October 2019

Life History Through the Eyes of a Hogfish: Evidence of Trophic Growth and Differential Juvenile Habitat Use

Meaghan E. Faletti
University of South Florida

Life History Through the Eyes of a Hogfish: Evidence of Trophic Growth and Differential Juvenile Habitat Use

by

Meaghan E. Faletti

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
with a concentration in Marine Resource Assessment
College of Marine Science
University of South Florida

Major Professor: Christopher Stallings, Ph.D.
Ernst Peebles, Ph.D.
Angela Collins, Ph.D.

Date of Approval:
October 29, 2019

Keywords: ontogeny, sclerochronology, connectivity, Carbon, Nitrogen, reef fish

DEDICATION

I'd like to dedicate this work to my Papa Jack and Nana for supporting my dreams since the day I found my first horseshoe crab on the beaches of Cape Cod. I also dedicate this project to my entire family, especially my parents: John; Carole; and Bob, my sister Danielle, my grandparents, my aunt Cathleen, and my uncle Mike. Thank you all for believing in me through even the toughest times, always reminding me to keep a positive outlook on life, and encouraging me to never give up. To my fiancé, Travis, for being the most incredible partner in life I could ever ask for, and for putting a smile on my face every single day. Last but certainly not least, I'd like to dedicate this to my nephew Owen, to encourage him to be infinitely curious about this big and beautiful world. Never stop exploring, buddy.

ACKNOWLEDGMENTS

I'd first like to recognize the funding sources who allowed me the opportunity to attend an incredible program to conduct this work. Thank you to the University of South Florida College of Marine Science, National Marine Fisheries Service, Tampa Bay Parrothead Club, the Guy Harvey Ocean Foundation, Florida Sea Grant, and the Florida Skin Divers Association.

I am incredibly grateful to the numerous spearfishers who donated fish for this project, including J. Bihari, N. Burko, R. Chaple, B. D'antuono, C. Enis, J. Falcone, W. Hardman, C.J. Hnlica, I. Hornik, C. Hunt, M. Jeanes, K. Ludwig, B. Mahardy, M. Miller, E. Muehlstein, H. Tillotson, C. Trier, R. Rindone, K. Spurgin, J. Thompson, H. Thoricht, and R. Zacker. I owe many thanks to J. Ostroff, T. Emory, and the rest of the Florida Fish and Wildlife Research Institute (FWRI) staff, including the Southeast Area Monitoring and Assessment Program (SEAMAP) crew for providing additional samples.

I also want to express my endless gratitude to everyone who helped me with sample processing, data analysis, and thesis review. I had a tremendous amount of assistance from A. Cison, J. Curtis, D. Chacin, R. Ellis, E. Goddard, J. Granneman, G. Helmueller, J. Kilborn, J. Vecchio, and I. Williams. Thank you to the entire Fish Ecology lab, especially J. Peake, M. Schram, and K. Wall for acting as my graduate school "family" and always being willing and available to offer help, guidance, and make this chapter of my life an absolute blast.

Thank you to my advisor C. Stallings, for your endless support and helping me gain the skills to become both a better scientist and communicator. To my committee members A. Collins

and E. Peebles, thank you for inspiring this work and sharing your knowledge to make this project possible. I can never express how much I appreciate all three of you for your bountiful guidance and enthusiasm you shared with me every step along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 an effective tool commonly used to investigate ecological questions regarding fish movement and habitat use. Here, I analyzed stable isotopes from the 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. I documented evidence of entire-life scale trophic growth through increases in $\delta^{15}N$. I also observed depth separation at the juvenile stage, evidenced by variation in $\delta^{13}C$. These results suggest that Hogfish inhabiting deeper adult 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 is a novel finding for eGOM Hogfish and contradicts prior literature that solely discuss seagrass as juvenile Hogfish habitat. A linear discriminant function analysis revealed the Cedar Key region to be the most highly used juvenile habitat by the Hogfish sampled in this study, but more evidence is needed to determine the status of this area as a Hogfish 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 in Hogfish can help to better manage the stock and preserve essential habitats.

LIFE HISTORY THROUGH THE EYES OF A HOGFISH: EVIDENCE OF TROPHIC GROWTH AND DIFFERENTIAL JUVENILE HABITAT USE

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, Gutierrez 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 (Chacin & Stallings 2016, Searcy & Sponaugle 2001). 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 fully understand the distribution of fishes. Tracing fish movements throughout their life history can help to distinguish among these influences and help us better understand population connectivity.

Movement studies often involve the use of conventional artificial tags (e.g., dart, satellite, acoustic tags). However, these studies are often conducted with limited spatial and temporal

resolution, and strongly 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 organismal movement. Trace elements and stable isotopes have successfully been used to investigate several ecological questions regarding fish movement and habitat use (see reviews by Trueman et al. 2012, Tzadik et al. 2017). For example, otolith microchemistry can be used to identify differences in trace element composition among otoliths from different habitats (Gillanders 2002) and even reveal changes in habitat use by a single individual (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 were 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 can also be used to examine coastal shelf movements. For example, 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, Curtis 2016**,** Kurth et al. 2019), 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 the 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), which allows the ability to gather a chronology of isotopic data. Thus, the lens allows for retrospective investigation of isotopic ontogeny.

Stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ along the West Florida Shelf in the 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 which selectively fix ^{12}C over ^{13}C resulting in lower $\delta^{13}C$ values (France 1995). Thus, $\delta^{13}C$ gradients may be primarily

influenced by light attenuation, which leads to differences in growth rates and fractionation (Radabaugh & Peebles 2014). Values of $\delta^{13}C$ therefore reflect whether basal resources are dominated by benthic or pelagic primary production. 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). Values of $\delta^{15}N$ vary with distance from nutrient inputs, with higher background $\delta^{15}N$ levels found close to nutrient sources such as the Mississippi River in the northern Gulf of Mexico, and lower ones found further south in 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 elevated ^{15}N levels with increasing trophic level, with enrichment of ~3‰ per trophic step (Deniro & Epstein 1978, Peterson & Fry 1987). In sum, variation in $\delta^{13}C$ sampled from marine fishes in the eGOM can be used to predict on-to-offshore geographic movement, while variation in $\delta^{15}N$ can be used as a trophic proxy. The patterns and predictability of isotopes in the eGOM provide an opportune landscape upon which to study life history of marine organisms using eye lens SIA by allowing measured isotope values to be translated into geographic locations.

Hogfish (*Lachnolaimus maximus*) are a reef fish in the eGOM for which some questions remain regarding life history and ontogenetic movement. Hogfish are harem-forming protogynous hermaphrodites (Collins & McBride 2015) and exhibit high site fidelity on short temporal scales (Colin 1982, Lindholm 2006, Cooper et al. 2013). However, movement patterns on longer, life-long (2 – 3 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

eastern Gulf of Mexico 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 are significantly larger than those captured nearshore 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 patterns are 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 juvenile densities (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 juvenile habitats is therefore critical for management and conservation of marine species and the habitats on which they rely. Beck et al. (2001) describe a nursery specifically as a habitat which 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 juvenile habitats to adult ones. 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.

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, as well as identify a potential nursery area for eGOM Hogfish. Recent advancements in eye lens SIA techniques provide a useful method by which to estimate movement patterns and juvenile habitat contributions to the adult Hogfish population. Here, I addressed the following questions: (1) How do values of $\delta^{13}C$ and $\delta^{15}N$ 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? (3) Which juvenile habitats contribute to the adult eGOM Hogfish population? These data will provide the first empirical evidence on Hogfish ontogeny and juvenile habitat use using techniques in stable isotope ecology.

Methods

Sample collection

A total of 251 Hogfish were collected at depths ranging from 1.3 m to 60.9 m between June 2016 – December 2018. Most specimens were donated by spearfishers, resulting in most sizes above the legal harvest size (30.5 cm fork length [FL] in 2016-2017, and 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 with permission from Florida Fish and Wildlife Conservation Commission (Special Activities License # SAL-15-1673A-SR) in order to obtain representation across a greater size range. Additional Hogfish samples were provided by the Florida Fish and Wildlife Research Institute (FWRI) Fisheries Independent Monitoring (FIM) program and the Southeast Monitoring and Assessment Program (SEAMAP). Inshore FWRI samples 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 four 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 region (North of latitude 28°N; Figure 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 shallow (<30 m) and deep (≥30 m) strata, resulting in a total of eight regions.

Eight potential juvenile habitats were identified within the eGOM study area based on previous observations of juvenile Hogfish presence (<15 cm; Switzer et al. 2013, Faletti *personal observations*, FWRI 2018). 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 four shallow nearshore areas were also selected as potential juvenile habitats between 5-30 m depth: Big Bend (SBB), Tampa Bay (STB), Charlotte Harbor (SCH), and Keys (SKE; Figure 1b).

Sample processing

For each fish, fork length (FL) was measured to the nearest 0.1 cm. Whole eyes and muscle tissue samples were extracted from each fish. Most spear-caught fish were gutted or filleted prior to processing, therefore liver samples and total mass were only collected when available. 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. 2015). Muscle tissue samples were freeze-dried at -40°C in a vacuum of 50-100 microbar for 48 hours, then pulverized with mortar and pestle for SIA preparation.

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

$$RM = (d_o - d_i)/2$$

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 hours.

Stable isotope analysis

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

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 1000.$$

Samples were combusted in a Carlo-Erba NA2500 Series-II Elemental Analyzer coupled to a ThermoFinnigan 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. Values of $\delta^{13}C$ were measured relative to a PeeDee Belemnite (PDB) standard, and $\delta^{15}N$ was measured relative to air. Samples were calibrated to NIST8573, NIST8574 L-glutamic acid Standard Reference Materials.

Statistical analyses

A subsample of 27 individuals was selected for eye lens SIA. The subsample included eight fish from the Big Bend region (five deep, three shallow), seven from the Tampa Bay region (four deep, three shallow), seven from the Charlotte Harbor region (one deep, six shallow), and five from the Keys region (five shallow). Not every region was equally represented due to sampling constraints, or errors during processing. Linear regression of lens radius and FL at time of capture was used to back-calculate estimated FL at each measured radial midpoint (RM) from the eye lens. 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 $\delta^{13}C$ and $\delta^{15}N$ were each plotted against estimated FL for each individual to analyze changes in habitat and basal resource dependency ($\delta^{13}C$) and trophic growth ($\delta^{15}N$). To analyze overall changes in isotope ratios across life, a linear model was fit to measure the relationship between $\delta^{13}C$ and RM, and a logarithmic model was fit to measure the relationship between $\delta^{15}N$ and RM.

Raw data were interpolated using a cubic spline function (Microsoft Excel add-on: SRS1 Software). This allowed for direct comparison of isotope values across depth and region at specific estimated sizes to determine the size(s) at which $\delta^{13}C$ or $\delta^{15}N$ may change. Sizes for analysis were biologically-relevant based on previous work in the eGOM: length at settlement; 2 cm (Cooper et al. 2013), size at maturity; 15 cm (Cooper et al. 2013), and median age at sexual transition; 30 cm nearshore, 60 cm offshore (Collins & McBride 2015). Several additional sizes were included to increase resolution of the analysis (10, 20, 40, and 50 cm). For each size of interest, permutational Multivariate Analysis of Variance (permMANOVA) was used to test whether isotope values differed among regions or capture depth, with depth and region as categorical variables, and $\delta^{13}C$ and $\delta^{15}N$ values as the multivariate responses. When significant differences were identified, pairwise permMANOVA was then used to explore pairwise differences.

To test whether isotope values differed by juvenile habitat, these analyses were limited to muscle tissue isotope values from small Hogfish (<25 cm) to prevent any isotopic effects of size. 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

permMANOVA, despite data transformations. Juvenile muscle tissue isotope values were therefore analyzed for overlap using the Stable Isotope Bayesian Ellipses (SIBER) package in R (R Core Team) 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. 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 juvenile habitat from which each adult individual originated. The function was tested with muscle tissue isotope data from juvenile Hogfish (for which capture region was known) by classifying each individual into their predicted juvenile habitat. 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. All analyses were conducted using the vegan (Oksanen et al. 2018) and MASS (Ripley et al. 2018) packages in R (R Core Team), and where applicable, based on 999 permutations.

Eye lens cores are representative of the earliest stages of life and can therefore be used to predict spawning location or natal origin. To determine if area of natal origin differed by region of capture, eye lens cores from 27 adult individuals were analyzed for differences using permMANOVA, with latitudinal regions (BB, TB, CH, KE) and depth strata (Shallow: <30 m, Deep: ≥30 m) as predictors and isotope values ($\delta^{13}C$, $\delta^{15}N$) as the responses. Eye lens core values were also analyzed for overlap using the SIBER package to identify isotopically distinct spawning areas. Eye lens core isotope values were also analyzed via linear regression to determine any potential relationship with latitude (i.e., $\delta^{13}C$~latitude, $\delta^{15}N$~latitude).

Results

Hogfish sampled for SIA in this study ranged from 6.4 cm to 85.2 cm FL (mean ± SE: 37.5 ± 0.97 cm). The number of layers peeled from each lens varied and ranged from seven to 16 (median: 10). Lamina thickness ranged from 0.05 to 0.85 mm (mean ± SE: 0.25 ± 0.006 mm). Values of $\delta^{13}C$ and $\delta^{15}N$ were plotted against radial midpoint (RM) for each eye lens layer sampled to illustrate individual isotope chronologies (Appendix I). Values of $\delta^{13}C$ for Hogfish eye lens layers ranged from -21.27 to -12.67‰. There was no significant relationship between eye lens $\delta^{13}C$ and RM ($F = 0.452$, $df = 1, 272$, $r^2 = 0.002$, $p = 0.502$; Figure 2). Values of $\delta^{15}N$ for Hogfish eye lens layers ranged from 4.74 to 12.57‰. The overall relationship between eye lens $\delta^{15}N$ and RM was fit to a logarithmic model (Figure 3), where $\delta^{15}N = 1.11 * \log(RM) + 9.08$ ($F = 165.6$, $df = 1, 272$, $r^2 = 0.38$, $p < 0.001$). There was a significant negative relationship between $\delta^{15}N$ and $\delta^{13}C$ across all measured eye lens data ($\delta^{13}C = -0.46 * \delta^{15}N + 1.34$, $F = 64.77$, $df = 1, 272$, $r^2 = 0.19$, $p < 0.0001$; Figure 4).

Values of $\delta^{13}C$ were significantly lower for Hogfish captured at deeper depths at sizes 10, 15, and 50 cm ($p < 0.05$; Figure 5a). There were also significant differences in $\delta^{13}C$ values among some regions (Figure 5b), with the Keys region being significantly higher in $\delta^{13}C$ across all sizes all sizes 20 cm and greater. Specifically, $\delta^{13}C$ values were significantly different between BB-KE at all sizes 20 cm and greater. $\delta^{13}C$ values were also significantly different between CH-KE at size 20 and 50 cm, and between KE-TB for sizes 40 and 50 cm (Table 1). There were no significant differences in $\delta^{15}N$ between capture depths (Figure 5b) or among regions (Figure 6b) across all size classes analyzed, but the Keys region had consistently lower $\delta^{15}N$ compared to all other regions across all sizes analyzed.

For juvenile muscle tissue values, the 95% CI ellipses generated in the SIBER analysis (Figure 7) reveal very little overlap among juvenile habitats. Overlap between SBB & SCH was 29.82%, SBB & SKE was 11.58%, and EBB & ECK was 11.28% (Table 2). Biologically significant overlap for ellipses is defined as overlap of 60% (Smith 1985) or higher, however the studies that use this distinction have focused on overlapping of trophic niches (Olson et al. 2007, Curtis et al. 2017) rather than geographic differences. According to this designation, all juvenile Hogfish habitats were significantly different from one another (Figure 7).

Since juvenile habitats are isotopically distinct from one another, with little overlap, the linear discriminant analysis (LDA) could be used to estimate from which habitat each adult individual is from. The LDA function accurately predicted 44.9% of individuals based on juvenile muscle tissue isotope data. Juvenile habitat was predicted for adult Hogfish based on isotope values from eye lens isotope data corresponding to when the fish were 10 cm in length (See results in Table 3). Overall, 51.9% of Hogfish (of the 27 individuals analyzed) in this study were predicted to be from ECK, 25.9% from ETB, 7.4% from EBB, and 14.8% from SKE.

Eye lens core values of $\delta^{15}N$ ranged from 5.71‰ to 9.06‰, while values of $\delta^{13}C$ ranged from -19.10‰ to -13.62‰. The permMANOVA revealed a significant interactive effect of latitudinal region*depth zone on eye lens core isotope values ($F = 4.01$, $df = 2, 19$, $r^2 = 0.23$, $p = 0.032$). There was no significant relationship between eye lens core $\delta^{15}N$ and capture latitude, however, core values of $\delta^{13}C$ did have a significant negative linear relationship with capture latitude ($F = 5.71$, $df = 1, 25$, $r^2 = 0.19$, $p = 0.025$). SIBER analysis revealed little overlap between groups: BB & TB (29.9%), BB & CH (6.9%), BB & KE (9.0%), TB & KE (4.1%), and CH & KE (3.1%; Table 4). Eye lens cores of fish captured in CH parsed out to be slightly higher

in $\delta^{15}N$ than the other three latitudinal regions and had much lower overlap with the other groups (Figure 8).

