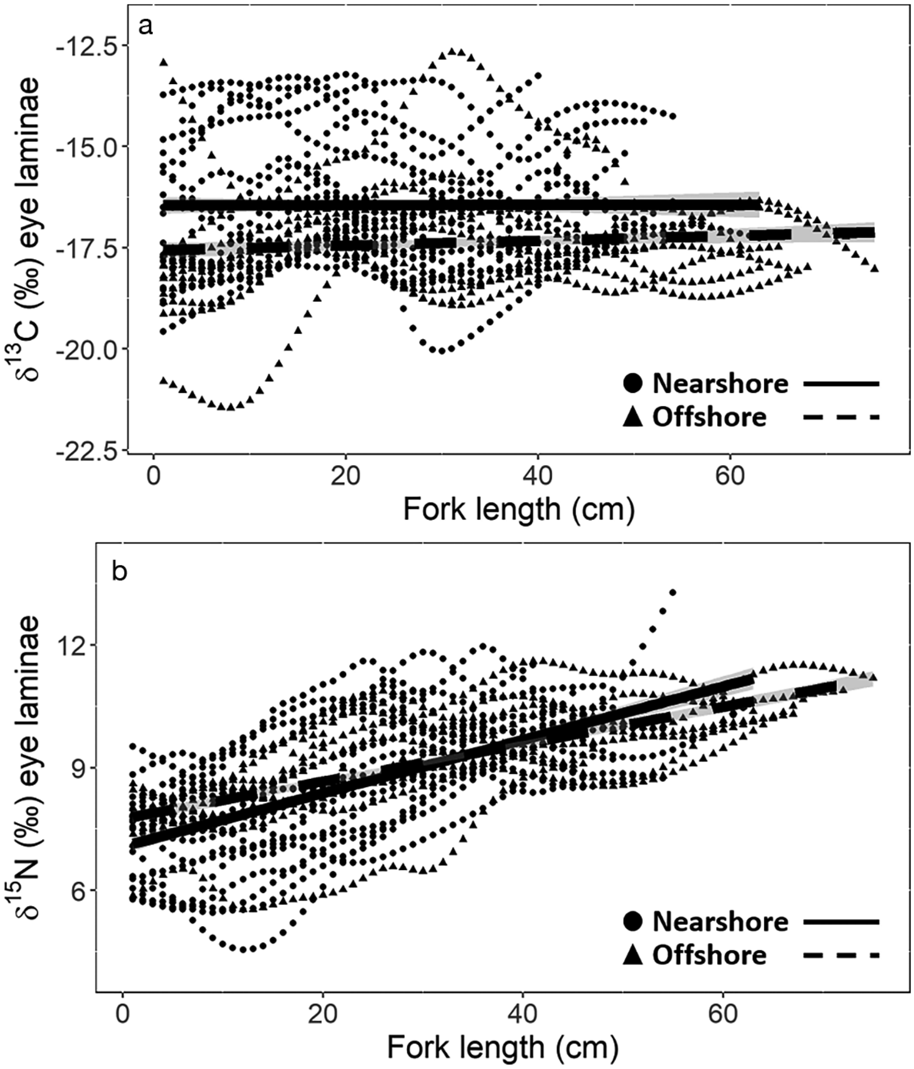

Fig. 6. Interpolated values of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ from hogfish eye lenses across estimated fork length with fitted generalized linear mixed model trendline (gray envelope = 95% CI), separated by depth from which the fish were caught

uals based on muscle tissue isotope data sampled from hogfish in juvenile habitats. Adult hogfish eye lens isotope data corresponding to when the fish were 10 cm in length were used to predict the habitat that each adult individual previously inhabited as a juvenile (Table 2). Overall, 51.9% of adult hogfish (of the 27 adults analyzed) in this study were predicted to have inhabited ECK as juveniles, 25.9% from ETB, 14.8% from the NKE, and 7.4% from EBB.

Eye lens core values of $\delta^{13}C$ ranged from −19.10 to −13.62‰ and $\delta^{15}N$ ranged from 5.71–9.06‰. The perMANOVA revealed a significant interaction of latitudinal region × depth zone on eye lens core isotope values ($F_{2,19}$ = 4.01, r^2 = 0.23, p = 0.032). Core values of $\delta^{13}C$ had a significant negative linear relationship with capture latitude ($F_{1,25}$ = 5.71, r^2 = 0.19, p = 0.025). There was no significant relationship between eye lens core $\delta^{15}N$ and capture latitude. SIBER analysis revealed little overlap between groups: BB and TB (29.9%), BB and CH (6.9%), BB and the KE (9.0%), TB and the KE (4.1%), and CH and KE (3.1%; Table 3). Eye lens cores of fish captured in CH parsed out to be slightly higher in $\delta^{15}N$ than the other 3 latitudinal regions and had much lower overlap with the other groups (Fig. 9). We also compared isotope values between the outer lens layer and fish muscle. There was no significant difference for $\delta^{15}N$ between muscle and outer eye lens layer, but a significant difference was found in $\delta^{13}C$, with values being on average 0.70% higher in the outer lens than in the muscle tissue (Fig. A1 in Appendix 1).

4. DISCUSSION

This study provides new insights into the ontogeny of hogfish in the eGOM, including changes in trophic

