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Population Ecology of Invasive Lionfish (Pterois volitans/miles) in the South Atlantic Bight

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POPULATION ECOLOGY OF THE INVASIVE LIONFISH (*Pterois volitans/miles*) IN THE SOUTH ATLANTIC BIGHT

by:

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TABLE OF CONTENTS

Chapter 1

Chapter 2

LIST OF TABLES

Chapter 1

Chapter 2

LIST OF FIGURES

Chapter 1

Chapter 2

ABSTRACT

Invasive species are a top threat to global biodiversity. Lionfish (*Pterois volitans/miles*) are a marine invasive predator that are now established in the Western Atlantic, Caribbean Sea and Gulf of Mexico. Studies that have documented impacts of lionfish suggest they can reduce fish recruitment and native species biomass by up to 80%. Research on lionfish impacts, biology and ecology is heavily focused in tropical and subtropical systems, with considerably less research effort occurring in the temperate regions of their invaded range, such as the South Atlantic Bight. Lionfish life history estimates are important to modeling lionfish population growth, modeling future dispersal and evaluating the efficacy of different removal strategies. Since lionfish life history will vary with a suite of environmental, biological and ecological factors, estimates of lionfish life history should be collected in all regions of their invaded range. The purpose of this study was to collect baseline information of lionfish biology and ecology in the southern portion of the South Atlantic Bight, an unstudied region for this species. As such, chapter one uses a quantitative approach to determine lionfish life history estimates important to management. Some important results of chapter one are: lionfish are recruiting at one main time throughout the year, growth changes seasonally and the population is relatively young (< 3 years of age). Chapter two takes the first step in determining lionfish impacts in this region by quantifying their diet. The main conclusion of this chapter is that round scad (*Decapterus punctatus*), sand perch (*Diplectrum formosum*) and black sea bass (*Centropristis striata*) are the most important prey items in the lionfish diet. Black sea bass

are an important fishery in the South Atlantic Bight, and lionfish could be negatively affecting recruitment. Together, these chapters provide important insight into lionfish ecology in this region and in general.

GENERAL INTRODUCTION

Invasive species are organisms that have been introduced to areas where they do not naturally occur, whose establishment may adversely affect native biotas and ecosystems, resulting in economic loss or adverse effects on human health. The introduction of invasive species is increasing with heightened global connectivity and poses a serious threat to ecosystems worldwide (Molnar et al. 2008). The establishment of an invasive species can have far reaching effects on invaded ecosystems through predation (Race 1982), competition for prey or habitat (Mills et al. 2004), or by introducing new diseases and parasites (Crowl et al. 2008) and can ultimately lead to declines in the abundance and diversity or even extinction of native organisms (Grosholz et al. 2000). In addition, invasive species cost the U.S. an estimated $137 million annually in eradication and mitigation (Pimentel et al. 2005). High profile examples of costly and environmentally destructive invasive species include the zebra mussel (*Dreissena polymorpha*) and sea lamprey (*Petromyzon marinus*) in aquatic systems, kudzu (*Pueraria lobata*) and Brazilian pepper (*Schinus terebinthifolius*) in terrestrial ecosystems, and the green crab (*Carcinus maenas*) and sea walnut (*Mnemiopsis leidyi*) in coastal communities.

A marine invader of particular concern in the western Atlantic Ocean is the Indo-Pacific lionfish (two species *Pterois volitans* and *Pterois miles*), the only one of many introduced marine fishes in the last decade to become established (Morris and Akins 2009). Although two invasive species, lionfish will hereto be referred to in these studies as *Pterois volitans* or *P. volitans* due to the high percentage that this species comprises of the invasive lionfish population (Hamner et al. 2007). Lionfishes are now established in the western Atlantic, Gulf of Mexico, and Caribbean Sea (Schofield 2011). Introduced by negligent aquarists, lionfish have long venomous spines that deter predation, and reproduce rapidly (Morris 2009), consume a generalist diet (Côté et al. 2013) and are capable of distant dispersal during egg and larval stages (Arenholz and Morris 2010). Also notable is this species' ability to grow faster, reach a larger maximum size, and maintain higher population densities than in their native range (Green and Côté 2009; Darling et al. 2011; Cure et al. 2014). This combination of life history characteristics has allowed this species to spread rapidly; lionfish are now among the most abundant predatory fishes in the region and one of Florida's most notorious examples of the growing global invasive species problem.

There is growing concern for the adverse effects lionfish are having on native ecosystems. Among the variety of problems caused by lionfish, one of the major impacts is predation on, and competition with, native fishes. Lionfish are voracious consumers of an array of reef fishes, and small-scale studies in The Bahamas have shown their capability of reducing the abundance of native fish recruitment nearly 80% (Albins and Hixon 2008) and overall native species biomass by 65% (Green et al. 2012). Further, although lionfish are too small to eat commercially important adult reef fishes (i.e. snappers and groupers), they do consume these species as juveniles and impact them indirectly through competition for food resources and have been shown to feed on the same trophic level as native apex predators such as snappers and groupers (Layman and Allgeier 2012).

Lionfish biology is a rapidly growing field of research with previous effort focusing heavily in well-studied systems like the Florida Keys, The Bahamas, and the Caribbean. However, since life history characteristics often vary with environmental (i.e. temperature) and biological and ecological (i.e. prey availability) factors, the population characteristics and local impacts of lionfish will differ by region. At present, few impacts have been rigorously studied and many remain poorly quantified for many regions, including the South Atlantic Bight (Morris 2012). The potential impact of lionfish in nearshore marine hard bottom habitats of northeast Florida is unknown, yet these coastal habitats harbor high densities of lionfish (Johnson and Swenarton unpubl. data) and serve as important juvenile nurseries for many commercially important fishes in the south Atlantic. For example, black sea bass (*Centropristis striata*) initially settle from the plankton in nearshore hard bottom habitats (ASFMC 2009). Similarly, after vermilion snapper (*Rhomboplites aurorubens*) recruit onto live-bottom habitats, they have strong site fidelity (Grimes and Huntsman 1980). Other important fishes that undergo the larval-juvenile transition in hard bottom include Atlantic menhaden (*Brevoortia tyrannus*), spotted sea trout (*Cynoscion nebulosus*) and weakfish (*Cynoscion regalis*) (Hare et al. 2007). In addition, lionfish may impede the recovery of several managed species of concern, such as the Warsaw grouper (*Epinephalus nigritis*) and speckled hind (*Epinephelus drummondhayi*). Data generated from this project will fill critical data gaps for this species (Morris 2012) and provide insight into regional population differences. These regional comparisons of life history are essential for managing invasive species on a local scale, the most effective method of invasive species mitigation (Engeman et al. 1998).

The overall goal of this project was to accumulate basic information on the population biology and ecology of the invasive lionfish in a historically understudied region, the lower South Atlantic Bight (SAB). Specifically, this project generated data on lionfish size- and age-structure, growth and diet in the lower SAB. Analyses of age, growth and population structure were conducted to provide more accurate life history inputs into models that assess population growth, invasion spread and evaluate the efficacy of removal strategies. Lionfish diet was also investigated as it is important for quantifying direct predatory impacts on native fish and potentially incorporating additional juvenile mortality estimates into fishery models of economically important species. This urgently needed information will fill large data gaps for the species (Morris 2012) and determine if lionfish management should differ on a regional scale, iding biologists to more effectively manage and control this established invasive species locally and to predict their impacts on native ecosystems.

CHAPTER 1

Population Demographics of Invasive Lionfish in the South Atlantic Bight

ABSTRACT

Analyses of population structure can be used to accurately estimate life history parameters for a population of interest. In invasive species management, the determination of life history characteristics is essential for modeling population growth, predicting rates of expansion and assessing the potential efficacy of removal strategies. Invasive lionfish (*Pterois volitans*) have rapidly invaded the Western Atlantic, Gulf of Mexico and Caribbean Sea and there is growing concern for the negative impacts they are having on native ecosystems. To better understand the life history of this species, statistical length-based modeling was performed on lionfish (n=2,836) captured off the coast of northeast Florida in 2013 and 2014. Different candidate models were compared that tested if significant seasonal growth and/or annual differences in growth were occurring in lionfish from this region. The main findings from this study were: (1) seasonal differences in growth were apparent in 2014, suggesting there is growth variability throughout the year that should be taken into account in future assessments, (2) distinct cohorts were present in the length frequency data, indicating lionfish are recruiting over a relatively short interval in this region and (3) the best model, which was verified with otolith ages, predicted that a majority of

the population was comprised of individuals that were age 1 or 2, demonstrating that older lionfish are likely moving to deeper water, which may provide them a deep water refuge from recreational spearfishing.

INTRODUCTION

Analysis of population structure is often applied to species of concern to estimate life history characteristics and better understand population dynamics, as well as identify the factors influencing those dynamics (Pauly 1987). In particular, examining population structure of invasive species can provide critical information on life history that is important for assessing removal and control strategies, understanding the progression of an invasion over time and identifying ecologically limiting factors in a system (Sakai et al. 2001; Mills et al. 1993). The serious threat that invasive species pose to ecosystems worldwide makes studies that improve invasion control and/or mitigate impacts to native systems exceedingly important (Abdelkrim et al. 2004; Molnar et al. 2008). Moreover, these studies are urgently needed given that the rate of introductions of invasive species is increasing with heightened global connectivity (Hobbs et al. 2006).