Discussion

This study provided some new insights on the ontogeny of Hogfish in the eGOM. The use of eye lens SIA provided a retrospective view on individual life histories and resource use. Variation in stable isotope values from Hogfish eye lens layers showed trophic growth throughout life, while differences in isotope values from early life suggested differences in juvenile habitat depth for shallow- versus deep-caught individuals. Furthermore, these values were also used to predict habitats used during the juvenile stage and can be used to quantify the relative contributions of various habitats to the adult population.

The increase in $\delta^{15}N$ observed across Hogfish lifetime was found to be consistent with a one- to two-step trophic level increase, as estimated by other studies (Minagawa et al. 1984, McCutchan et al. 2003). Trophic growth with increasing body size is common and has been observed in chronological isotope studies of other species (Estrada 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 $\delta^{15}N$ followed a pattern of trophic growth, this was not the case for $\delta^{13}C$. The relationship between $\delta^{15}N$ and $\delta^{13}C$ for a trophic level increase is expected to have a 3‰ increase in $\delta^{15}N$ per 1‰ increase in $\delta^{13}C$ (McCutchan et al. 2003), however this trend was not observed in this study. In fact, $\delta^{13}C$ had an inverse relationship with $\delta^{15}N$. The observed, inverse relationship in $\delta^{13}C$ relative to $\delta^{15}N$ suggests that as Hogfish are undergoing this trophic growth, they are likely switching their diet from a more benthically-derived source to

a more planktonic one. The findings of trophic growth and basal resource shift is consistent with recent dietary findings. These results indicate a potential ontogenetic shift in Hogfish diet from benthic-based invertebrates to a broader diet that even includes small fishes that rely on planktonically-derived resources (FWRI, *unpublished data*). This shift would explain both the increases in $\delta^{13}C$ and the inverse relationship of $\delta^{13}C$ relative to $\delta^{15}N$.

The classic hypothesis regarding Hogfish life history and ontogeny is that individuals settle in estuarine seagrass or shallow 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, nearshore depths (Collins & McBride 2011). A continuous ontogenetic migration offshore would manifest as a gradual decrease in $\delta^{13}C$ over the lifespan of Hogfish. However, these gradual trends were not consistently observed in this study, contradicting the theory of gradual ontogenetic migration. In fact, there were no significant overall trends in $\delta^{13}C$ over the lifespan of individual Hogfish. This could potentially be in part due to very low $\delta^{13}C$ values found in the eye lens cores, followed by a jump in $\delta^{13}C$ 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-day duration) when Hogfish are planktivorous (Colin 1982) and influenced by phytoplankton-based primary production. Isotope values outside the core tended to be higher, indicating settlement to benthic habitats. This drastic increase in $\delta^{13}C$ could lead to difficulty determining patterns as both the core values and settlement values may disproportionately affect the overall trend in $\delta^{13}C$. 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 have resulted in $\delta^{13}C$ values being elevated throughout life due to the strong influence of benthic primary production. For example, Pinnegar & Polunin (2000) found no difference in $\delta^{13}C$ 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, $\delta^{13}C$ may be elevated enough to obscure any depth-related decreases in $\delta^{13}C$ that would be expected with offshore movement and increased dependency on planktonic basal resources.

Hogfish that were caught in deep habitats as adults had significantly lower $\delta^{13}C$ values in early life stages (<20 cm estimated FL) compared with individuals caught in shallow habitats as adults. This could indicate that adult fish caught in deeper water likely inhabited deeper habitats as juveniles (i.e., nearshore reefs). In contrast, fish that were captured in shallow waters as adults likely settled in shallower (perhaps estuarine) habitats. These patterns are consistent with fisheries-independent data, as Hogfish <20 cm are indeed captured offshore in depths between 10-30 m (GSMFC 2018). Collins & McBride (2011) found that Hogfish found in offshore habitats were significantly larger than those found in nearshore reefs, which could be attributed to differences in resource quality, disturbances, density-dependent effects, or higher fishing intensity on 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 shallow water (<25 m; Smith 1975, Dupont et al. 2010), leading to higher mortality in shallower areas compared to deeper ones. 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*, as $\delta^{13}C$ did not differ in the eye lens data corresponding to 2 cm. However, differences at 10-15 cm suggested that Hogfish that inhabited nearshore waters as juveniles were more likely to reach deeper, perhaps more suitable, habitats as adults. The use of deep 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 and live to older ages (Collins & McBride 2011). Thus, inhabiting deeper juvenile habitats may allow fish to reach deepwater refugia sooner. This could allow for higher overall success compared to shallow-water individuals.

The size-specific differences in eye lens $\delta^{13}C$ values across regions of capture show that the Hogfish in the Keys region incorporated higher $\delta^{13}C$ values across multiple life stages compared to other regions. Greater water clarity in the KE region leads to greater light penetration (i.e., lower light attenuation) and therefore lead to more enriched $\delta^{13}C$ values due to the domination of benthic primary production (Fry 2006). In fact, the KE region had the highest $\delta^{13}C$ values for all size classes analyzed, although these were only significantly higher at larger sizes. Values of $\delta^{15}N$ were consistently lower in the Keys region across all sizes analyzed. Although this difference was not significant, these low values are likely due to the oligotrophic nature of these waters, with greater contribution of Nitrogen from atmospheric deposition rather than nutrient inputs. The differences in isotope values for Hogfish in the Keys compared to other

regional groups suggest that these fish are likely staying within this area throughout their life. The other regions exhibited isotopic overlap throughout life, indicating that the fish moved across these three regional boundaries, or that the regions are isotopically similar to one another.

The SIBER analysis of juvenile muscle tissue isotopes suggest that each juvenile habitat is isotopically distinct, according to the traditionally-accepted 60% cutoff (Smith 1985). However, this designation has been used in studies on trophic niche overlap, and not for geographic distinctions. Therefore, although "significantly" different, the overlap among these groups can still lead to confusion in estimating juvenile habitats based on isotope values. The discriminant analysis was able to predict the juvenile habitat from which each adult originated. These results suggest that although Hogfish in the eGOM can originate from various regions, most of the individuals in this study originated from the ECK region. This is consistent with the findings of Switzer et al. (2013) who found high juvenile Hogfish densities in seagrass habitats near Cedar Key (ECK). 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. 2015b). However, the contribution of these areas to adult reef fish populations have yet to be quantified, 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, I cannot confirm the presence of a nursery habitat. However, these results do suggest that ECK serves as effective juvenile habitat for Hogfish in the eGOM. Despite this region's significance as juvenile habitat, designation as an Aquatic Preserve (FDEP

2019), and importance in transporting energy and nutrients from inshore to offshore food webs in the Gulf of Mexico (Nelson et al. 2012, Nelson et al. 2013), environmental protections in this region are currently not very restrictive.

The LDA revealed ETB (Tampa Bay) as the juvenile habitat with the second highest predicted usage for 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 EBB (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 Tampa Bay contain hardbottom, rocky limestone ledges, and artificial reefs that could also provide suitable juvenile habitat (Savercool & Lewis III 1994). Moreover, a study on the otoliths of 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 Tampa Bay would not be captured by the seine and trawl gears used by FWRI, which cannot be deployed over hardbottom or depths >1 m (McMichael Jr 2009). In addition, fisheries-dependent samples are rarely captured from inside the bay (Faletti, *personal observations*) and were difficult to target during this study. This lack of detectability highlights another benefit to isotope studies such as this, since they can retrospectively identify these potentially important habitats while reducing the challenges associated with sampling in inaccessible areas. Although the LDA did have some confusion classifying individuals between ETB and SKE, neither of these two regions have been previously discussed in the literature as potential juvenile habitat for Hogfish, warranting further research about their presence in these areas.

It is important to note that the habitats analyzed here are not an inclusive list. In fact, only four of the eight habitats of interest for this study had adequate sample sizes to predict habitat usage. 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 better predict juvenile habitat usage. The low capture rate of Hogfish during this time period does not necessarily reflect the lack of alternative juvenile habitats. In fact, juvenile (<15 cm) Hogfish have been captured across all eight separate areas along the Florida Gulf Coast in recent years (FWRI 2018). In addition, juvenile Hogfish have also been observed on shallow nearshore reefs in depths 5-30 m (Faletti *personal observations;* GSMFC 2018), which is consistent with the isotopic findings of this study that suggest nearshore reefs are likely used by juvenile Hogfish in addition to 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.

The high variability and degree of overlap in eye lens core isotope values suggest that spawning locations are widely distributed across the West Florida Shelf. Since $\delta^{15}N$ is known to have a decreasing North-South gradient along the shelf (Radabaugh et al. 2013), the lack of relationship between $\delta^{15}N$ core values and capture latitude indicates that fish are not necessarily remaining close to where they are spawned. The fish captured within the CH region had very little overlap with the other groups, mainly due to higher $\delta^{15}N$ core values than the other 3 latitudinal zones (BB, TB, and KE). This suggests that fish settling out or migrating to this area as adults may potentially be spawned in a different area perhaps close to stronger nutrient inputs.

Higher $\delta^{15}N$ in CH Hogfish core values could also indicate higher trophic level of the spawning adults, or least likely, a more northerly spawning area.

The high degree of overlap in the eye lens cores from the other three regions suggests overlap in spawning area or natal origin. This evidence is consistent with the known spawning behavior of Hogfish, as they spawn within small harems across the shelf as opposed to aggregating at small, geographically and temporally distinct spawning sites (McBride & Johnson 2007). Hogfish have a pelagic larval duration of ~30 days before settlement during which their movements are driven by physical oceanographic processes (Colin 1982). 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). Hogfish also have protracted spawning periods (4-8 months, up to 11 months) and there is evidence that individual females can spawn daily throughout this period (Collins & McBride 2015). This extended spawning season can leave larval Hogfish to be exposed to seasonally variable physical processes. The timing and direction of these events can therefore 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 Big Bend 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. 2015b). These processes likely extend to Hogfish spawned along the West Florida Shelf, especially since 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 Keys fish overlap with those from other regions along the WFS. Therefore, it is more likely that this genetic mixing is due to some of the Keys Hogfish being spawned in similar areas and settling out in the southern part of the WFS.

This study provides new insights on 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 elucidate knowledge gaps on other species' ontogenies. Though highly variable across individuals, these data revealed significant trophic growth across Hogfish lifetimes. Significant differences in $\delta^{13}C$ values at early life stages suggests differential habitat use at early life stages for deep- versus shallow-caught Hogfish. Isotope values from adults caught offshore suggest they inhabited deeper habitats as juveniles, while adults caught nearshore have isotope values that suggest they used estuarine habitats as juveniles. This could indicate that Hogfish inhabiting deeper juvenile habitats are able to reach reefs further offshore where they are less accessible to fishing activities. Hogfish that can reach these greater depths, and potentially grow larger and live longer, are critical components to supporting a long-term sustainable population in the face of higher fishing intensity nearshore. In addition, several regions were identified as potential juvenile habitats for 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 should be analyzed from other estuaries

and shallow reef habitats in order to more accurately estimate the importance of other juvenile habitats and potentially help in the identification of Hogfish nurseries.

TABLES AND FIGURES

Table 1. Size specific comparisons of $\delta^{13}C$ values among regions.

Size class (cm)	*Comparison*	*$\delta^{13}C$ p-value*	
2	BB-CH	0.160	
	BB-KE	0.160	
	BB-TB	0.750	
	CH-KE	0.850	
	CH-TB	0.850	
	KE-TB	0.850	
10	BB-CH	0.629	
	BB-KE	0.074	.
	BB-TB	0.598	
	CH-KE	0.598	
	CH-TB	0.724	
	KE-TB	0.629	
15	BB-CH	0.636	
	BB-KE	0.051	.
	BB-TB	0.418	
	CH-KE	0.418	
	CH-TB	0.636	
	KE-TB	0.636	
20	BB-CH	0.393	
	BB-KE	0.002	**
	BB-TB	0.117	
	CH-KE	0.013	*
	CH-TB	0.393	
	KE-TB	0.124	

*Note: P-values reported are corrected via Holm's method for multiple comparisons. BB – Big Bend; TB – Tampa Bay; CH – Charlotte Harbor; KE – Keys. Significance codes: ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, . p ≤ 0.1*

Table 1 Continued

Size class (cm)	*Comparison*	*$\delta^{13}C$ p-value*	
30	BB-CH	0.120	
	BB-KE	0.010	*
	BB-TB	0.110	
	CH-KE	0.553	
	CH-TB	0.932	
	KE-TB	0.553	
40	BB-CH	0.674	
	BB-KE	0.011	*
	BB-TB	0.875	
	CH-KE	0.118	
	CH-TB	0.674	
	KE-TB	0.023	*
50	BB-CH	0.268	
	BB-KE	< 0.001	***
	BB-TB	0.246	
	CH-KE	0.003	**
	CH-TB	0.941	
	KE-TB	0.001	**

Table 2. Proportion of overlap for Bayesian ellipses of small Hogfish (<25 cm) muscle tissue stable isotope values from juvenile habitats.

Habitat Comparison	*Proportion of Overlap*
SBB-SCH	0.140
SBB-SKE	0.043
SBB-ECK	0
SBB-EBB	0.004
SCH-SKE	0
SCH-ECK	0
SCH-EBB	0
SKE-ECK	0
SKE-EBB	0.009
ECK-EBB	0.030

Note: SBB – Shallow Big Bend; SCH – Shallow Charlotte Harbor; SKE – Shallow Keys; EBB – Estuarine Big Bend; ECK – Estuarine Cedar Key.

Table 3. Results from the linear discriminant analysis (LDA).

Predicted Juvenile Habitat	***Capture Region***						
	DCH	***DBB***	***DTB***	***SBB***	***SCH***	***SKE***	***STB***
EBB		1	1				
ECK	1	3	3	3	2	2	
ETB		1			1	3	2
SKE					3		1

Note: Columns indicate capture region for adult Hogfish, while rows represent potential juvenile habitats. Numbers in the table represent the number of individual Hogfish predicted to be from each of the four juvenile habitats tested, based on eye lens stable isotope data corresponding to the time at which each individual was 10cm FL (e.g., the single individual captured in the DCH 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; SKE – Shallow Keys; DCH – Deep Charlotte Harbor; DBB – Deep Big Bend; DTB – Deep Tampa Bay; SBB – Shallow Big Bend; SCH – Shallow Charlotte Harbor; SKE – Shallow Keys; STB – Shallow Tampa Bay.

Table 4. Proportion of overlap between Bayesian ellipses of juvenile muscle tissue stable isotope values from juvenile habitats.

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

Note: . BB – Big Bend; TB – Tampa Bay; CH – Charlotte Harbor; KE – Keys.

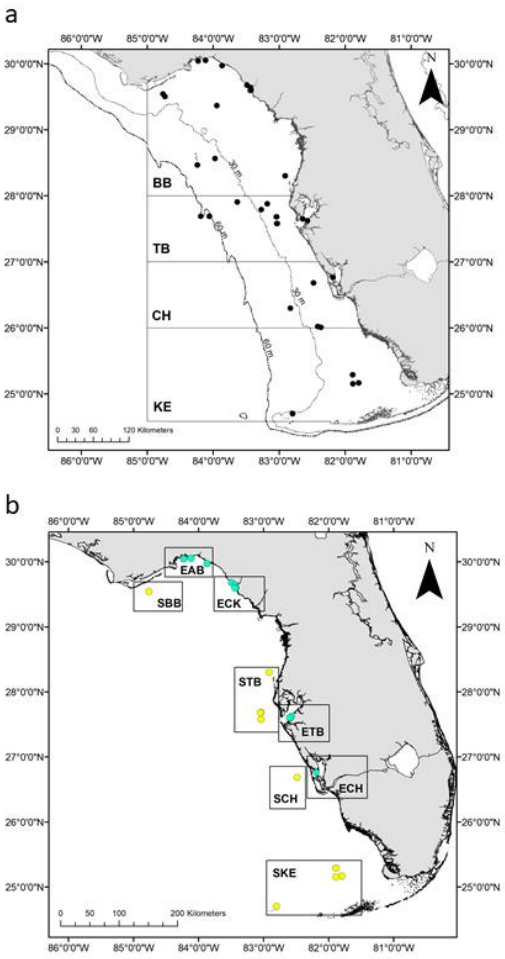


Figure 1. Map of Hogfish capture locations. **a.** Map displaying capture locations for Hogfish used in the stable isotope analysis, latitudinal regions, and depth breaks (30 m and 60 m). West Florida Shelf was divided into four latitudinal regions: BB – Big Bend; TB – Tampa Bay; CH – Charlotte Harbor; KE – Keys.

b. Map of all Hogfish capture locations used in juvenile analysis. Polygons encompass juvenile habitat breaks used in linear discriminant analysis for retrospective habitat usage predictions. Sampling locations displayed in yellow (nearshore) and green (estuarine) Hogfish used in this study. EBB – Estuarine Big Bend; SBB – Shallow Big Bend; ECK – Estuarine Cedar Key; SCK – Shallow Cedar Key; ETB – Estuarine Tampa Bay; STB – Shallow Tampa Bay; SCH – Shallow Charlotte Harbor; SKE – Shallow Keys.

