Resource Use Overlap in a Native Grouper and Invasive Lionfish

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Resource Use Overlap in a Native Grouper and Invasive Lionfish

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
College of Marine Science
University of South Florida

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ABSTRACT

Invasive species can severely disrupt biological communities through their interactions with native organisms, yet little is known about the response of marine predators to the establishment of a competitive invasive fish. In the western Atlantic, invasive Indo-Pacific lionfishes (*Pterois spp.*)) may represent a novel competitor to several commercially and ecologically important native species. However, there is a scarcity of empirical research documenting comparative resource use of cohabitant lionfish and native fishes, as well the physiological consequences that may result from interspecific interactions with the invasive species. For this thesis, I conducted two studies designed to elucidate the strength of resource use overlap and potential competition among invasive lionfish and an ecologically similar serranid, the Graysby (*Cephalopholis cruentata*), along a contiguous coral-reef ledge in Biscayne National Park, South Florida.

My first study aimed to determine whether lionfish and Graysby could be classified as competitors through comparisons of Graysby population size, diet, and condition across a range of ambient lionfish biomass. Using stable isotope and gut content analyses, I measured a difference in Graysby diet on sites with larger populations of lionfish, specifically a smaller breadth of resource use and lower consumption of teleost fish prey. Despite a shift in diet, Graysby condition did not vary with lionfish biomass, and thus this study did not provide unequivocal evidence of competition between the two species. However, based on a high amount of apparent overlap in interspecific resource use, competitive interactions between lionfish and species such as Graysby remain likely in systems with more limiting prey or shelter.
For my second study, I measured stable isotope values of muscle, liver, and eye lens layers in lionfish and Graysby to further compare individual and population-level patterns of diet and habitat use. The use of eye lenses as metabolically stable chronological recorders of stable isotopes has vast potential to provide insight about animal life history, but has not yet been applied to describe trends in resource use among invasive and native species. To aid these analyses I created a rudimentary map of spatial isotopic variation along the reef ledge of Biscayne National Park, which could serve as a frame of reference to study local-scale animal movements. Isotopic differences between liver and muscle samples suggested a broader range of movement in lionfish than Graysby, important for understanding the relative scale of habitat use in these species. In eye lenses, stable isotope values increased logarithmically with lens radius (i.e. fish size), likely reflecting patterns of trophic growth. There was a high amount of variability among the shapes of eye lens isotopic chronologies, particularly those of lionfish, yielding further information about movement and individual resource use specificity in these species.

The results of this thesis are the first to compare native predator diet and condition across a range of invasive lionfish biomass, as well as the first to measure size-structured trends in the resource use of individual lionfish. Together, these results enhance our understanding of the potential for competition among lionfish and native mesopredators, an important objective for researchers studying how this highly invasive species interacts with surrounding ecological communities.
CHAPTER ONE:

GENERAL INTRODUCTION

Invasive species pose a threat to biodiversity on a global scale, and can severely disrupt biological communities through their interactions with native organisms (Zavaleta et al. 2001, Strayer et al. 2006). Invaders can alter the dynamics of natural ecosystems via top-down and bottom-up influences (Dolman & Wäber 2008, Kurle et al. 2008, Gallardo et al. 2016), but can also can devastate native populations through intense resource competition (Mandrak & Cudmore 2010, Morales et al. 2013). However, little is known about the response of marine systems to the establishment of a competitive invasive fish. In the western Atlantic, invasive Indo-Pacific lionfishes (*Pterois miles* and *P. volitans*; hereafter, lionfish) have caused substantial depletions of prey communities (Albins & Hixon 2008, Green et al. 2014, Albins 2015) and demonstrated the potential to affect the habitat use of other fishes (Raymond et al. 2015). Thus, lionfish may represent a particularly potent competitor to native predators. However, the amount of resource use overlap and resultant competition among lionfish and other species has been sparsely documented within the invaded range (Layman & Allgeier 2012, Albins 2013, O’Farrell et al. 2014).

For this thesis, I measured patterns of resource use and the strength of competition between lionfish and a native mesopredator, the Graysby (*Cephalopholis cruentata*). Because mesopredators can regulate the abundance and behavior of other reef species, any influence of
lionfish on Graysby could strongly affect invaded communities (Stallings 2008, Feeney et al. 2012). Therefore, the results of my work provide useful insight to conservation scientists and resource managers working to predict the effects of lionfish on native predators as the species continues to integrate into western Atlantic ecosystems.

In Chapter Two, *Diet Shifts in a Native Mesopredator across a Range of Invasive Lionfish Biomass*, I studied the strength and nature of resource use overlap and competition between lionfish and Graysby. Data were collected along the reef ledge of Biscayne National Park, Florida, in conjunction with a Before-After-Control-Impact (BACI) lionfish removal experiment (Stallings & Albins 2016). Surveys of lionfish and Graysby populations allowed description of the relationship between biomass and density of these species, which could indicate whether interspecific interactions led to displacement from preferred habitat (Hardin 1960, Robinson & Wilson 1994, Bøhn et al. 2008). Additionally, using a combination of gut content and stable isotope analyses, I measured whether variation in the biomass of ambient lionfish was associated with differences in Graysby diet and resource use (Bearhop et al. 2004, Layman et al. 2007, Jackson et al. 2011). Finally, by quantifying Graysby condition across a range of lionfish biomass, I examined whether the two species could be considered competitors in Biscayne National Park (Brian 1956, Petren & Case 1996). The results of this chapter will be submitted for publication to the journal *Marine Ecology Progress Series* in late 2016.

In Chapter Three, *Life History Patterns of Invasive Lionfish and a Native Mesopredator Described using Stable Isotopes of Muscle, Liver, and Eye Lenses*, I performed analyses of stable isotopes to further compare patterns of population- and individual-level resource use between lionfish and Graysby. The use of eye lenses as metabolically stable chronological recorders of stable isotopes has vast potential to provide insight about animal life history, yet has only
recently been applied to the study of fish ecology (Wallace et al. 2014, Nielsen et al. 2016). This chapter represents the first use of such isotopic data-series to describe the life history of individual invasive lionfish, as well as the first study to compare the spatiotemporal resource use of an invasive and native species using chemical information from eye lenses. Using the same specimens from Chapter Two, I created a rudimentary map of spatial isotopic variation along the reef ledge of Biscayne National Park, which could be useful for interpreting local-scale movements of lionfish, Graysby, and other species of management interest (Barnes et al. 2009, Radabaugh et al. 2013). By studying eye lens isotopic chronologies, I described general life history features of lionfish and Graysby, including the relationship of trophic level to size, relative patterns of movement, and level of individual resource use specificity (Kim et al. 2012, Wallace et al. 2014, Acosta-Pachon et al. 2015). I corroborated interpretations about population-level movement of both species by measuring stable isotope values in two tissues with different metabolic turnover rates, liver and white muscle (Hobson 1999, Fry et al. 2003, Davis et al. 2015). The results of this chapter are being prepared for submission to a peer-reviewed journal.

In combination, these two studies yield important insight into patterns of interspecific resource use and interaction among invasive lionfish and native predators in south Florida. Additionally, my work demonstrates the power of integrating diverse datasets and methodologies to describe the ecology of an invasive species, particularly through the application of sophisticated stable isotopic analyses. This thesis not only enhances our understanding of the effects that invasive lionfish may have on native predators, but also informs the efforts of conservation scientists working to measure and predict the implications of biological invasions across diverse ecosystems.


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Stallings CD, Albins MA (2016). Final report to Florida Sea Grant on Project # R/LR-B-66H


CHAPTER TWO:

DIET SHIFTS IN A NATIVE MESOPREDATOR ACROSS A RANGE OF INVASIVE LIONFISH BIOMASS

Abstract

In marine ecosystems, little is known about how competition with invasive fishes may affect the resource use of native predators. Throughout the western Atlantic, invasive Indo-Pacific lionfishes (*Pterois* spp.) are likely to compete with native mesopredators such as the Graysby (*Cephalopholis cruentata*), an ecologically similar serranid. In conjunction with a Before-After-Control-Impact lionfish removal experiment, this study measured whether Graysby population size, diet, and condition varied in relation to cohabitant lionfish biomass. Lionfish, Graysby, and prey populations were surveyed and sampled along a contiguous reef ledge in Biscayne National Park, south Florida. Mesopredator diet was measured with stable isotope (δ13C and δ15N) and gut content analyses, and isotopic niches were plotted to compare patterns of inter- and intraspecific resource use diversity. The isotopic niches of Graysby and lionfish overlapped by 67%, indicating similar population-level resource use. On sites with higher lionfish biomass, Graysby isotopic niche was 33% smaller and overlapped 25% less with that of lionfish, suggesting both a narrower breadth of resource use and associated interspecific niche segregation. Although 25% fewer Graysby stomachs contained fish on high lionfish biomass sites, prey fish populations did not vary accordingly, potentially signifying interference by lionfish on Graysby foraging
behavior. However, Graysby condition did not relate to lionfish biomass, so the two species ultimately could not be classified as competitors. By discussing potential influences of lionfish on Graysby resource use, my research contributes important information to the study of how invasive lionfish may affect native predator communities.

**Introduction**

Interspecific competition is a critical component of invasive species ecology, and can impart broad, long lasting effects on native species and communities through multiple mechanisms (Zavaleta et al. 2001, Strayer et al. 2006). When a novel invader depletes common resources, exploitative competition can force native organisms to rely on sub-optimal prey and habitat (Park 1954, Petren & Case 1996, Bøhn & Amundsen 2001). Invasive species can also preclude access to preferred resources through non-consumptive interactions, constituting interference competition (Brian 1956, Kiesecker et al. 2001, Warnock & Rasmussen 2013). Both forms of competition can lead to reductions in fitness, physiological condition, or population growth of the native competitor (Park 1954, Schoener 1982, Petren & Case 1996). In extreme cases, successful invaders can completely displace indigenous species, causing local extirpations and losses of biodiversity (Mandrak & Cudmore 2010, Morales et al. 2013). Alternatively, adaptation can drive niche partitioning as species optimize the use of new resources to ameliorate the effects of competition (Schoener 1974, Ross 1986, Cherel et al. 2008). Studying the nature and outcome of competitive interactions between invasive and native species is therefore key to predicting the long term effects of biological invasions and informing subsequent management efforts.

In marine ecosystems, little is known about how competition may shape the resource use of native fishes following the establishment of an invasive species. As prominent marine invaders, Indo-Pacific lionfishes (*Pterois miles* and *P. volitans*; hereafter, lionfish) have received
considerable attention from both the scientific community and the public following their precipitous spread throughout the western Atlantic. Due to their high consumption rates, lionfish can reduce the abundance and diversity of native prey (Albins & Hixon 2008, Green et al. 2014, Albins 2015), potentially resulting in exploitative competition with similarly sized native predatory fishes. Lionfish diet has been found to overlap with that of native predators (Layman & Allgeier 2012, O’Farrell et al. 2014), a necessary precursor of exploitative competition.

Additionally, lionfish occupy a broad range of habitats including seagrass beds (Jud & Layman 2012), mangrove roots (Morris & Akins 2009), artificial structures (Dahl & Patterson 2014), patch reefs (Layman & Allgeier 2012), and complex contiguous reefs (Albins 2015), and thus are likely to encounter a wide diversity of commercially and ecologically important species. Given that lionfish can engage in aggressive, territorial behavior (Fishelson 1975) and may incur avoidance in other predators (Raymond et al. 2015), interspecific interactions also have the potential to result in interference competition. Despite the high likelihood of competition, only Albins (2013) has examined the physiological consequences of interaction between invasive lionfish and a native predator, finding no effect of the invasive species on the growth rate of Coney (*Cephalopholis fulva*). However, the author noted that the duration of that experiment may have been too short to allow detection of the long-term effects of interspecific competition.

To date, no study has measured population-level variation in the diet and condition of predators native to the western Atlantic across a range of lionfish biomass.