An invasive species of particular concern is the lionfish (*Pterois volitans*), now established in the western Atlantic, Gulf of Mexico, and Caribbean Sea (Schofield 2009; Morris 2012). Introduced by negligent aquarists, lionfish have long venomous spines that deter predation, exhibit rapid growth (Barbour et al. 2011), mature early and reproduce year-round (Morris 2009), consume a generalist diet (Côté et al. 2013), and are capable of distant dispersal during egg and larval stages (Ahrenholz and Morris 2010). Also notable is this species' ability to grow faster, reach a larger maximum size and maintain higher population densities than in their native range (Green and Côté 2009; Darling et al. 2011; Cure et al. 2014). This combination of life history characteristics has allowed this species to spread rapidly; lionfish are now among the most abundant predatory fishes in their invaded range (Whitfield et al. 2007). Lionfish are consumers of an array of reef fishes, and studies in The Bahamas have shown their capability of reducing the abundance of native fish recruitment nearly 80% (Albins and Hixon 2008), reducing overall native species biomass by 65% (Green et al. 2012) and feeding on the same trophic level as native apex predators like snappers and groupers (Layman and Allgeier 2012). Thus, there is growing concern for the negative impacts lionfish are having on native ecosystems.

Previous studies suggest extremely high levels of effort are necessary to decrease spawning biomass to levels that induce recruitment overfishing in the invasive lionfish population (Barbour et al. 2011; Morris et al. 2011; Albins and Hixon 2013). Many of these studies employ models that rely on key input parameters (e.g., growth, mortality) that contain considerable uncertainty and may vary by region and across time. Since these models assess the effects of removal effort (Morris et al. 2011) and generate management scenario predictions (Chagaris et al. 2016), accurate estimates of model parameters are critically important for evaluating the efficacy of various control and harvest strategies. Previous age and growth estimates for lionfish have been generated using otolith analysis (Barbour et al. 2011; Edwards et al. 2014), but with limited success due to the difficulty in ageing otoliths of this species. Moreover, life history characteristics may vary by region

(Conover 1992), sex (Poole and Reynolds 1996), season (Francis 1994) or year (Campana 1996) and understanding these differences can aid in improving the accuracy of model inputs for future analyses. Accurate estimates of growth and population demographics aid in the approximation of other important biological characteristics for lionfish, including natural mortality (Pauly 1980, Lorenzen 1996), yield per recruit (Lai and Gunderson 1987) and generation time (Beverton 1982). The purpose of this study was to assess lionfish growth and population demographics in an understudied region, the southern South Atlantic Bight, to reveal which factors influence size and age structure, and to generate population level estimates of life history characteristics to inform management.

METHODS

Sample Collection

Lionfish samples were collected from locations offshore of northeast Florida by trained spearfishermen (Figure 1-1). Sampling occurred during several large-scale public removal events in 2013 and 2014 (April and August) and by opportunistic sampling by recreational spearfishermen in 2014 (July, September, October, November) and 2015 (January). Lionfish in this region are restricted to offshore hardbottom and artificial reef habitats; inshore waters fall below their lower lethal temperature in the winter (Kimball et al. 2004). All fish were captured offshore (>15km) at approximately 25-50 m of depth. All fish were measured for total length (TL) to the nearest 1mm in the field. A subset of fish from each tournament, and all fish from the opportunistic samples, were transported to the University of North Florida for further processing. In the laboratory, fish were measured (standard length and total length), weighed to the nearest 0.1g and sexed. Some fish under 180mm were difficult to accurately sex and were considered immature (Morris 2009). A random subset of the laboratory fish had their sagittal otoliths removed, which were used to determine fish age directly and in model validation

Statistical length-based modeling

Lionfish TL data were used to construct length-frequency histograms for the observed data from 0 to 450 mm (TL) using 10 mm increments (46 length bins) for each collection month and year separately (Figure 2). Growth and population age structure were estimated by fitting a statistical length-based, age-structured model to the observed length-frequency data.

The statistical length-based, age structured model uses length as a proxy for age and estimates the proportion of fish in each age class using a maximum likelihood approach, fitting a predicted length frequency distribution to the observed data (similar to MULTIFAN; Fournier et.al. 1990, Johnson 2004). To generate the predicted length frequency distribution, the mean size-at-age was first estimated using either the traditional (von Bertalanffy 1934) or seasonalized (Gayalino and Pauly 1997) von Bertalanffy growth function (VBGF) which expresses fish length as a function of age. The traditional formulation of the VBGF is given in *Equation* 1:

$$(Eq.1) \quad L_t = L_\infty\left[1 - e^{-(k(t-t_0))}\right]$$

where L_t is the length of a fish at age t, L_∞ is the asymptotic maximum length, k is the Brody growth coefficient, and (t_0) is time at which a fish was length 0. The seasonalized VBGF (Pauly and Gaschutz 1979, Equation 2) extends the traditional VBGF to allow the growth rate to vary seasonally, and may better reflect the growth of fish inhabiting temperate regions with pronounced seasonal temperature fluctuations. The seasonalized VBGF is given in *Equations* 2-4:

$$(Eq. 2)\quad L_t = L_\infty\left[1 - e^{-(k(t-t_0)+S(t)-S(t_0))}\right]$$

$$(Eq. 3)\quad S(t) = \left(\frac{Ck}{2\pi}\right) Sin\pi(t - t_s)$$

$$(Eq. 4)\quad S(t_0) = \left(\frac{Ck}{2\pi}\right) Sin\pi(t_0 - t_s)$$

where L_t, L_∞, k, t_0 are the same as previously defined (*Equation* 1), C is the intensity of seasonal growth oscillation, and t_s is the timing of seasonal growth oscillations relative to t_0. The VBGF only estimates the mean size-at-age over time; variation in the size of individuals in each age class was estimated directly within the model structure by including variance in size-at-age (σ_a^2) as a model parameter. The proportion of lionfish in each age class during each sampling month and year was also estimated within the model ($P_{a,t}$). The expected number of individuals of age *a* in size class *i* in month *t* ($n_{i,a,t}$) was then calculated using *Equation* 5:

$$(Eq. 5)\ n_{i,a,t} = N_t \cdot P_{a,t} \cdot P\left(L_{lower} \le L_i \le L_{upper} \,\middle|\, N(\bar{L}_{a,t}, \sigma_a^2)\right)$$

where N_t is the total number of individuals captured in month *t*, $P_{a,t}$ is the probability of a fish captured in month *t* being age *a*, L_{lower} and L_{upper} are the lower and upper bounds of a predicted size class (e.g., 230 and 240 mm), and $N(\bar{L}_{a,t}, \sigma_a^2)$ defines a normal probability density function with the mean length $\bar{L}_{a,t}$ of fish of age *a* in month *t* estimated from the VBGF (*Equation* 1 or 2-4), and a model estimated variance at age, σ_a^2. Because size distributions overlap across ages, the total number of expected fish of size *i* in month *t* was then calculated by summing the expected contributions to size bin *i* from each age:

$$(Eq. 6) \qquad N_{i,t} = \sum_{a=0}^{3} N_{i,a,t}$$

Five different candidate models were compared: (1) no differences in individual growth rates by year and non-seasonal growth, (2) no differences in individual growth rates by year and seasonal growth, (3) annually explicit individual growth rates and seasonal growth, (4) annually explicit individual growth rates and non-seasonal growth, and (5) annually explicit individual growth rates, non-seasonal growth in 2013 and seasonal growth in 2014 (Table 2). In all cases, model fit was assessed by freely estimating model parameters to minimize the log-likelihood between observed and predicted (*Equation* 5) monthly length-frequency data using the SOLVER optimization routine in Microsoft Excel (MS Excel 2013, Microsoft, Inc. Seattle, WA). Akaike's Information Criterion corrected for sample size (AIC_c) was used to select the best model from the candidate set and quantify the relative support of each model given the data (ω_i).

The assumptions all candidate models were as follows:

(1) Predicted length-at-age follows a normal distribution with mean $\bar{L}_a$ and variance σ_a^2.

(2) There are only four age classes present in the observed length-frequency samples (age 0, 1, 2, and 3). This assumption is supported by aging of sagittal otoliths from a subset of lionfish (n=100) which found that only 8% of individuals were age three (despite non-random sampling that was biased to select larger individuals), and no individuals were found to be age four or older (see *Age Validation* below).

(3) Lionfish recruitment is assumed to occur a single point during the year and was estimated in the model by the parameter, t_r, the estimated recruitment date of an annual cohort.

(4) Diver effort varied across time and the pattern of selectivity for divers of lionfish of varying ages is unknown, thus the proportion of lionfish in each age class is freely estimated. No attempt is made to make quantitative inferences regarding relative changes in abundance of cohorts over time (e.g., recruitment strength, natural mortality).

Model performance and sensitivity

Two types of analyses were conducted to examine the robustness of model solutions and associated parameter estimates. The first test was a randomized grid search to evaluate the robustness of the model; that is, the ability of the model to converge on a consistent, stable solution from randomly generated sets of biological plausible model parameters. Second, the sensitivity of the model to various model inputs was assessed by fixing individual model parameters at ± 10% of their best fit values, allowing the model to converge on a new constrained solution, and examining the resulting effect on model fit and parameter estimates.