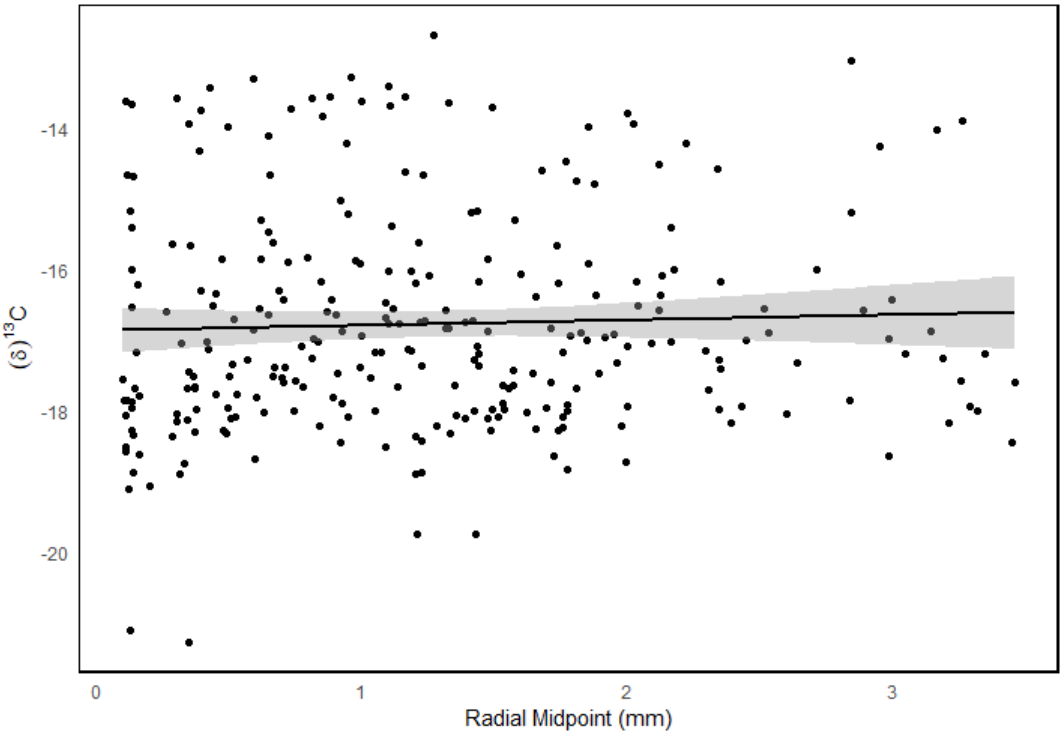


Figure 2. Raw $\delta^{13}C$ values plotted by radial midpoint (mm) for all individuals sampled (n=27 fish, n=277 layers). The solid black line represents a linear model fit to these data with gray shaded area representative of the 95% confidence interval.

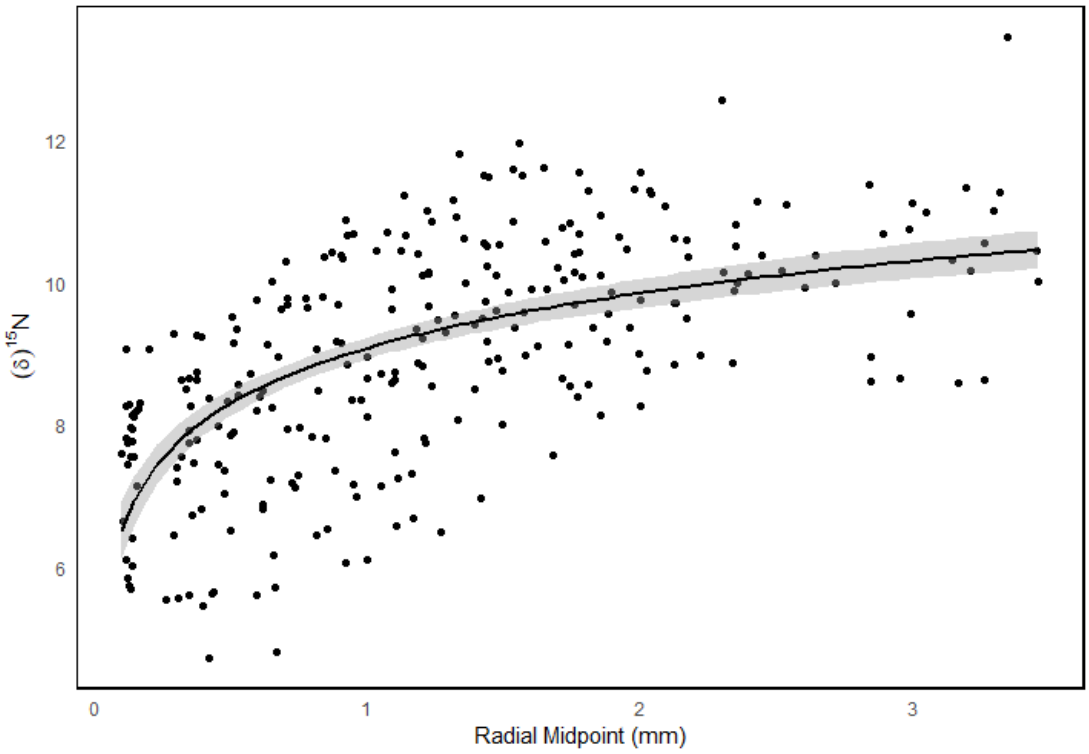


Figure 3. Raw $\delta^{15}N$ values plotted by radial midpoint (mm) for all individuals sampled (n=27 fish, n=277 layers). The solid black line represents a logarithmic model fit to these data with gray shaded area representative of the 95% confidence interval.

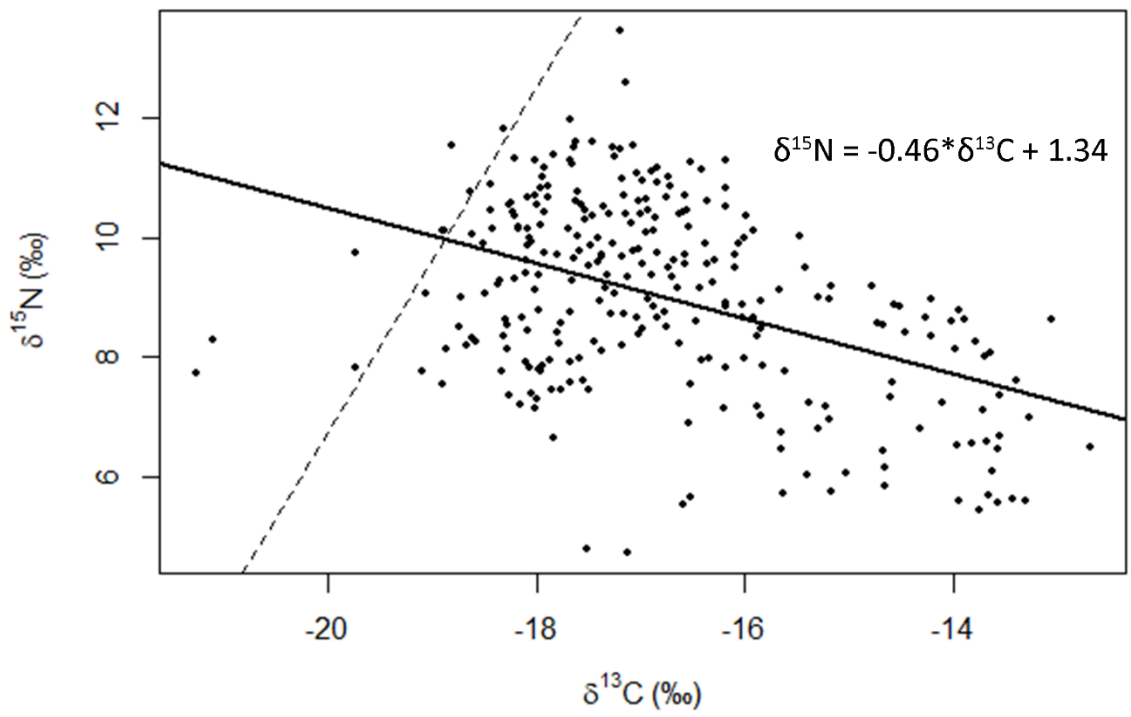


Figure 4. $\delta^{15}N$ plotted against $\delta^{13}C$ for each eye lens layer sampled. The dashed line represents the 3:1 relationship expected due to trophic increase, while the solid line represents the observed relationship between $\delta^{15}N$ and $\delta^{13}C$ ($r^2 = 0.19$, $p < 0.0001$).

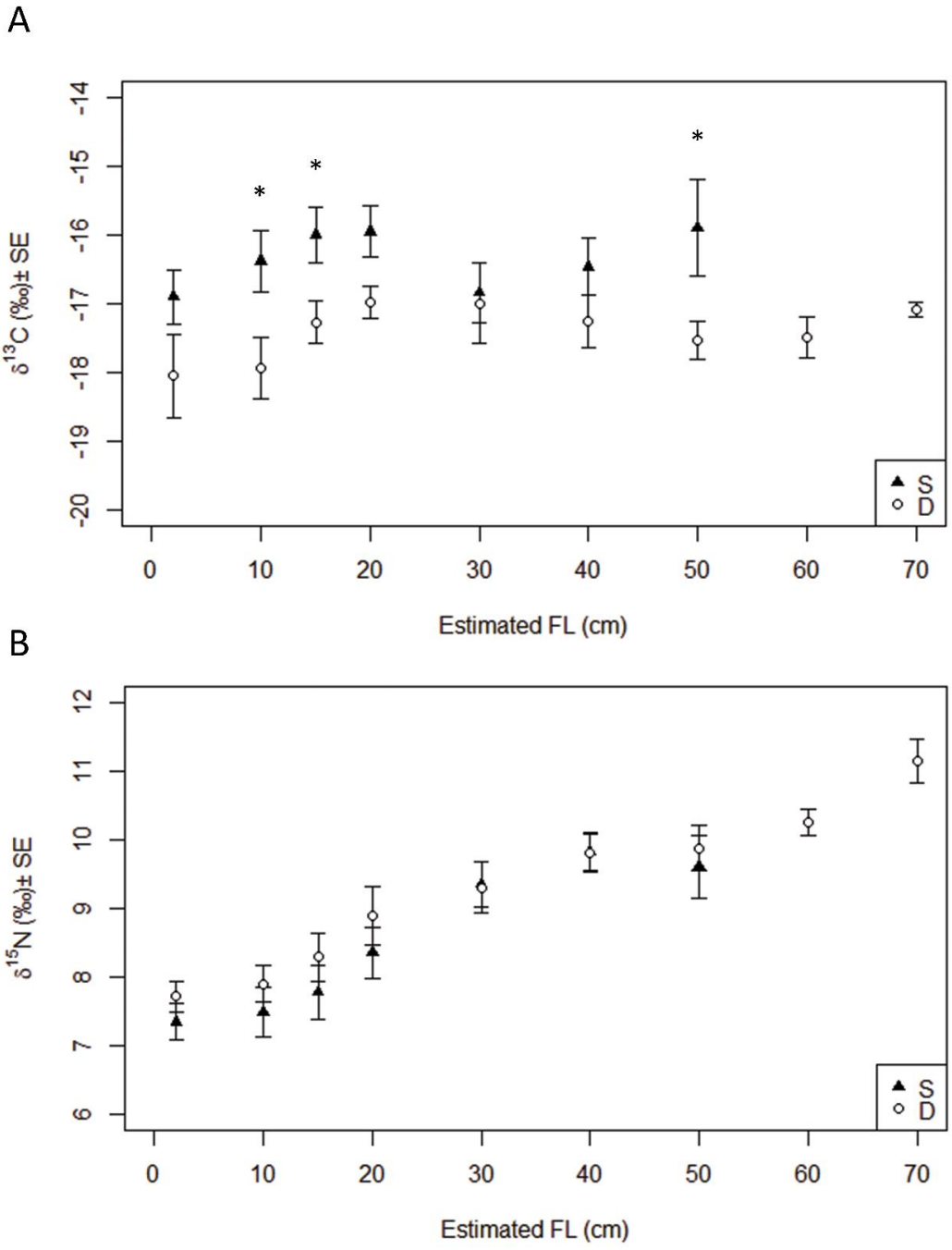


Figure 5. Mean isotope values by depth for each size of interest. Mean $\delta^{13}C$ (**A**) and $\delta^{15}N$ (**B**) plotted against estimated FL for shallow-(S; <30m) versus deep-caught (D; >30m) Hogfish within estimated sizes of interest. Asterisks (*) indicate significant differences using Holm's method for multiple comparisons.

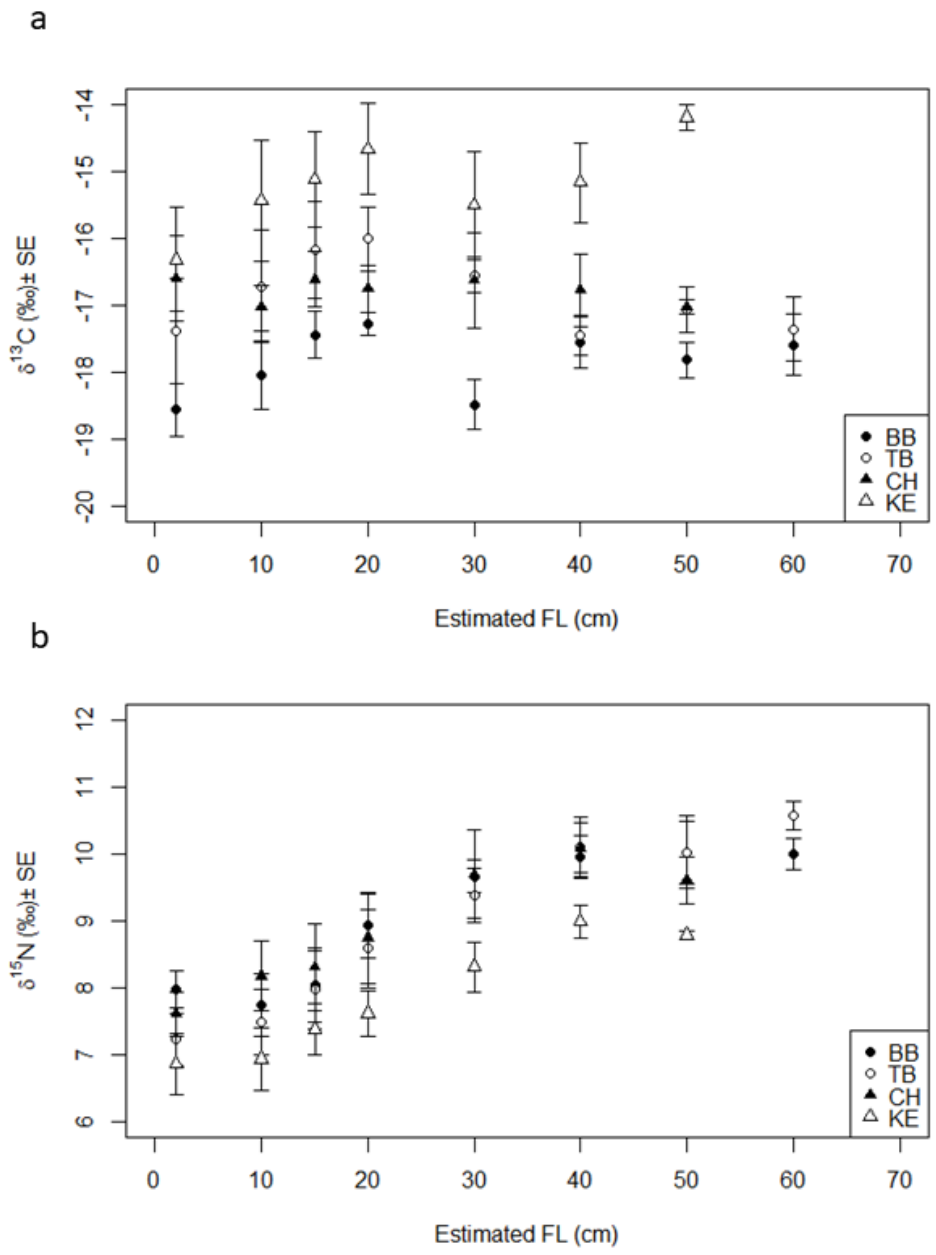


Figure 6. Mean isotope values by region for each size of interest. Mean $\delta^{13}C$ (**A**) and $\delta^{15}N$ (**B**) plotted against estimated FL for Hogfish by capture region, within estimated sizes of interest. Significant relationships are noted in Table 1. BB – Big Bend; TB – Tampa Bay; CH – Charlotte Harbor; KE – Keys.