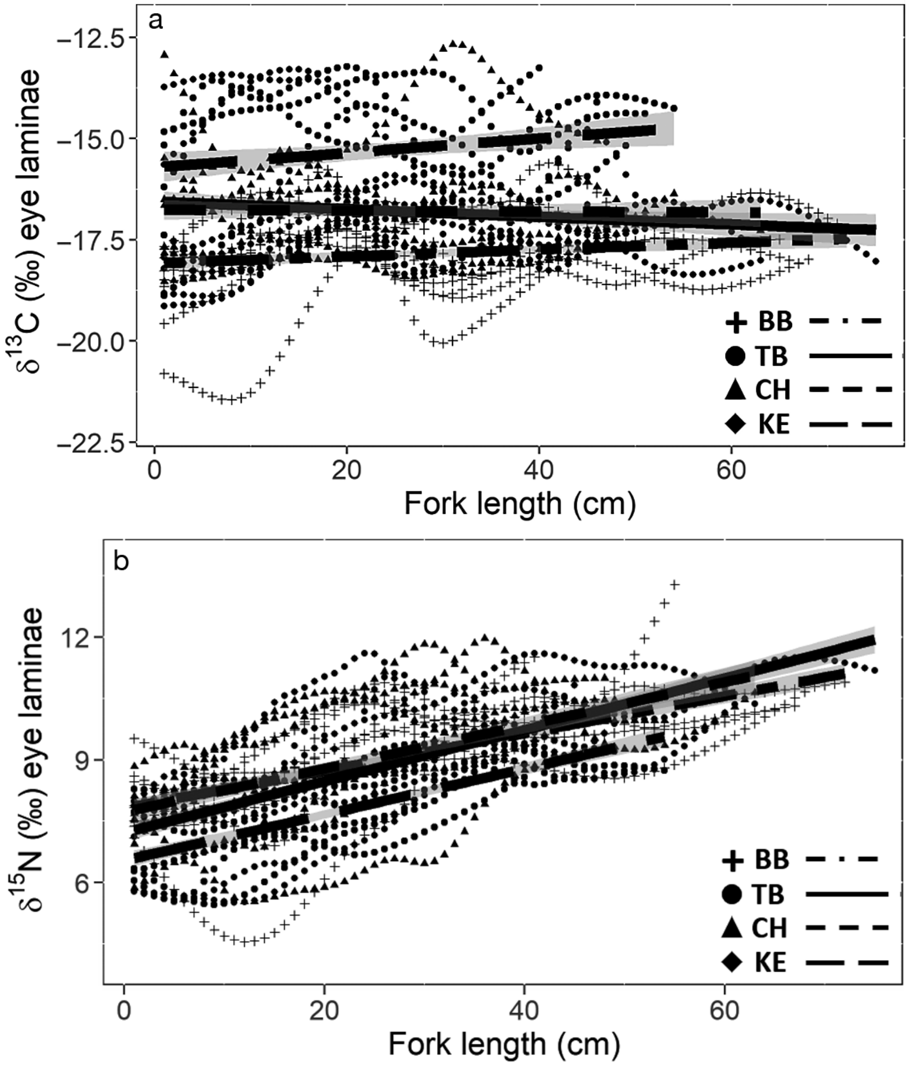

Fig. 7. Interpolated values of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ from hogfish eye lenses across estimated fork length with fitted generalized linear mixed model trendline (gray envelope = 95% CI), separated by study region from which the fish were caught

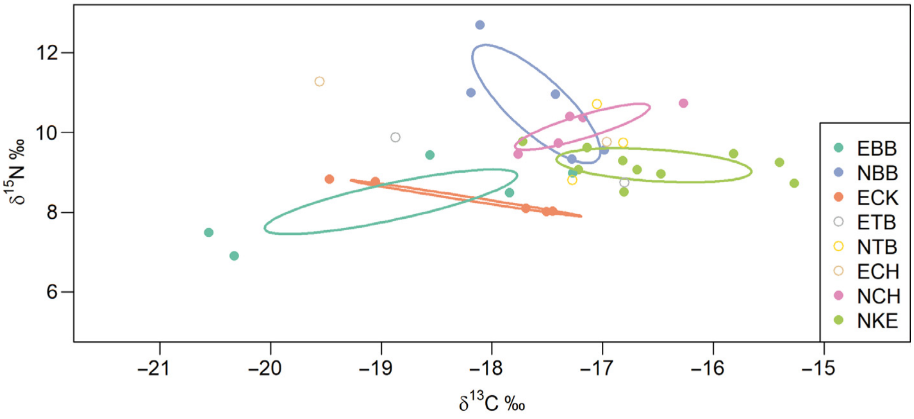

Fig. 8. Stable isotope biplot for hogfish (<25 cm fork length; n = 37) muscle tissue data from habitats of juveniles. Ellipses: 95% confidence intervals for 8 potential juvenile habitats; solid circles: individual data points from regions with adequate sample size for SIBER analysis; hollow circles: additional data points from regions without adequate sample size for SIBER analysis. EBB: estuarine Big Bend (n = 5); ECK: estuarine Cedar Key (n = 5); NBB: nearshore Big Bend (n = 5); NCH: nearshore Charlotte Harbor (n = 5); NKE: nearshore Keys (n = 10); NTB: nearshore Tampa Bay (n = 3); ECH: estuarine Charlotte Harbor (n = 1); ETB: estuarine Tampa Bay (n = 3)

Table 1. Proportion of overlap for Bayesian ellipses of small hogfish (<25 cm) muscle tissue stable isotope values from habitats of juveniles in the eastern Gulf of Mexico. NBB: nearshore Big Bend; NCH: nearshore Charlotte Harbor; NKE: nearshore Keys; EBB: estuarine Big Bend; ECK: estuarine Cedar Key

Habitat comparison	Proportion of overlap
NBB–NCH	0.140
NBB–NKE	0.043
NBB–ECK	0
NBB–EBB	0.004
NCH–NKE	0
NCH–ECK	0
NCH–EBB	0
NKE–ECK	0
NKE–EBB	0.009
ECK–EBB	0.030

Table 2. Linear discriminant analysis of adult hogfish eye lens isotope data corresponding to when each fish was in the juvenile stage (10 cm fork length). Columns: capture region for adult hogfish; rows: potential habitats they inhabited as juveniles; numbers: individual hogfish predicted to be from each of the 4 habitats of juveniles tested (e.g. the single individual captured in the OCH region was predicted by the model to inhabit the ECK region when it was in the juvenile stage). EBB: estuarine Big Bend; ECK: estuarine Cedar Key; ETB: estuarine Tampa Bay; NKE: nearshore Keys; OCH: offshore Charlotte Harbor; OBB: offshore Big Bend; OTB: offshore Tampa Bay; NBB: nearshore Big Bend; NCH: nearshore Charlotte Harbor; NKE: nearshore Keys; NTB: nearshore Tampa Bay

Predicted	Capture region						
habitat	OBB	OTB	OCH	NBB	NTB	NCH	NKE
EBB	1	1					
ETB	1				2	1	3
ECK	3	3	1	3		2	2
NKE					1	3	

Table 3. Proportion of overlap between Bayesian ellipses of adult hogfish eye lens core stable isotope values by region. BB: Big Bend; TB: Tampa Bay; CH: Charlotte Harbor; KE: Keys

Habitat comparison	Proportion of overlap
BB–TB	0.30
BB–CH	0.07
BB–KE	0.09
TB–CH	0
TB–KE	0.41
CH–KE	0.03

level and basal resource use potentially driven by movement and differences in habitat use at early life stages. The use of eye lens SIA provided a retrospective track of 27 individual hogfish life histories and resource use. Although a small subsample of the eGOM hogfish population, these data provide high-resolution, life-long sequential isotopic data for individuals across a large geographic area. The obtention of these chronologies demonstrates a common trade off of high-resolution data versus greater sample sizes. While larger sample sizes help to better infer population-level information for data with low temporal resolution (i.e. snapshots in time), studies that investigate chronological data at high resolution can better answer specific questions about ontogeny (e.g. Kim et al. 2012, Wallace et al. 2014, Kurth et al. 2019, Curtis et al. 2020, Hane et al. 2020).

The increase in $\delta^{15}N$ observed across hogfish lifetime indicated an increase in trophic level until sizes above 40 cm FL. As hogfish fed at increasing tropic levels, $\delta^{13}C$ also increased, which could indicate a shift from benthic to pelagic basal resources, either influenced by a shift in diet, ontogenetic migration offshore, or a combination of the two. There was considerable variability in isotope patterns across individual eye lenses (Fig. S1), which indicates heterogeneity