Age validation

Ageing of a 100 fish subsample using sagittal otolith analysis was performed to verify ages and validate model outputs. Otoliths were extracted by first making a transverse cut into the brain cavity, and removing the otoliths from outpockets under the brain cavity. Otoliths were rinsed and stored dry in envelopes. Ageing was completed by the Florida Fish and Wildlife Research Institute (FWRI) in St. Petersburg, FL following the procedures outlined in VanderKooy and Guindon-Tisdel (2003). A singular otolith from each fish was embedded in casting resin and 500μm sections were cut using a Buehler low-speed Isomet saw. Sections were then mounted on glass slides with histomount and viewed under reflected light with a dissecting microscope at 32x magnification. Ages were assigned to fish using a January birthdate (Jearld 1983). Marginal increment analysis was used to validate ages and the distance from the annulus to the margin was scored 1-4. Two readers aged the otoliths independently. If the ages did not agree, the otolith was removed from further analysis. Plotting of these ages verified validates that the model was accurately predicting fish size-at-age (Figure 1-5).

RESULTS

General

Fish total lengths (n= 2,836) ranged from 41-448 mm in northeast Florida over the study period. Maximum length (448mm) and minimum length (41mm) were both recorded in August 2014. Some fish were not sexed and some fish were immature, but of the fish that were sexed there were 466 females present and 727 males. This approximates to a 2:3 female to male sex ratio.

Model Selection

There was considerable support for model 1 as the best fit model (ω_i= 0.81), which assumed annual differences in growth, seasonal variability in growth in 2014 and non-seasonal growth in 2013 (Table 1-1). The predicted length frequency distribution fits the observed length frequency distribution exceptionally well (Figures 1-2, 1-3), particularly in months with large sample sizes. The model converged on realistic values for life history parameters (Table 1-4). The Akaike weight ($P(model|data)$) of the best fit model was 0.81, but next best model differed by only two parameters and also fit the data reasonably well with a model weight of 0.19 (Table 1-1). Models that did not assume annual differences in growth or seasonal variability in growth fit the data poorly ($\omega_i \approx 0$).

Growth

The best fit model as chosen by AIC allowed for seasonal growth in 2014 (C=0.59, t_s=0.71) and non-seasonal growth (C = 0, t_s = 0) in 2013 (Table 1-1, Figure 1-2, 1-3). Brody growth coefficients for fish from 2013 (k= 0.62) were higher than those in 2014 (k= 0.47), with the same L_∞. Because our dataset had relatively few old fish, there was little information about maximum size and L_∞ was difficult to estimate precisely. Therefore, we fixed L_∞ at 448, the largest fish in our dataset. We also fixed t_0 at 0; this nuisance parameter was not required since the time at recruitment (t_r) was freely estimated (FAO 2016; see below). The estimated VBGF from the model in 2013 was:

$$(Eq.7)\quad L_t = 448\text{mm}\left[1 - e^{-0.62(t-0)}\right]$$

The estimated VBGF from the model in 2014 was:

$$(Eq.8)\quad L_t = 448\text{mm}\left[1 - e^{-0.47(t-0)+\left[\frac{(0.59*0.47)}{2\pi}Sin\pi(t-0.71)\right]-\left[\frac{0.59*0.47}{2\pi}Sin\pi(0-0.71)\right]}\right]$$

Population Age Structure

Aged otoliths ranged from age 0 to age 3 (Figure 1-5). Otolith data supported the model assumption of three age classes in the population and generally agreed with the predicted age class distribution (Figure 1-5). Direct quantitative comparisons of age composition between otolith samples and model outputs are not possible because we selectively targeted larger fish, for which age is more uncertain, for ageing analysis. For all months sampled across both years, the highest proportion of the fish fell into the age 1 age class (Table 1-3, 1-4). The highest proportion of age 0 fish occurred in April of each year, before the fish became age 1 (2013 recruitment date t_r= 0.64 or ~ August 20th; 2014 recruitment date t_r= 0.42 or ~ June 2nd).

Recruitment

Lionfish are recruiting to northeast Florida over a relatively short period during summer (t_r= August 20th for 2013; June 2nd for 2014). Recruitment in the model was assumed to happen one time during the year (t_r) and the data largely support that assumption. For instance, there was a distinct bimodal distribution of total lengths in both 2013 and 2014 (Figures 1-2, 1-3). Because recruitment occurs on a single date in the model, all variance in size-at-age is assumed to result from individual differences in growth rates and not timing of recruitment. Despite this, there was not overlap between age 1 and age 2 lengths in 2013 and very little overlap between age 1 and age 2 lengths in 2014. There was not large variance (σ_a^2) among total lengths of fish that were the same age (Table 1-2).

DISCUSSION

This study used a length-based age-structured model to predict size-at-age in lionfish and is the first to validate the statistical method using otoliths for this species. Length-based modeling of age and growth is a more practical method for these species than ageing by otoliths which is time consuming and can be imprecise (Edwards et al. 2014), particularly for tropical species which often lack defined annuli. Although a large sample size of lengths representative of the sampled population is necessary for ageing by length based methods (Fournier et al. 1998) and this information is commonly collected for lionfish in derby tournaments; many single day tournaments exceed 1000 fish. Theoretically, this method could be applied to many regions; however, more protracted recruitment could create more uncertainty in the model outputs. Nevertheless, the model generated important life history information for this region quickly and cost effectively in this region and could be applied to other regions to make inferences about regional differences in life history.

Our results generally suggest that lionfish have very fast growth rates in this region, mirroring the findings of many other studies (Barbour et al. 2011; Jud and Layman 2012; Albins 2013; Benkwitt 2013; Akins et al. 2014; Edwards et al. 2014;; Rodriguez-Cortez et al. 2015). Although it is not possible to make robust comparisons of growth coefficients with earlier studies because of differing sampling time, sampling methods, and overall project goals, we can postulate reasons for differences between our study and others. Several studies have targeted juvenile lionfish (<age1) which will be growing at their fastest rate (Jud and Layman 2012; Benkwitt 2013) and may overestimate growth rates when extrapolated to predict length in older fish for which growth slows markedly as they approach maximum size. Using the estimated VBGF parameters from the best fit model (Table 1-1), it was estimated that the maximum daily growth rate in our study was 0.81mm/day (occurring in summer of age 0). This is comparable to the findings of Jud and Layman (2012) in South Florida; however, significantly lower than the daily growth rates of Benkwitt (2013), perhaps due to differing environmental factors (warmer temperature), ecological factors (prey availability) or sampling error (measuring fish to the 1mm while alive). Our findings were similar to those from otolith ageing studies (Barbour et al. 2011; Edwards et al. 2014), although these studies had 8 age classes and 5 age classes, respectively, present in their sample and fish from the current study are all age 3 or younger. This study reflects the most current maximum size estimates for lionfish.

Throughout the progression of the invasion and increased popularity of lionfish “hunting,” maximum size estimates for lionfish have steadily grown (Akins pers. comm.). Lionfish are now frequently caught at or above 450mm (Akins, Johnson and Swenarton unpubl. data). The L_∞ values from earlier studies, especially Edwards et al. 2014 (L_∞=382mm males) may be outdated and inaccurate. In addition, studies that rely on otolith ageing have limitations that this study did not. For example, Barbour et al. (2011) were forced to fix t_0 because of gear bias and Edwards et al. (2014) was only able to age 42% of the otoliths collected because annuli were not discernable. As a result of these inconsistencies, the length-based estimation of growth presented in this study may be particularly useful in tropical regions, where annuli are typically more difficult to discern due to relatively consistent growth rates, than in subtropical and temperate areas where annuli are more apparent given the seasonal difference in growth (Green et al. 2009). Further, growth information from this study indicates that lionfish are growing extremely fast; this fact coupled with other life history information (Morris 2009; Ahrenholz and Morris 2010; Côté et al. 2013) is concerning for potential lionfish impacts in this region through both competition and predation. Other fish in the region that are competing on the same trophic level as lionfish (Layman and Allgeier 2012) such as black sea bass and vermillion snapper, take a longer time to grow to reproductive size (Hood et al. 1994; Zhao et al. 1997). With both a low size at maturity and fast growth rates, lionfish have the potential to reach a large size and reproduce well before their native competitors.

In this study, AIC selected a model that fit life history data differently by year. Lionfish grew more quickly in 2013 (k=0.62) than in 2014 (k=0.47) and the recruitment date in 2013 is also predicted to be later (mid-August) than in 2014 when the recruitment date is estimated to be during early-June. These discrepancies could be due to several factors. First, length-frequency data in 2013 was only available for two months (April and August), whereas the sampling in 2014 was expanded, with sampling occurring over a period of 10 months (April 2014-January 2015). Because data was limited in 2013, during the period of most rapid growth during summer, the model predicted rapid growth and was not constrained by the slower growth rates that occur during fall and winter. Ultimately, more rigorous sampling and larger sample sizes occurred in 2014 and the model predictions from that year are the most robust. Although we assume the annual differences predicted by the model to be due to sampling to some extent, annual differences in recruitment and year class survival could also be contributing to the observed patterns. Abiotic factors like temperature and currents and biotic factors like food availability and predation (Swearer et al. 1999) are subject to change year to year. It is beyond the scope of this study to determine which factors drove our observed patterns; however, studies that make inferences about lionfish age and growth either collected fish all in one year (Benkwitt 2013; Edwards et al. 2014; Jud and Layman 2012) or pooled fish from many years (Barbour et al. 2011), so it may be prudent for future studies to estimate life history parameters at finer temporal scale to determine if annual variability in population demographics exists.