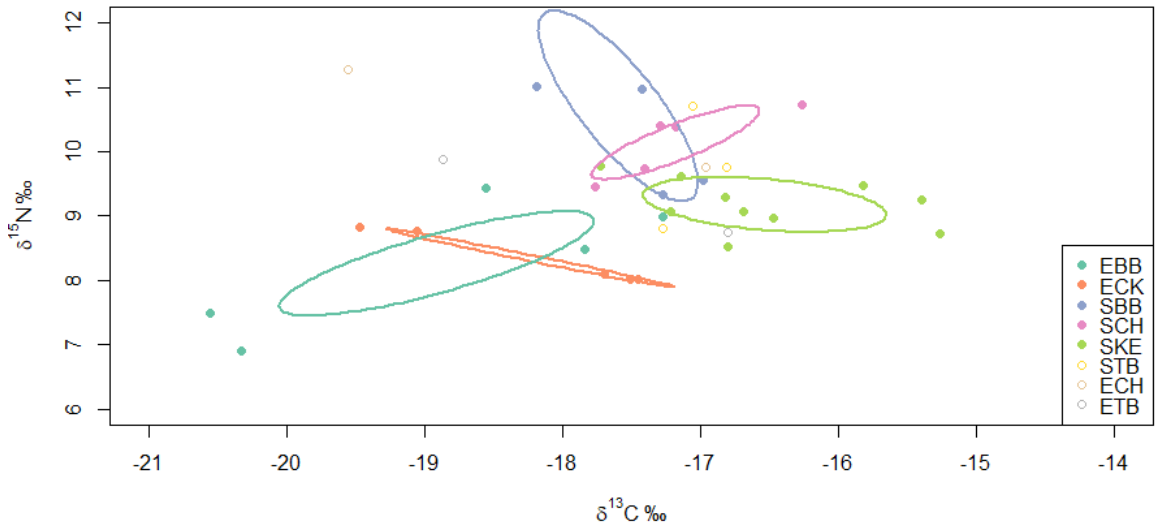


Figure 7. Stable isotope biplot for juvenile Hogfish muscle tissue data. Ellipses display 95% confidence intervals for eight potential juvenile habitats. Solid circles represent individual data points from regions with adequate sample size for SIBER analysis. Hollow circles are additional data points from regions without adequate sample size for SIBER analysis. EBB – Estuarine Big Bend; ECK – Estuarine Cedar Key; SBB – Shallow Big Bend; SCH – Shallow Charlotte Harbor; SKE – Shallow Keys; STB – Shallow Tampa Bay; ECH – Estuarine Charlotte Harbor; ETB – Estuarine Tampa Bay.

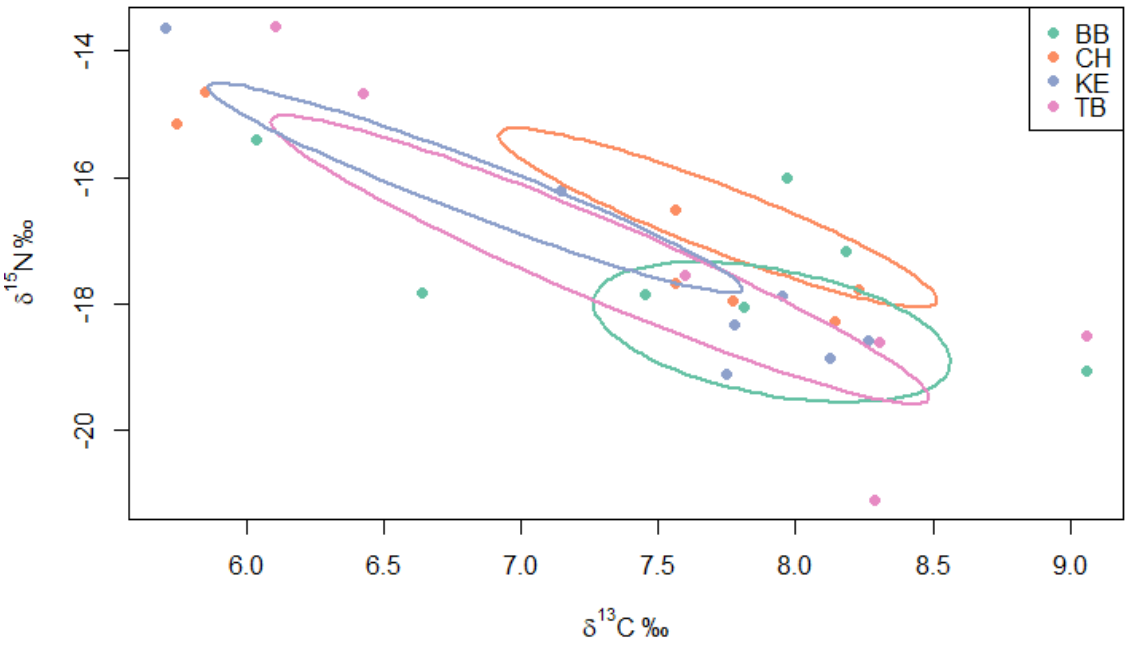


Figure 8. Stable isotope biplot for Hogfish eye lens core isotope data. Ellipses display 95% confidence intervals for four regions of capture. Solid circles represent individual Hogfish eye lens cores. BB – Big Bend; TB – Tampa Bay; CH – Charlotte Harbor; KE – Keys.

REFERENCES

1. Andley UP (2008) The lens epithelium: Focus on the expression and function of the α-crystallin chaperones. The International Journal of Biochemistry & Cell Biology 40:317-323
2. Beck MW, Heck KL, Able KW, Childers DL et al. (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51:633-641
3. Bron AJ, Vrensen G, Koretz J, Maraini G, Harding JJ (2000) The ageing lens. Ophthalmologica 214:86-104
4. Chacin DH, Stallings CD (2016) Disentangling fine- and broad-scale effects of habitat on predator-prey interactions. Journal of Experimental Marine Biology and Ecology 483:10-19
5. Collins AB, McBride RS (2011) Demographics by depth: spatially explicit life-history dynamics of a protogynous reef fish. Fishery Bulletin 109:232-242
6. 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, Florida
7. Curtis JS (2016) Resource Use Overlap in a Native Grouper and Invasive Lionfish. Thesis, University of South Florida

8. Curtis JS, Wall KR, Albins MA, Stallings CD (2017) Diet shifts in a native mesopredator across a range of invasive lionfish biomass. Marine Ecology Progress Series 573:215-228
9. Dahlgren CP, Kellison GT, Adams AJ, Gillanders BM et al. (2006) Marine nurseries and effective juvenile habitats: concepts and applications. Marine Ecology Progress Series 312:291-295
10. Davis JC (1976) Biology of the Hogfish, *Lachnolaimus maximus* (Walbaum), in the Florida Keys. University of Miami
11. Deniro MJ, Epstein S (1978) Influence of diet on distribution of carbon isotopes in animals. Geochima et Cosmochima Acta 42:495-506
12. 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. Marine Ecology Progress Series 415:189-200
13. FDEP (2019) Florida Department of Environmental Protection Aquatic Preserve Program. www.floridadep.gov/rcp/aquatic-preserve. Accessed 2019.
14. France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae - Foodweb implications. Marine Ecology Progress Series 124:307-312
15. Frank KT, Petrie B, Leggett WC, Boyce DG (2018) Exploitation drives an ontogenetic-like deepening in marine fish. Proceedings of the National Academy of Sciences of the United States of America 115:6422-6427
16. Fry B, Mumford PL, Robblee MB (1999) Stable isotope studies of pink shrimp (*Farfantepenaeus duorarum* Burkenroad) migrations on the southwestern Florida shelf. Bulletin of Marine Science 65:419-430

17. FWRI (2018) FL Fisheries Independent Monitoring Inshore Stratified-Random Sampling Records. In: Fisheries Independent Monitoring Program Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission
18. Gannon DP, McCabe EJB, Camilleri SA, Gannon JG et al. (2009) Effects of *Karenia brevis* harmful algal blooms on nearshore fish communities in southwest Florida. Marine Ecology Progress Series 378:171-186
19. 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. Marine Ecology Progress Series 141:13-20
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. Marine Ecology Progress Series 247:281-295
21. GMFMC (2018) Modification of Gulf of Mexico Red Snapper and West Florida Hogfish Annual Catch Limits, Gulf of Mexico Fishery Management Council
22. Gutierrez 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
23. Hazen EL, Maxwell SA, Bailey H, Bograd SJ, Hamann M, Gaspar P, Godley, BJ, Shillinger, GL (2012) Ontogeny in marine tagging and tracking science: technologies and data gaps. Marine Ecology Progress Series 457: 211-240
24. Heck KL, Hays G, Orth RJ (2003) Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series 253:123-136

25. Heppell SA, Semmens BX, Archer SK, Pattengill-Semmens CV et al. (2012) Documenting recovery of a spawning aggregation through size frequency analysis from underwater laser calipers measurements. Biological Conservation 155:119-127
26. Herzka SZ (2005) Assessing connectivity of estuarine fishes based on stable isotope ratio analysis. Estuarine Coastal and Shelf Science 64:58-69
27. Iverson RL, Bittaker HF (1986) Seagrass distribution and abundance in eastern Gulf of Mexico coastal waters. Estuarine Coastal and Shelf Science 22:577-602
28. Jackson AL, Parnell AC. Stable Isotope Bayesian Ellipses in R. 2.1.4 ed2019.
29. 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 7:11
30. Lara MR, Jones DL, Chen Z, Lamkin JT, Jones CM (2008) Spatial variation of otolith elemental signatures among juvenile gray snapper (*Lutjanus griseus*) inhabiting southern Florida waters. Marine Biology 153:235-248
31. 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. Bulletin of Marine Science 66:929-956
32. 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), U.S. Department of Commerce, Silver Spring, Maryland

33. Longmore C, Trueman CN, Neat F, Jorde PE et al. (2014) Ocean-scale connectivity and life cycle reconstruction in a deep-sea fish. Canadian Journal of Fisheries and Aquatic Sciences 71:1312-1323
34. 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 3:3
35. Matheson RE, Flaherty-Walia KE, Switzer TS, McMichael RH (2017) The importance of time of day in structuring demersal ichthyofaunal assemblages on the West Florida Shelf. Bulletin of Marine Science 93:407-437
36. McBride RS, Murphy MD (2003) Current and Potential Yield per Recruit of Hogfish, *Lachnolaimus maximus*, in Florida. Proceedings of the Fifty-Fourth Annual Gulf and Caribbean Fisheries Institute 54:513-525
37. 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. Bulletin of Marine Science 80:401-417
38. McConnaughey T, McRoy CP (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Marine Biology 53:257-262
39. 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
40. McMichael Jr R (2009) Fisheries-independent monitoring program 2008 annual data summary report. Report to the Florida Fish and Wildlife Conservation Commission from the Florida Fish and Wildlife Research Institute, Florida Fish and Wildlife Research Institute

41. 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. Continental Shelf Research 174:76-84
42. Minagawa M, Winter DA, Kaplan IR (1984) comparison of Kjeldahl and combustion methods for measurement of nitrogen isotope ratios in organic matter. Analytical Chemistry 56:1859-1861
43. 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. Bulletin of Marine Science 86:93-116
44. 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. Marine Biology 159:365-372
45. Nelson JA, Stallings CD, Landing WM, Chanton J (2013) Biomass Transfer Subsidizes Nitrogen to Offshore Food Webs. Ecosystems 16:1130-1138
46. Nielsen J, Hedeholm RB, Heinemeier J, Bushnell PG et al. (2016) Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*). Science 353:702-704
47. Oksanen J, Blanchet FG, Friendly M, Kindt R et al. (2018) Package 'vegan' Community Ecology Package
48. Olson NW, Guy CS, Koupal KD (2007) Interactions among three top-level predators in a polymictic Great Plains reservoir. North American Journal of Fisheries Management 27:268-278

49. Orth RJ, Heck KL, 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
50. Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology, Evolution, and Systematics 18:293-320
51. Pinnegar JK, Polunin NVC (2000) Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. Oecologia 122:399-409
52. 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. Marine Ecology Progress Series 302:233-243
53. 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 6:26
54. R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
55. Radabaugh KR, Hollander DJ, Peebles EB (2013) Seasonal delta C-13 and delta N-15 isoscapes of fish populations along a continental shelf trophic gradient. Continental Shelf Research 68:112-122
56. Radabaugh KR, Peebles EB (2014) Multiple regression models of delta C-13 and delta N-15 for fish populations in the eastern Gulf of Mexico. Continental Shelf Research 84:158-168
57. Randall JE, Warmke GL (1967) The food habits of the hogfish *Lachnolaimus maximus* a labrid fish from the Western Atlantic. Caribbean Journal of Science 7:141-144

58. Rester JK (2017) Environmental and biological atlas of the Gulf of Mexico 2016, Gulf States Marine Fisheries Commission, Ocean Springs, Mississippi
59. Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D (2018) Package 'mass' Functions and datasets to support Venables and Ripley, "Modern Applied Statistics with S"
60. Rooker JR, Secor DH, DeMetrio G, Kaufman AJ, Rios AB, Ticina V (2008) Evidence of trans-Atlantic movement and natal homing of bluefin tuna from stable isotopes in otoliths. Marine Ecology Progress Series 368:231-9.
61. Savercool DM, Lewis III RR (1994) Hard Bottom Mapping of Tampa Bay, Tampa Bay National Estuary Program, St. Petersburg, Florida
62. 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. Fishery Bulletin 113:442-455
63. Schloesser RW, Neilson JD, Secor DH, Rooker JR (2010) Natal origin of Atlantic bluefin tuna (*Thunnus thynnus*) from Canadian waters based on otolith delta C-13 and delta O-18. Canadian Journal of Fisheries and Aquatic Sciences 67:563-569
64. GSMFC. SEAMAP Trawl Data 2018. Available from: https://seamap.gsmfc.org/.
65. Searcy SP, Sponaugle S (2001) Selective mortality during the larval-juvenile transition in two coral reef fishes. Ecology 82:2452-2470
66. Shulman MJ (1985) Recruitment of coral-reef fishes - effects of distribution of predators and shelter. Ecology 66:1056-1066

67. Simpson SJ, Sims DW, Trueman, CN (2019) Ontogenetic trends in resource partitioning and trophic geography of sympatric skates (Rajidae) inferred fromstable isotope composition across eye lenses. Marine Ecology Progress Series 624:103-116
68. Smith EP (1985) Estimating the reliability of diet overlap measures. Environmental Biology of Fishes 13:125-138.
69. Smith GB (1975) 1971 Red tide and its impact on certain reef communities in mid-eastern Gulf of Mexico. Environmental Letters 9:141-152
70. SRS1 Software L SRS1 Cubic Spline for Excel
71. Stallings CD, Nelson JA, Rozar KL, Adams CS, Wall KR, Switzer TS, Winner BL, Hollander DJ (2015) 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
72. Stallings CD, Mickle A, Nelson JA, McManus MG, Koenig CC (2015b) Faunal communities and habitat characteristics of the Big Bend seagrass meadows, 2009–2010. Ecology 96:304-304
73. Switzer TS, MacDonald TC, McMichael RH, 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. Transactions of the American Fisheries Society 141:707-719
74. Switzer TS, Keenan SF, McMichael Jr RH, Fischer KM. Fisheries-independent data for juvenile Hogfish (*Lachnolaimus maximus*) from polyhaline seagrasses of the Florida Big Bend, 2008-2012, Fish and Wildlife Research Institute; 2013. SEDAR 37-08.