One likely competitor with lionfish throughout much of the invaded range is the Graysby (*Cephalopholis cruentata*), a reef-dwelling serranid with similar maximum size, habitat use, diet, and life history patterns as the invasive species (Nagelkerken 1979, Sluka et al. 1998, Morris & Akins 2009). In the Red Sea, native lionfish densities increased following removal of
Cephalopholis spp., suggesting competition with Graysby congeners (Shpigel & Fishelson 1991a). As mesopredators, Graysby can mediate the abundance and behavior of other reef species, particularly through top-down effects on prey density and diversity (Stallings 2008, Feeney et al. 2012). Any influence of lionfish on Graysby could therefore disrupt invaded communities, especially in southeast Florida where Graysby are among the most abundant grouper species (Sluka et al. 1998). Thus, understanding how the lionfish invasion can affect Graysby and other mesopredators is a pertinent objective for resource managers and conservation ecologists working to protect and restore invaded western Atlantic ecosystems.

This study examined whether Graysby population metrics, diet, and physiological condition varied across naturally occurring and experimentally-induced gradients of lionfish biomass, allowing assessment of the potential effects of interspecific competition. I compared lionfish and Graysby diet via stable isotope analysis (SIA), a useful tool for describing resource use overlap between two species (Iken et al. 2001, Jackson et al. 2011, Knickle & Rose 2014). I also applied a combination of SIA and gut content analyses to assess whether Graysby diet composition related to ambient lionfish biomass. Concurrent measurements of Graysby condition allowed me to discuss how changes in diet were reflected in population-level physiology, and thus whether lionfish and Graysby could be classified as competitors in the study area.

**Materials and Methods**

**Study Region and Sites**

My study was performed in Biscayne National Park (BNP) in southeast Florida (Figure 1), where a complex, contiguous reef ledge supports comparable densities of both lionfish and Graysby. Data was collected from eighteen 1000m² sites (20 - 25m depth) distributed along 25km of reef as part of an associated lionfish removal experiment (Stallings & Albins 2016).
Each site was randomly assigned to one of three levels of a lionfish removal treatment: control (no removal, n = 6 sites), tri-annual removal (n = 6 sites), and monthly removal (n = 6 sites). Sites were surrounded by a 500m buffer zone where lionfish were not removed for the duration of the experiment. Although recreational anglers had unrestricted access to the sites, creel surveys have suggested that the catch of lionfish and Graysby was extremely low along BNP’s reef ledge during the study period (BNP, unpublished data).

**Surveys and Removals**

The accompanying two-year removal experiment used a Before-After-Control-Impact (BACI) study design, with one year of surveys before (January, May, July, September 2014) and after (December 2014, May, July, September 2015) the initiation of lionfish removal in late September 2014. No data from May 2014 were included for analysis, as inclement weather prevented the completion of surveys. The BACI study structure allowed for potential analyses of Graysby diet and condition across both natural (spatial) gradients of lionfish biomass and removal-driven (temporal) gradients. On each sampling trip, scientific SCUBA divers measured populations of lionfish, native predators, and native prey on all sites. Estimates of abundance and length of lionfish and native predators, including Graysby, were made over the entire site using a roving protocol designed to enhance lionfish detection (Green et al. 2013). Subsequently, two divers estimated abundance and length of all native fishes along adjacent permanent belt transects (25m x 2m). All surveys took place from 0800 – 1900 and were directed into the prevailing current.

Lionfish removals were performed by BNP scientific staff, following the completion of surveys in months when the two activities overlapped. On removal sites, all individuals were collected with a polespear, placed on ice, frozen at the end of the day, and eventually transported
to the University of South Florida (USF) for further analysis. At the conclusion of the study, lionfish were collected from all sites, including those in the control treatment. Graysby were sampled during July and September of both years, after the completion of native fish surveys. To avoid causing local depletions or strongly affecting Graysby population dynamics, a maximum of three individuals per sampling effort were removed from each site prior to the final survey of the experiment. Graysby were collected via polespear, when necessary with the assistance of a 20% solution of Quinaldine ($C_{10}H_9N$), a commonly used fish anesthetic (Gibson 1967). Sampled Graysby were stored on ice after each dive and dissected the same day.

**Dissections**

Before dissection, all frozen lionfish were thawed at room temperature. Thawing was not required for Graysby, which were dissected on the day of capture. Standard length (cm), total length (cm), and total mass (g) of each individual were measured. The viscera of each fish was then removed from the anterior end of the esophagus to the posterior end of the digestive tract and weighed. When intact, the liver was separated and weighed, followed by the eviscerated soma of each fish. Graysby viscera were preserved in a 30% Formalin solution for a minimum of 48 hours before transfer to a 50% isopropanol solution. Lionfish viscera were frozen for future analyses not included in this study.

**Stable Isotope Analyses (SIA)**

For SIA in both lionfish and Graysby, approximately 1 cm$^3$ of tissue was removed from the musculature posterior to the dorsal fin, the same location used in previous measurements of lionfish stable isotopes (Layman & Allgeier 2012). Freezing was chosen as the preferred method for fish tissue storage, as it does not impart preservation-driven offsets in stable isotope values (Stallings et al. 2015). I measured the two stable isotopes most commonly used in diet studies:
$^{13}\text{C}$, which reflects basal resource use of the sampled organism, and $^{15}\text{N}$, which describes relative food web positioning and increases with trophic level (Peterson & Fry 1987). Muscle tissue was freeze dried at -40°C for at least 36 hours and mechanically ground until homogenized. For analysis, 400 - 1000µg of material was collected and weighed on a Mettler-Toledo precision micro-balance, wrapped in tin capsules and loaded into a Costech Technologies Zero-Blank Autosampler. Samples were combusted at 1050°C in a Carlo-Erba NA2500 Series-II Elemental Analyzer coupled in continuous-flow mode to a Finnigan Delta Plus XL isotope ratio mass spectrometer. Measurements of molar C:N were calibrated and isotopic measurements were normalized to the AT-Air and VPDB scales, respectively, using NIST 8573 and NIST 8574 L-glutamic acid Standard Reference Materials. Measurements were expressed in units per mil (‰) using δ notation, where $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, and R is the isotopic ratio of interest (e.g. $^{13}\text{C}$:$^{12}\text{C}$). Analytical precision, estimated by replicate measurements of a working standard (NIST 1577b, Bovine Liver, n = 176) was ±0.16‰ ($\delta^{15}\text{N}$), ±0.13‰ ($\delta^{13}\text{C}$), and ±0.27‰ (C:N). Two replicate samples were analyzed for each fish, and the results were averaged for further statistical comparison. The mean difference (±SD) between replicate samples was 0.00±0.15‰ for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, indicating a high degree of consistency among isotopic measurements of the same material.

**Stomach Contents**

SIA provides an integrated measurement of isotopic input from diet over the turnover period of sampled tissue, which for fish muscle takes weeks to months (Trueman et al. 2005, Ankjærø et al. 2012, Mohan et al. 2016). Therefore, it is also informative to consult stomach contents to obtain a snapshot of feeding trends and ground-truth stable isotope data (Overman & Parrish 2001, Condini et al. 2014, Knickle & Rose 2014). However, to robustly compare fine-scale
patterns in consumption, larger sample sizes are usually required than were available for this study (Ferry & Cailliet 1996, Cortés 1997). Additionally, delays between capture and dissection led to stomach contents frequently being digested beyond the point of high taxonomic resolution. Therefore, I limited my analysis to quantification of the presence/absence of invertebrate and teleost prey, which was performed via examination of Graysby gut contents under a dissecting microscope.

**Condition Indices**

I used four physiological metrics to assess whether Graysby condition varied across a range of lionfish biomass: Ricker’s condition index, the hepatosomatic index (HSI), muscle C:N ratios, and gut fullness. Ricker’s condition index can be applied to readily compare the condition of two populations experiencing different environmental conditions (Weatherley 1972), and is described by the equation $K = \frac{W}{aL^b}$, where $W =$ observed weight (g), $L =$ length (cm), and $a, b$ are species-specific growth parameters (Le Cren 1951). Parameters for Graysby ($a = 0.0079$, $b = 3.22$) were calculated using non-linear least squares regression on morphometric data measured in specimens collected for this experiment (Figure 2). These parameters may better describe weight-length relationships of Graysby in southeast Florida than those derived in the Caribbean (Nagelkerken 1979, Potts & Manooch 1999) due to regional differences in maximum size and growth patterns. The HSI describes energy reserves and fish condition, as the liver is a primary site for lipid deposition in teleost fishes (Delahunty & De Vlaming 1980, Stallings et al. 2010). HSI values were calculated as $100 \times \frac{W_l}{W_s}$, where $W_l =$ liver weight, and $W_s =$ somatic weight, or weight of the fish after removal of the viscera (Jensen 1979). Because Graysby were collected only in the summer, relative comparisons of HSI values were assumed to be unaffected by seasonal fluctuations which can influence lipid deposition and confound analyses of condition.
(Adams & McLean 1985). Measurements of muscle C:N ratios can also serve as a proxy for lipid content (Post et al. 2007), and were calculated as a component of SIA. Samples with larger C:N ratios are higher in fat content, as lipids are richer in carbon than the amino acids predominant in lean muscle (Woodland & Secor 2011). Gut fullness was assessed to link physiological condition indices to the ecological process of foraging. Graysby stomach fullness was measured using a metric described by Haram & Jones (1971). Each stomach was assigned a fullness value based on the following rubric: 0 (completely empty stomach), 0.5 (trace of food present), 1 (1/4 full), 2 (1/2 full), 3 (3/4 full), 4 (full), 5 (stomach completely distended).

**Statistical Analysis**

For all frequentist analyses in this study, statistics were calculated with distribution-free, non-parametric tests derived from the Fathom Toolbox for MATLAB (Jones 2015). Significance was assessed using p-values calculated by comparing the position of test statistics derived from the original data relative to a distribution of the same statistic calculated from n = 1000 randomized permutations of the dataset (Moore et al. 2009). Statistics that were more extreme than 95% of permuted values (2-tailed) were considered to be significant (α = 0.05). Although these methods allowed me to relax some of the strict assumptions of parametric statistical tests, particularly that of normally distributed errors, they can be sensitive to the inclusion of dependent or spatially structured data (Anderson 2001). Therefore, for tests involving multiple individuals collected from the same study site, which represent subsamples rather than independent replicates, randomization was constrained to within study sites (Legendre & Legendre 1998, Økland 2007).

After a review of preliminary results, I determined that there was too much variability in the success of lionfish removals for temporal comparison of Graysby diet and condition; not enough sites showed rapid and persistent decreases in lionfish population size for statistically powerful
analyses using the BACI study structure. Therefore, for several subsequent analyses sites were
grouped into two lionfish biomass categories (LBCs) based on each site’s average lionfish
biomass relative to the mean value of the study region (Figure 3). Estimates of lionfish and
Graysby biomass were derived from visual diver surveys using the same weight-length
relationship used to calculate Ricker’s condition index ($W = aL^b$). Growth parameters for lionfish
($a = 0.0082, b = 3.18$) were derived from local specimens collected through BNP’s lionfish
removal program (Stallings & Albins 2015, unpublished data). To ensure that the size structure
of collected individuals did not systematically vary between LBCs, I used a 2-way Kolmogorov-
Smirnov test to compare distributions of both species’ standard lengths. There was no evidence
that the size distribution of either lionfish ($ks$-stat = 0.13, $p = 0.220$) or Graysby ($ks$-stat = 0.07,
$p = 0.986$) differed between LBCs.

To measure evidence of potential interspecific habitat exclusion or population declines driven
by competitive interactions, I examined trends among site-averaged lionfish and Graysby
population metrics (density and biomass) with tests of Pearson’s correlation coefficient.
Calculations were performed separately for both years of the study (before and after lionfish
removal), as well as among treatment groups (control vs. removal sites), to assess whether
systematic culling may have affected interspecific population dynamics. Both density and
biomass values were estimated based on information from visual diver surveys.

Similarly, linear regressions were performed to describe how prey abundance and species
richness varied with site-wise lionfish biomass. Although all native fishes were surveyed, only
individuals ≤10cm were classified as potential prey, as larger teleosts do not contribute
substantially to the diet of either lionfish (Morris & Akins 2009, Muñoz et al. 2011) or Graysby
(Nageklerken 1979, Stallings 2008). Site-wise averages of predator and prey community compositions were compared across LBCs using one-way non-parametric MANOVAs.

Relationships between stable isotope values and standard length were assessed using linear regressions to measure trophic trends with growth of both species. Patterns of change in stable isotope values related to size are inconsistent in marine fishes, but can be associated with ontogenetic shifts in diet and habitat (Rau 1982, Cocheret de la Morinière et al. 2003, Nakamura et al. 2008). Comparisons of mean stable isotope values were made with non-parametric t-tests, and 95% confidence intervals were calculated for differences among means via bootstrapping with n = 1000 permutations (Moore et al. 2009).