Temperature has a large effect on growth in fishes, and for that reason, growth generally varies seasonally in temperate areas and is more uniform throughout the year in tropical areas (Pauly 1980). This study ascertained that lionfish were exhibiting seasonal growth in this temperate area, with a strength of seasonal oscillation of 0.59 (out of a maximum of 1) and the winter point (slowest growth point) occurring sometime in mid-February. Although our best model only fit seasonal parameters in 2014, the 2013 dataset consisted of only two months (April and August, Figure 1-2), so without winter months it is difficult for the model to determine the occurrence of seasonal growth. For the 2014 study period sampled (April 2014-January 2015), sea surface temperatures were 12˚C at the coldest and 32˚C at the warmest (NOAA). These fluctuations are predicted to be accompanied by reciprocal physiological, biological, and ecological changes in this region. Occasionally, seasonal growth estimated from lengths can be falsely estimated due to migration (Sparre 1980), but since lionfish have high site fidelity (Jud and Layman 2012), this is not likely to be the case in our study. This study is the first to account for seasonal differences in growth in lionfish. It is important to consider seasonal aspects of growth when modeling population demographics (Sparre 1990); thus, seasonal VBGF parameters may need to be included in future population assessments and removal modeling, especially in temperate areas.

A very clear bimodal distribution is evident in the data from both 2013 and 2014 (Figure 1-2; 1-3). This pattern indicates that lionfish are likely recruiting at one time during the year. The presence of a single annual recruitment event could be due to several factors:

spawning could be occurring at one peak time during late spring/summer, although literature suggests lionfish reproduce year-round (Ahrenholz and Morris 2010; Morris et al. 2011; Gardner et al. 2015), pre-settlement mortality as larvae could be occurring, post-settlement juvenile mortality could be occurring, or a combination of these processes. Recent reproductive assessments in temperate areas indicate reproduction is more seasonal (Fogg et al. 2015) than originally predicted in Ahrenholz and Morris (2010) and Morris et al. (2011), so that could be a contributing factor. In addition, the presence of clear cohorts and low variability in size-at-age is indicative of low variability in individual growth rates, perhaps as a result of low genetic variability in this invasive population (Hamner et al. 2007). Further, preliminary length-based modeling of derby data from the Florida Keys (Akins unpubl. data) indicates increased variance in fish length-at-ages from south Florida and suggests fish from the Keys are recruiting over a broader time scale relative to their northern conspecifics. Preliminary modeling of lionfish length-frequencies from Sarasota, FL and the northern Gulf of Mexico (Dahl and Patterson 2014) shows similar patterns of distinct cohorts that have been observed in this study and suggests the factors driving these differences may be latitudinal. The observed differences between regions are not expected to be the result of genetic differences between the two invasive species of lionfish, *Pterois volitans* and *Pterois miles*, because *P.miles* comprises such a small portion (~5%) of the abundance of lionfish in their invaded range (Hamner et al. 2007). Although identifying the causative factors driving this pattern is not possible from our current data, identifying factors that may limit lionfish larval supply or survival in northern latitudes is inherently important to management and future control efforts.

Both the otolith ages and the predicted ages from the dataset show that a majority of the sampled population is three years of age or younger. Lionfish live for decades in aquaria (Potts et al. 2011) and older fish have been aged in North Carolina (Barbour et al. 2011), so this trend is unexpected. Further, lionfish first arrived in the South Atlantic Bight in 2000, following their dispersal north from their initial introduction in South Florida (Schofield 2009). This pattern could be explained by high natural mortality at a young age, although that seems unlikely given the lack of significant documented predation and resiliency of the species. If mortality is occurring, it could be due to periodic cold winters in the region, which may lower temperatures below lethal limits; temperature is one of the only abiotic factors limiting the survival of this naturally tropical fish (Kimball et al. 2004). Far more likely is the presence of older ages at depth, either as a result of an ontogenetic habitat shift or culling in shallow areas, allowing fish in deeper areas to become older (Andrari-Brown 2015). The presence of deep water refuges of lionfish is a major concern for management and control, since they occur beyond recreational diving depth and culling efforts in shallow depths can be easily replenished due to the high reproductive rates of the species (Morris 2009). Further research in this area should include long-term tagging studies and aging of lionfish retrieved at greater depths.

CONCLUSION

Overall, the key findings from this study are:

(1). Clear age classes are present in lionfish size structure, indicating lionfish larval supply or survival is limited during certain times of the year in northeast Florida and potentially other northern parts of the lionfish range. As a result, efforts to control lionfish populations in this region may be less than in more tropical areas where larval supply and/or survival is not limited.

(2). Annual differences in population structure and growth parameters may indicate that growth parameters from studies only lasting one year are inaccurate and parameters need to be collected at a finer temporal scale for accurate modeling.

(3). Only 3 age classes are present in this region, despite field and captive studies that show lionfish are relatively long-lived. This finding suggests lionfish may be undergoing an ontogenetic habitat shift to a deep water refuge; thus, intercepting lionfish before their migration to depth is of the upmost importance.

(4). The significance of seasonal growth indicates that there is some variability throughout the year and models that rely heavily on growth parameters should consider this variability.

In the future, continued observation of changes in population structure will demonstrate the progression of lionfish population dynamics over time. More reproductive assessments and larval survival studies need to be done to decipher what factors are leading to restricted recruitment in this area. Finally, these data can be used directly to lessen uncertainty in estimating growth in the varying ecosystems of Florida.

TABLES AND FIGURES

Table 1-1. Akaike Information Criteria (AIC), corrected AIC (AIC_c) values, and model weights for the 5 candidate model comparisons in the study of lionfish population demographics in the South Atlantic Bight.

Model #	Seasonalized 2013	Seasonalized 2014	Annual	AIC	AIC_c	ΔAIC_c	ω
1	N	Y	Y	1435.4	1443.5	0	0.81
2	Y	Y	Y	1437.5	1446.4	2.9	0.19
3	N	N	Y	1454.1	1461.3	17.9	0
4	Y	Y	N	1460.9	1467.8	24.3	0
5	N	N	N	1486.5	1492.3	48.8	0

Table 1-2. von Bertalanffy growth parameters for lionfish caught in the South Atlantic Bight, estimated from the best fit model (Model 1; see Table 1-1).

Year	K	t_0	$L\infty$ (mm)	T_r	C	t_s	σ_a^2
2013	0.62	0	448	0.64	0	0	20.23
2014	0.47	0	448	0.42	0.59	0.71	26.57

Table 1-3a. The estimated proportion of the population in each age class (P_{at}) at each sampling period in 2013; data derived from the best fit model (Table 1-1).

Month	P_{a0}	P_{a1}	P_{a2}	P_{a3}
April	0.58	0.37	0	0
August	0	0.86	0.09	0

Table 1-3b. The estimated proportion of the population in each age class (P_{at}) at each sampling period in 2014; data derived from the best fit model (Table 1-1).

Month	P_{a0}	P_{a1}	P_{a2}	P_{a3}
April	0.37	0.61	0.01	0.02
July	0	0.62	0.38	0
August	0.01	0.49	0.44	0.06
October	0.19	0.68	0.11	0.02
November	0.09	0.46	0.44	0.01
January	0	1	0	0

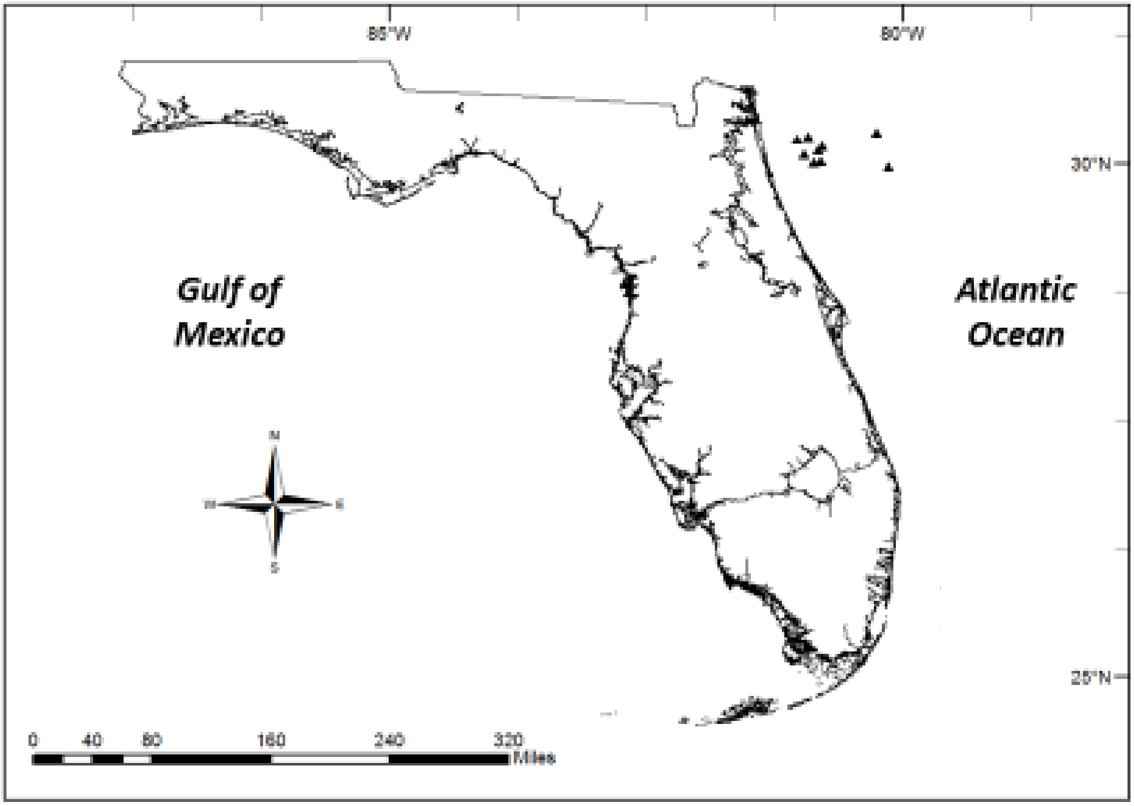

Figure 1-1. Dive sites where lionfish were collected off the coast of northeast Florida in 2013 and 2014.