75. 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. Bulletin of Marine Science 11:552-649

76. Todd AC, Morey SL, Chassignet EP (2014) Circulation and cross-shelf transport in the Florida Big Bend. Journal of Marine Research 72:445-475

77. Trueman CN, MacKenzie KM, Palmer MR (2012) Identifying migrations in marine fishes through stable-isotope analysis. Journal of Fish Biology 81:826-847

78. 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. Environmental Conservation 29:484-492

79. Tzadik OE, Goddard EA, Hollander DJ, Koenig CC, Stallings CD (2015) Non-lethal approach identifies variability of delta N-15 values in the fin rays of Atlantic Goliath Grouper, *Epinephelus itajara*. PeerJ 3:22

80. Tzadik OE, Curtis JS, Granneman JE, Kurth BN et al. (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. Limnology and Oceanography: Methods 15:238-263

81. Vasconcelos RP, Reis-Santos P, Tanner S, Fonseca V et al. (2007) Discriminating estuarine nurseries for five fish species through otolith elemental fingerprints. Marine Ecology Progress Series 350:117-126

82. Victor BC (1986) Larval Settlement and Juvenile Mortality in a Recruitment-Limited Coral Reef Fish Population. Ecological Monographs 56:145-160

83. Vihtelic TS (2008) Teleost lens development and degeneration. In: Jeon KW (ed) International Review of Cell and Molecular Biology, Vol 269. Elsevier Academic Press Inc, San Diego, p 341-373

84. Wallace AA, Hollander DJ, Peebles EB (2014) Stable Isotopes in Fish Eye Lenses as Potential Recorders of Trophic and Geographic History. PLoS One 9:8

85. Weisberg RH, Zheng LY, Liu YG (2016) West Florida Shelf upwelling: Origins and pathways. Journal of Geophysical Research – Oceans 121:5672-5681

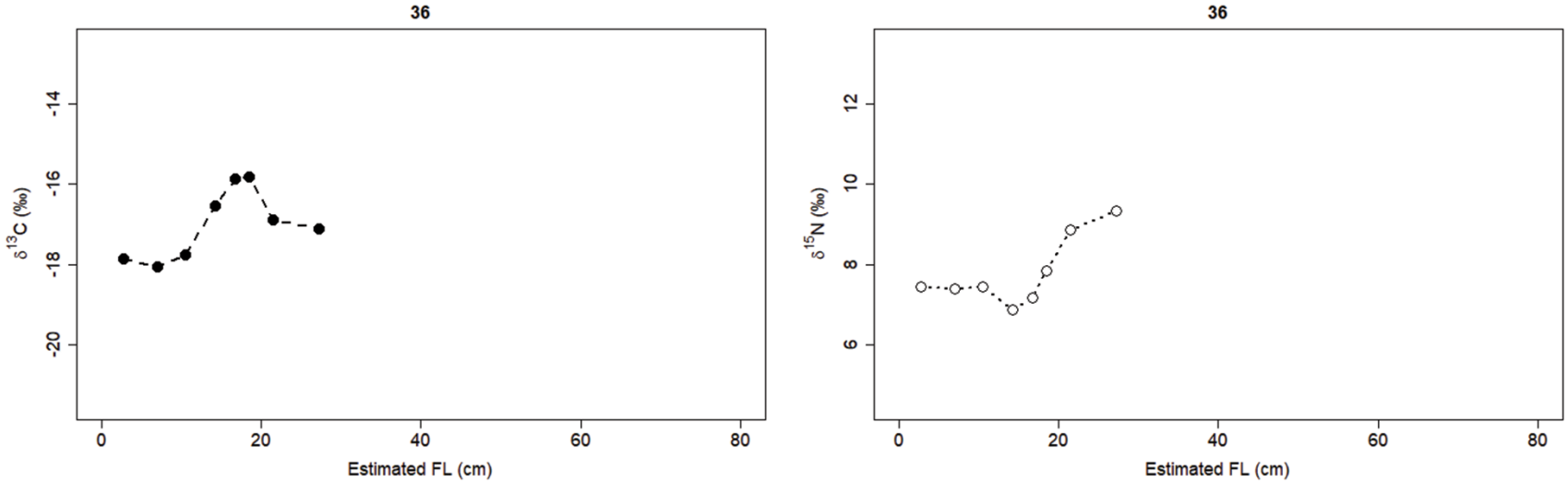
86. Weisberg RH, Zheng LY, Peebles E (2014) Gag grouper larvae pathways on the West Florida Shelf. Continental Shelf Research 88:11-23

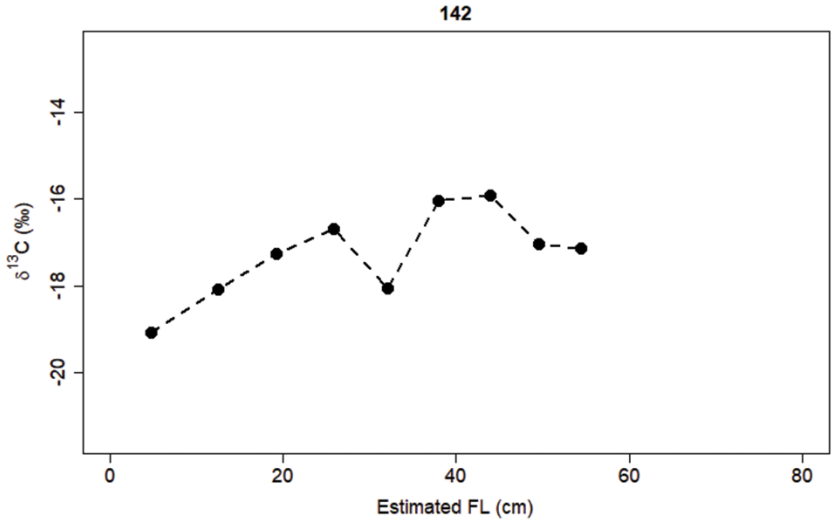
87. Zieman JC & Zieman RT (1989) The ecology of the seagrass meadows of the west coast of Florida USA: a community profile. US Fish and Wildlife Service Biological Report 85

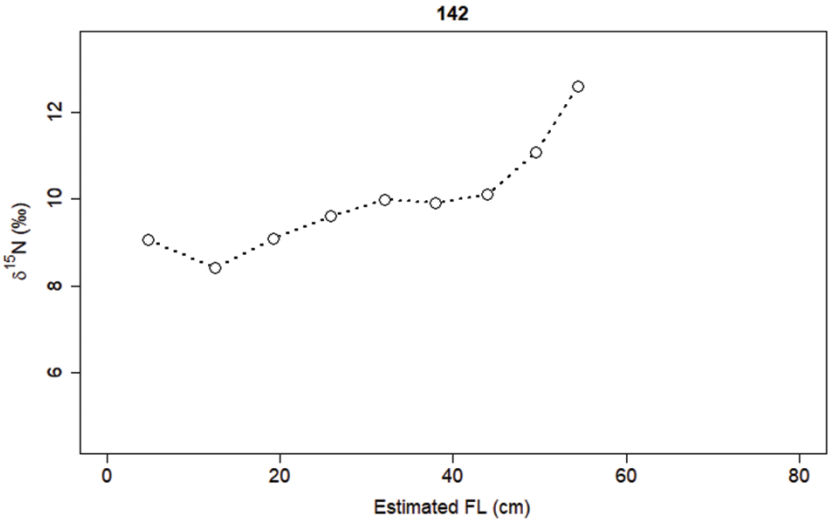
APPENDIX I: INDIVIDUAL HOGFISH STABLE ISOTOPE CHRONOLOGIES

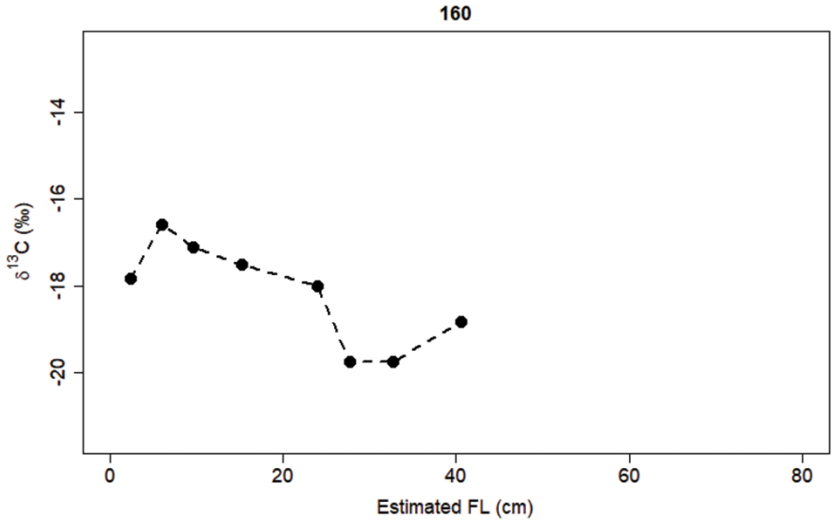
Plots displaying individual stable isotope value chronologies for all fish sampled. Isotope values are plotted against estimated fork length (cm), for $\delta^{13}C$ (left panel) and $\delta^{15}N$ (right panel). Groups are separated by geographic regions.