In addition to calculating mean stable isotope values, I compared the dispersion of lionfish and Graysby isotopic measurements to provide information on inter- and intraspecific patterns of resource use diversity (Bearhop et al. 2004, Layman et al. 2007). Analyses of isotopic variation have previously been used to assess population-level diet in terrestrial and marine species (Fry et al. 1978, Newsome et al. 2009), including lionfish (Layman & Allgeier 2012). Comparing the relative width, position, and overlap of lionfish and Graysby isotopic niches can help indicate the extent to which the two species share prey and habitat, a precursor to competition if those resources become limiting (Tilman 1977, Feiner et al. 2013, O’Farrell et al. 2014). Describing differences in Graysby isotopic niche width across a range of lionfish biomass may additionally suggest whether the invasive species can affect the breadth of Graysby resource use. Lionfish and Graysby stable isotope dispersions were quantified using SIBER analysis (Stable Isotope Bayesian Ellipses in R), which describes aspects of a population’s isotopic niche by plotting and measuring the bi-variate standard deviation, or standard ellipse area (SEA), of isotope bi-plots (Jackson et al. 2011). The value used for statistical comparisons of isotopic niche width was
SEA_b, or the mode of n = 20,000 standard ellipses generated via Bayesian permutation, with a significance cutoff for the test set at Bayesian probability = 95%. Before applying SIBER analysis, multivariate outliers were identified via the MVN package in R using a Mahalanobis measure of distance from the multivariate centroid (Korkmaz et al. 2014). Because isotopic outliers can still yield important information about individual foragers (Layman et al. 2007), only the most extreme values (Mahalanobis distance >10) were removed from SIA calculations. In total, four lionfish and two Graysby were excluded as multivariate isotopic outliers.

Proportions of full (fullness index ≥4) and empty (fullness index ≤0.5) stomachs, as well as stomach content presence/absence data, were compared using a Pearson’s 2x2 Chi-Square Test. Mean condition indices were compared across LBCs using non-parametric t-tests, and were tested for a relationship with site-wise lionfish biomass via linear regressions.

**Results**

*Population Measurements*

Out of all possible correlations of interspecific population metrics, the only nearly significant relationship was a negative trend between Graysby density and lionfish biomass on sites in the removal treatment following the initiation of experimental culling (n = 12, r = -0.53, p = 0.074, Figure 4). Because the trend appeared somewhat non-linear, data were log-transformed for further analysis. Post-transformation, a potential outlier was identified (BNP 02) using a Grubb’s test on residuals generated by linear regression (α = 0.05). BNP 02 was geographically distant from most other sites and featured the lowest lionfish recolonization rate in the study area following removal, demonstrating unusual site-specific qualities. Thus, BNP 02 was excluded from this analysis and the test was repeated on the remaining removal sites, which yielded a significant correlation (n = 11, r = -0.68, p = 0.019). The same negative correlation between
Graysby abundance and lionfish biomass was not measured on sites in the removal treatment before the initiation of lionfish culling (n = 12, r = 0.08, p = 0.805), or on non-culled (control) sites after lionfish removals had begun (n = 6, r = -0.30, p = 0.513). Despite being negatively correlated with lionfish biomass, Graysby density was not related to lionfish density on removal sites after the start of lionfish culling (n = 12, r = 0.01, p = 0.982). Therefore, it appears that size, but not quantity, of lionfish that either avoided removal or recolonized culled sites was correlated with Graysby density.

There was no evidence of a difference in Graysby density (t₁₆ = 0.78, p = 0.221) or biomass (t₁₆ = 0.87, p = 0.205) between LBCs. Given comparable population sizes, the strength of Graysby intraspecific competition should not have varied across LBCs, and thus is not likely to have strongly influenced comparisons of diet and condition made using this analytic structure.

**Stable Isotope Analysis**

Both lionfish and Graysby δ¹⁵N values were positively related to length (Table 1, Figure 5). Using a bootstrap analysis (n = 1000 permutations), I determined that the 95% confidence intervals (CI) of regression slopes overlapped between lionfish and Graysby (Lionfish 95% CI: 0.0275 to 0.0372‰/cm, Graysby 95% CI: 0.0226 to 0.0478‰/cm), and therefore the rate of increase of δ¹⁵N values with size was not significantly different between species. However, the relationship of δ¹³C values with fish size was not consistent between species. Lionfish δ¹³C values were lower in larger individuals, while Graysby δ¹³C values were not related to standard length (Table 1). Although the regression was significant, lionfish size explained <10% of variation among δ¹³C values, indicating a high amount of variability in size-based trends of lionfish basal resource use.
Examining all sampled individuals, mean $\delta^{13}$C was 0.33‰ higher in lionfish than in Graysby (95% CI = 0.23 to 0.40, $t_{443} = 6.82$, $p = 0.001$). However, the magnitude of this difference was small relative to the range of measured stable isotope values (Table 2). Although mean lionfish $\delta^{15}$N was 0.07‰ lower than in Graysby (95% CI = 0.01 to 0.13, $t_{443} = 2.03$, $p = 0.017$), this difference was smaller than instrumentation error. Overall, SIBER analysis measured a 67.5% overlap in Graysby isotopic niche with that of lionfish (Figure 6). In previous studies of diet, an overlap of 60% has been used as the threshold of biological significance (Zaret & Rand 1971, Smith 1985).

Stable isotope values on low LBC sites showed similar trends to those measured in the full dataset (Table 2). Lionfish $\delta^{13}$C values were 0.29‰ higher than measured in Graysby (95% CI = 0.17 to 0.42, $t_{228} = -4.30$, $p = 0.001$) and SIBER analysis measured a 60.9% interspecific isotopic niche overlap (Figure 7). In contrast, data from high LBC sites showed potential signs of resource use differentiation between the two species. Although the interspecific difference in mean $\delta^{13}$C values was still small (Difference = 0.38‰, 95% CI = 0.28 to 0.51, $t_{213} = -5.42$, $p = 0.001$), its magnitude increased by 32% relative to low LBC sites. Mean Graysby $\delta^{13}$C did not differ between LBCs ($t_{149} = -1.22$, $p = 0.106$) and mean $\delta^{15}$N differed by an amount smaller than measurement error ($t_{149} = -1.72$, $p = 0.041$). Despite having the same average $\delta^{13}$C and $\delta^{15}$N values, Graysby isotopic niche width was 32.6% smaller on high LBC sites (Low $\text{SEA}_B = 0.408‰^2$, High $\text{SEA}_B = 0.275‰^2$, Probability = 99.2%, Figure 8). Conversely, lionfish isotopic niche width did not differ between low and high LBCs (Low $\text{SEA}_B = 0.562‰^2$, High $\text{SEA}_B = 0.587‰^2$, Probability = 64.0%). In addition, the amount of isotopic niche overlap between lionfish and Graysby was 25% lower on high LBC sites.
than on low LBC sites (0.186‰). Similarly, the proportion of lionfish isotopic niche occupied by that of Graysby was 45.8% on low LBC sites, but only 31.3% on high LBC sites.

**Stomach Contents**

Among stomachs with identifiable contents, the proportion of gut contents containing teleost prey was 25% lower on high LBC sites ($\chi^2 = 3.96$, $p = 0.046$). However, the proportion of stomachs containing invertebrate prey did not differ between LBCs ($\chi^2 = 0.82$, $p = 0.365$), nor did the proportion of full ($\chi^2 = 0.08$, $p = 0.775$) or empty ($\chi^2 = 0.02$, $p = 0.885$) Graysby stomachs.

**Prey and Predator Communities**

At the site-level, neither prey fish abundance ($n = 18$, $F = 0.29$, $p = 0.607$) nor species richness ($n = 18$, $F = 0.50$, $p = 0.480$) were related to lionfish biomass. Additionally, non-parametric MANOVAs measured no difference in the community composition of prey ($F_{1,17} = 1.72$, $p = 0.172$) or predatory fishes ($F_{1,17} = 0.60$, $p = 0.748$) between LBCs.

**Condition Indices**

Although I observed a difference in Graysby diet associated with higher lionfish biomass, none of the measured condition indices varied between LBCs (Table 3). Similarly, measurements in Graysby of Ricker’s condition index ($n = 18$, $F = 0.009$, $p = 0.91$), viscerosomatic index ($n = 18$, $F = 0.003$, $p = 0.96$), and hepatosomatic index ($n = 18$, $F = 1.75$, $p = 0.221$) were not related to site-wise averages of lionfish biomass. Unexpectedly, Graysby C:N values were positively related to ambient lionfish biomass ($n = 18$, $F = 4.50$, $p = 0.478$). However, the range of average C:N values among sites was small (~0.1), so this relationship is likely not physiologically meaningful (Post et al. 2007).
Discussion

The strength and outcome of competition between lionfish and native predators is an important, yet relatively unexplored aspect of this marine fish invasion. The results of this study are the first to describe changes in the resource use of a native mesopredator across a range of lionfish biomass and provide insight into the possible consequences and mechanisms of interaction among lionfish and ecologically similar predatory fishes.

Correlations of lionfish and Graysby population metrics yielded mixed evidence regarding potential interspecific competition. Generally, biomass and density of both species were not related across the study region, and thus did not show the negative trend expected under a strong competitive regime (Hardin 1960, Robinson & Wilson 1994, Bøhn et al. 2008). However, this result may not reflect shifts in microhabitat use or small-scale spatial distribution that could have followed interspecific interactions (Grossman et al. 1998, Eagle et al. 2001), which would not have materialized as site-level correlations in biomass or density. Conversely, the lower biomass of lionfish measured on sites with higher Graysby densities following culling events may indicate that Graysby can, to some degree, deter habitat occupation by large lionfish, possibly through competitive mechanisms. In the Red Sea, Shpigel & Fishelson (1991a, 1991b) determined that Cephalopholis groupers are capable of excluding other predators, including lionfish, from their home ranges. Therefore, given an average home range of 2000m² (Popple & Hunte 2005), Graysby could plausibly slow immigration of conspicuous competitive invaders at the scale of the BNP study sites. An alternative explanation for the correlation between lionfish biomass and Graysby density following lionfish removal could be increases in Graysby population size on sites where lionfish were more successfully culled. However, differences in Graysby density following the initiation of lionfish removal were comparable across removal and
control treatments, so a reduction in lionfish biomass does not appear to spur short-term population growth in Graysby.

The high amount of isotopic overlap between lionfish and Graysby suggests similarity in population-level resource use, and strongly indicates a potential for competition (Tilman 1977, Bearhop et al. 2004, Layman et al. 2007). This finding contrasts with a report by O’Farrell et al. (2014) that lionfish stable isotope values were higher than in native mesopredators, including *C. fulva*. Thus, the strength of resource use overlap and resultant competition between lionfish and Graysby may vary geographically and among study systems. Both lionfish and Graysby $\delta^{15}$N values rose similarly with size, measuring comparable growth-driven increases in trophic level that could result in overlapping resource use across multiple life stages and size classes (France et al. 1998). This result matches descriptions of dietary transitions from invertebrate to teleost prey based on gut content analyses in both lionfish (Morris & Akins 2009, Muñoz et al. 2011) and Graysby (Nagelkerken 1979). While Dahl & Patterson (2014) also found an increase of $\delta^{15}$N with length in lionfish, several other studies did not detect this relationship (Muñoz et al. 2011, Layman & Allgeier 2012, O’Farrell et al. 2014). The size range sampled for this study (4.4 - 32.3cm SL) was wider than those used in most previous analyses of lionfish stable isotopes, and thus may have included individuals foraging at trophic extremes that facilitated measurement of a length-$\delta^{15}$N relationship.