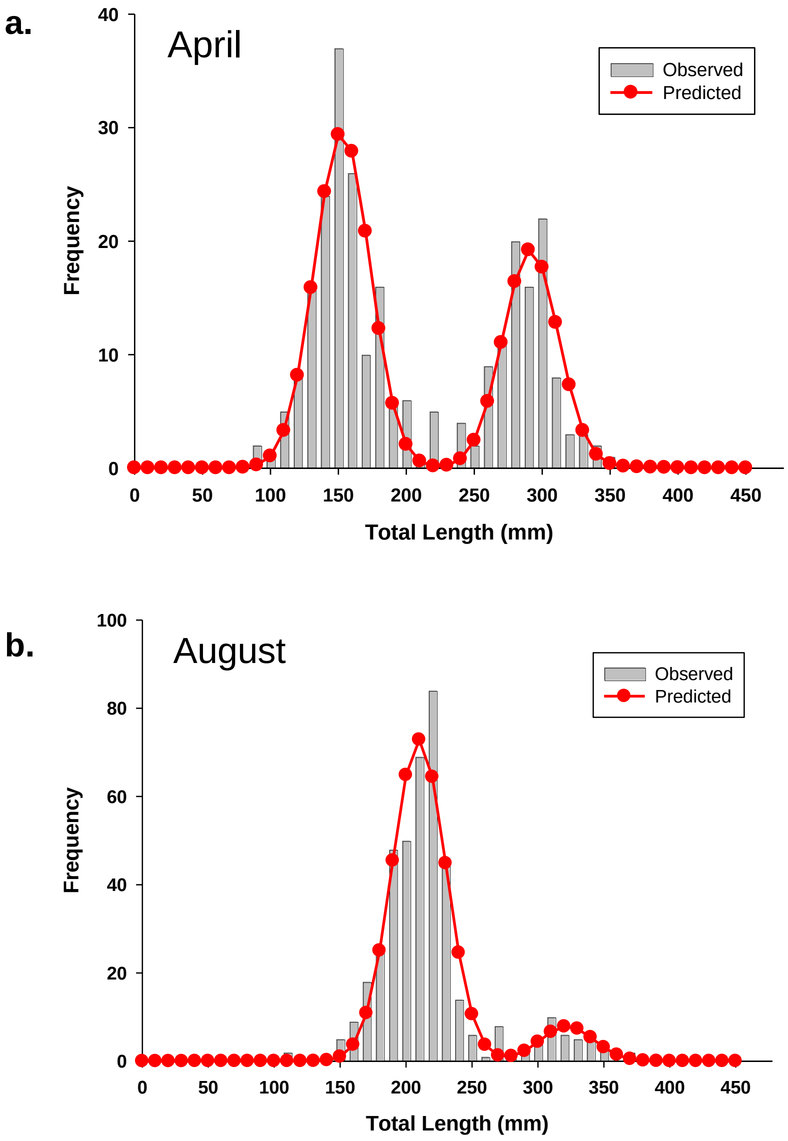

Figure 1-2. Length frequency histograms of lionfish collected from northeast Florida by derby events in (a) April 2013 and (b) August 2013 (gray bars). The red line symbolizes the predicted size-at-age distribution of lionfish from the best statistical length-based model (see Table 1-1).

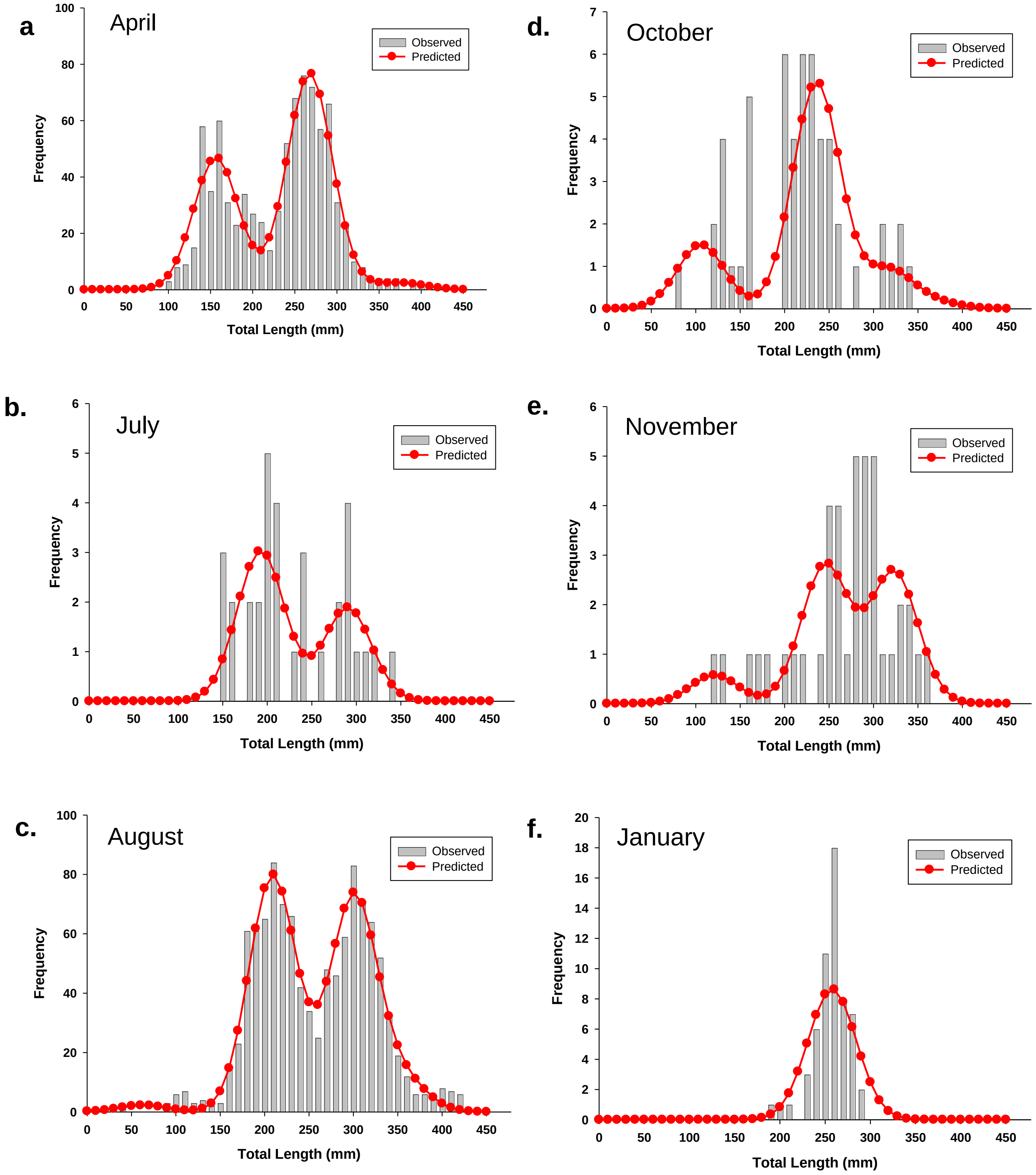

Figure 1-3. Length frequency histograms of lionfish collected from northeast Florida by derby events in (a) April 2014 (b) July 2014 (c) August 2014 (d) October 2014 (e) November 2014 and (f) January 2015 (grey bars). The red line symbolizes the predicted size-at-age distribution of lionfish from the best statistical length-based model (see Table 1-1).