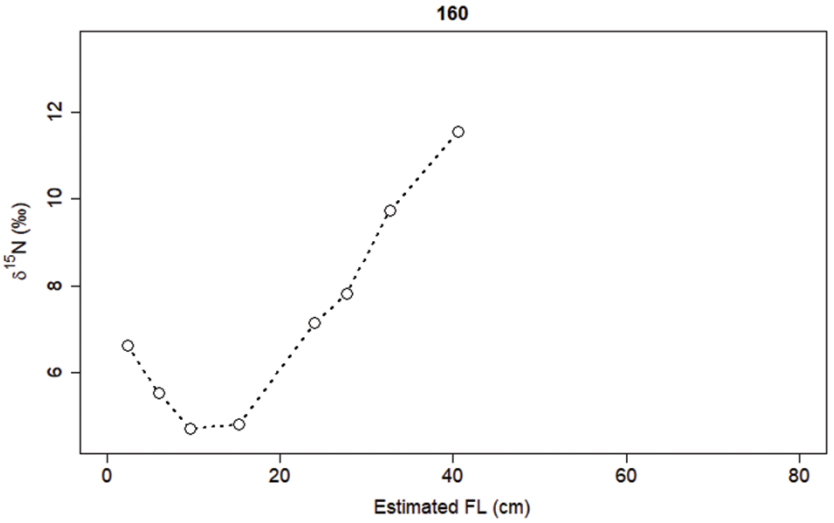
Shallow Big Bend



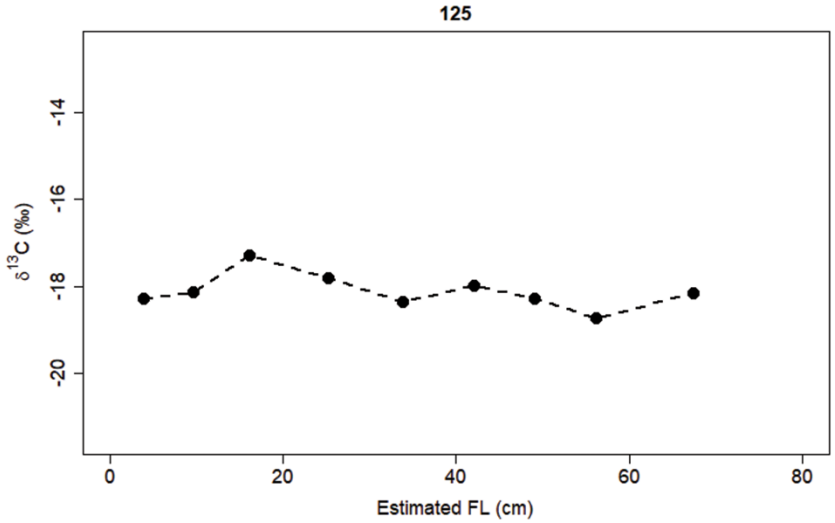

142
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

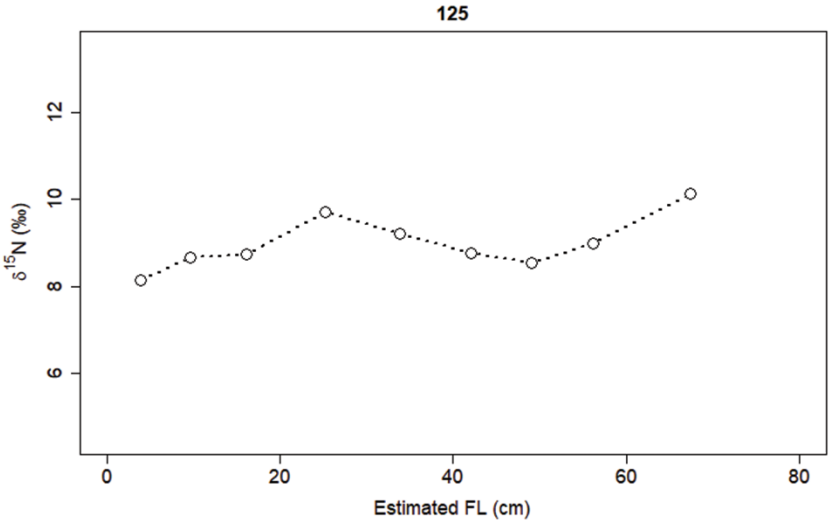

142
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

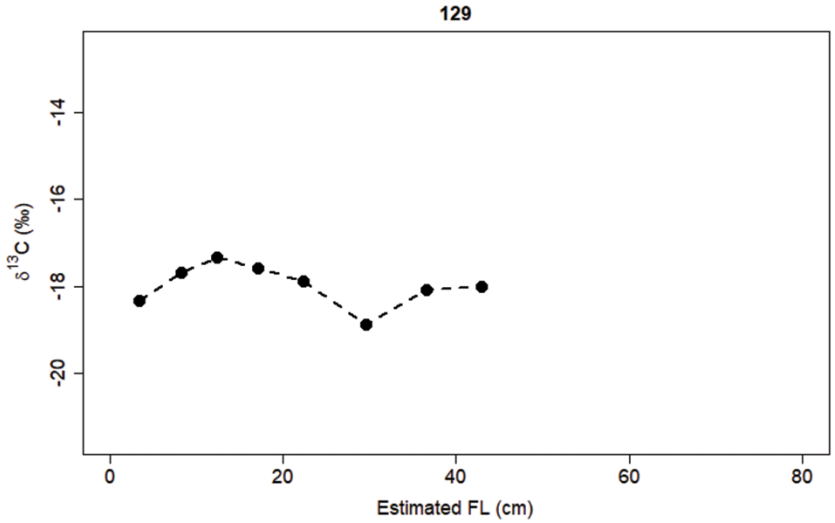

160
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

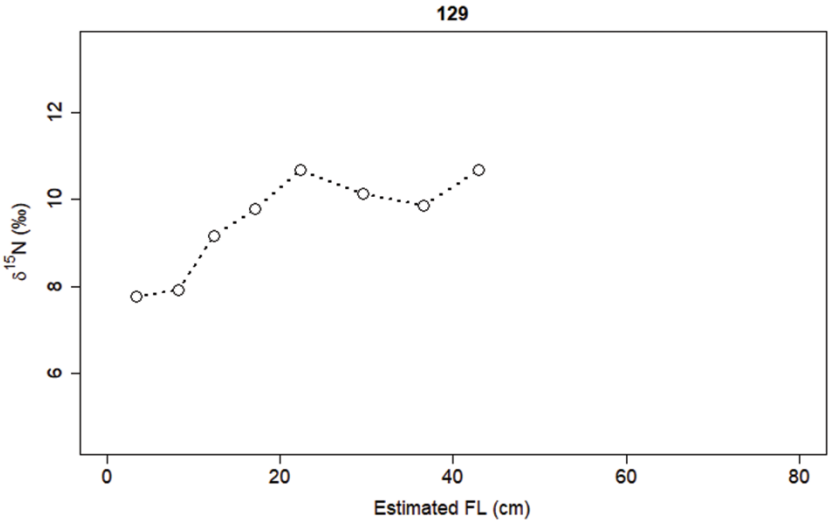

160
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

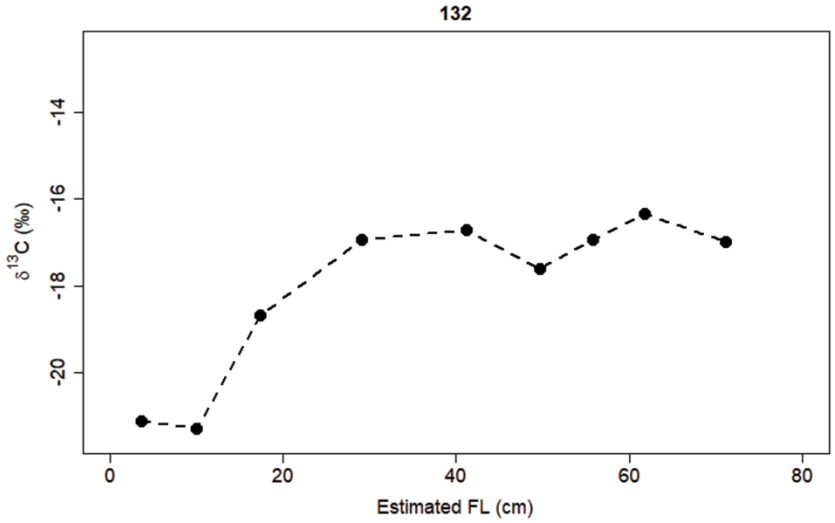
Deep Big Bend

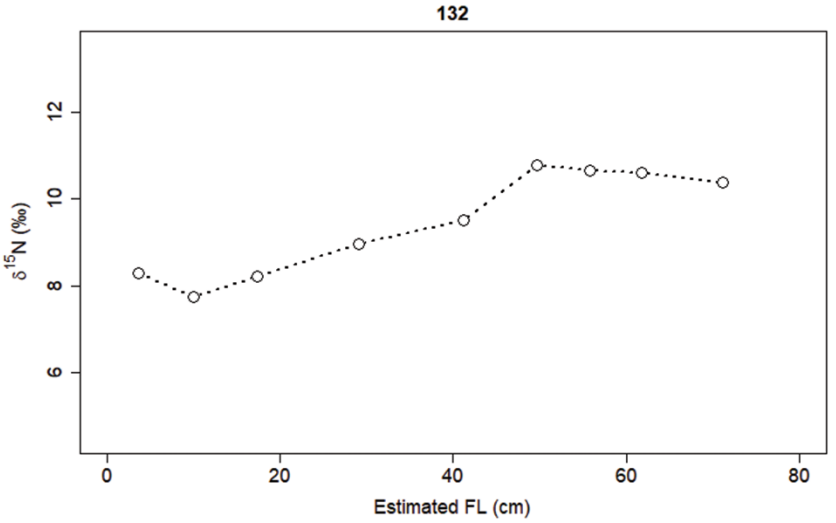


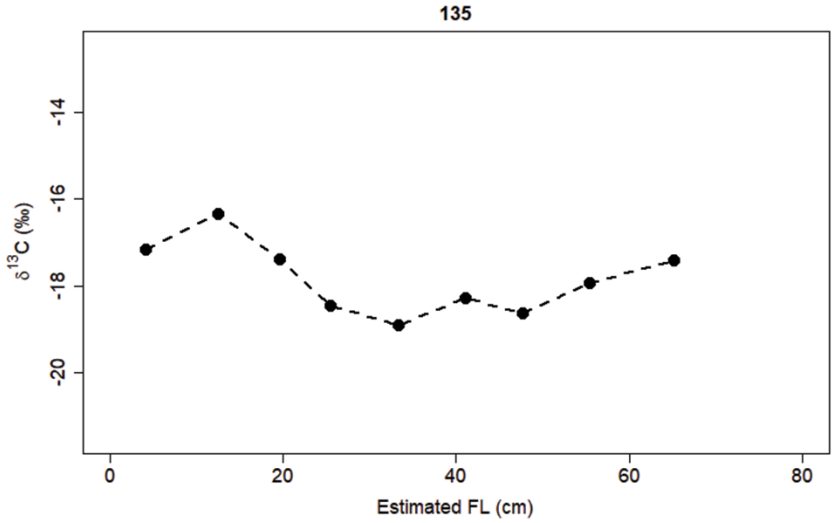


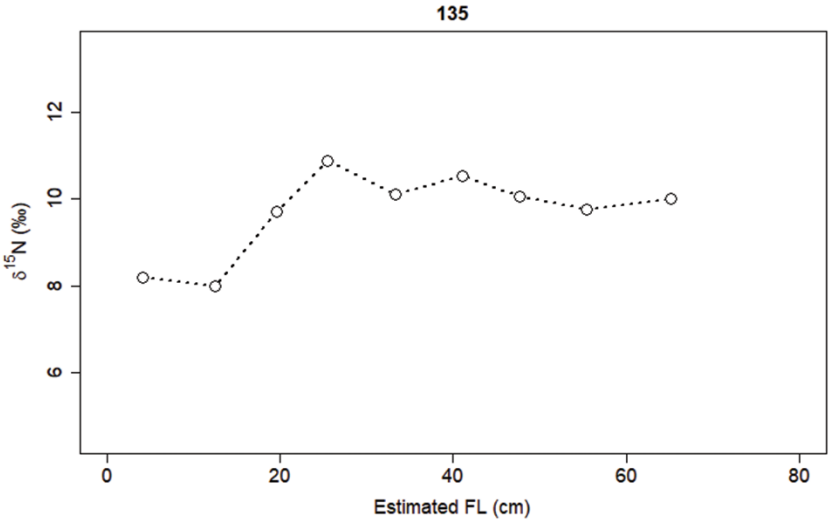


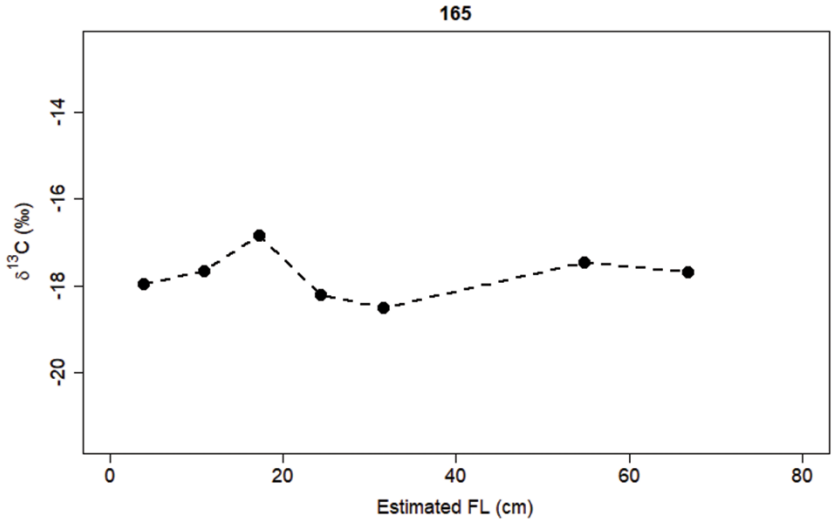


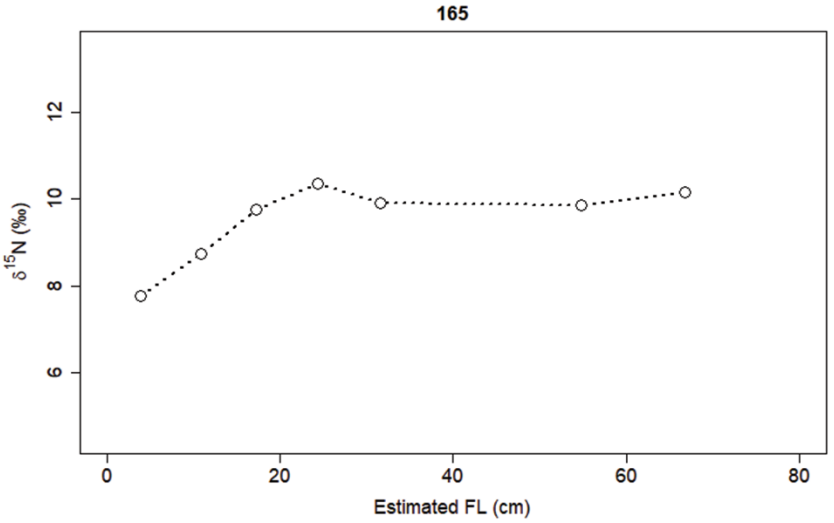

132
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)