The observed change in Graysby isotopic niche across LBCs may provide insight on how invasive lionfish could affect the resource use of native mesopredators. Based on the reduced amount of interspecific isotopic overlap on high LBC sites, Graysby appear to differentiate diet or habitat use from that of lionfish where populations of the invasive species are larger. This result resembles findings from other systems, which have been similarly interpreted as niche
segregation in response to competitive interspecific interactions (Cherel et al. 2008, Páez-Rosas et al. 2014, San Sebastián et al. 2015). Additionally, Graysby isotopic niche width was 33% smaller on high LBC sites, possibly reflecting a narrowing of resource use diversity in the presence of larger populations of lionfish. Lower dispersion of Graysby stable isotope values on sites with presumably stronger interspecific competition is consistent with the niche variation hypothesis, or NVH (Van Valen 1965). According to the NVH, release from interspecific competition at the population level should lead to niche expansion, while increased interspecific competitive interactions should cause niche contraction (Araújo et al. 2011, Kernaléguen et al. 2015). The mechanism driving the NVH is the degree of individual diet specialization, which is expected to increase as intraspecific interactions become more dominant following weakened interspecific competition (Bolnick et al. 2010, Araújo et al. 2011). In the Bahamas, Layman & Allgeier (2012) found that lionfish, usually described as a generalist species, displayed a surprisingly high capacity for individual specialization. Therefore, similar individual specialization of Graysby resource use, which has not yet been empirically measured, may play a role in shaping the species’ response to any competitive interactions with invasive lionfish. My findings add to the body of empirical support for the NVH in natural systems, which includes several other studies of fish diet across competitive regimes (Harmelin-Vivien et al. 1989, Knudsen et al. 2007, Bolnick et al. 2010).

Considering the lower proportion of Graysby stomachs containing fish material on high LBC sites, the narrowing of Graysby isotopic niche width may stem from reduced foraging on teleost prey. The primary difference in Graysby isotopic distributions between LBCs was the presence of Graysby with relatively high $\delta^{13}$C values (-14 to -14.5‰) on low LBC sites (Figure 9). Combining these results, it appears that reduced consumption of prey fish in the benthic food
web, which is less depleted in $^{13}$C than planktonic food webs (Fry 2006), may be a potential outcome for Graysby in areas heavily invaded by lionfish. Site depth may have been a confounding variable for this result, as the two low LBC sites where the majority of Graysby with high $\delta^{13}$C values were captured were also among the shallowest in the study area. In tissues of marine consumers, $\delta^{13}$C values often decrease with depth independently of diet composition due to changes in the metabolic rate and isotopic fractionation of primary producers (Muscatine et al. 1989, Barnes et al. 2009, Radabaugh et al. 2013). However, had depth-driven $\delta^{13}$C gradients contributed to patterns in Graysby stable isotope values, lionfish isotopic dispersion should have changed similarly across LBCs, which was not observed. Furthermore, the same two shallow sites of interest hosted among the lowest lionfish densities in the study region, maintaining at least an association of high Graysby $\delta^{13}$C values with smaller lionfish populations. Refined measurements of $\delta^{13}$C values in a variety of taxa, especially primary producers, would have to be made across the study area to elucidate how spatial variables such as depth may have influenced Graysby stable isotope dispersions.

Given a difference in Graysby resource use between LBCs, the lack of a relationship between lionfish biomass and prey communities among study sites may allow me to infer the dominant mode (exploitative vs. interference) of interspecific competition. If fish communities had been depleted on high LBC sites due to greater lionfish consumption, exploitative competition would likely have affected Graysby diet as preferred prey became unavailable (Park 1954, Petren & Case 1996, Davey et al. 2006). Although invertebrate communities were not surveyed, the consistency in the proportion of Graysby stomachs containing invertebrate prey suggests that lionfish may not influence the amount of opportunities for consumption of non-teleost taxa. Competition for habitat would also be considered exploitation if shelter were a limiting resource.
(Park 1954, Petren & Case 1996), but the complex reefs of BNP’s reef ledge likely offered enough space for Graysby potentially displaced by lionfish. Given the apparent lack of exploitation of any obviously limiting resource, lionfish might have more strongly affected Graysby diet by altering patterns of foraging behavior, either through passive avoidance of the invasive species (Raymond et al. 2015) or aggressive, territorial interactions (Fishelson 1975, Nagelkerken 1979), both of which would constitute interference competition (Brian 1956).

Although an interference mechanism may be more dominant in BNP, there remains high potential for exploitative competition between lionfish and native mesopredators throughout the invaded range, especially given the strength of evidence that lionfish consumption can locally deplete prey communities (Albins & Hixon 2008, Green et al. 2014, Albins 2015).

Despite a change in diet associated with higher lionfish biomass, Graysby condition did not vary between LBCs. Because competition must result in a negative physiological effect on involved species (Birch 1957, Schoener 1982, Petren & Case 1996), my results do not allow unequivocal classification of lionfish and Graysby as competitors. Possibly, the ratio of consumer demand to resource availability was low enough to preclude interspecific interactions from manifesting as competition. The maximum lionfish density on BNP study sites was 113 per hectare, substantially lower than the 400 - 1500 per hectare reported elsewhere in the invaded range (Albins & Hixon 2013, Dahl & Patterson 2014). Also, Graysby were sampled during the summer, a season which features high amounts of juvenile fish recruitment to coral reefs (Williams & Sale 1981, Booth & Beretta 1994), plausibly elevating prey densities past the point of resource limitation. Finally, BNP’s rugose reef ledge may have offered enough shelter for cohabitant lionfish and Graysby, effectively reducing the strength of interspecific competition by providing unexploited space for occupation by both predator and prey taxa (Holt 1987, Almany
2004). Studies of native mesopredator diet and condition in an environment with higher lionfish biomass, more depleted prey, and less complex habitat could help identify the environmental and ecological thresholds at which resource use overlap with invasive lionfish transitions into empirically demonstrable interspecific competition.

An assumption for my analyses and interpretations is that both lionfish and Graysby were resident species that moved and foraged similarly within the 1000m² study sites. Graysby dwell in home ranges comparable in scale to the size of the study sites and demonstrate high site fidelity (Popple & Hunte 2005), meeting both criteria. Although lionfish have also been described as having high site fidelity (Jud & Layman 2012), rapid and broad translocations have been repeatedly observed (Green et al. 2011, Bacheler et al. 2015, Tamburello & Cote 2015) with a maximum reported travel distance of 2km in a single day (Kletou et al. 2016). While conducting predator surveys, divers frequently observed individual lionfish swimming across the 100m length of study sites (personal observation). Thus, lionfish have a demonstrated capacity to move and forage on a larger spatial scale than considered by this study, potentially weakening the categorization of sites into LBCs and any statistical analyses based on site-wise lionfish biomass. Movement of either species could also have influenced measurements of stable isotope values, as the diet of mobile organisms may incorporate prey from several isotopically distinct habitats (Fry et al. 2003, Haas et al. 2009). Further measurement of invasive lionfish movement thus remains a critical objective for future research, and could not only clarify my findings but also inform predictions of resource use overlap and resultant competition with resident native mesopredators.
Summary and Conclusions

Considering the extent and severity of the lionfish invasion, total eradication of the species across the invaded range is unrealistic. Measuring the non-consumptive effects of lionfish on native species as they continue to integrate into western Atlantic ecosystems is therefore a necessary objective for concerned resource managers and conservation scientists. I did not detect a change in Graysby condition associated with lionfish biomass, and thus cannot classify the two species as competitors in Biscayne National Park. However, the high amount of interspecific overlap in stable isotope distributions suggests that competition between these two species, and possibly other reef predators, remains likely in resource limited environments. Additionally, I found a reduction of Graysby isotopic niche width and teleost consumption coincident with larger lionfish populations. Given the lack of response in prey communities to lionfish population size, as well as the high availability of habitat likely offered by BNP’s complex reef ledge, my results suggest that invasive lionfish may interfere with patterns of resource use in native mesopredators. Future studies in environments with more depleted resources or higher lionfish biomass (or both) could reveal the circumstances under which these species can be considered classical competitors, as well as the physiological consequences associated with interspecific interaction. Furthermore, characterization of Graysby habitat association and behavior along a gradient of lionfish population size may demonstrate the strength and nature of interference by the invasive species on native mesopredator foraging habits. Finally, comparative studies of movement in lionfish and species such as Graysby could indicate the spatial scale of resource use overlap among lionfish and native predators, critical information for managers designing removal programs intended to alleviate the effects of lionfish on invaded ecosystems.


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Tables and Figures

Table 2.1. Statistics for linear regressions of Graysby and lionfish standard length and muscle stable isotopes ($\delta^{13}$C, $\delta^{15}$N). * indicates a significant p-value ($\alpha = 0.05$)

<table>
<thead>
<tr>
<th>Regression</th>
<th>n</th>
<th>Slope</th>
<th>Intercept</th>
<th>F</th>
<th>$R^2$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graysby Standard Length x $\delta^{13}$C</td>
<td>170</td>
<td>-0.00</td>
<td>-15.0</td>
<td>0.02</td>
<td>0.0001</td>
<td>0.891</td>
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<tr>
<td>Lionfish Standard Length x $\delta^{13}$C</td>
<td>311</td>
<td>0.25</td>
<td>-15.3</td>
<td>28.5</td>
<td>0.085</td>
<td>0.001*</td>
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<tr>
<td>Graysby Standard Length x $\delta^{15}$N</td>
<td>170</td>
<td>0.04</td>
<td>8.58</td>
<td>31.6</td>
<td>0.158</td>
<td>0.001*</td>
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<tr>
<td>Lionfish Standard Length x $\delta^{15}$N</td>
<td>311</td>
<td>0.03</td>
<td>8.39</td>
<td>134</td>
<td>0.303</td>
<td>0.001*</td>
</tr>
</tbody>
</table>
Table 2.2. Stable isotope ($\delta^{13}$C, $\delta^{15}$N) means and ranges from Graysby and lionfish across sites, and separated between low and high lionfish biomass categories (LBCs)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>$\delta^{13}$C (Mean±SE)</th>
<th>$\delta^{13}$C (Min)</th>
<th>$\delta^{13}$C (Max)</th>
<th>$\delta^{15}$N (Mean±SE)</th>
<th>$\delta^{15}$N (Min)</th>
<th>$\delta^{15}$N (Max)</th>
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</thead>
<tbody>
<tr>
<td>Graysby – All sites</td>
<td>151</td>
<td>-15.05 ± 0.03</td>
<td>-15.92</td>
<td>-13.92</td>
<td>9.17 ± 0.02</td>
<td>8.59</td>
<td>9.94</td>
</tr>
<tr>
<td>Lionfish – All sites</td>
<td>294</td>
<td>-14.72 ± 0.03</td>
<td>-16.32</td>
<td>-13.13</td>
<td>9.10 ± 0.02</td>
<td>8.00</td>
<td>10.35</td>
</tr>
<tr>
<td>Graysby – Low LBC</td>
<td>84</td>
<td>-15.02 ± 0.05</td>
<td>-15.90</td>
<td>-13.92</td>
<td>9.20 ± 0.03</td>
<td>8.59</td>
<td>9.94</td>
</tr>
<tr>
<td>Lionfish – Low LBC</td>
<td>146</td>
<td>-14.73 ± 0.04</td>
<td>-15.89</td>
<td>-13.42</td>
<td>9.09 ± 0.03</td>
<td>8.18</td>
<td>10.14</td>
</tr>
<tr>
<td>Graysby – High LBC</td>
<td>67</td>
<td>-15.10 ± 0.04</td>
<td>-15.92</td>
<td>-14.59</td>
<td>9.12 ± 0.03</td>
<td>8.60</td>
<td>9.67</td>
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<tr>
<td>Lionfish – High LBC</td>
<td>148</td>
<td>-14.71 ± 0.04</td>
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<td>-13.13</td>
<td>9.11 ± 0.03</td>
<td>8.00</td>
<td>10.35</td>
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</table>
Table 2.3. Comparisons of Graysby condition indices between low and high lionfish biomass categories (LBCs) using non-parametric, randomization based t-tests. * indicates a significant p-value ($\alpha = 0.05$)