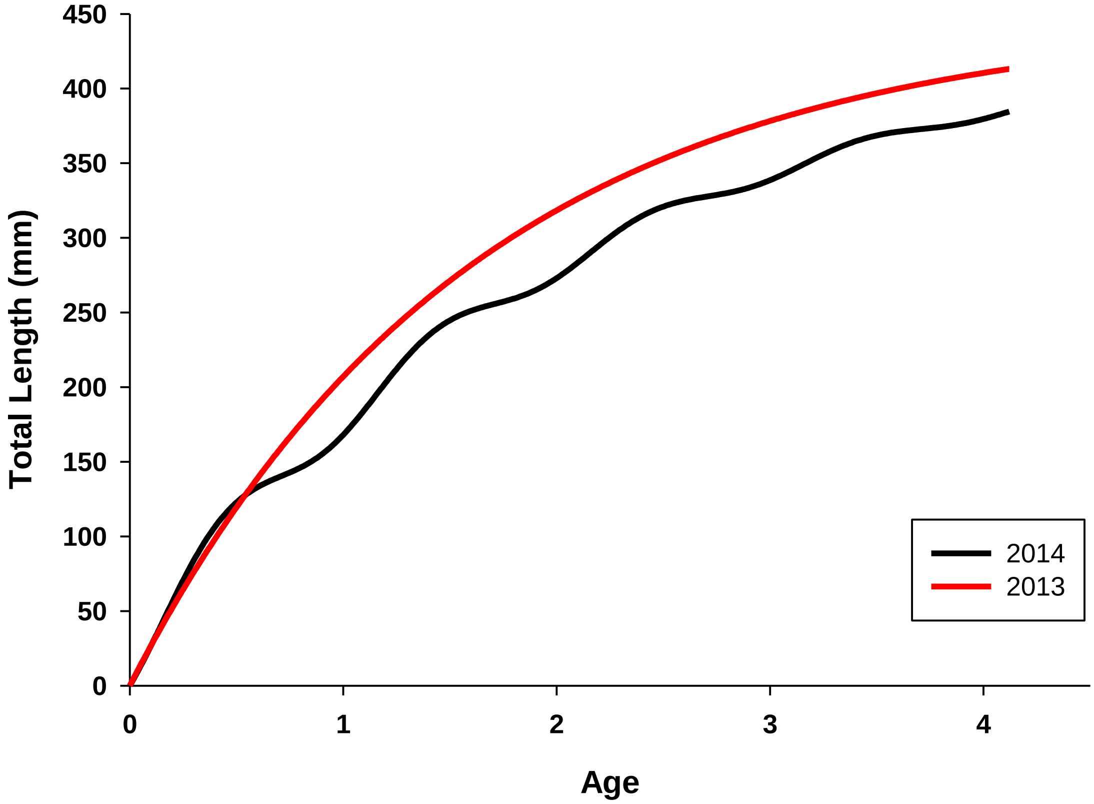

Figure 1-4. Von Bertalanffy growth functions generated from the best model outputs. (red) Nonseasonalized curve for lionfish caught in 2013 and (black) seasonalized curve for lionfish caught in 2014.

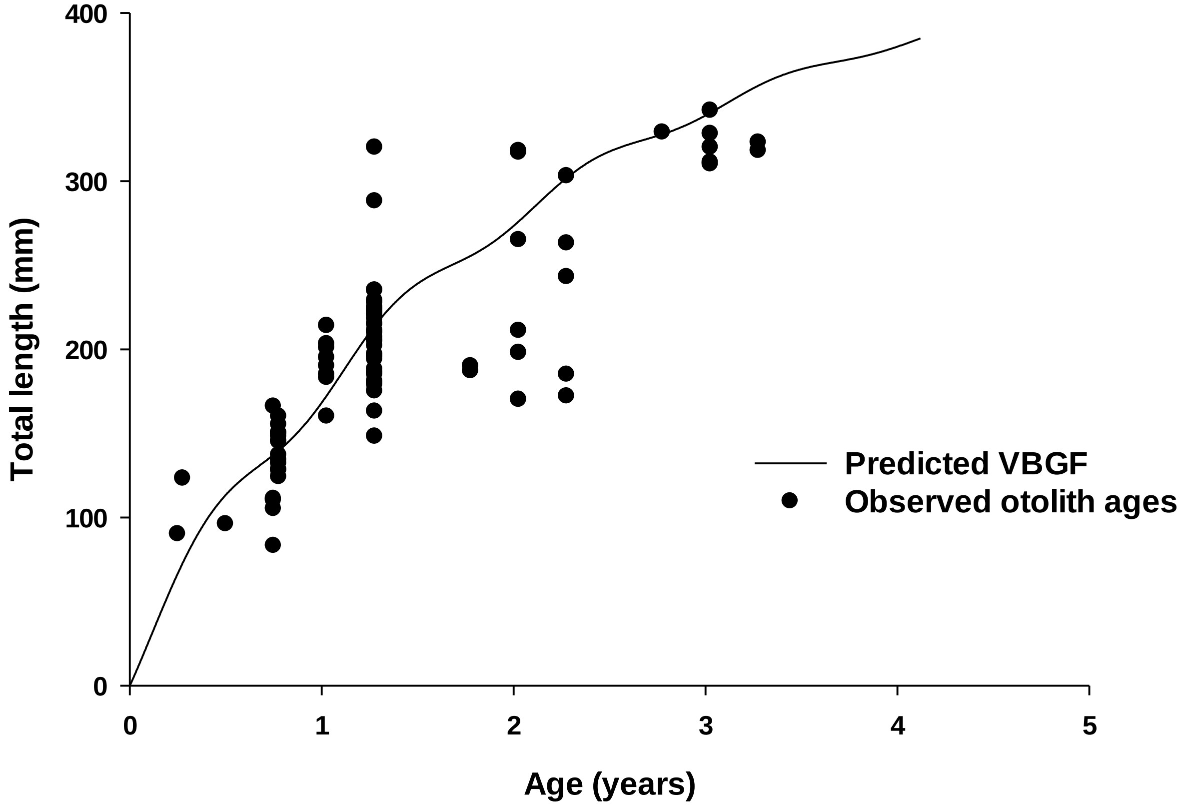

Figure 1-5. The seasonalized von Bertalanffy growth function predicting size-at-age, generated from the best model output (see Table 1-2) in 2014. The ages of otoliths from lionfish caught during the same period.

CHAPTER 2

Diet of Invasive Lionfish in the South Atlantic Bight

ABSTRACT

The impacts of invasive predators on native systems can be drastic. A marine invasive predator of particular concern is the invasive lionfish (*Pterois volitans*), which has been shown to drive declines in native species biomass and recruitment. Studies of lionfish diet, and most other biology and ecology, have been centered in subtropical systems. This study employed DNA barcoding to characterize the diet of lionfish from offshore of northeast Florida, a temperate system. Eighteen species were identified, belonging to 11 families, indicating that lionfish are generalist consumers at the population level. Although not as diverse as other described diets for lionfish, a prey accumulation curve showed this study accurately estimated the diet of lionfish in this region. Diet metrics and the Index of Relative Importance ranked the same prey species as being the most important: round scad (*Decapterus punctatus*), black sea bass (*Centropristis striata*) and sand perch (*Diplectrum formosum*). The components of the lionfish diet in this region overlapped with documented diets of native species, including scamp grouper (*Mycteroperca phenax*) and vermillion snapper (*Rhomboplites aurorubens*). This study discovered a commercially important species, *C. striata*, as an important component in the lionfish diet and increased juvenile mortality in the species should be considered by managers. DNA barcoding was a useful

technique for diet estimation, particularly because this species has a high proportion of unidentifiable prey contents in their gut. Overall, the results presented herein suggest high potential species-specific and ecological impacts in the South Atlantic Bight.

INTRODUCTION

Among the array of problems caused by invasive species, one major impact is the alteration of competitive and predatory interactions among species within native communities. Since predators themselves have profound effects on marine community structure (Paine 1966), invasive predators can fundamentally alter food web functioning (Molnar et al. 2008) and in the worst cases, cause native species declines and extinctions (Ruiz et al. 1997; Mack et al. 2000). The effects of invasive predators can be facilitated directly by predation, or indirectly through competition with, or exclusion of, native organisms. Moreover, the consumption of ecologically important species can cause cascading effects throughout the ecosystem that are difficult to predict. Understanding how and what invasive species are consuming in their invaded range is the first of many steps towards understanding species-specific and ecosystem level impacts.

Lionfish (*Pterois volitans*) are invasive marine predators native to the Indo-Pacific. Introduced into their invaded range by negligent aquarists, lionfish are now established in the Western Atlantic, Caribbean and Gulf of Mexico (Schofield 2009; Morris 2012). Lionfish are generalist consumers of small-bodied fishes and invertebrates and studies in The Bahamas have shown they are capable of reducing the recruitment and biomass of prey species by up to 79%, and 65%, respectively (Albins and Hixon 2006; Green et al. 2012;

Black et al. 2013). These predatory impacts may be resulting in cascading indirect effects because many of these small prey species play important ecological roles. For example, lionfish are known to feed on several species of parrotfish (Morris and Akins 2009; Côté et al. 2013), which heavily graze macroalgae which compete with reef-building corals, indirectly benefitting corals (Mumby et al. 2007). Although economically important species are infrequent in lionfish diets, the presence of vermillion snapper (*R. aurorubens*) in the diets of lionfish from the Gulf of Mexico indicates that lionfish may be consuming a higher proportion of economically important species in temperate regions (Dahl and Patterson 2014).