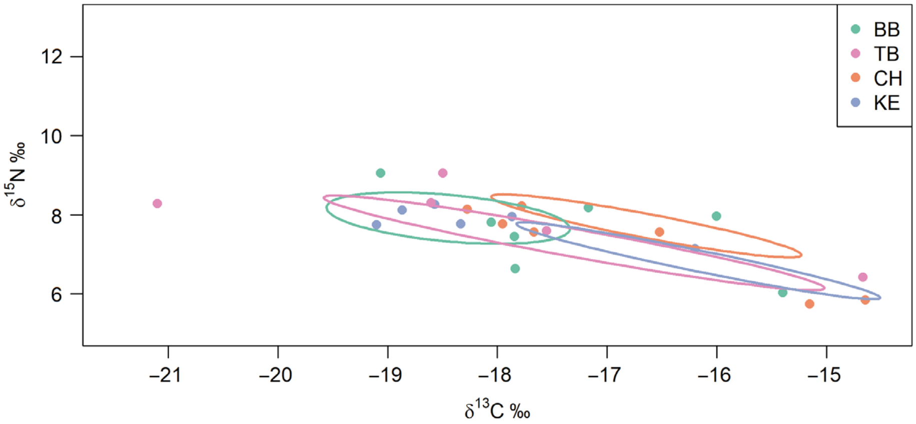

Fig. 9. Stable isotope biplot for adult hogfish eye lens core isotope data (n = 27). Ellipses: 95% confidence intervals for 4 regions of capture; points: individual hogfish eye lens cores. BB: Big Bend (n = 8); TB: Tampa Bay (n = 7); CH: Charlotte Harbor (n = 7); KE: Keys (n = 5)

in ontogenetic migrations or diet shifts. Differences in isotope values from early life suggest that nearshore and offshore hogfish inhabited different depths as juveniles. Eye lens isotopes were also used to infer potential habitats that may have been used during the juvenile stage. With greater sampling, these data could be used to quantify the relative contributions of habitats to the adult population, illustrating another benefit to the collection of high-resolution, ontogenetic isotope data from individual organisms.

The increase in δ^{15}N observed across hogfish lifetime was found to be consistent with a 1 to 2 step trophic level increase, as estimated by other studies (Minagawa et al. 1984, McCutchan et al. 2003). Increases in trophic level with increasing body size is common and has been observed in chronological isotope studies of other species (Estrada et al. 2006, Kurth et al. 2019). However, this significant trophic increase was unexpected in hogfish, given that their diets consist primarily of small, low trophic level benthic invertebrates throughout their life (Randall & Warmke 1967, Davis 1976). Although δ^{15}N followed a pattern of increasing trophic level, this was not the case for δ^{13}C. The relationship between δ^{15}N and δ^{13}C for a trophic level increase is typically expected to have a 3‰ increase in δ^{15}N per 1‰ increase in δ^{13}C (McCutchan et al. 2003); however, this trend was not observed in this study. In fact, δ^{13}C had an inverse relationship with δ^{15}N. The observed inverse relationship in δ^{13}C relative to δ^{15}N could be attributed to shifts in dominant basal resources — either through diet shifts or movement. The findings of trophic growth and basal resource shift were surprising, but may be explained further by recent dietary findings. Preliminary hogfish gut content analysis from the FWRI (K. Thompson unpubl. data) indicates that differences in small versus large hogfish diets were driven primarily by smaller fish consuming bivalves, brachyuran crabs, and majid crabs, with larger fish consuming some fish, portunid crabs, and echinoids. When analyzing the data by depth and area, they saw little difference in the diets, which indicates that age and length are driving shifts in diets rather than spatial differences. These results indicate a potential ontogenetic shift in hogfish diet from benthic-based invertebrates to a broader diet that includes small fishes that rely on planktonically derived resources. This shift would explain both the decreases in δ^{13}C and the inverse relationship of δ^{13}C relative to δ^{15}N, and could also contribute to the high variability observed in δ^{13}C observed across life histories (Fig. S1) and obscure the true relationship between δ^{13}C relative to δ^{15}N. Additionally, the expected 3:1 ratio of δ^{15}N:δ^{13}C with trophic growth is commonly referenced in trophic stable isotope studies conducted on individuals rather than on chronologies. Higher resolution isotopic chronologies such as this study and others (e.g. Kurth et al. 2019, Curtis et al. 2020, Vecchio & Peebles 2020) have the potential to disprove or challenge this expected pattern, since entire life histories may prove to be more complex than a simple linear relationship between isotope values. With the addition of these new findings, it is becoming increasingly clear that the generalization of the 3:1 trophic relationship may need to be reevaluated, considered on a species-specific basis, or perhaps applied to specific life history stages rather than generalizing across entire life histories.

The classic hypothesis regarding hogfish life history and ontogeny is that individuals settle in estuarine seagrass habitats or nearshore reefs and gradually migrate offshore with increasing size or age (Davis 1976, Cooper et al. 2013). This life history theory was based mainly on landings data (Switzer et al. 2013) that showed increasing size with increasing depth (Collins & McBride 2011). These patterns could also be driven by selective fishing mortality in areas that are easily accessible and closer to shore (Frank et al. 2018), but this is contradicted by the higher densities that are found in shallower depths nearshore (Collins & McBride 2011). The results in this study refute the hypotheses presented of either gradual offshore migrations or abrupt habitat shifts. Due to near- to offshore gradients of δ^{13}C in the eGOM, a continuous ontogenetic migration offshore would manifest as a gradual decrease in δ^{13}C over the lifespan of hogfish (i.e. Fig. 2a). These hypothesized gradually decreasing trends were not consistently observed across the entire life of hogfish in this study. Following maturity, a slight steady decrease was observed for some (but not all) individuals, which could indicate that some individuals do migrate deeper with growth. However, there were no significant overall trends in δ^{13}C over the entire lifespan of hogfish in this study (Fig. 3), indicating that there is high variability in the life histories of eGOM hogfish. These results suggest an alternative scenario — that post-settlement movement of eGOM hogfish varies greatly among individuals and does not follow a clear, predictable pattern.

One pattern that did emerge was the consistently low δ^{13}C values found in many of the eye lens cores, followed by a jump in δ^{13}C shortly after. The eye lens core is formed near the time of hatching, and therefore likely reflects the ambient isotopic values during the pelagic larval stage (~34 d duration) when hogfish are planktivorous (Colin 1982) and influenced by phytoplankton-based primary production, or could reflect maternal isotopic contributions to the egg. Notably, isotope values outside the core tended to be higher, which could indicate settlement to benthic habitats or, particularly, to seagrass beds, which are known to have δ^{13}C values in the range of −12 to −10‰ (Moncreiff & Sullivan 2001, Fry 2006). The jumps in δ^{13}C from this study were in the range of −16 to −14‰, which may be explained by the influences of epiphytic algae that frequently grows on seagrasses and exhibits δ^{13}C values closer to 17.5‰ (Moncreiff & Sullivan 2001). The phenomenon of a peak in δ^{13}C has been observed in eye lens stable isotope data for several eGOM reef fish species and has been referenced as the 'carbon bump', indicating settlement to benthic habitats (Vecchio 2019). These peaks in δ^{13}C could lead to difficulty determining any overall trends, as both the low core values and high settlement values may disproportionately affect the overall trend in δ^{13}C. In addition, post-settlement hogfish diets are tightly linked to benthic production, as their feeding takes place directly in sediments rather than in the water column (Randall & Warmke 1967). This feeding behavior could explain the elevated δ^{13}C values throughout life due to the strong influence of benthic primary production. For example, Pinnegar & Polunin (2000) found no difference in δ^{13}C between benthic invertivores and carnivorous fishes; however, these isotope values were different from those of planktivorous fishes. Since hogfish diets are tightly linked to the benthos, any depth-related decreases in δ^{13}C that may have been observed with offshore movement may be obscured by this elevated δ^{13}C.