<table>
<thead>
<tr>
<th>Comparison (Low x High LBC)</th>
<th>$n$ (Low LBC)</th>
<th>$n$ (High LBC)</th>
<th>Mean±SE (Low LBC)</th>
<th>Mean±SE (High LBC)</th>
<th>$t$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ricker’s Condition Index</td>
<td>85</td>
<td>67</td>
<td>1.01 ± 0.11</td>
<td>1.00 ± 0.12</td>
<td>0.80</td>
<td>0.224</td>
</tr>
<tr>
<td>Hepatosomatic Index (HSI)</td>
<td>55</td>
<td>44</td>
<td>0.70 ± 0.94</td>
<td>0.62 ± 0.94</td>
<td>-0.94</td>
<td>0.197</td>
</tr>
<tr>
<td>Gut Fullness</td>
<td>85</td>
<td>67</td>
<td>1.69 ± 0.18</td>
<td>1.64 ± 0.20</td>
<td>0.18</td>
<td>0.430</td>
</tr>
<tr>
<td>C:N</td>
<td>85</td>
<td>68</td>
<td>3.15 ± 0.34</td>
<td>3.16 ± 0.38</td>
<td>-1.11</td>
<td>0.126</td>
</tr>
</tbody>
</table>
Figure 2.1. Map of Biscayne National Park and the study area
Figure 2.2. Graysby total length vs. wet weight. The fitted curve was used to calculate parameters for the equation $W = aL^b$ ($a = 0.0079$, $b = 3.22$) using non-linear least squares regression.
Figure 2.3. Mean lionfish biomass (±SE) on each study site in ascending order. The dashed line indicates the mean biomass across all sites (19.1kg/ha)
Figure 2.4. Relationship between site-averaged lionfish biomass (kg/ha) and Graysby density (no./m²) on sites in the removal treatment in the second year of the study (post-lionfish removal). Site BNP 02 was removed as an outlier prior to analysis. Dashed lines are 95% confidence bands for the regression.
Figure 2.5. Regressions of Graysby (top) and lionfish (bottom) $\delta^{15}\text{N}$ with standard length (solid lines). Dashed lines are 95% confidence bands for the regression.
Figure 2.6. Stable isotope bi-plot of all sampled Graysby (black circles) and lionfish (open triangles). Standard ellipses created by SIBER analysis (SEA_B) are overlaid on the data. Graysby SEA_B (0.392‰) is depicted as a black line, lionfish SEA_B (0.561‰) as a grey line. Interspecific SEA_B overlap was 67.5%, indicating a similar isotopic niche.
Figure 2.7. Stable isotope bi-plot of Graysby (black circles) and lionfish (white triangles) from low (top) and high lionfish biomass sites (bottom). Standard ellipses created by SIBER analysis \((\text{SEA}_B)\) are overlaid on the data. Graysby \(\text{SEA}_B\) (low: \(0.275^{\%}_{oo}^2\), high: \(0.408^{\%}_{oo}^2\)) is depicted as a black line, lionfish \(\text{SEA}_B\) (low: \(0.562^{\%}_{oo}^2\), high: \(0.587^{\%}_{oo}^2\)) as a grey line. The amount of interspecific isotopic niche overlap was 25% lower on high LBC \((0.248^{\%}_{oo}^2)\) than low LBC sites \((0.186^{\%}_{oo}^2)\)
Figure 2.8. Stable isotope biplots of Graysby (top, circles) and lionfish (bottom, triangles) from low (open) and high (filled) lionfish biomass categories (LBCs). Standard ellipses created by SIBER analysis (SEA) are overlaid on the data (low LBC = dashed line, high LBC = solid line). Graysby SEA was 32.6% smaller on high vs. low LBC sites (Probability = 99.2%). Lionfish SEA did not differ between LBCs (Probability = 64%).
CHAPTER THREE:

LIFE HISTORY PATTERNS OF INVASIVE LIONFISH AND A NATIVE MESOPREDATOR DESCRIBED USING STABLE ISOTOPES OF MUSCLE, LIVER, AND EYE LENSES

Abstract

Records of stable isotopes retained in metabolically stable, serially deposited structures such as eye lenses can yield robust descriptions of resource use across the life history of fishes. I performed stable isotope analysis of muscle, liver, and eye lenses sampled from invasive lionfishes (*Pterois spp.*) and a potentially competitive native mesopredator, the Graysby (*Cephalopholis cruentata*), to compare patterns of diet and movement on a coral reef ledge in Biscayne National Park, Florida. To serve as a reference frame for interpretation of isotopic measurements in fish tissues, I described variation in $\delta^{13}$C and $\delta^{15}$N values with sampling location. Differences in $\delta^{15}$N values between liver and muscle samples were greater in lionfish than Graysby, possibly due to broader ranges of movement by the invasive species. In both lionfish and Graysby, stable isotope values increased logarithmically with eye lens radius, likely reflecting similar changes in trophic level with growth. Intermediate lens layers were the most isotopically similar between species, suggesting that resource use overlap of lionfish and Graysby may be strongest in mid-sized fish. Despite the underlying logarithmic trend, there was substantial variation among individual isotopic chronologies, potentially driven by both diet
specialization and fish movement. Eye lens isotopic records were more variable in lionfish, further suggesting that the invasive species may undertake larger movements than cohabitant Graysby. These results enhance our understanding of size-structured resource use in lionfish and native mesopredators, an important objective for researchers studying how this highly invasive species interacts with surrounding ecological communities.

**Introduction**

Stable isotope analysis (SIA) has emerged as a prominent and powerful tool for ecological research, informing studies of diet, movement, and species interactions in many taxa including teleost fishes (Fry 2006, West et al. 2006). The two most commonly sampled isotopes are $^{13}$C, which reflects basal resource use (DeNiro & Epstein 1978, Peterson & Fry 1987), and $^{15}$N, which can serve as a proxy for trophic level (DeNiro & Epstein 1980, Gannes et al. 1998). Stable isotope values integrate dietary input over the metabolic turnover period of sampled tissue, which ranges from days to months (Tieszen et al. 1983). Thus, SIA can be applied to describe spatiotemporal patterns in consumer resource use, providing information about both foraging (Peterson & Fry 1987, Araújo et al. 2007, Kurle 2009) and movement among isotopically diverse habitats (Hobson 1999, Fry et al. 2003, Davis et al. 2015). Particularly useful for SIA-based studies of animal ecology are sequentially deposited, metabolically stable tissues, which can record of stable isotope values across the lifespan of individual organisms (Campana 1999, Estrada et al. 2006, McMahon et al. 2011). Such isotopic time-series provide information about ontogenetic shifts in diet and habitat, individual resource use specificity, and other important aspects of consumer life history (Kim et al. 2012, Hanson et al. 2013, Tzadik et al. 2015).

Historically, calcified structures such as otoliths, scales, and bones have been used to construct isotopic chronologies (Campana 1999, Clarke et al. 2007). However, these “hard parts”
contain high proportions of inorganic material, which can complicate or confound measurement of the organic stable isotopes most frequently studied to provide information about consumer diet and movement (Pinnegar & Polunin 1999, Sinnatamby et al. 2008). The eye lens has recently been proposed as an alternate, uncalcified isotopic recorder that may be consulted to describe animal life histories (Hunsicker et al. 2010, Wallace et al. 2014, Nielsen et al. 2016).

Eye lenses are structured by concentric layers (laminae), which are composed of cells that undergo a modified version of apoptosis shortly after formation (Nicol 1989, Dahm et al. 2007, Lynnerup et al. 2008). Following the cessation of metabolic activity, laminae retain the chemical composition associated with their period of deposition (Vihtelic 2008, Wride 2011). Because lenses can be readily extracted and mechanically separated into individual laminae, this structure has the potential to be highly valuable for the construction of isotopic chronologies. However, chemical records in eye lenses are infrequently consulted for marine ecological research. Until now, eye lens delamination and SIA has only been conducted for a pair of studies in squids (Parry 2003, Hunsicker et al. 2010) and fishes (Wallace et al. 2014, Nielsen et al. 2016).

One potential use for eye lens SIA is to help describe the ecology and spread of invasive species. Among the most well-known marine invaders, Indo-Pacific lionfishes (*Pterois miles* and *P. volitans*; hereafter, lionfish), have become a pervasive presence throughout the western Atlantic, and pose a considerable threat to native species (Albins & Hixon 2013, Côté et al. 2013). Although lionfish diet (Morris & Akins 2009, Muñoz et al. 2011, Layman & Allgeier 2012) and movement (Akins et al. 2014, Bacheler et al. 2015, Tamburello & Côté 2015) have been characterized at the population level, more detailed measurements across the lifespan of individual fish could yield additional insight for scientists and resource managers working to predict patterns of the species’ resource use in invaded ecosystems. Comparative analysis of
isotopic life history records in lionfish and native mesopredators could also refine understanding of diet and habitat overlap between the invasive species and potential competitors, a critically important yet relatively unquantified aspect of lionfish ecology in the western Atlantic (Layman & Allgeier 2012, O’Farrell et al. 2014).

Eye lens SIA could be a particularly useful tool for assessing size-driven shifts in diet of both lionfish and native predators. Increases in trophic level following somatic growth are prevalent in generalist piscivorous taxa such as lionfishes (Romanuk et al. 2010), primarily due to a widening of gape size which enables consumption of larger prey (Hairston & Hairston 1993, Arim et al. 2010). However, empirical demonstration of a relationship between size and diet in lionfish has not been consistent (Morris & Akins 2009, Muñoz et al. 2011, Layman & Allgeier 2012, Dahl & Patterson 2014, O’Farrell et al. 2014). Thus, refined descriptions of trophic growth based on eye lens stable isotopes may clarify the nature of size-based diet shifts in individual lionfish. Similar measurements in cohabitant native predators could help predict the relative trophic level and amount of interspecific diet overlap across multiple life stages, important to assessing the potential for size-structured competitive interactions.

Isotopic information in eye lenses may also allow better descriptions of movement patterns in invasive lionfish. Although lionfish generally show high site fidelity and forage at relatively small spatial scales (Akins et al. 2014, Bacheler et al. 2014), some individuals have been observed to undertake broad, rapid translocations (Green et al. 2011, Tamburello & Côté 2015), with a maximum reported travel distance of 2km in a single day (Kletou et al. 2016). Refined understanding of individual and population-level lionfish movements is essential for predicting not only the spatial scale of foraging activities and patterns of dispersion throughout invaded ecosystems, but also the potential for habitat overlap and interaction with native mesopredators.
Recently, stable isotopes in sequentially deposited tissues have been used to describe movement of fishes ranging in scale from localized habitat shifts to global migrations (McMahon et al. 2011, Kim et al. 2012, Acosta-Pachon et al. 2015). More commonly, however, SIA-based movement studies rely on measurements in tissues with different turnover rates, under the assumption that a greater amount of chemical difference (disequilibrium) indicates a larger or more recent transition across isotopically distinct environments (Hobson 1999, Fry et al. 2003, Davis et al. 2015). Frequently plotted in conjunction with such movement studies are isoscapes, maps of spatial variation in baseline stable isotope values (Barnes et al. 2009, Jaeger et al. 2010, Radabaugh et al. 2013). Isotopic mapping provides a reference frame that can aid accurate interpretation of spatiotemporal foraging behavior from patterns in consumer stable isotope values.

I applied various SIA-based approaches, including the relatively novel analysis of eye lens laminae, to compare life history patterns of lionfish and a native mesopredator, the Graysby (Cephalopholis cruentata), on a south Florida coral reef. Graysby are a small grouper that is likely to compete with lionfish due to similarities in maximum size, diet, and habitat use (Nagelkerken 1979, Sluka et al. 1998, Popple & Hunte 2005). In the previous chapter of this thesis, I described population-level overlap in stable isotope values of lionfish and Graysby that suggested interspecific similarities in resource use. I also measured parallel increases in $\delta^{15}$N values with standard length that could indicate comparable patterns of trophic growth in these species. Through this study, I aimed to expand upon those findings and further describe the amount of resource use overlap and potential for competition between lionfish and Graysby, especially at the individual level and across multiple life stages. To serve as a reference frame for my analyses, I first created a rudimentary map of spatial isotopic variation within my study area.
By analyzing stable isotopes in multiple tissues with different turnover rates (liver and muscle), I then estimated the comparative amount of movement by lionfish and Graysby along environmentally-driven isotopic gradients. Finally, I created eye lens isotopic chronologies for both lionfish and Graysby, enabling comparison of trophic growth patterns, movement, and other aspects of the ecology and life history of these invasive and native mesopredators.