Previous characterization of lionfish diet and trophic impacts have been focused predominantly in tropical ecosystems, and on coral reefs where local fish assemblages are well characterized and lionfish are abundant (Albins and Hixon 2008; Green et al. 2012; Côté et al. 2013). However, because fish abundance and diversity varies with a suite of environmental (e.g., salinity, temperature), physical (e.g., wind, currents), biological (size, life history stage) and ecological (e.g., habitat, predation) factors, the impact of lionfish will vary regionally and among ecosystems. Lionfish have been increasingly observed within alternative habitats, in particular on artificial reefs and structures (Smith 2010) and within shallow mangrove and seagrass ecosystems (Barbour et al. 2008; Jud et al. 2011; Claydon et al. 2012). Similarly, the potential impact of lionfish in nearshore marine hard bottom habitats of the southern Atlantic Bight (SAB) is unknown, yet these coastal habitats harbor high densities of lionfish (Swenarton and Johnson, see Chapter 1) and serve as important juvenile nurseries for many fishes in the south Atlantic. For example, black sea bass (*Centropristis striatus*) initially settle from the plankton in nearshore hard-bottom habitats, where lionfish are frequently congregated. Other important fishes that undergo the larval-juvenile transition in hard-bottom include Atlantic menhaden (*Brevoortia tyrannus*), spotted sea trout (*Cynoscion nebulosus*) and weakfish (*Cynoscion regalis*) (Hare et al. 2007). Lionfish also impact many economically important large-bodied reef predators (e.g., snappers and groupers) directly by preying on them in their juvenile stages, or indirectly by competing with them for food resources as adults, and may possibly impede the recovery of certain species of concern in the south Atlantic (e.g, Warsaw grouper and speckled hind) (Morris 2012).

This study sought to quantify the diet of lionfish in northeast Florida and assess temporal variability in diet as a function of seasonal changes in environmental conditions (e.g., temperature) and prey abundance and diversity. We employed DNA barcoding, an exciting new approach that provides increased taxonomic resolution (typically to species for fishes), and is particularly valuable for predators such as lionfish in which a high frequency of prey items are not identifiable (Morris and Akins 2009; Johnson et al. unpublished data). This approach has been shown to be effective in recent studies of lionfish trophic dynamics conducted in the Bahamas (Côté et al. 2013) and Mexico (Valdez-Moreno et al. 2012). We used this novel technique to accomplish two main objectives:

(1) To characterize lionfish diet and identify individual prey species that are highly important in the lionfish diet in this region and

(2) Better understand the potential indirect and direct impacts of lionfish on native prey species and ecosystems.

METHODS

Sample Collection

Lionfish samples were collected from numerous locations offshore of northeast Florida by trained spearfishermen (Figure 1-1). Sampling occurred during several large-scale public removal events in 2013-15 and by opportunistic sampling by recreational spearfishermen in 2014-15. Lionfish were collected throughout the year at artificial and natural reef sites ranging from 20-45m. Lionfish in this region are restricted to offshore hardbottom and artificial reef habitats; inshore waters fall below their lower lethal temperature in the winter (Kimball et al. 2004). Lionfish were placed on ice and transported to the UNF Fisheries Biology Laboratory in Jacksonville, FL, where lionfish were dissected immediately or placed in freezers at -20°C for later dissection.

Laboratory Procedures

Lionfish were measured for total length (TL) and standard length (SL) to the nearest 1mm, weighed to the nearest 0.1g, sexed, and had their sagittal otoliths removed. Six-hundred twenty-one stomachs were randomly selected and removed. Two-hundred ninety-four stomachs contained prey; these items were enumerated, measured for total length or carapace width (if applicable) and weighed. Each prey item was given a digestion score (Green et al. 2012). Prey items were visually identified if possible and then preserved in 95% ethanol for later identification using DNA barcoding.

Overall, 381 prey items (vertebrates, invertebrates, or unknown) from the 294 stomachs were sent for identification using DNA barcoding. Because most prey items were in late stages of digestion and too degraded for adequate visual identification, samples were taxonomically identified using DNA barcoding approaches.

A 1mm piece of tissue was removed (from the right side if possible) from the 381 prey items and placed in Autogen M2 Tissue Digestion Fluid. All tools were rinsed in 95% ethanol and flame sterilized in between samples. All DNA barcoding analysis was conducted at the Smithsonian Institution's Laboratories of Analytical Biology (SI-LAB). DNA was extracted from fish tissue via an automated phenol: chloroform extraction. Approximately 650bp of the COI gene was amplified by polymerase chain reaction (PCR). The PCR 10 µL mixes included 1µL of the genomic extract, 0.4 µL $MgCl_2$ (50 nM), 1µL 10X PCR buffer, 0.5 µL mM deoxyribonucleic triphosphate (dNTP), 0.05 µL Bioline Taq polymerase (Bioline USA, Boston, MA), 0.3 µL of each primer (FISH-BCL, FISH-BCH) and 2µL DNA template. The thermal conditions for PCR included: 1 cycle for 5 min at 95°C; 35 cycles for 0.5 min at 95°C, 0.5 min at 52°C and 0.75 min at 72°C; 1 cycle for 5 min at 72°C; and a hold at 10°C.

Sequencing reactions were performed using 1 µL of the PCR product with 0.5 µL primer, 1.75 µL BigDye buffer, and 0.5 µL BigDye (Applied Biosystems Inc., Foster City, CA) and run for 30 cycles of 0.5 min at 95°C, 0.5 min at 50°C, 4 min at 60°C; then held at 10°C products were labelled using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA), sequenced bidirectionally using an ABI 3730XL automated DNA sequencer (Applied Biosystems Inc., Foster City, CA). Sequences were trimmed and aligned using Sequencher 4.7 (Genecodes, Ann Arbor, MI).

Barcodes were matched to species in the using the ID engine at the Barcode of Life Database (BOLD; www.boldsystems.org) and used in subsequent dietary analyses if a sequence similarity of over 98 % was reached. To verify the accuracy of barcoding, 20 samples were sent that had a digestion score of 1 or 2 (not digested) and had been visually identified.

Data Analysis

Three diet metrics: frequency of occurrence (%F), percent composition by number (%N) and percent composition by weight (%W) (Hyslop 1980), and one index of importance, the Index of Relative Importance (IRI, equation 1) (Pinkas et al. 1971, George and Hadley 1979) were used to assess the importance of individual prey species in lionfish diets.

$$(1)\ IRI_a = \frac{100 \times (F_a + N_a + W_a)}{\sum_{a=1}^{n}(F_a + N_a + W_a)}$$

where *n* is the number of species, F_a is the frequency of occurrence of species *a*, N_a is the percent composition by number of species *a* and W_a is the percent composition by wet weight of species *a*.

To assess ontogenetic diet changes with respect to invertebrate composition of the diet, lionfish total lengths were binned into 20mm bins and the mean proportion of invertebrates (with respect to number and respect to weight) was calculated for each bin. Non-linear regression (exponential decline model) was fit to the observed data to determine if the proportion of invertebrates in the diet varied as a function of lionfish size (TL).

A cumulative prey curve was used to assess whether adequate sample size to characterize the lionfish diet has been reached. Although previous studies have indicated a large number of samples is necessary (Morris and Akins 2009, Côté et al. 2013), we expected the sample size required to be reduced relative to previous studies given the reduced diversity of fish and invertebrate assemblages in more temperate latitudes and the use of DNA barcoding which greatly enhances the number positive identifications of prey items from stomachs. We used the program Estimate S (Version 9.1; Colwell et al. 2012) to approximate the asymptotic prey diversity of our samples and specifically, the Chao 2 species diversity estimator. The Chao 2 estimator uses occurrence data from multiple samples to calculate diversity (Chao 2005).

RESULTS

General

Stomachs from 621 lionfish, ranging from 103 mm to 435 mm, were removed and dissected. Of these stomachs, 327 (53%) were empty. The remaining stomachs contained between 1 and 8 prey items. Of the 381 vertebrate tissue samples sent out for DNA barcoding, to date, 188 vertebrate barcodes were returned. Invertebrate barcodes were not included in this chapter and will be the subject of future papers. Thirty-nine of the vertebrate barcodes positively matched with lionfish (*Pterois volitans*), were assumed to result from contamination and were excluded from all further analysis.

Ontogenetic diet changes

The same 294 stomachs that were used for DNA barcoding analysis were used to examine the ontogenetic change in diet. An exponential decrease occurred in the proportion of invertebrates in the gut, both as a function of number (r^2= 0.63, p= 0.0001) and weight (r^2= 0.63, p= 0.0001), as lionfish size (TL) increased (Figure 2-1, Figure 2-2). The stomachs of lionfish between 120 mm and 140 mm were composed 60% of invertebrates; this proportion declined to 0% in the largest fish (360-450 mm TL).

Diet composition

A total of 18 species from 11 families were identified by DNA barcoding analysis. An additional 3 species were described by visual identification; these were not included in any quantitative analyses of diet but are listed in Table 2-1. The same three species were highest in frequency of occurrence (%F), percent number (%N), and the most important in the index of relative importance (IRI): round scad (*Decapterus punctatus*), black sea bass (*Centropristis striata*) and sand perch (*Diplectrum formosum*). The most important prey in terms of percent weight (%W) also included *D. punctatus* and *D. formosum* as important, but included bank sea bass as the third most important (*Centropristis ocyurus*). The cumulative number of observed prey species increased with sample size. The Chao 2 non-parametric estimator of species diversity reached an asymptote (Figure 2-1); however, the upper 95% confidence interval of the Chao 2 estimator suggested there could be as many as 29 species comprising the lionfish diet in this region.