Offshore adult hogfish had significantly lower δ^{13}C values in eye lens layers, particularly in early life stages (<20 cm estimated FL) compared with nearshore adult hogfish (Fig. 10). This could indicate that adult fish caught offshore likely inhabited nearshore habitats as juveniles. In contrast, adult fish that were captured nearshore likely settled in shallower (perhaps estuarine) habitats. These patterns are consistent with fisheries-independent data, as hogfish <20 cm are indeed captured offshore at depths between 10 and 30 m (GSMFC 2018). Collins & McBride (2011) found that hogfish found in offshore habitats were significantly larger than those found on nearshore reefs, which could be attributed to differences in resource quality, disturbances, density-dependent effects, or higher fishing intensity at nearshore sites. Nearshore and estuarine abundance of prey items can be more vulnerable to disturbances such as eutrophication and anoxia (Powers et al. 2005) and lead to lower prey availability for predators. Thus, hogfish that inhabit shallower depths may be limited to lower quality or less consistent prey and therefore be less successful than deep-water hogfish. In addition, disturbances can also have direct effects on hogfish, such as the toxic effects of red tide *Karenia brevis* blooms (Gannon et al. 2009) in nearshore waters (<25 m; Smith 1975, Dupont et al. 2010), leading to higher mortality in nearshore areas compared to offshore. Inhabiting deeper areas could also reduce competition for resources due to lower densities (Collins & McBride 2011) or allow fish to escape the fishing intensity of nearshore reefs (McBride & Richardson 2007), and lead to their ability to grow to larger sizes. The results here did not suggest a difference in post-larval settlement depth per se, but differences of δ^{13}C in the inner eye lens layers corresponding to early life suggest hogfish that inhabited nearshore waters as juveniles were more likely to reach deeper offshore habitats as adults that could perhaps influence individual fitness. The use of deeper juvenile habitats could have important implications for the growth and success of the eGOM hogfish population. Hogfish in deeper habitats are known to grow to larger sizes, live to older ages, and have higher reproductive output (Collins & McBride 2011, 2015). Thus, inhabiting deeper habitats as juveniles may allow fish to reach deepwater refugia sooner, and allow for higher overall fitness compared to shallow-water individuals.

The differences in eye lens δ^{13}C values across regions of capture show that the hogfish in the KE region incorporated higher δ^{13}C values across life compared to other regions. The differences in isotope values for hogfish in the KE compared to other regional groups suggest that these fish likely stayed within this area throughout their life. The other regions exhibited isotopic overlap throughout life, indi-

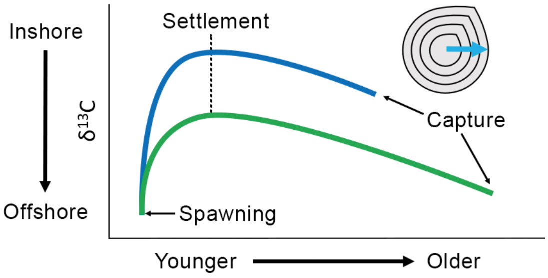

Fig. 10. Conceptual model of δ^{13}C patterns in eye lenses of nearshore (blue) and offshore (green) hogfish observed in this study associated with new hypothesized ontogenetic migrations, indicating where settlement, spawning, and capture occur within the eye lens data

cating either that fish moved across these 3 regions or that the regions are isotopically indistinct from one another. The elevated δ^{13}C values observed in fish from the KE could be due to the environmental differences of this ecosystem compared to more northerly areas of the West Florida Shelf. Greater water clarity in the KE region leads to greater light penetration (i.e. lower light attenuation) and therefore to more enriched δ^{13}C values due to the domination of benthic primary production (Fry 2006). The δ^{15}N values were consistently lower in hogfish eye lenses from the KE region across all sizes analyzed, likely due to the oligotrophic nature of these waters, with greater contribution of nitrogen from atmospheric deposition rather than nutrient inputs.

Muscle tissue samples from fish in potential habitats of juveniles were not from hogfish specifically in the juvenile size range, but were all <25 cm FL to avoid any isotopic effects of size. The SIBER analysis of juvenile muscle tissue isotopes suggested that each juvenile habitat was isotopically distinct, according to the traditionally accepted 60% cutoff (Smith 1985). However, this designation has been used in studies on trophic niche overlap (Olson et al. 2007, Curtis et al. 2017) and not for geographic distinctions. Therefore, although 'significantly' different, the small sample size and slight overlap among these groups could still lead to confusion in estimating juvenile habitats based on isotope values. Furthermore, it is important to keep in mind that understudied areas could also exhibit isotopic backgrounds similar to other areas not sampled in this study. The LDA was still useful for inferring the juvenile habitat from which each adult originated, although only a subsample of potential habitats of juvenile hogfish was sampled in this study. These results lack representation from the nearshore habitats, as the fish sampled for eye lens SIA from these sites were larger adults and therefore their muscle tissue was not analyzed due to isotope values potentially being influenced by trophic growth. With greater representation of juveniles from all potential habitats, we could better estimate the habitats used most frequently by juvenile hogfish in the eGOM. The results suggest that although hogfish in the eGOM can originate from various regions, most of the individuals in this study were predicted to originate from the ECK region. This is consistent with Switzer et al. (2013) who found high juvenile hogfish densities in seagrass habitats near Cedar Key. The expansive seagrass beds in this area are among the largest in the world (Iverson & Bittaker 1986) and are known to serve as juvenile habitat for numerous other reef fish species (Zieman & Zieman 1989, Switzer et al. 2012, Stallings et al. 2015a).

The contribution of juvenile fishes from Cedar Key seagrass beds to adult reef fish populations have yet to be quantified, however, leaving the status of this area as a 'nursery' habitat undefined. Building upon the concept of a nursery habitat from Beck et al. (2001), a broader definition of 'effective juvenile habitats' has been defined as those that contribute a greater proportion of individuals to the adult population, regardless of area coverage (Dahlgren et al. 2006). Since the per-area contribution of each habitat was not quantified in this study, we cannot confirm the presence of a nursery habitat. However, these results do suggest that ECK could serve as effective juvenile habitat for hogfish in the eGOM. Despite this region's importance as juvenile habitat, designation as an Aquatic Preserve (FDEP 2019), and contribution of energy and nutrients from estuarine to offshore food webs in the Gulf of Mexico (Nelson et al. 2012, 2013), environmental protections in this region are currently not very restrictive.

The LDA predicted ETB to be the habitat with the second highest contribution to adult hogfish sampled in this study. This result was unexpected since catches of hogfish in this region by FWRI are relatively low compared to other estuaries, especially when compared to the BB estuary (FWRI 2018). Despite low catch rates of hogfish in this region, it could potentially act as juvenile habitat for more individuals than expected. Many areas within TB contain hardbottom, rocky limestone ledges, and artificial reefs that could also provide suitable juvenile habitat (Savercool & Lewis 1994). Moreover, a study on the otoliths of the blue groper *Achoerodus viridis*, a Pacific labrid, indicated that a large portion of the population inhabited rocky reefs as juveniles rather than the hypothesized seagrass habitats (Gillanders & Kingsford 1996). Juvenile hogfish occupying ledges or artificial reefs within TB would not be captured by the seine and trawl gears used by FWRI, which cannot be deployed over hardbottom or depths >1 m (McMichael 2009). In addition, hogfish are seldom captured from inside the bay (M. E. Faletti pers. obs.) and were difficult to target during the course of this study. This lack of detectability highlights another benefit of isotope studies such as this, since they can retrospectively identify these potentially important habitats while reducing the challenges associated with sampling inaccessible areas. Although the LDA did have some confusion classifying individuals between ETB and NKE, neither of these 2 regions have been previously discussed in the literature as potential juvenile habitat for hogfish, warranting further research about their presence in these areas.