132
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

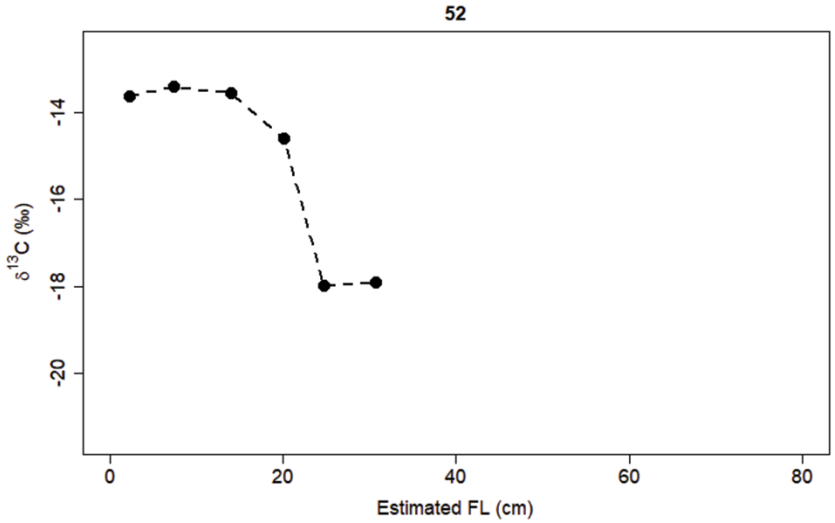

135
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

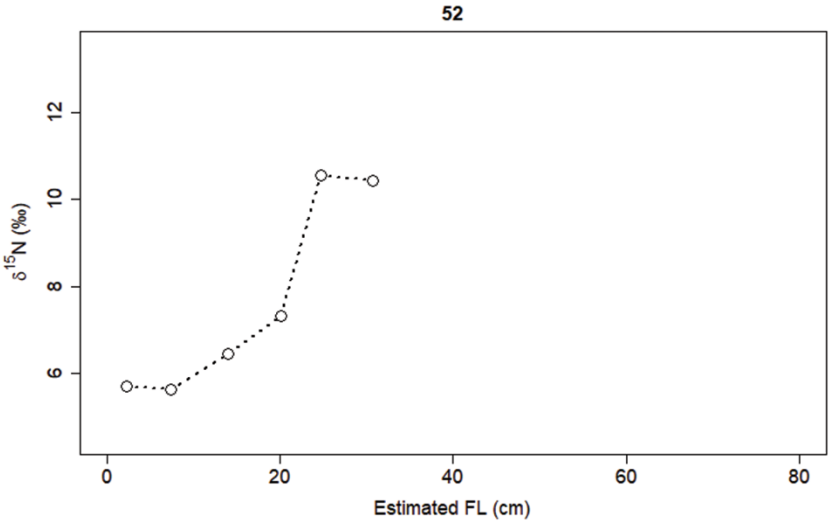

135
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

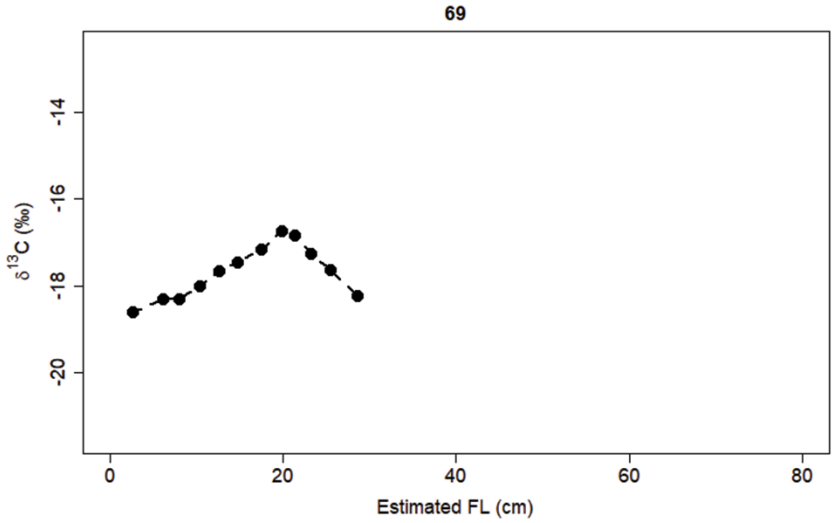


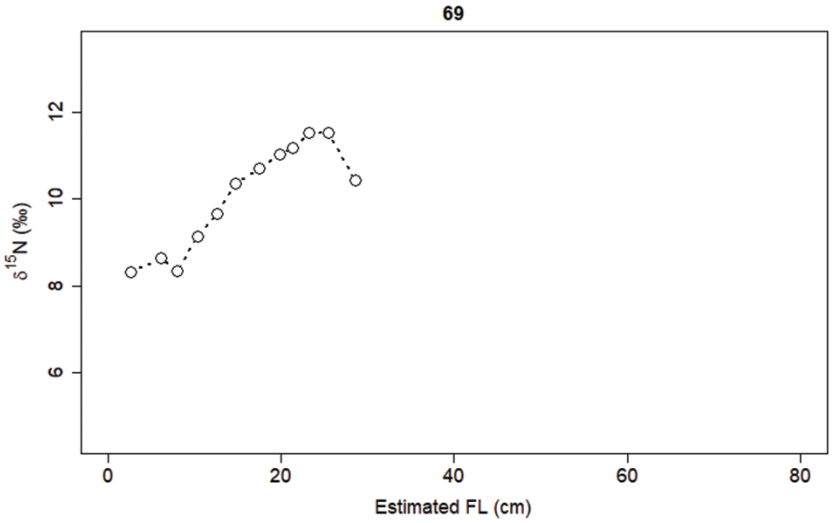


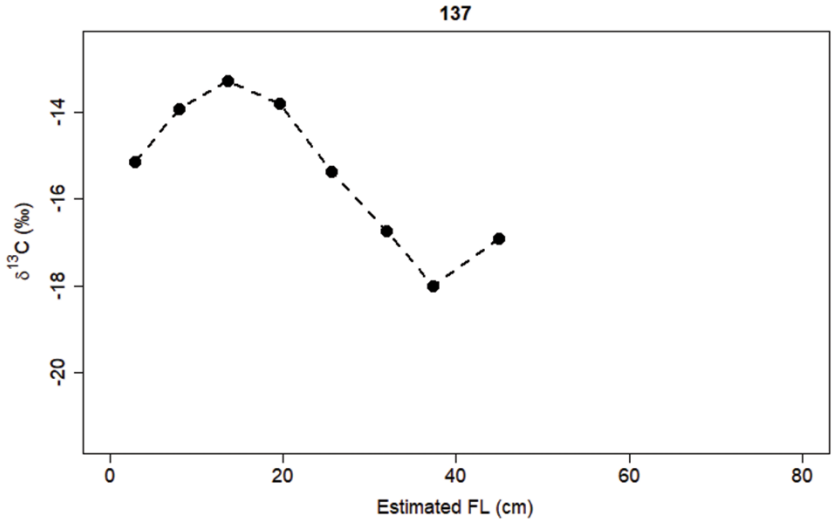
Shallow Tampa Bay

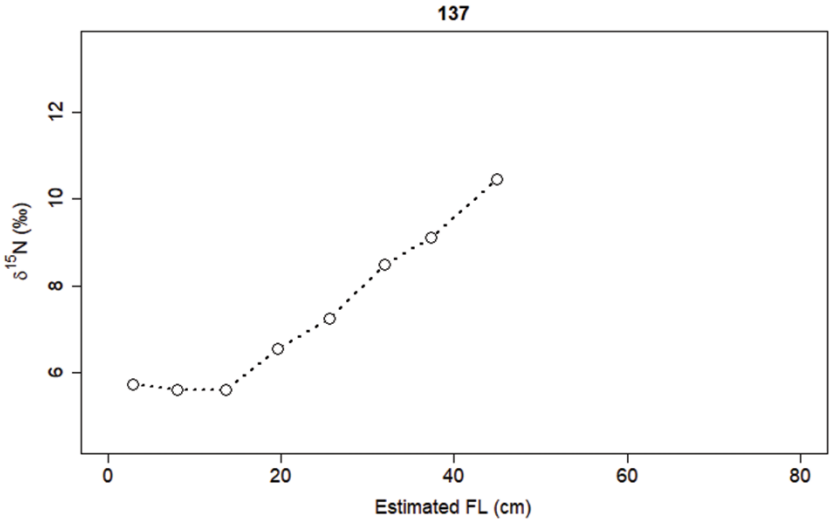




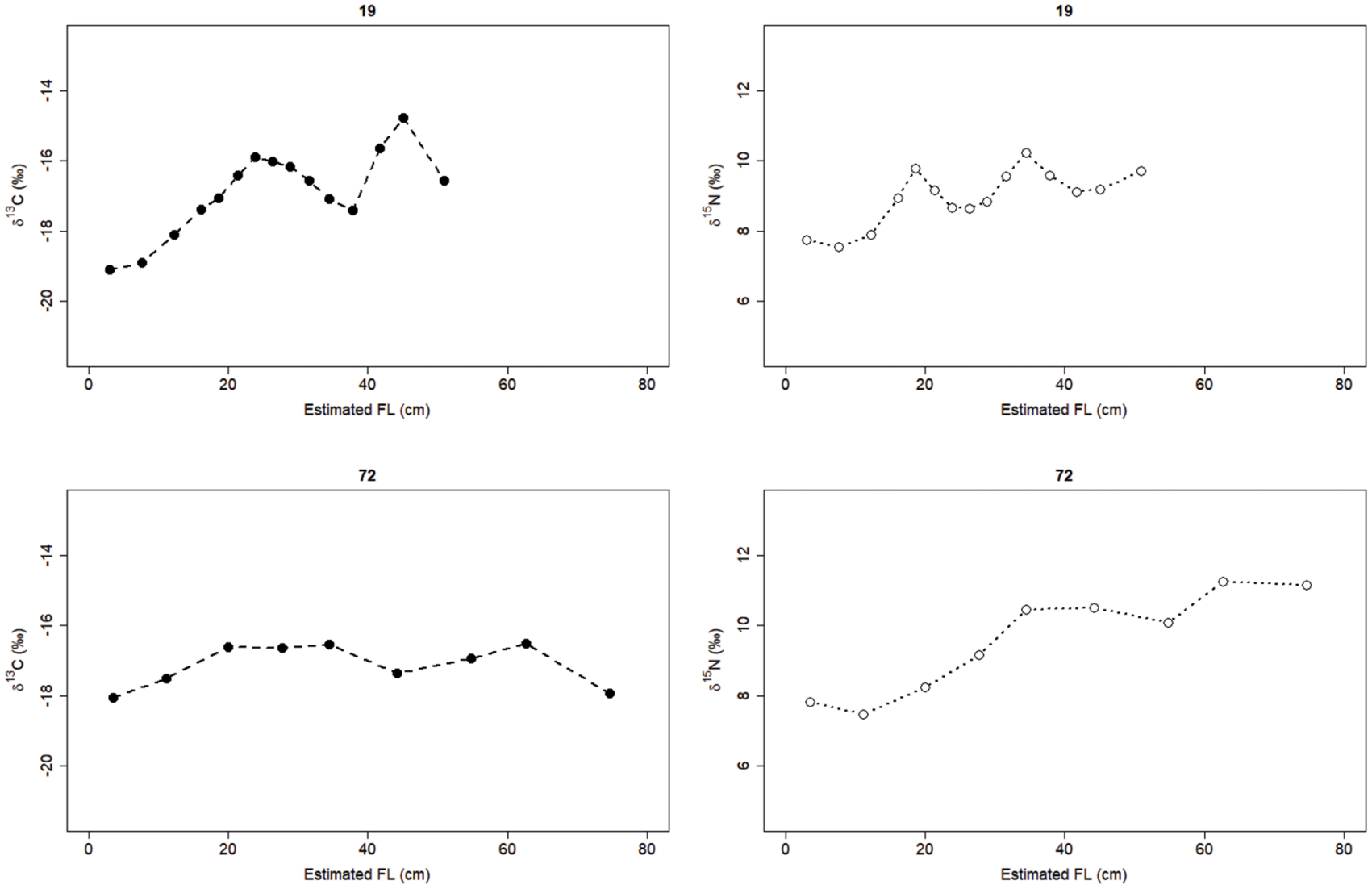

69
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

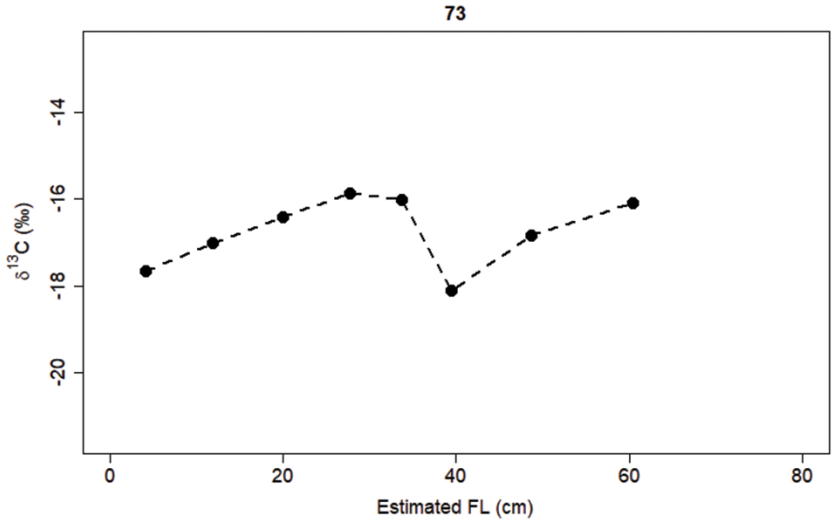

69
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

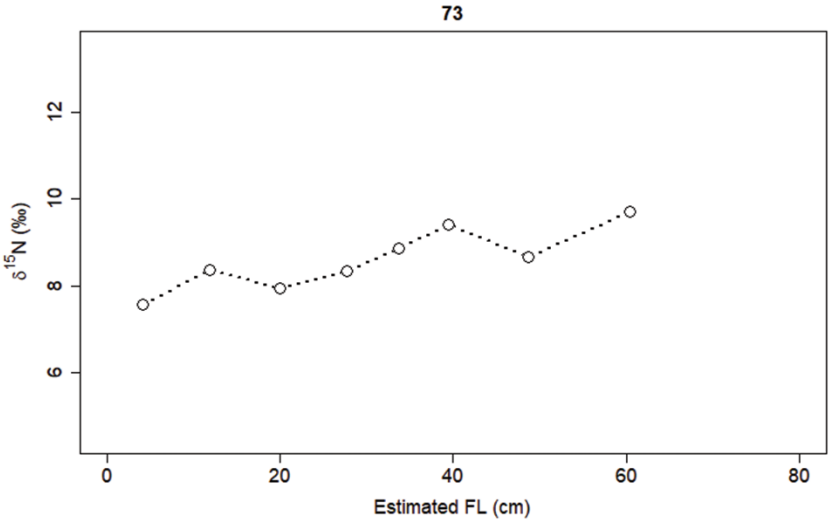

137
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

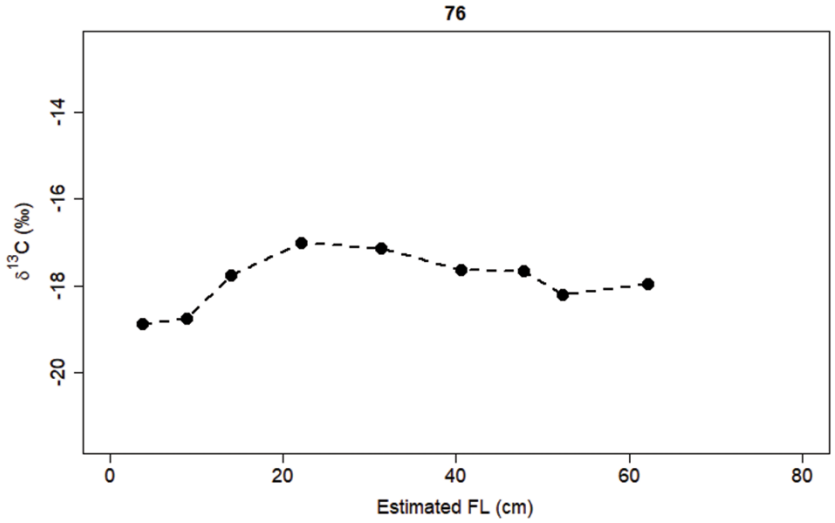

137
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

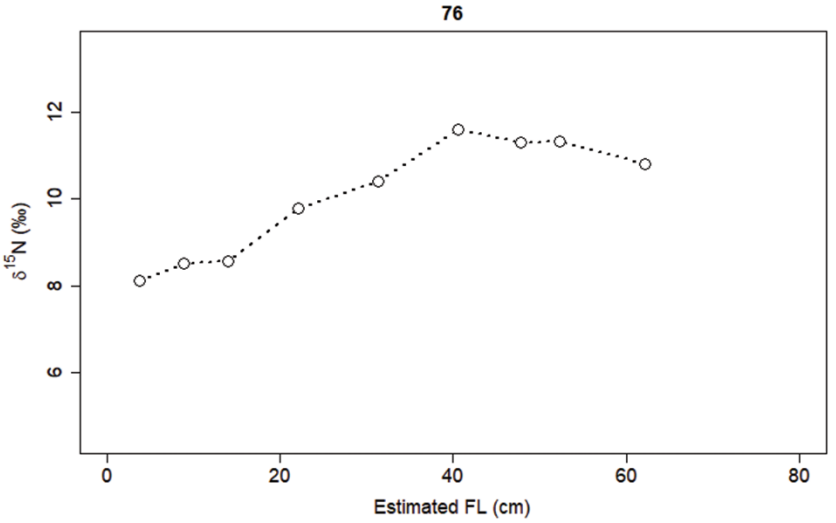
Deep Tampa Bay



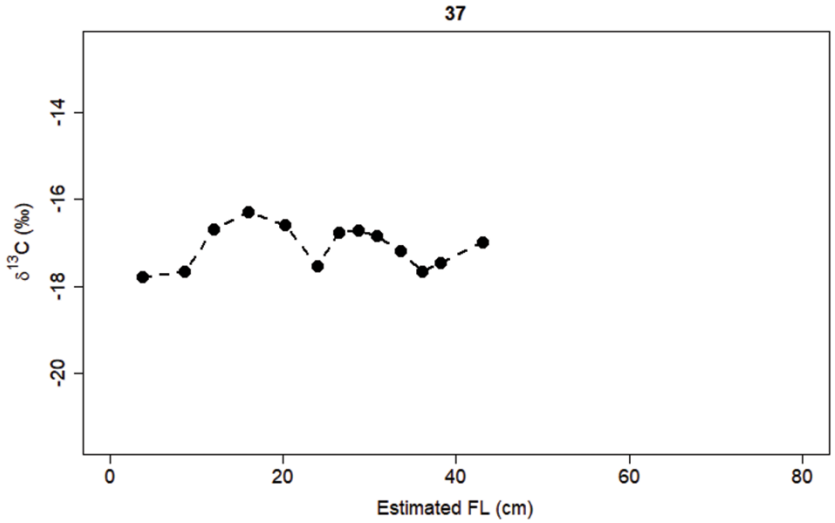

73
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

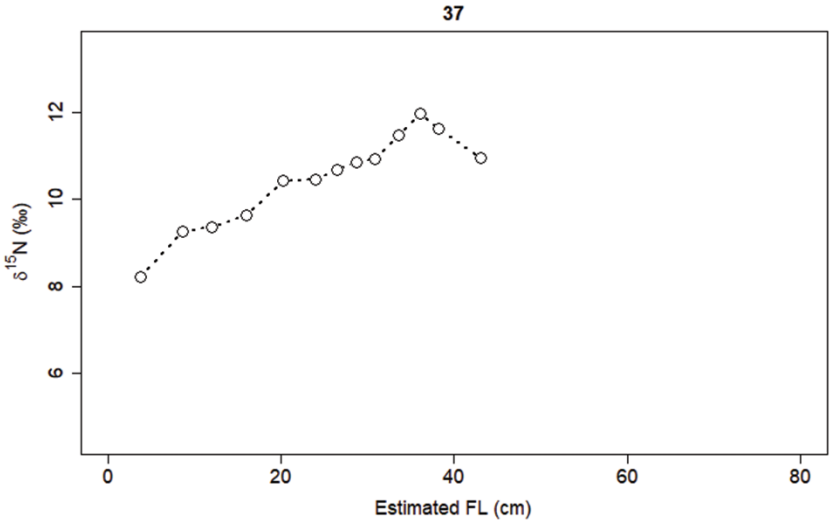

73
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

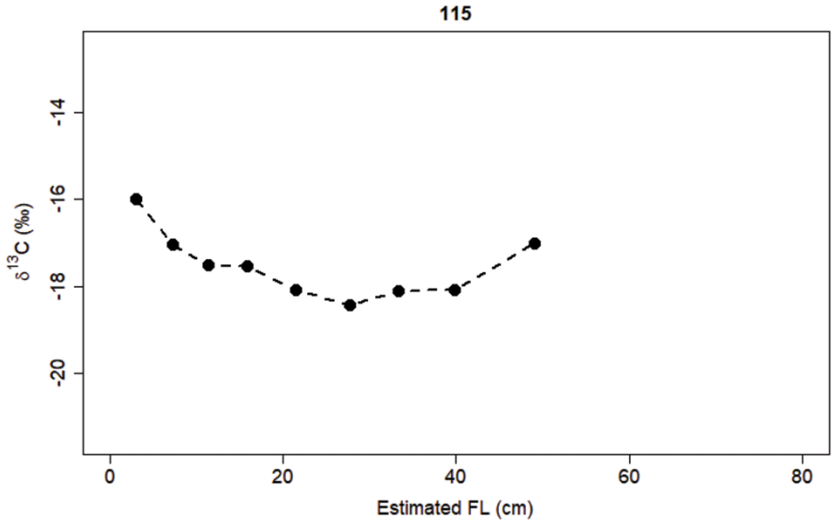

76
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

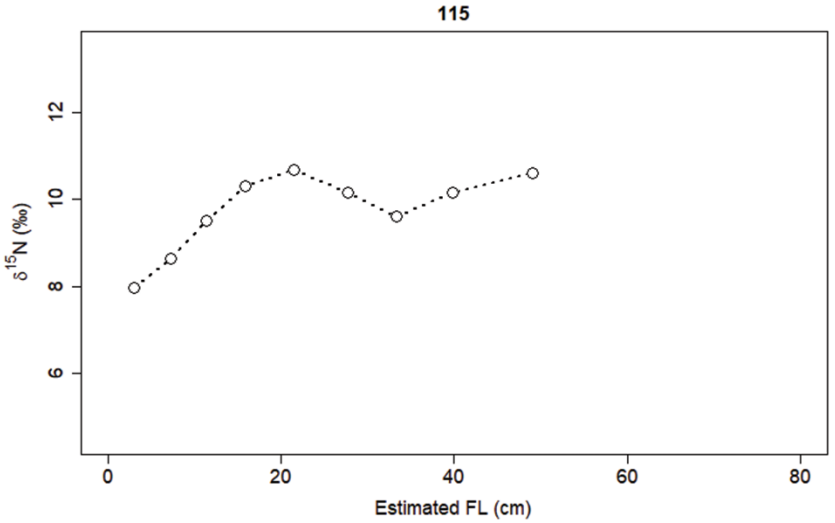

76
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

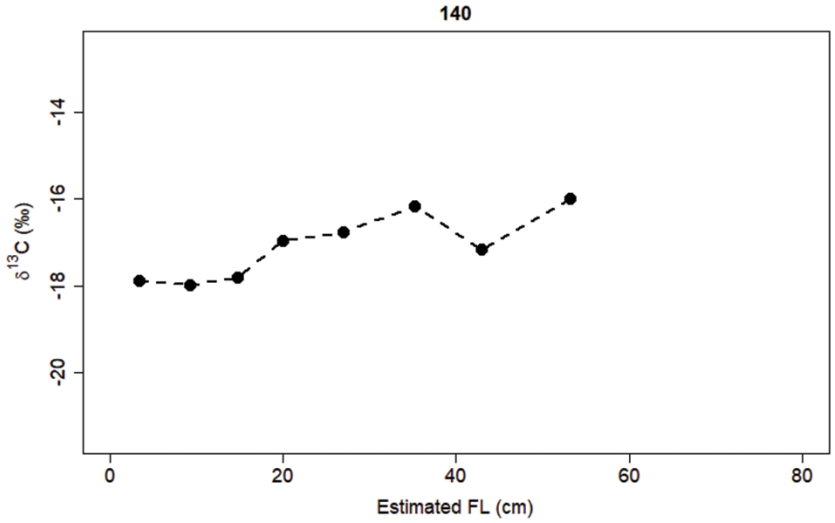
Shallow Charlotte Harbor