**Materials and Methods**

**Study Region and Sites**

This study took place in Biscayne National Park (BNP) in south Florida (Figure 1), where complex contiguous reefs support resident populations of both lionfish and Graysby. Data were collected from twenty 1000m² sites distributed along 25km of reef ledge habitat in association with a Before-After-Control-Impact lionfish removal experiment (Stallings & Albins 2016). Lionfish removals began in September 2014 and proceeded for one year. Each site was randomly assigned to one of three levels of a lionfish removal treatment: control (no removal, n = 6 sites), tri-annual removal (n = 6 sites) and monthly removal (n = 8 sites). Lionfish collections were performed by BNP scientific staff using a polespear, after which sampled individuals were frozen and transported to the University of South Florida for further analysis. At the conclusion of the study, lionfish were collected from all sites including those in the control treatment. Graysby were sampled during July and September of 2014 (pre-lionfish removal) and 2015 (post-lionfish removal), and were collected with polespears using the assistance of a 20% Quinaldine solution (C₁₀H₉N), a common fish anesthetic (Gibson 1967). Sampled Graysby were stored on ice after each dive and dissected the same day.
**Muscle and Liver SIA**

Before dissection, all frozen lionfish were thawed at room temperature. Thawing was not required for Graysby, which were dissected on the day of capture. The standard length (cm) of each individual was measured, and approximately 1cm³ of liver and muscle tissue was removed and frozen for SIA. Freezing was chosen as the preferred method for fish tissue storage, as it does not impart preservation-driven offsets in stable isotope values (Stallings et al. 2015). White muscle was sampled posterior to the dorsal fin, the same location used in previous measurements of lionfish stable isotopes (Layman & Allgeier 2012). Liver samples are often chemically treated to remove lipids before SIA, as lipids are disproportionately depleted in ^13^C (DeNiro & Epstein 1977, Post et al. 2007). Because I analyzed samples with unaltered lipid composition, I only considered liver δ^15^N values for isotopic comparisons with muscle samples. Lipids generally do not contain nitrogen, and thus do not strongly affect fractionation or measurement of δ^15^N values (Bodin et al. 2007, Logan et al. 2008).

Muscle and liver samples were freeze dried at -40°C for 36 - 72 hours and mechanically homogenized using a mortar and pestle. For SIA, 400 - 1000µg of material was collected and weighed on a Mettler-Toledo precision micro-balance, wrapped in a tin capsule and loaded into a Costech Technologies Zero-Blank Autosampler. Samples were combusted at 1050°C in a Carlo-Erba NA2500 Series-II Elemental Analyzer coupled in continuous-flow mode to a Finnigan Delta Plus XL isotope ratio mass spectrometer. Measurements of C:N ratios were calibrated and isotopic measurements were normalized to the AT-Air and VPDB scales, respectively, using NIST 8573 and NIST 8574 L-glutamic acid Standard Reference Materials. Measurements were expressed in units per mil (‰) using δ notation, where δ = [(R_sample/R_standard) − 1] × 1000, and R is the isotopic ratio of interest (e.g. ^13^C:^12^C). Analytical precision, estimated by replicate
measurements of a working standard (NIST 1577b, Bovine Liver, n = 253) was ±0.17‰ ($\delta^{15}$N) and ±0.13‰ ($\delta^{13}$C). Two samples were analyzed for each fish, and the results were averaged for further statistical use. The mean difference (±SD) between replicate samples of muscle was 0.00±0.15‰ for both $\delta^{13}$C and $\delta^{15}$N, indicating a high degree of consistency in isotopic measurements of the same material.

**Eye Lens Delamination and SIA**

To plot a series of stable isotope values across the lifespan of individual fish, I measured $\delta^{13}$C and $\delta^{15}$N in sequentially sampled laminae from lionfish and Graysby eye lenses. Specimens were selected to represent a range of lionfish removal conditions both spatially (control vs. removal sites) and temporally (pre- and post-removal). In total, thirteen lionfish and fourteen Graysby were sampled from a standard length range chosen to offer the largest number of similarly sized fishes (Lionfish: 16.0 - 22.0cm, Graysby: 15.8 - 22.1cm). Although stable isotope retention does not vary between the left and right eye (Wallace et al. 2014), the left eye lens was preferentially used for analysis unless it had been damaged during capture. Lens delamination methodology was based on the thorough description provided in Wallace et al. (2014). Intact eyes were removed by severing the optic nerve and connective tissue between the eye and surrounding skeletomusculature, then wrapped in aluminum foil and frozen for preservation. Before delamination, each eye was thawed at room temperature. Lenses were then extracted and rinsed in deionized water to remove surrounding vitreous material. Lens laminae were sequentially peeled under a dissecting microscope using a pair of fine-tipped forceps. Before and after the removal of each lamina, the diameter of the lens was measured at its widest point (rounded to the nearest 0.05mm) using an ocular micrometer. The distance of each lamina relative to the center of the lens was defined as the radial midpoint, or the average of the lens diameter before and
after each delamination. Between laminar removals, tools were rinsed and dried with a lint free cloth, and a small amount of deionized water was applied to the lens to facilitate subsequent delamination. This process was repeated until the lens diameter was \( \leq 1 \text{mm} \), the size at which each individual lamina no longer provided enough material for unique isotopic analysis. Laminae were air-dried at room temperature for an hour, then processed for SIA using the same equipment, standards, and procedure as for muscle and liver tissue. When enough material was available, 300 – 600\( \mu \)g of material was sampled from each lamina, and replicate measurements were made and averaged. If laminar mass was <300\( \mu \)g, it was combined with equal material from the adjacent interior lamina for SIA and their radial midpoints were averaged for further analysis.

**Isoscape Measurements**

To describe spatial isotopic trends along BNP’s reef ledge, I examined changes in site-averaged stable isotope values of both lionfish and Graysby muscle across gradients in depth (18 - 25m) and latitude (25°N 18' 9.7122" to 25°N 31' 25.626'”). Only lionfish from removal sites were used for these measurements, as collections from control sites generally consisted of low sample sizes or had skewed size distributions. Graysby samples from all twenty study sites were included for analysis. For depth, I used the maximum value recorded during the set-up of each site. For latitude, I used GPS coordinates measured at the center of each site with a Garmin GPSMAP 4210. Because sites were oriented in a line running approximately from north to south, latitude was an appropriate descriptor of site position.

For all frequentist analyses in this study, statistics were calculated with distribution-free, non-parametric tests derived from the Fathom Toolbox for MATLAB (Jones 2015). Significance was assessed using p-values calculated by comparing the position of test statistics derived from the
original data relative to a distribution of the same statistic calculated from \( n = 1000 \) randomized permutations of the dataset (Moore et al. 2009). Statistics that were more extreme than 95\% of permuted values (2-tailed) were considered to be significant (\( \alpha = 0.05 \)). Although these methods allowed me to relax some of the strict assumptions of parametric statistical tests, particularly that of normally distributed errors, they can be sensitive to the inclusion of dependent or spatially structured data (Anderson 2001). Therefore, for tests involving multiple individuals collected from the same study site, which represent subsamples rather than independent replicates, randomization was constrained to within study sites (Legendre & Legendre 1998, Økland 2007).

Isotopic trends with depth and latitude (in decimal notation) were analyzed using linear regressions. To measure the strength of potentially confounding spatial and biological relationships, I examined the correlation between site depth and latitude using Pearson’s \( r \), as well as the relationship between fish length and site position using linear regressions.

**Muscle-Liver Disequilibria**

To compare population-level movement patterns of lionfish and Graysby, I analyzed muscle and liver \( \delta^{15}N \) values and measured the amount of isotopic disequilibria between the two tissues (Hobson 1999, Fry et al. 2003, Davis et al. 2015). Teleost liver turnover generally takes place over 1-3 weeks, whereas muscle turnover takes 1-3 months, although rates vary among species and environments (Perga & Gerdeaux 2005, Carleton & Del Rio 2010, Ankjaerø et al. 2012). To avoid an influence of seasonality, only individuals sampled in the final month of the removal experiment (September 2015) were included in this analysis. To calculate multiple-tissue isotopic disequilibria (\( \delta^{15}N_{M-L} \)), liver \( \delta^{15}N \) values were subtracted from those measured in muscle samples. The difference between average lionfish and Graysby \( \delta^{15}N_{M-L} \) was then compared using a non-parametric \( t \)-test. To assess the potential influence of lionfish removal on \( \delta^{15}N_{M-L} \)
measurements, I used a one-way non-parametric ANOVA with the three levels of removal frequency as a treatment factor.

**Eye Lens Chronologies**

Analyses of eye lens chronologies were performed with a mix of quantitative tests and qualitative observations. To describe general trends in lionfish and Graysby eye lens chronologies, I used non-linear least-squares regression on the aggregated data collected from all eye lenses of both species. Specifically, I measured the fit of a logarithmic curve with the equation $y = a + b \cdot \log(x)$, where $y$ = the isotopic value of interest, $x$ = radial midpoint, $a$ = the parameter controlling curve location on the $y$-axis and $b$ = the parameter controlling curve shape. This model was chosen to represent a simplified version of growth equations commonly used in fish, such as von Bertalanffy and logistic curves (von Bertalanffy 1938, Ricker 1975). The regression was used to characterize trends in eye lens stable isotope values with lens radius (i.e. size and age), which could theoretically be attributable to changes in trophic level following growth. Although isotopic fractionation with trophic level is smaller in magnitude and less consistent in $\delta^{13}$C values than $\delta^{15}$N values (~1‰ vs. 3‰ enrichment per trophic step), a trend should still be evident in both isotopic species if lionfish and Graysby trophic level relates to size (DeNiro & Epstein 1978, 1980, Fry 2006). As multiple eye lens samples from the same individual represent subsamples and not truly independent replicates, the randomization-based method used to assess significance was constrained to individual fish (Legendre & Legendre 1998, Økland 2007). Confidence intervals (95%) were calculated around model parameters using a bootstrapping method with $n = 1000$ permutations (Moore et al. 2009). A lack of overlap between 95% CIs was used to distinguish parameters which significantly differed between
regressed curves. One lionfish was excluded as an outlier from regression analysis, based on its extremely elevated $\delta^{15}$N values.

Data from eye lenses were qualitatively compared both within and among spatiotemporal categories to assess the influence of year, site, and removal treatment on both species’ isotopic records. To determine whether any consistent life-history events explained patterns in isotopic chronologies across multiple individuals, I plotted stable isotope values against laminar distance from the center of the lens (radial midpoint). Similar fluctuations at consistent radial midpoints could theoretically be attributable to shifts in diet or habitat during specific developmental stages (Kim et al. 2012, Wallace et al. 2014). Any lens for which material $<$1mm was lost during delamination was excluded from this analysis. Similarly, by plotting stable isotope values “backwards”, against radial distance from the outermost lens layer (maximum lens radius - radial midpoint), I could examine whether any consistent environmental influence, such as seasonal variation, may have influenced patterns in recently deposited laminae. Only individuals collected in September 2015 were used in this analysis to avoid inclusion of potentially confounding inter-annual variation.

**Results**

**Isoscape Measurements**

In both lionfish and Graysby, $\delta^{13}$C values increased with depth, while $\delta^{15}$N values did not (Table 1, Figure 2). Conversely, although $\delta^{13}$C values did not vary with latitude, $\delta^{15}$N values decreased in both species from north to south (Table 1, Figure 3). Further inspection of the regression of Graysby $\delta^{13}$C values with latitude showed that the southernmost site appeared isotopically distinct from nearby sampling locations, and may have skewed the analysis by preventing measurement of a trend among the other sites (Figure 4). Repeating the regression
without the southernmost site, a negative trend was detected between latitude and Graysby $\delta^{13}C$
values ($n = 19$, $F = 7.38$, $R^2 = 0.30$, $p = 0.021$). There was no correlation between site latitude
and depth ($n = 20$, $r = 0.15$, $p = 0.526$) or relationship between fish size and site position
(Table 1).

**Muscle-Liver Disequilibria**

In all except two of 142 individuals ($n = 73$ lionfish, $n = 69$ Graysby), liver $\delta^{15}N$ values were
lower than measured in muscle. On average, $\delta^{15}N_{M-L}$ (±SD) was 70% greater in lionfish than
Graysby (Lionfish: $1.45 \pm 0.52\%$, Graysby: $0.85 \pm 0.37\%$, $t_{140} = -7.92$, $p = 0.001$, Figure 5). A
non-parametric ANOVA showed no relationship between lionfish removal treatment and $\delta^{15}N_{M-L}$
values in lionfish ($F_{2,70} = 4.79$, $p = 0.487$) and only marginal evidence of a relationship in
Graysby ($F_{2,66} = 5.05$, $p = 0.061$). Despite a lack of statistical significance, there was a trend in
both species for lower $\delta^{15}N_{M-L}$ values on sites in the control treatment.