This study provides an example of a useful methodology that can be used to retrospectively identify juvenile habitats based on known isoscapes. However, it is important to note that the habitats of juveniles analyzed here were not an inclusive list, as many habitats are difficult to sample. In fact, only 4 of the 8 juvenile habitats of interest for this study had adequate sample sizes for predicting habitat use. Juvenile hogfish were rarely captured from several of the estuaries throughout the period of this study, and the resulting low sample sizes likely contributed to the uncertainty in the LDA model. Model accuracy may be improved with greater sample sizes, which would help to better infer habitat use. The low capture rate of hogfish during this time period does not necessarily reflect the lack of alternative habitats for juveniles. In fact, juvenile hogfish (<15 cm) have been captured across all 8 of the areas of interest in this study along the Florida Gulf Coast in recent years (FWRI 2018). In addition, juvenile hogfish have also been observed on nearshore reefs in depths 5–30 m (GSMFC 2018 , M. E. Faletti pers. obs.), which is consistent with the isotopic findings of this study that suggest nearshore reefs are likely used by juvenile hogfish in addition to estuarine seagrass beds. A quantitative, spatial analysis of juvenile hogfish contribution to the adult population per unit area would be needed to specifically designate any of these areas as hogfish nursery habitat. Future studies including larger sample sizes could better answer this question.

SIBER analysis conducted on eye lens core data was used to determine potential overlap in spawning areas. These groupings, if distinct from one another, would indicate that the fish in each region were spawned in a similar location to each other, but would not necessarily reflect self-recruitment or natal homing. Eye lens cores from hogfish in the CH region had very little overlap with the other groups (BB, TB, and KE). This distinction was primarily driven by higher eye lens core δ^{15}N values in CH fish. This contrast in δ^{15}N could be driven by several factors, including higher trophic levels of the spawning adults, a more northerly spawning area, or most likely, a stronger influence of nutrient inputs on the ambient δ^{15}N. The CH region is subjected to inputs from the Caloosahatchee River watershed, which is influenced by agricultural and urban runoff that have caused general declines in water quality (Liu et al. 2009) and increases in nutrients (e.g. bioavailable dissolved organic nitrogen; Pisani et al. 2017). These inputs to the Caloosahatchee River estuary and the nearshore CH region could indeed be reflected in enrichment of δ^{15}N values, driving differences in δ^{15}N values from the fish studied here.

The high variability and degree of overlap in eye lens core isotope values from the other 3 regions suggested that spawning locations were widely distributed and possibly overlapped across the West Florida Shelf in the eGOM. Since δ^{15}N is known to have a decreasing north–south gradient along the shelf (Radabaugh et al. 2013), the lack of a relationship between core δ^{15}N values and capture latitude suggests that fish were not necessarily remaining in the same region in which they were spawned. This evidence is consistent with the known spawning behavior of hogfish, as they spawn within small harems across the shelf (McBride & Johnson 2007). Hogfish have a pelagic larval duration of ~30 d before settlement, during which their movements are driven by physical oceanographic processes (Colin 1982). Hogfish also have protracted spawning periods (4 mo nearshore, 8 mo nearshore, up to 11 mo) and there is evidence that individual females can spawn daily throughout this period (Collins & McBride 2015). Along the West Florida Shelf, reef fish larvae can be transported inshore by loop current eddies and nearshore transport during protracted upwelling events (Weisberg et al. 2016) or by the bottom Ekman layer via remote forcing when the Loop current interacts with the shelf (Weisberg et al. 2014). The extended spawning season can leave larval hogfish exposed to seasonally variable physical processes. The timing and direction of these events can have a direct effect on reef fish settlement location, and perhaps a stronger influence than adult spawning locations. Specifically, cross-shelf flow creates a pathway by which gag *Mycteroperca microlepis* spawned in the northern Gulf of Mexico are transported to BB seagrass beds in the spring (Todd et al. 2014). This mechanism could be a contributing factor in this area's high densities of juvenile fishes (Stallings et al. 2015a). These processes likely extend to hogfish spawned along the West Florida Shelf, especially given that the spring (February–April) coincides with peak spawning season for eGOM hogfish (Collins & McBride 2015). Previous research shows that hogfish in the eGOM are genetically distinct from those in the Keys, with an area of genetic mixing corresponding to the region in which the fish from this study were sampled (Seyoum et al. 2015). This genetic mixing is not likely due to individuals moving across these boundaries as adults, due to the isotopically distinct eye lens data discussed above. The eye lens core values from the KE fish overlap with those from other regions along the West Florida Shelf.

Therefore, it is more likely that this genetic mixing is due to some of the KE hogfish being spawned in similar areas and settling out in the southern part of the West Florida Shelf.

5. CONCLUSIONS

This study provides new insights into the ontogeny of an important fishery species in the eGOM using eye lens SIA. These methods are a practical technique for providing a retrospective view on fish life history and resource use which could help address knowledge gaps on other species' ontogenies. Although highly variable across individuals, these data revealed significant increases in trophic level across hogfish lifetimes. Significant differences in $\delta^{13}C$ values across the lifespan, especially at early life stages (<20 cm), suggests differential habitat use at early life stages for offshore versus nearshore hogfish. Eye lens isotope values from offshore adults suggest they inhabited deeper habitats as juveniles (i.e. nearshore reefs), while nearshore adults have isotope values that suggest they used estuarine habitats as juveniles. This could indicate that hogfish inhabiting deeper habitats as juveniles are able to reach reefs further offshore where they are less accessible to fishing activities. Given potential for large hogfish in deeper waters to contribute greater reproductive output to the population (Collins & McBride 2015), the use of deeper habitats by juvenile hogfish could have important implications for the sustainability of the eGOM hogfish population. This is especially critical in the face of higher fishing intensity nearshore with recent increases in hook-and-line fishing activities (A. Collins pers. comm.). Several regions were identified as potential habitats for juvenile hogfish, including some nearshore areas, likely shallow reefs. This is a novel finding for eGOM hogfish and contradicts prior theories that solely discuss estuaries as juvenile hogfish habitat. The majority of adult hogfish sampled in this study were predicted to come from the Cedar Key region, which is consistent with previous research. Additional samples from other estuaries and nearshore reef habitats could help to more accurately estimate the importance of other juvenile habitats and potentially identify hogfish nurseries.