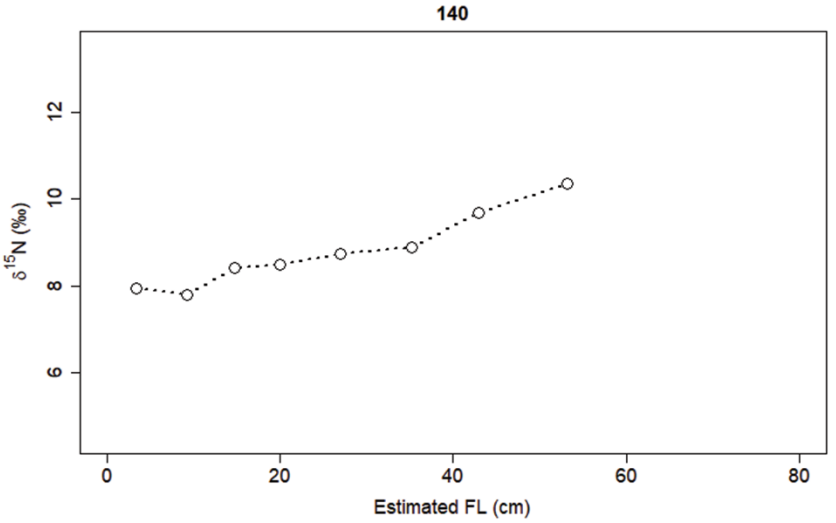


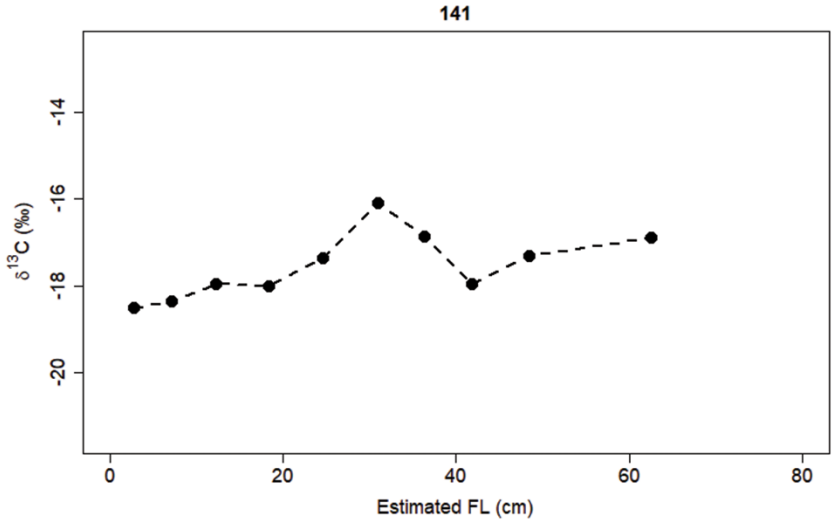


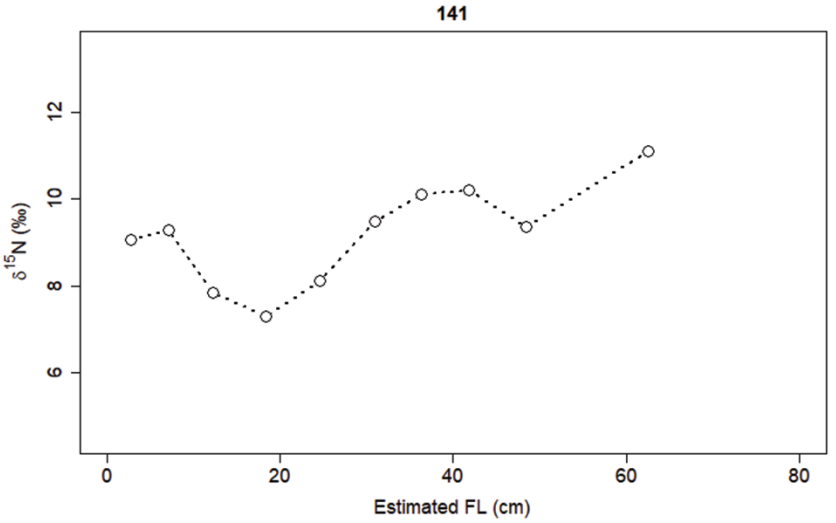




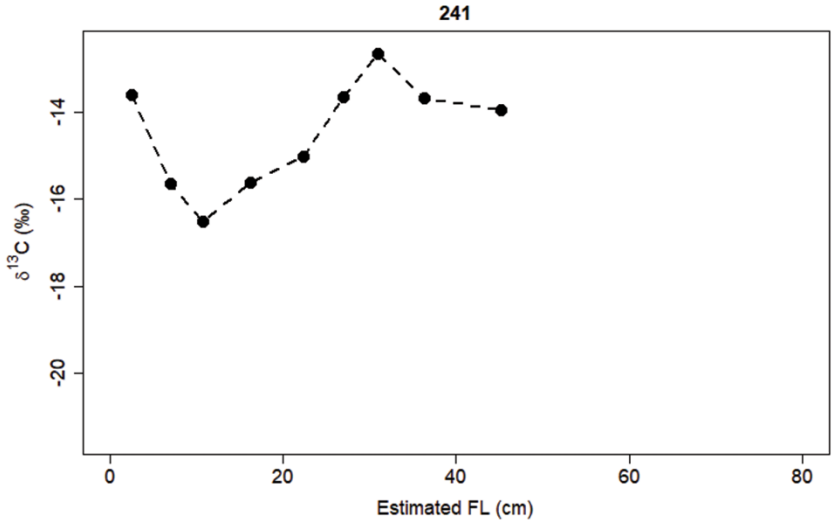

140
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

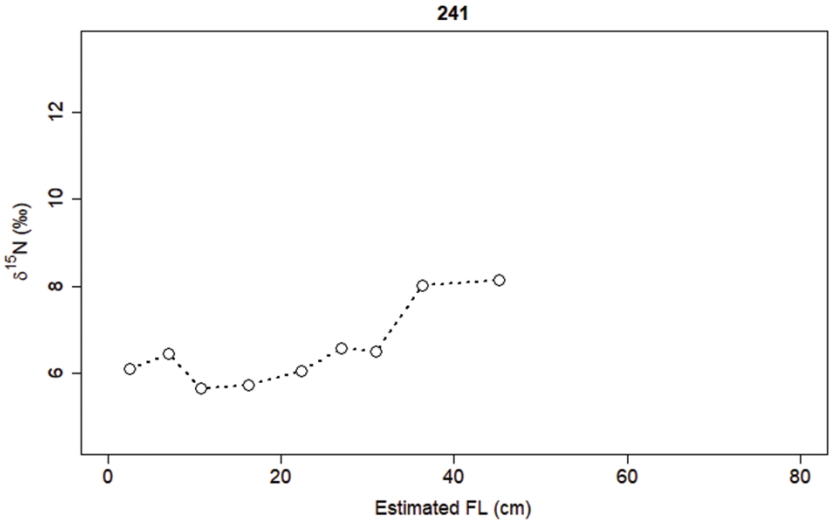

140
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)


141
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

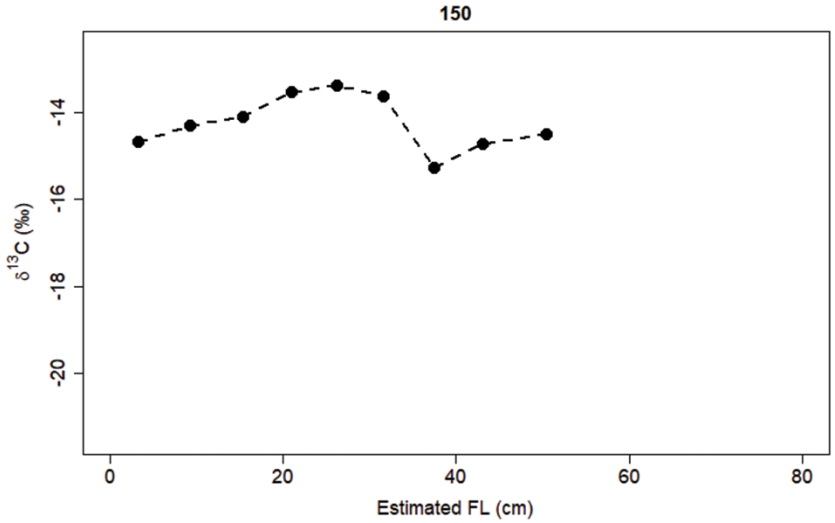

141
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

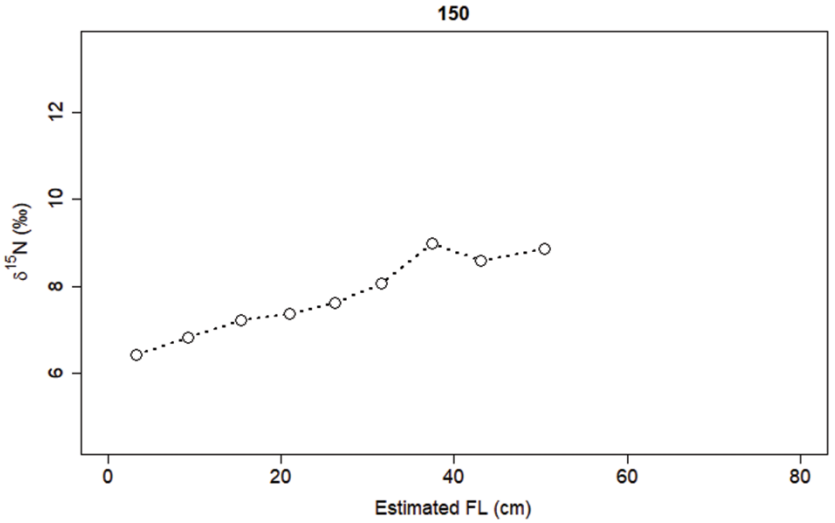
Deep Charlotte Harbor

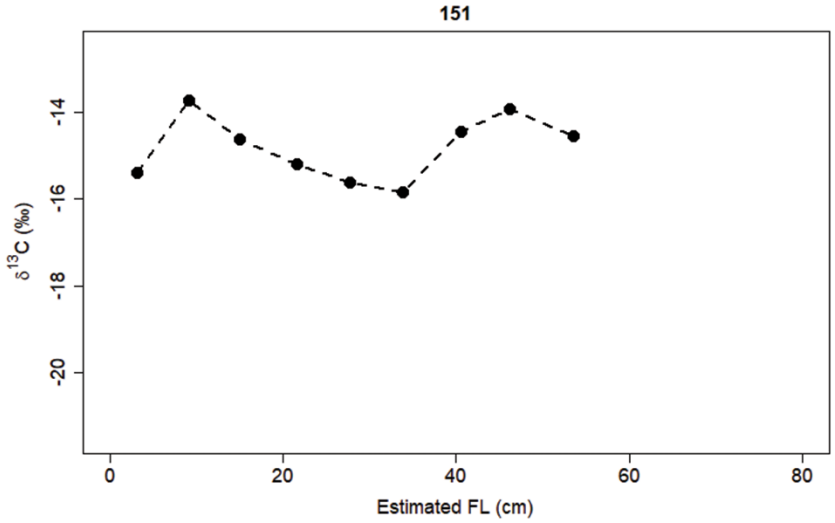


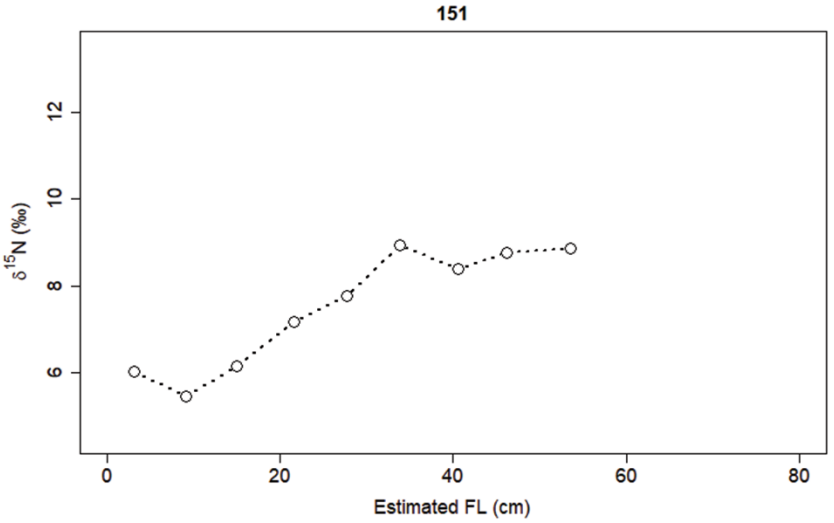


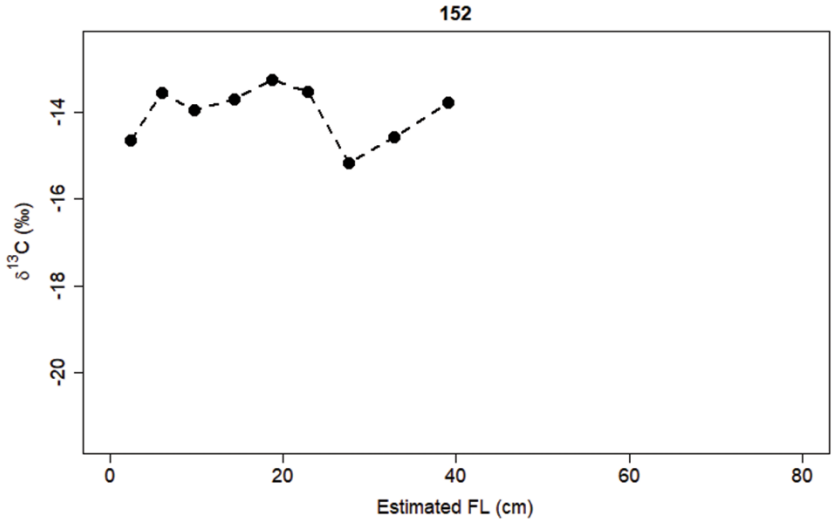
Shallow Keys

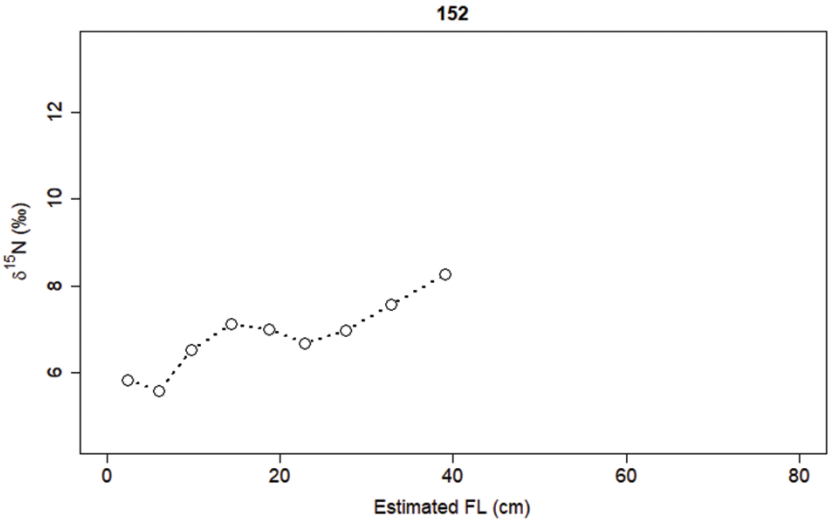


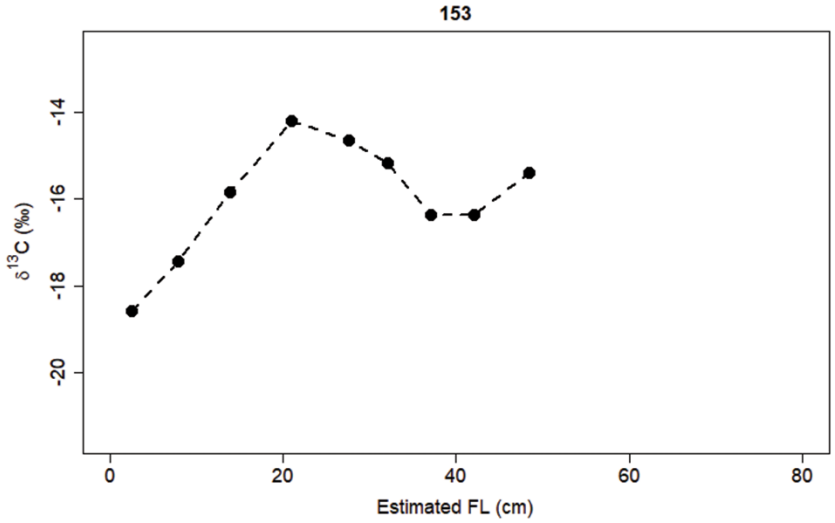


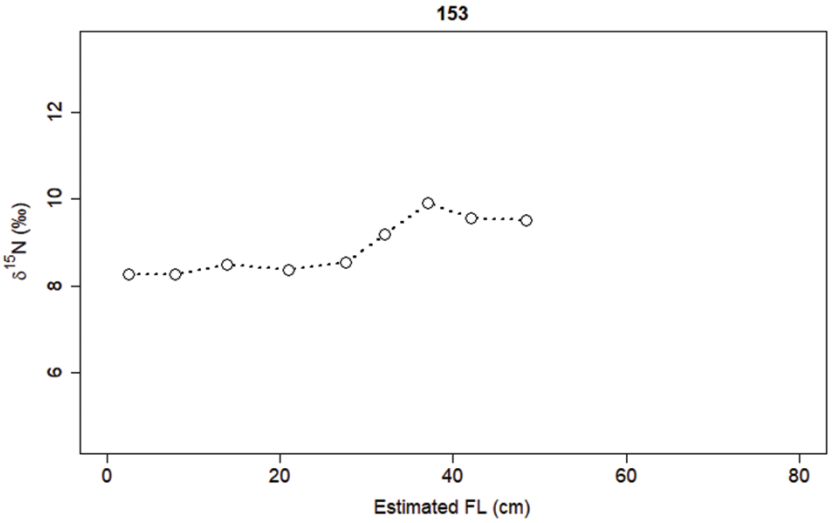

151
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)

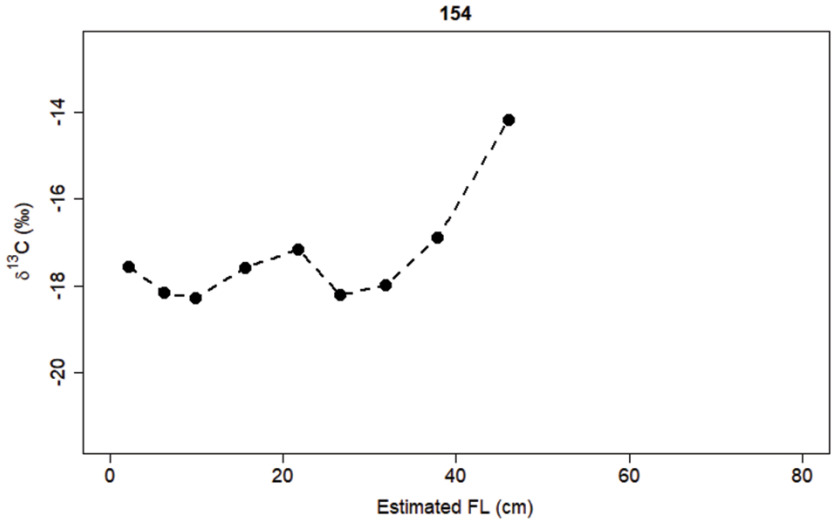

151
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)

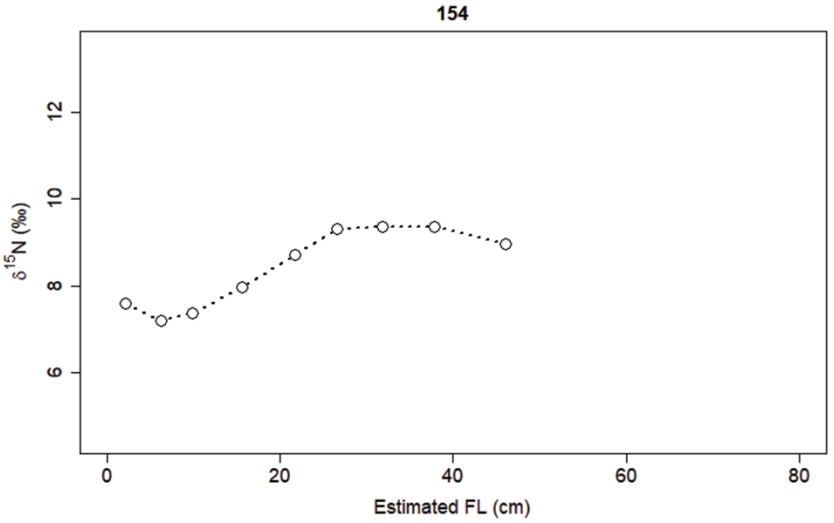

152
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)


152
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)


153
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)


153
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)


154
δ13C (‰)
-14
-16
-18
-20
0
20
40
60
80
Estimated FL (cm)


154
δ15N (‰)
12
10
8
6
0
20
40
60
80
Estimated FL (cm)