**Eye Lens SIA**

Non-linear regression revealed a logarithmic trend in the aggregated chronologies of $\delta^{13}C$ and
$\delta^{15}N$ in both lionfish and Graysby (Table 2, Figures 6 and 7). Based on respective $R^2$ values, the
model was a better fit for trends in $\delta^{15}N$ values than $\delta^{13}C$ values. Similarly, this trophic growth
model explained more variation in Graysby eye lens chronologies than those of lionfish. All
regression parameters were different from one another except for the location parameter (a), in
lionfish and Graysby $\delta^{15}N$ chronologies (Table 2). Qualitatively, there was substantial variation
in shapes, patterns, and features of individual eye lens chronologies (Figure 8). Many of the data-
series were characterized by sharp deviations, peaks, and valleys of different magnitude and
periodicity, with no obvious consistency within spatiotemporal categories. Whether plotted
“forwards” (Figure 9) or “backwards” (Figure 10), fluctuations in the curves did not appear to coincide with any common position within the eye lens.

**Discussion**

In combination, measurements of stable isotopes in the environment, tissues with different turnover rates, and concentric eye lens laminae can provide useful insight on the resource use of potentially competitive species such as lionfish and Graysby. To my knowledge, this study provides the first descriptions of foraging and movement patterns across the lifespan of individual invasive lionfish, and is the first to apply SIA of metabolically stable, sequentially deposited tissues to compare the life histories of invasive and native fishes.

The detection of spatial isotopic gradients within BNP may be useful for ecological inference, particularly the decline of $\delta^{15}\text{N}$ values with latitude measured in both lionfish and Graysby. Due to the small magnitude of variation across the study area (~0.4‰), deterministic tracing of movement and foraging along BNP’s reef ledge using $\delta^{15}\text{N}$ values may not be possible (Authier et al. 2012). However, the manifestation of the trend in both species indicates a consistent $\delta^{15}\text{N}$-latitude relationship that may be more geographically expansive, and at least provides a plausible orientation of underlying regional isotopic variation. Because anthropogenic wastewater and urban runoff can cause enrichment of $\delta^{15}\text{N}$ values (McClelland et al. 1997, Lake et al. 2001), higher values on northern sites are likely driven by proximity to the heavily developed coastline of Miami. As an example of the potential utility of mapping this isoscape, muscle $\delta^{15}\text{N}$ values in two sampled lionfish were four standard deviations higher than the average for the study area, which suggests recent translocation from an area with a highly enriched $\delta^{15}\text{N}$ baseline. Refined measurements of nearby stable isotope values could help identify how far these individuals may
have traveled during the season preceding capture, providing valuable empirical descriptions of longer-term movement in individual lionfish.

The negative depth-$\delta^{13}C$ relationship measured in both species is consistent with patterns found in multiple marine taxa, including teleost fishes (Barnes et al. 2009, Nerot et al. 2012, Radabaugh et al. 2013). Generally, this trend can be explained by a combination of enhanced fractionation in primary producers experiencing lower light availability (Wefer & Killingley 1986, Muscatine et al. 1989) and reduced trophic input from benthic primary producers with depth (Sierszen et al. 2014). Proximity to the extensive seagrass beds of Biscayne Bay may have also contributed to higher $\delta^{13}C$ values on shallower sites, as seagrasses are markedly less depleted in $\delta^{13}C$ than other marine primary producers (Hemminga & Mateo 1996, Jennings et al. 1997).

Conversely, the apparent relationship between Graysby $\delta^{13}C$ values and latitude on such a small scale is more unusual than the observed depletion with depth. Similarly to the latitude-$\delta^{15}N$ relationship, the most likely explanation for a latitudinal gradient in $\delta^{13}C$ values is anthropogenically driven eutrophication and enhanced planktonic production near Miami (Brand 1988, Caccia & Boyer 2007), which could drive localized depletions in consumer $\delta^{13}C$ values (Voss et al. 2000, Vadeboncoeur et al. 2003). If these measurements in Graysby do reflect a latitudinal trend in baseline $\delta^{13}C$ values, the lack of its materialization in lionfish could indicate that the invasive species forages at a wider spatial scale than Graysby, effectively “averaging out” the underlying signal of spatial isotopic variation (Radabaugh et al. 2013). However, inference about consumer movement along BNP’s reef ledge based on stable carbon isotopes is complicated by the bi-directional nature of the apparent isoscape (depth and latitude).
Given the presence of a latitudinal gradient in $\delta^{15}$N values, my analyses of muscle and liver stable isotopes may provide insight on the relative amount of lionfish and Graysby movement along BNP’s reef ledge. The detection of an isotopic difference between liver and muscle in both species suggests temporal variation in $\delta^{15}$N intake, because liver has a shorter isotopic turnover rate than muscle (Tieszen et al. 1983, Ankjaerø et al. 2012). However, it is difficult to establish the exact timespan encapsulated by liver-muscle isotopic disequilibria, as the duration of metabolic turnover varies among species and can be influenced by factors such as growth rate and environmental conditions (Perga & Gerdeaux 2005, Trueman et al. 2005, Sweeting et al. 2007, Michaud et al. 2013). Even so, the finding of greater $\delta^{15}$N$_{M-L}$ values in lionfish than Graysby could plausibly indicate broader-scale habitat use on the part of the invasive species. An obvious potential influence on this result is the study design, as these data were collected in conjunction with a lionfish removal experiment. Although there was a trend of higher $\delta^{15}$N$_{M-L}$ values on removal sites, which may suggest that samples from repeatedly culled populations tended to consist of individuals prone to broader movements, this effect was not statistically significant. Additionally, even on control sites, average $\delta^{15}$N$_{M-L}$ was nearly twice as large in lionfish as in Graysby, demonstrating that the higher amount of isotopic disequilibria measured in the invasive species was not wholly attributable to the study design. It is worth noting that there was substantial variation in the $\delta^{15}$N$_{M-L}$ values of both species among study sites, including controls, possibly indicating a range of habitat suitability and mesopredator site fidelity along BNP’s reef ledge.

In both species, liver samples almost always yielded lower $\delta^{15}$N values than muscle, which could indicate recent transitions from foraging grounds with relatively enriched baseline $\delta^{15}$N values (Fry et al. 2003, Haas et al. 2009, Gelpi et al. 2013). Considering the observed
relationship between $\delta^{15}$N values and latitude, average long-term movement of lionfish and Graysby along BNP’s reef ledge may therefore be orientated from north to south. Although intriguing, this interpretation is tenuous, and requires corroboration with tagging studies and more robust measurements of regional isotopic baselines. An alternative physiological mechanism could also explain the relative elevation of $\delta^{15}$N$_{M-L}$ values in lionfish. In teleost species reared in a constant environment with a steady diet, liver tissue has been observed to contain lower $\delta^{15}$N values than white muscle (Trueman et al. 2005, Sweeting et al. 2007, Davis et al. 2015), likely due to differences in the concentration of nitrogen-bearing amino acids (Pinnegar & Polunin 1999, Michaud et al. 2013). Variation between liver and muscle $\delta^{15}$N values can be further influenced by species-specific metabolic rates of tissue formation, as well as a suite of biological and environmental factors such as growth rate and temperature (Pinnegar & Polunin 1999, Perga & Gerdeaux 2005, Madigan et al. 2012, Michaud et al. 2013). Therefore, measurements in reared lionfish and Graysby of baseline multiple-tissue isotopic disequilibria would be necessary to verify whether my results actually reflect relatively broader scale habitat use on the part of the invasive species. Finally, isotopic disequilibria could be caused by persistent changes in the diet of a resident species rather than movement across isotopically variable habitat. Therefore, information derived from more traditional tagging methodologies could help determine to what degree my measurements of $\delta^{15}$N$_{M-L}$ values in Graysby and lionfish actually reflect differences in foraging ranges and relative site fidelity.

In addition to multiple-tissue SIA, measurements of stable isotopes in eye lens laminae yielded potentially useful information about the life histories of lionfish and Graysby. The logarithmic trend in $\delta^{13}$C and $\delta^{15}$N values of both species with lens radius could theoretically characterize how trophic level changes with age and size in these generalist, piscivorous
mesopredators. The observed patterns broadly match size-related diet trends previously measured in lionfish and Graysby (Nagelkerken 1979, Morris & Akins 2009), and are similar in shape to curves derived from serially sampled isotopic records in other fishes known to switch diet and increase trophic level with size (Kim et al. 2012, Madigan et al. 2012, Wallace et al. 2014, Tzadik et al. 2015). The logarithmic trend resembled traditional models of fish growth, although lacking in a horizontal asymptote (von Bertalanffy 1938, Ricker 1975). In part, the fact that isotopic values did not generally level out in the outermost laminae may be due to the size range of sampled fish, which was smaller than the maximum potential length of both lionfish and Graysby. Similar measurements in larger fish, which had attained their highest size and trophic level, may demonstrate whether isotopic values reach a horizontal asymptote in the eye lenses of full-grown adults.

Comparing modeled trophic growth curves between species may help identify the life stages during which lionfish and Graysby are most likely to overlap in diet. Based on the fitted regressions, Graysby isotopic values are lower than those of lionfish at the interior of the eye lens, but increase more rapidly with distance from the nucleus, resulting in higher values in the outer laminae (Figure 7). Because the trendlines intersect at a radial midpoint of 1.0mm ($\delta^{15}$N) and 1.75mm ($\delta^{13}$C) out of a ~4mm lens, it appears that resource use overlap, and thus potential for competition, may be highest in mid-sized, likely sub-adult fish. It is important to note that Graysby eye lenses were smaller than those of lionfish, and lens growth rates may differ between taxa, so the same radial midpoints could correspond to different ages and life stages in both species. To translate radial midpoints into more concrete age or size estimates would require intensive measurements in conjunction with rearing experiments, one of the most critical
avenues of future research to the developing use of teleost fish eye lenses as isotopic recorders of life histories.

The fitted logarithmic curves explained twice as much variation in eye lens $\delta^{15}$N values compared to $\delta^{13}$C measurements. In lionfish eye lenses in particular, lens radius explained only 12% of variation in $\delta^{13}$C values, demonstrating a high level of variability in size-driven patterns of basal resource use. The finding that $\delta^{15}$N values corresponded better with modeled trophic growth is not surprising, because $\delta^{15}$N relates more predictably with trophic level than $\delta^{13}$C (DeNiro & Epstein 1978, 1980, Pinnegar & Polunin 1999). Despite a high amount of population-level variability, features of individual $\delta^{13}$C chronologies may still yield useful life history information. For example, in an individual lionfish found to have extremely enriched $\delta^{15}$N values in both eye lens and muscle tissues, likely signifying a recent translocation, I observed a dramatic elevation of $\delta^{13}$C values in the outermost lens laminae (Figure 10). Although it is not impossible that this fish uniquely underwent such a large shift in basal resource use, rapid movement across an environmentally-driven isotopic gradient offers a more plausible explanation for this particular fluctuation. More refined measurements of regional environmental stable isotope variation, as well as a methodology for translating eye lens radius into robust estimates of size and age, could allow the use of similarly distinguished eye lens chronologies to derive information about the duration and scale of movements by individual invasive lionfish.

Collectively as well as individually, eye lens isotopic chronologies can provide information about the relative movement of lionfish and Graysby. Because average trophic level should be determined mostly by gape size in generalist foragers such as lionfish and Graysby (Hairston & Hairston 1993, Arim et al. 2010, Romanuk et al. 2010), variation of $\delta^{15}$N values around the modeled trophic growth curve may be more readily explained by transitions among habitats with
variable isotopic backgrounds than rapid switches in diet. In the context of an observed $\delta^{15}$N-latitude gradient, one possible explanation for such deviations in nitrogen isotopic chronologies would be movement along BNP’s reef ledge. The high amount of variability among lionfish stable isotope chronologies may therefore stem from lower site fidelity or broader long-term foraging ranges than Graysby, supporting the same conclusion drawn from analyses of liver-muscle isotopic disequilibria.