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LITERATURE CITED

Andley UP (2008) The lens epithelium: focus on the expression and function of the α-crystallin chaperones. Int J Biochem Cell Biol 40:317–323

Beck MW, Heck KL Jr, Able KW, Childers DL and others (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51:633–641

Bron AJ, Vrensen G, Koretz J, Maraini G, Harding JJ (2000) The ageing lens. Ophthalmologica 214:86–104

Chacin DH, Stallings CD (2016) Disentangling fine- and broad- scale effects of habitat on predator–prey interactions. J Exp Mar Biol Ecol 483:10–19

Colin PL (1982) Spawning and larval development of the hogfish, *Lachnolaimus maximus* (Pisces, Labridae). Fish Bull 80:853–862

Collins AB, McBride RS (2011) Demographics by depth: spatially explicit life-history dynamics of a protogynous reef fish. Fish Bull 109:232–242

Collins AB, McBride RS (2015) Variations in reproductive potential between nearshore and offshore spawning contingents of hogfish in the eastern Gulf of Mexico. Fish Manag Ecol 22:113–124

Cooper W, Collins AB, O'Hop J, Addis D (2013) The 2013 stock assessment report for hogfish in the South Atlantic and Gulf of Mexico. Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL

Curtis JS, Wall KR, Albins MA, Stallings CD (2017) Diet shifts in a native mesopredator across a range of invasive lionfish biomass. Mar Ecol Prog Ser 573:215–228

Curtis JS, Albins MA, Peebles EB, Stallings CD (2020) Stable isotope analysis of eye lenses from invasive lionfish yields record of resource use. Mar Ecol Prog Ser 637: 181–194

Dahlgren CP, Kellison GT, Adams AJ, Gillanders BM and others (2006) Marine nurseries and effective juvenile habitats: concepts and applications. Mar Ecol Prog Ser 312:291–295

Davis JC (1976) Biology of the hogfish, *Lachnolaimus maximus* (Walbaum), in the Florida Keys. MSc thesis, University of Miami, Coral Gables, FL

Deniro MJ, Epstein S (1978) Influence of diet on distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

Dupont JM, Hallock P, Jaap WC (2010) Ecological impacts of the 2005 red tide on artificial reef epibenthic macroinvertebrate and fish communities in the eastern Gulf of Mexico. Mar Ecol Prog Ser 415:189–200

Estrada JA, Rice AN, Natanson LJ, Skomal GB (2006) Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. Ecology 87: 829–834

FDEP (Florida Department of Environmental Protection) (2019) Florida Department of Environmental Protection Aquatic Preserve Program. www.floridadep.gov/rcp/aquatic-preserve (accessed January 2019)

France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 124:307–312

Frank KT, Petrie B, Leggett WC, Boyce DG (2018) Exploitation drives an ontogenetic-like deepening in marine fish. Proc Natl Acad Sci USA 115:6422–6427

Fry B (2006) Stable isotope ecology, 3rd edn. Springer Science+Business Media, New York, NY

Fry B, Mumford PL, Robblee MB (1999) Stable isotope studies of pink shrimp (*Farfantepenaeus duorarum* Burkenroad) migrations on the southwestern Florida shelf. Bull Mar Sci 65:419–430

FWRI (Fish and Wildlife Research Institute) (2018) FL fisheries independent monitoring inshore stratified-random sampling records. Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, St Petersburg, FL

Gannon DP, Berens McCabe EJ, Camilleri SA, Gannon JG and others (2009) Effects of *Karenia brevis* harmful algal blooms on nearshore fish communities in southwest Florida. Mar Ecol Prog Ser 378:171–186

Gillanders BM (2002) Connectivity between juvenile and adult fish populations: Do adults remain near their recruitment estuaries? Mar Ecol Prog Ser 240:215–223

Gillanders BM, Kingsford MJ (1996) Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Mar Ecol Prog Ser 141:13–20

Gillanders BM, Able KW, Brown JA, Eggleston DB, Sheridan PF (2003) Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries. Mar Ecol Prog Ser 247:281–295

GMFMC (Gulf of Mexico Fishery Management Council) (2018) Modification of Gulf of Mexico red snapper and west Florida hogfish annual catch limits. Gulf of Mexico Fishery Management Council, Tampa, FL

GSMFC (2018) Gulf States Marine Fisheries Commission SEAMAP trawl data 2018. https://seamap.gsmfc.org/

Gutiérrez L (1998) Habitat selection by recruits establishes local patterns of adult distribution in two species of damselfishes: *Stegastes dorsopunicans* and *S. planifrons.* Oecologia 115:268–277

Hane Y, Kimura S, Yokoyama Y, Miyairi Y and others (2020) Reconstruction of temperature experienced by Pacific bluefin tuna *Thunnus orientalis* larvae using SIMS and microvolume CF-IRMS otolith oxygen isotope analyses. Mar Ecol Prog Ser 649:175–188

Hazen EL, Maxwell SM, Bailey H, Bograd SJ and others (2012) Ontogeny in marine tagging and tracking science: technologies and data gaps. Mar Ecol Prog Ser 457: 221–240

Heck KL Jr, Hays G, Orth RJ (2003) Critical evaluation of the nursery role hypothesis for seagrass meadows. Mar Ecol Prog Ser 253:123–136

Heppell SA, Semmens BX, Archer SK, Pattengill-Semmens CV and others (2012) Documenting recovery of a spawning aggregation through size frequency analysis from underwater laser calipers measurements. Biol Conserv 155:119–127

Herzka SZ (2005) Assessing connectivity of estuarine fishes based on stable isotope ratio analysis. Estuar Coast Shelf Sci 64:58–69

Iverson RL, Bittaker HF (1986) Seagrass distribution and abundance in eastern Gulf of Mexico coastal waters. Estuar Coast Shelf Sci 22:577–602

Jackson AL, Parnell AC (2019) SIBER: stable isotope Bayesian ellipses in R. R package version 2.1.4

Kim SL, Tinker MT, Estes JA, Koch PL (2012) Ontogenetic and among-individual variation in foraging strategies of northeast Pacific white sharks based on stable isotope analysis. PLOS ONE 7:e45068

Kurth BN, Peebles EB, Stallings CD (2019) Atlantic tarpon (*Megalops atlanticus*) exhibit upper estuarine habitat dependence followed by foraging system fidelity after ontogenetic habitat shifts. Estuar Coast Shelf Sci 225: 106248

Lindeman KC, Pugliese R, Waugh GT, Ault JS (2000) Developmental patterns within a multispecies reef fishery: management applications for essential fish habitats and protected areas. Bull Mar Sci 66:929–956

Lindholm J, Knight A, Kaufman L, Miller S (2006) A pilot study of hogfish (*Lachnolaimus maximus* Walbaum 1792) movement in the conch reef research only area (Northern Florida Keys National Marine Sanctuary). National Marine Sanctuary Program NMSP-06-06. US Department of Commerce, Silver Spring, MD

Liu ZJ, Choudhury SH, Xia M, Holt J and others (2009) Water quality assessment of coastal Caloosahatchee River watershed, Florida. J Environ Sci Health Part A Tox Hazard Subst Environ Eng 44:972–984

Lynnerup N, Kjeldsen H, Heegaard S, Jacobsen C, Heinemeier J (2008) Radiocarbon dating of the human eye lens crystallines reveal proteins without carbon turnover throughout life. PLOS ONE 3:e1529

Matheson RE Jr, Flaherty-Walia KE, Switzer TS, McMichael RH Jr (2017) The importance of time of day in structuring demersal ichthyofaunal assemblages on the West Florida Shelf. Bull Mar Sci 93:407–437

McBride RS, Johnson MR (2007) Sexual development and reproductive seasonality of hogfish (Labridae: *Lachnolaimus maximus*), an hermaphroditic reef fish. J Fish Biol 71:1270–1292

McBride RS, Murphy MD (2003) Current and potential yield per recruit of hogfish, *Lachnolaimus maximus*, in Florida. Proc Annu Gulf Caribb Fish Inst 54:513–525

McBride RS, Richardson AK (2007) Evidence of size-selective fishing mortality from an age and growth study of hogfish (Labridae: *Lachnolaimus maximus*), a hermaphroditic reef fish. Bull Mar Sci 80:401–417

McConnaughey T, McRoy CP (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar Biol 53:257–262

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390

McMichael R Jr (2009) Fisheries-independent monitoring program 2008 annual data summary report. Report to the Florida Fish and Wildlife Conservation Commission, Florida Fish and Wildlife Research Institute, St. Petersburg, FL

Meath B, Peebles EB, Seibel BA, Judkins H (2019) Stable isotopes in the eye lenses of *Doryteuthis plei* (Blainville

1823): exploring natal origins and migratory patterns in the eastern Gulf of Mexico. Cont Shelf Res 174:76–84

Minagawa M, Winter DA, Kaplan IR (1984) comparison of Kjeldahl and combustion methods for measurement of nitrogen isotope ratios in organic matter. Anal Chem 56: 1859–1861

Moncreiff CA, Sullivan MJ (2001) Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. Mar Ecol Prog Ser 215:93–106

Munoz RC, Burton ML, Brennan KJ, Parker RO (2010) Reproduction, habitat utilization, and movements of hogfish (*Lachnolaimus maximus*) in the Florida Keys, USA: comparisons from fished versus unfished habitats. Bull Mar Sci 86:93–116

Nelson J, Wilson R, Coleman F, Koenig C, Devries D, Gardner C, Chanton J (2012) Flux by fin: fish-mediated carbon and nutrient flux in the northeastern Gulf of Mexico. Mar Biol 159:365–372

Nelson JA, Stallings CD, Landing WM, Chanton J (2013) Biomass transfer subsidizes nitrogen to offshore food webs. Ecosystems 16:1130–1138

Nielsen J, Hedeholm RB, Heinemeier J, Bushnell PG and others (2016) Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*). Science 353:702–704

Oksanen J, Blanchet FG, Friendly M, Kindt R and others (2018) vegan: community ecology package version 2.5-3. https://github.com/vegandevs/vegan

Olson NW, Guy CS, Koupal KD (2007) Interactions among three top-level predators in a polymictic Great Plains reservoir. N Am J Fish Manage 27:268–278

Orth RJ, Heck KL Jr, Vanmontfrans J (1984) Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator–prey relationships. Estuaries 7:339–350

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Evol Syst 18:293–320

Pinnegar JK, Polunin NVC (2000) Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. Oecologia 122:399–409

Pisani O, Boyer JN, Podgorski DC, Thomas CR, Coley T, Jaffe R (2017) Molecular composition and bioavailability of dissolved organic nitrogen in a lake flow-influenced river in south Florida, USA. Aquat Sci 79:891–908

Powers SP, Peterson CH, Christian RR, Sullivan E, Powers MJ, Bishop MJ, Buzzelli CP (2005) Effects of eutrophication on bottom habitat and prey resources of demersal fishes. Mar Ecol Prog Ser 302:233–243

Quaeck-Davies K, Bendall VA, MacKenzie KM, Hetherington S, Newton J, Trueman CN (2018) Teleost and elasmobranch eye lenses as a target for life-history stable isotope analyses. PeerJ 6:e4883

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Radabaugh KR, Peebles EB (2014) Multiple regression models of $\delta^{13}C$ and $\delta^{15}N$ for fish populations in the eastern Gulf of Mexico. Cont Shelf Res 84:158–168

Radabaugh KR, Hollander DJ, Peebles EB (2013) Seasonal $\delta^{13}C$ and $\delta^{15}N$ isoscapes of fish populations along a continental shelf trophic gradient. Cont Shelf Res 68:112–122

Randall JE, Warmke GL (1967) The food habits of the hogfish *Lachnolaimus maximus*, a labrid fish from the Western Atlantic. Caribb J Sci 7:141–144

Rester JK (2017) Environmental and biological atlas of the Gulf of Mexico 2016. Gulf States Marine Fisheries Commission, Ocean Springs, MS

Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D (2018) mass: support functions and datasets for Venables and Ripley's MASS version 7.3-49. http://www.stats.ox.ac.uk/pub/MASS4/

Rooker JR, Secor DH, DeMetrio G, Kaufman AJ, Belmonte Rios A, Ticina V (2008) Evidence of trans-Atlantic movement and natal homing of bluefin tuna from stable isotopes in otoliths. Mar Ecol Prog Ser 368:231–239

Sand-Jensen K, Borum J (1991) Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat Bot 41:137–175

Savercool DM, Lewis RR III (1994) Hard bottom mapping of Tampa Bay. Tampa Bay National Estuary Program, St. Petersburg, FL

Schloesser RW, Neilson JD, Secor DH, Rooker JR (2010) Natal origin of Atlantic bluefin tuna (*Thunnus thynnus*) from Canadian waters based on otolith $\delta^{13}C$ and $\delta^{18}O$. Can J Fish Aquat Sci 67:563–569

Searcy SP, Sponaugle S (2001) Selective mortality during the larval–juvenile transition in two coral reef fishes. Ecology 82:2452–2470

Seyoum S, Collins AB, Puchulutegui C, McBride RS, Tringali MD (2015) Genetically determined population structure of hogfish (Labridae: *Lachnolaimus maximus*) in the Southeastern United States. Fish Bull 113:442–455

Shulman MJ (1985) Recruitment of coral-reef fishes: effects of distribution of predators and shelter. Ecology 66: 1056–1066

Simpson SJ, Sims DW, Trueman CN (2019) Ontogenetic trends in resource partitioning and trophic geography of sympatric skates (Rajidae) inferred from stable isotope composition across eye lenses. Mar Ecol Prog Ser 624:103–116

Smith GB (1975) 1971 Red tide and its impact on certain reef communities in mid-eastern Gulf of Mexico. Environ Lett 9:141–152

Smith EP (1985) Estimating the reliability of diet overlap measures. Environ Biol Fishes 13:125–138

SRS1 Software (2015) SRS1 cubic spline for Excel version 2.5.1.0. SRS1 Software, Boston, MA

Stallings CD, Mickle A, Nelson JA, McManus MG, Koenig CC (2015a) Faunal communities and habitat characteristics of the Big Bend seagrass meadows, 2009–2010. Ecology 96:304

Stallings CD, Nelson JA, Rozar KL, Adams CS and others (2015b) Effects of preservation methods of muscle tissue from upper-trophic level reef fishes on stable isotope values ($\delta^{13}C$ and $\delta^{15}N$). PeerJ 3:e874

Switzer TS, MacDonald TC, McMichael RH Jr, Keenan SF (2012) Recruitment of juvenile gags in the eastern Gulf of Mexico and factors contributing to observed spatial and temporal patterns of estuarine occupancy. Trans Am Fish Soc 141:707–719