Despite the underlying logarithmic trend, no two eye lens chronologies were completely alike in either species. The asynchronous fluctuations in stable isotope chronologies of both lionfish and Graysby suggest that the timing and strength of diet or habitat shifts can differ dramatically among individuals. The inconsistency of patterns observed in lionfish eye lens chronologies may also constitute evidence of a high amount of individual resource use specialization in the invasive species (Kim et al. 2012, Hanson et al. 2013), corroborating measurements made by Layman & Allgeier (2012). Furthermore, the periodic, sinusoidal variations which appear in many of the isotopic time-series could characterize ecological events of interest such as seasonal variation in the local prey community (Michaud et al. 2013), transitions into and out of spawning cycles (Doucett et al. 1999), or variation in growth rates (Perga & Gerdeaux 2005), all of which can affect isotopic values in consumer tissues.

**Summary and Conclusions**

Overall, the results of this study demonstrate the ability of SIA to measure aspects of invasive species ecology, and could enhance the understanding of resource managers working to mitigate the effects of lionfish in the western Atlantic. I found rudimentary patterns of spatial isotopic variation along BNP’s reef ledge that may be reflective of broader geographic trends, especially a consistent increase of $\delta^{15}$N values with latitude. If elaborated, a well-mapped isoscape between
Miami and the Florida Keys could become an invaluable tool for regional marine conservation efforts by enabling natural tracking of not only invasive lionfish, but also of other species transitioning among nearby zones under different management regimes. Additionally, I measured higher isotopic disequilibria between muscle and liver in lionfish than a native mesopredator, as well as spatial variation in $\delta^{15}$N$_{M-L}$ values. Consulting such isotopic indicators of comparative habitat use and site fidelity in lionfish and Graysby could help organize and optimize the spatial structuring of future lionfish removal efforts. Finally, I used eye lens isotopic chronologies to describe aspects of individual and population-level life histories of Graysby and lionfish, including size-trophic level relationships, patterns in movement, and individual specificity of resource use. Especially in concert with a well-defined isoscape, these isotopic records could be referenced to compare size-structured patterns of diet and habitat use among invasive lionfish and potentially competitive mesopredators. Critical to future applications of eye lens SIA, the timing of laminar deposition must be determined in a variety of teleost taxa. Studies using reared fish of a known age exposed to changes in diet, in conjunction with well-established aging procedures such as otolith analysis, would help translate patterns found in eye lens stable isotopes into more readily interpretable life history timelines. Similar research could also refine models describing trophic growth in eye lens isotopic chronologies, allowing better parsing of signals from diet, movement, and the environment and a stronger subsequent ability to make accurate ecological inferences based on these data.

**Literature Cited**


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


Stallings CD, Albins MA (2016). Final report to Florida Sea Grant on Project # R/LR-B-66H.


Tables and Figures

Table 3.1. Statistics for linear regressions of stable isotope measurements ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in muscle of lionfish and Graysby with spatial variables (latitude and depth) along the reef ledge of Biscayne National Park. Significant p-values ($p \leq 0.05$) are indicated by *.

<table>
<thead>
<tr>
<th>Regression</th>
<th>n</th>
<th>Slope</th>
<th>Intercept</th>
<th>$F$</th>
<th>$R^2$</th>
<th>p</th>
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<tbody>
<tr>
<td>Depth x Graysby $\delta^{13}\text{C}$</td>
<td>20</td>
<td>-0.06</td>
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<td>4.72</td>
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<td>-213</td>
<td>1.24</td>
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Table 3.2. Statistics for non-linear least squares regressions of stable isotope measurements ($\delta^{13}$C and $\delta^{15}$N) from eye lenses of lionfish and Graysby with laminar radial midpoint (mm). Regressions were made to test the fit of a logarithmic growth equation, $y = a + b*\log(x)$, to aggregated measurements from all sampled eye lenses. Significant p-values ($p \leq 0.05$) are indicated by *

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<th>Regressed w/ Radial Midpoint</th>
<th>$n$</th>
<th>$a$ (95% CI)</th>
<th>$b$ (95% CI)</th>
<th>$F$</th>
<th>$R^2$</th>
<th>$p$</th>
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<td>220</td>
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<td>78.4</td>
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<td>0.001*</td>
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<td>(1.16 to 1.54)</td>
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<td>139</td>
<td>-15.05</td>
<td>0.44</td>
<td>9.76</td>
<td>0.125</td>
<td>0.001*</td>
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<td></td>
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<td>(-15.18 to -14.91)</td>
<td>(0.23 to 0.62)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Graysby $\delta^{15}$N</td>
<td>220</td>
<td>8.22</td>
<td>1.97</td>
<td>501</td>
<td>0.821</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8.13 to 8.32)</td>
<td>(1.84 to 2.09)</td>
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<td></td>
</tr>
<tr>
<td>Lionfish $\delta^{15}$N</td>
<td>139</td>
<td>8.27</td>
<td>0.84</td>
<td>70.2</td>
<td>0.506</td>
<td>0.001*</td>
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<td></td>
<td></td>
<td>(8.15 to 8.36)</td>
<td>(0.71 to 1.00)</td>
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</table>
Figure 3.1. Map of Biscayne National Park and the study area
Figure 3.2. Linear regressions (solid lines) of average $\delta^{13}$C values from muscle samples of Graysby (top, circles) from all sites ($n = 20$) and lionfish (bottom, triangles) from removal sites ($n = 12$) with maximum site depth. Dashed lines are 95% confidence bands for the regression.
Figure 3.3. Linear regressions (solid lines) of average $\delta^{15}$N values from muscle samples of Graysby (top, circles) from all sites ($n = 20$) and lionfish (bottom, triangles) from removal sites ($n = 12$) with latitude. Dashed lines are 95% confidence bands of the regression.
Figure 3.4. Linear regressions of average $\delta^{13}$C values from muscle samples of Graysby (top, circles) from all sites except the southernmost site, which was removed from analysis ($n = 19$) and lionfish (bottom, triangles) from removal sites ($n = 12$) with latitude. Solid trendlines (black) indicate a statistically significant regression ($p \leq 0.05$). Curved dashed lines are 95% confidence bands of the regression.
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Figure 3.6. Non-linear regressions (solid lines) of Graysby $\delta^{13}\text{C}$ (top) and $\delta^{15}\text{N}$ (bottom) values with laminar radial midpoint (mm) using a logarithmic model. Dashed lines depict the 95% CI calculated by bootstrapping of regression parameters ($n = 1000$ permutations)
Figure 3.6 (cont). Non-linear regressions (solid lines) of lionfish $\delta^{13}$C (top) and $\delta^{15}$N (bottom) with laminar radial midpoint (mm) using a logarithmic model. Dashed lines depict the 95% CI calculated by bootstrapping of regression parameters ($n = 1000$ permutations)
Figure 3.7. Logarithmic trendlines of $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) values from aggregated Graysby (black) and lionfish (grey) eye lens isotopic chronologies.
Figure 3.8. Individual chronologies of $\delta^{13}\text{C}$ values vs. radial midpoint of lionfish eye lenses collected from a) Removal sites in September 2014 b) Removal sites in September 2015 and c) Control sites in September 2015. * denotes an individual for which axes are differently scaled than the other graphs in the same category
Figure 3.8 (cont). Individual chronologies of $\delta^{15}$N values vs. radial midpoint of lionfish eye lenses collected from a) Removal sites in September 2014 b) Removal sites in September 2015 and c) Control sites in September 2015. * denotes an individual for which axes are differently scaled than the other graphs.
Figure 3.8 (cont). Individual chronologies of $\delta^{13}$C values vs. radial midpoint of Graysby eye lenses collected from a) Removal sites in September 2014 b) Removal sites in September 2015 and c) Control sites in September 2015. * denotes an individual for which axes are differently scaled than the other graphs.
Figure 3.8 (cont). Individual chronologies of $\delta^{15}$N values vs. radial midpoint of Graysby eye lenses collected from a) Removal sites in September 2014 b) Removal sites in September 2015 and c) Control sites in September 2015. * denotes an individual for which axes are differently scaled than the other graphs.
Figure 3.9. Graysby $\delta^{13}$C (top) and $\delta^{15}$N (bottom) chronologies within eye lens laminae plotted against distance of laminar radial midpoint (mm) from the lens center
Figure 3.9 (cont). Lionfish $\delta^{13}$C (top) and $\delta^{15}$N (bottom) chronologies from eye lens laminae plotted against the distance of laminar radial midpoint (mm) from the lens center.
Figure 3.10. Graysby $\delta^{13}$C (top) and $\delta^{15}$N (bottom) chronologies from eye lens laminae plotted against the distance of laminar radial midpoint (mm) from the outermost lens layer in each individual. Only individuals collected in September 2015 were included for this figure.
Figure 3.10 (cont). Lionfish $\delta^{13}$C (top) and $\delta^{15}$N (bottom) values from eye lens laminae plotted against the distance of laminar radial midpoint (mm) from the outermost lens layer in each individual. Only individuals collected in September 2015 were included for this figure.
CHAPTER FOUR:

GENERAL CONCLUSIONS

Accurately predicting the long-term ecological effects of a successful invasive species is necessary for effective management of ecosystems threatened by anthropogenic biological introductions. For this thesis, I used a diverse array of methodologies, in particular sophisticated applications of stable isotope analysis, to measure the nature of resource use overlap and subsequent competition between an invasive and ecologically important native mesopredator.

In Chapter Two, I attempted to determine whether interaction with lionfish affected the resource use and condition of Graysby, which could allow classification of the two species as competitors. I found a high amount of isotopic niche overlap between lionfish and Graysby, suggesting that competition between these species, and possibly other reef predators, is a likely outcome in resource limited environments. Additionally, I measured a smaller isotopic niche width and lower teleost consumption in Graysby on sites with higher lionfish biomass, the first documentation of diet shifts in a native predator associated with invasive lionfish. However, I did not detect any relationship between Graysby condition and lionfish biomass, and therefore was unable to describe these two species as competitors within the study area. I recommend that similar studies be conducted in environments with more depleted resources and higher lionfish biomass. Such research efforts may reveal the environmental and ecological thresholds for
competition among *Cephalopholis* spp. and lionfish, as well as the nature of physiological declines in native mesopredators that may result from encounters with the invasive species.

In Chapter Three, I applied several isotopic approaches to describe both individual and population-level patterns of diet and movement in lionfish and cohabitant Graysby. To aid this work, I created rudimentary descriptions of spatial isotopic variation along BNP’s reef ledge which, if elaborated, could become an invaluable natural method of tracking animal movement in the region. I also measured several isotopic indications that lionfish may have broader ranges of movement than Graysby, information that could help spatially structure effective lionfish removal efforts. Finally, I used eye lens isotopic chronologies to describe aspects of lionfish and Graysby life histories, including size-trophic level relationships and individual specificity of resource use. This study provides the first measurements of diet across the lifespan of individual invasive lionfish, and demonstrates the potential value of eye lenses to the study of animal ecology and invasive species. Critical to future analyses of eye lens stable isotopes, the timing of laminar deposition must be determined in a variety of taxa to enable translations of these data into readily interpretable life history timelines.

By applying a diverse array of study techniques, especially novel methodologies such as the measurement of isotopic niches or chronologies from metabolically stable eye lenses, I have expanded the base of scientific knowledge describing potential effects of invasive lionfish on native predatory fishes. As a whole, the results of my work may be of interest not only to those seeking to understand and manage the invasion of lionfish into the western Atlantic, but to any ecologist studying patterns of resource use overlap and interspecific interactions among potentially competitive species.
APPENDIX I:

IACUC DETERMINATION LETTER

MEMORANDUM

TO: Christopher Stallings,

FROM: Farah Moulvi, MSPH, IACUC Coordinator
Institutional Animal Care & Use Committee
Research Integrity & Compliance

DATE: 4/17/2015

PROJECT TITLE: Population and community dynamics of marine fishes in the Gulf of Mexico and western Atlantic Ocean

FUNDING SOURCE: NOAA

IACUC PROTOCOL #: W IS00001150
PROTOCOL STATUS: APPROVED

The Institutional Animal Care and Use Committee (IACUC) reviewed your application requesting the use of animals in research for the above-entitled study. The IACUC APPROVED your request to use the following animals in your protocol for a one-year period beginning 4/17/2015:

Cephalopholis cruentata (0-5, both, 5-35cm TL)
Pterois volitans (0-5, both, 3-45cm TL)