Switzer TS, Keenan SF, McMichael RH Jr, Fischer KM (2013) Fisheries-independent data for juvenile hogfish (*Lachnolaimus maximus*) from polyhaline seagrasses of the Florida Big Bend, 2008–2012. Fish and Wildlife Research Institute, St. Petersburg, FL

Tabb DC, Manning RB (1961) A checklist of the flora and fauna of northern Florida Bay and adjacent brackish waters of the Florida mainland collected during the period July, 1957 through September, 1960. Bull Mar Sci 11:552–649

Todd AC, Morey SL, Chassignet EP (2014) Circulation and cross-shelf transport in the Florida Big Bend. J Mar Res 72:445–475

Trueman CN, MacKenzie KM, Palmer MR (2012) Identifying migrations in marine fishes through stable-isotope analysis. J Fish Biol 81:826–847

Tupper M, Rudd MA (2002) Species-specific impacts of a small marine reserve on reef fish production and fishing productivity in the Turks and Caicos Islands. Environ Conserv 29:484–492

Tzadik OE, Curtis JS, Granneman JE, Kurth BN and others (2017) Chemical archives in fishes beyond otoliths: a review on the use of other body parts as chronological recorders of microchemical constituents for expanding interpretations of environmental, ecological, and life-history changes. Limnol Oceanogr Methods 15:238–263

US Geological Survey (2020) National water information system. https://waterdata.usgs.gov/fl/nwis/current/?type=flow&group_key=county_cd (accessed 15 Oct 2020)

Vasconcelos RP, Reis-Santos P, Tanner S, Fonseca V and others (2007) Discriminating estuarine nurseries for five fish species through otolith elemental fingerprints. Mar Ecol Prog Ser 350:117–126

Vecchio JL (2019) Isotope-based methods for evaluating fish trophic geographies. PhD dissertation, University of South Florida, Tampa, FL

Vecchio JL, Peebles EB (2020) Spawning origins and ontogenetic movements for demersal fishes: an approach using eye-lens stable isotopes. Estuar Coast Shelf Sci 246:107047

Vecchio JL, Ostroff JL, Peebles EB (2021) Isotopic characterization of lifetime movement by two demersal fishes from the northeastern Gulf of Mexico. Mar Ecol Prog Ser 657: 161–172

Victor BC (1986) Larval settlement and juvenile mortality in a recruitment-limited coral reef fish population. Ecol Monogr 56:145–160

Vihtelic TS (2008) Teleost lens development and degeneration. Int Rev Cell Mol Biol 269:341–373

Wallace AA, Hollander DJ, Peebles EB (2014) Stable isotopes in fish eye lenses as potential recorders of trophic and geographic history. PLOS ONE 9:e108935

Weisberg RH, Zheng LY, Peebles E (2014) Gag grouper larvae pathways on the West Florida Shelf. Cont Shelf Res 88:11–23

Weisberg RH, Zheng LY, Liu YG (2016) West Florida Shelf upwelling: origins and pathways. J Geophys Res Oceans 121:5672–5681

Zhang J, Huang X (2008) Interactions between seagrass and its epiphytic algae: a review. Shengtaixue Zazhi 27: 1785–1790

Zieman JC & Zieman RT (1989) The ecology of the seagrass meadows of the west coast of Florida USA: a community profile. Biological Report 85(7.25). US Fish and Wildlife Service, Washington, DC

Appendix 1

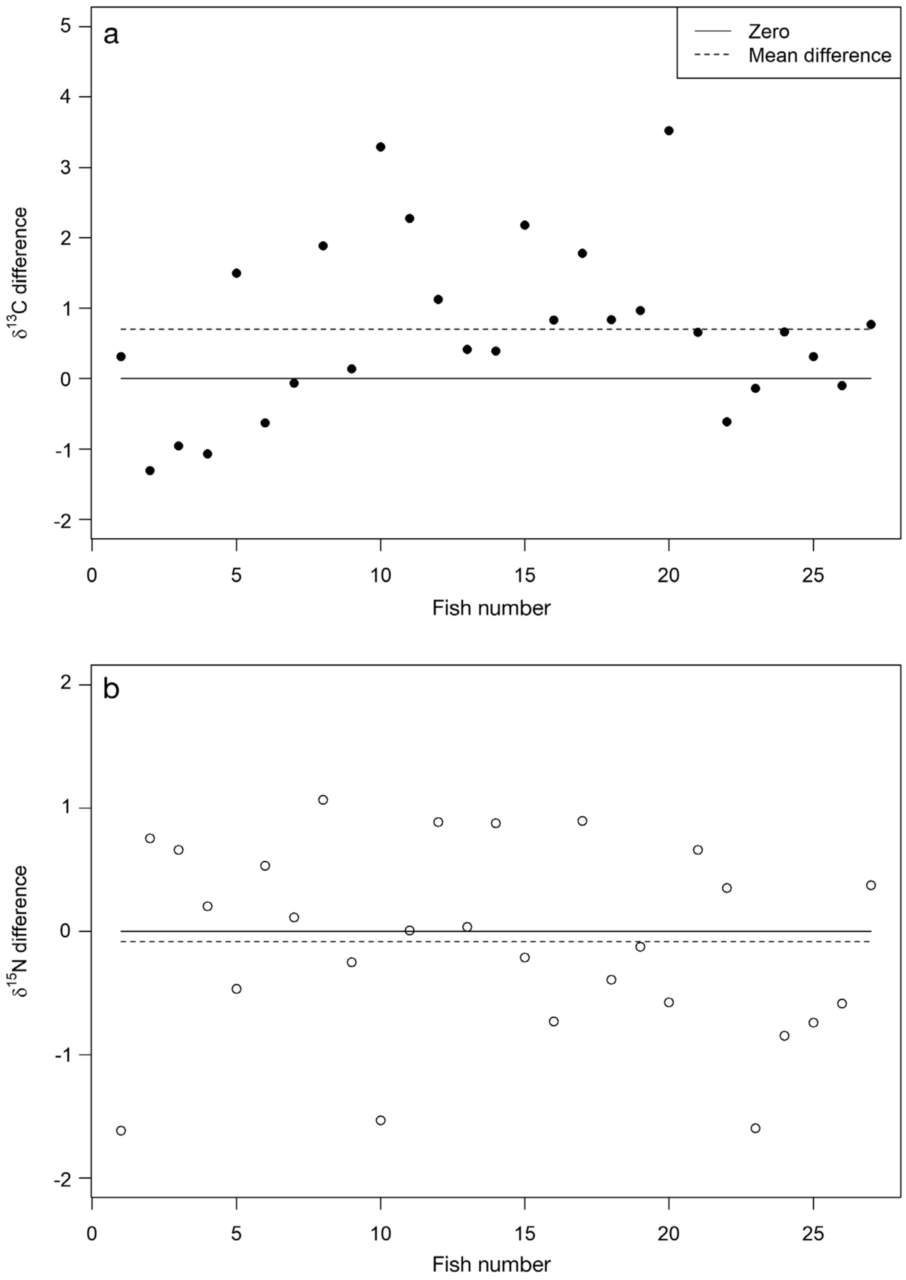

Fig. A1. Isotope value differences observed between the outer lens layer and fish muscle. There was no significant difference for $\delta^{15}N$ between muscle and outer eye lens layer, but a significant difference was found in $\delta^{13}C$, with values being on average 0.70 % higher in the outer lens than in the muscle tissue

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