Seasonal variability in diet

The composition of lionfish diets varied by season. For example, *C. striata*, *Serranus subligarius* (belted sandfish), *Mullus auratus* (red goatfish), *Starksia ocellata* (checkered blenny) were only present in the gut in the spring (March-May, n=76). *Apogon affinis* (bigtooth cardinalfish), *Phaeoptyx pigmentaria* (Dusky cardinalfish), and *Halichoeres dispilus* (chameleon wrasse) were only present in the gut in the summer (June- August, n=53). In the spring, *C. striata* was the most frequent, the highest by number, while *D. formosum* was the highest by weight and the most important (IRI). In summer, *D. punctatus*, *Haemulon aurolineatum* (tomtate grunt) and *D. formosum* were highest in frequency, number, weight and importance. Barcodes identified from specimens caught in fall and winter were low (n=20), limiting inference from these seasons at this time, however, *D. punctatus* was highest in frequency, number, weight and importance.

DISCUSSION

The main finding from this study is that lionfish in the South Atlantic Bight are feeding primarily on small-bodied, reef-associated fishes. Although not as diverse as the diets found in other regions (Morris and Akins 2009; Valdez-Moreno et al. 2012; Côté et al. 2013), our findings in the South Atlantic Bight still indicate an overall generalist diet. Our study added 15 additional species to known prey species of lionfish in this region, as the only other published study in the SAB is Muñoz et al. (2011). Contrary to Muñoz et al. (2011), this study found three species to be important in most dietary metrics: sand perch (*D. formosum*), round scad (*D. punctatus*) and black sea bass (*C. striata*). Black sea bass, a recently recovered fishery, is one of the first commercially important species recorded to be important in the lionfish diet.

Lionfish were found to exhibit an ontogenetic shift from a diet composed mainly of invertebrates to a diet composed mainly of teleosts. This finding is in accordance with other published lionfish diet studies (Morris and Akins 2009, Muñoz et al. 2011, Dahl and Patterson 2014). This ontogenetic shift in diet is common in many other teleosts, including red snapper (Szedlmayer and Lee 2004), Nassau grouper (Eggleston et al. 1998) and snook (Luczkovich et al. 1995). Ontogenetic diet shifts are generally prevalent when feeding is a function of gape width (Scharf et al. 2000). Interestingly, invertebrates in this study were not absent from the diets of larger lionfish. Other studies have found that lionfish diet is dependent on prey availability (Muñoz et al. 2011, Côté et al. 2013), so invertebrates are likely opportunistically consumed when available but not the primary food source in larger lionfish.

Round scad (*D. punctatus*), sand perch (*D. formosum*) and black sea bass (*C. striata*) were the most important prey on the basis of %F, %N, and the Index of Relative Importance (IRI). Bank sea bass (*C. ocyurus*) was important in terms of %W, but in no other dietary metric. Interestingly, scad are generally considered a pelagic species, although they tend to school both in the mid water column and around reefs (Smith-Vaniz et al. 2015). Other studies have found that lionfish diets are composed of mostly demersal and benthic species (Côté et al. 2013; Dahl and Patterson 2014), and the high prevalence of scad in the lionfish diet may indicate they are feeding away from the reef structures. Round scad was also found in the diets of lionfish from the Gulf of Mexico (Dahl and Patterson 2014), but have not been reported in previous assessments of lionfish diet in the South Atlantic Bight (Muñoz et al. 2011). The absence of scad from earlier work may be due to sampling season; Muñoz et al. (2011) only collected lionfish in the summer, and scad are differentially distributed by season (Smith-Vaniz et al. 2015). The majority of the samples in this study came from artificial reef habitats with high lionfish densities (Figure 1-1), thus density-dependent competition for prey (Svanbäck and Bolnick 2007) may cause lionfish to forage further from the reef, where they encounter pelagic species. Lionfish have the potential to impact abundances of this species, which is especially worrisome because scad are recreationally important and commonly used as a baitfish throughout their range (Smith-Vaniz et al. 2015). In contrast, sand perch, sea basses, and other fishes found in this study such as wrasses and cardinalfish, are demersal species and substrate associated and their presence as prey items is in accordance with other lionfish diet studies (Morris and Akins 2009; Valdez-Moreno et al. 2012; Côté et al. 2013). Although not commercially important, many of these species fill important ecological roles. For example, goatfishes are considered ecosystem engineers because their feeding strategies locate and uncover buried prey using barbels, which oxygenates the sediment (Uiblein 2007). Damselfish actively feed on algal mats in their territories, which promotes algal diversity, invertebrate abundance (Ferreira et al. 1998) and nitrogen fixation (Lobel 1980). Other small-bodied, demersal species like blennies and gobies are detritivores, quickly turning over biomass and making nutrients accessible in the system (Wilson 2004). Thus, lionfish feeding on small demersal species could have negative indirect ecological effects, although these are difficult to quantify.

Our study found that lionfish, like many other successful invasive species (Olden et al. 2004), are generalist consumers on the population level, consuming 18 species from 11 families (Table 2-1). Although diet breadth in this region is not as substantial as recorded in the Caribbean (Morris and Akins 2009, Valdez-Moreno et al. 2012, Côté et al. 2013); this occurrence is most likely a function of the lower diversity of prey in this region (Schobernd and Sedberry 2009). An asymptote was reached in the cumulative prey curve for this study (Figure 2-1), indicating that this study closely estimated diet for lionfish in this region. We also found interesting indications of individual specialization, when a generalist population is actually made of many specialists, which has been suggested for lionfish by Layman and Allgeier (2012). One individual lionfish consumed 8 prey items, all of which were barcoded as black sea bass (*C. straita*). Another individual had consumed 5 wrasses from the *Halichoeres* genus. Layman and Allgeier (2012) suggested individual specialization in lionfish is the result of prey availability and lionfish site fidelity, which may also be the mechanism underlying the pattern in our dataset. Individual specialization within invasive lionfish could result in small-scale extirpation of specialized prey items and may have larger evolutionary consequences such as rapid diversification (Bolnick et al. 2003). More research into intraspecific variation in lionfish diet, and other components of their biology, such as growth (see Chapter 1), is necessary to accurately predicting the predatory impacts of lionfish.

The prevalence and importance of cannibalism in lionfish is controversial. Although every step was taken to avoid contamination in this study, 29 of 138 barcodes amplified and identified were lionfish (*Pterois volitans*). Because of the high risk of contamination and the fact that several of the items identified as lionfish were visually identified as belonging to other families, for example, Bothidae, all lionfish barcodes were excluded from analysis, although there is probably some level of cannibalism occurring in this region. Anecdotal evidence and visual identification evidence (Fishelson 1997; Valdez-Moreno 2012; Côté et al. 2013; Dahl and Patterson 2014) have shown that lionfish do cannibalize one other, but the extent of cannibalism in the wild is unclear. Cannibalism is common in marine fishes has been reported in over 36 teleost families, including salmonids and gadids (Smith and Reay 1991) and may be a density-dependent behavior. Cannibalism may occur simply as a function of prey availability; thus, we should expect areas with dense populations of lionfish, like the northern Gulf of Mexico (Dahl and Patterson 2014) to have higher rates of cannibalism. If so, cannibalism may function in population regulation at high densities (Smith and Reay 1991) and act in concert with previously described density-dependent mechanisms such as density-dependent growth, which has been documented for lionfish in the Caribbean (Benkwitt 2013). However, it is possible that the importance of cannibalism in lionfish may be overestimated, especially in studies that employ DNA barcoding. Contamination can occur in one or many steps in the DNA barcoding process (Radulavici et al. 2010, Barba et al. 2013, Pompanon et al. 2012). Studies that use DNA barcoding to assess diet are more likely to incur contamination because all prey items have traces of DNA from their predators. This DNA can be partially avoided by removing the outside tissue of the prey sample that has come into contact with the predator stomach before sampling, but, especially for more digested items, this technique is imperfect. We do not feel that contamination was adequately addressed in previous work (Valdez-Moreno et al. 2012; Côté et al. 2013) and cannibalism may have been overestimated in this species. Best laboratory practices, replicated sampling and the corroboration of barcodes with visual ID is necessary to assess contamination frequency in the future.

Managers in the South Atlantic Bight are concerned about the predatory and competitive effect lionfish are having on economically important fishery stocks. Besides the prevalence of *R. auroruebens* in the guts of lionfish from artificial reefs in the northern Gulf of Mexico (Dahl and Patterson 2014), this study is the first to find the prevalence of a commercially important species in lionfish diets. Black sea bass were historically overfished with overfishing occurring and the stock has since been rebuilt following successful management. Black sea bass undergo their larval-juvenile transition on offshore reefs (Adams 1993), where lionfish are abundant (Swenarton and Johnson unpubl. data), before moving inshore to estuary and coastal nursery areas (Stiemle et al. 1999). Black sea bass also frequent artificial reefs, where lionfish are more abundant than on natural reefs (Swenarton and Johnson, unpubl. data, Dahl and Patterson 2014). This initial settlement makes them more susceptible to lionfish predation than other fish species that undergo their larval-juvenile transition directly in estuaries and shallow coastal areas. The prey items that were barcoded as *C. striata* were all under 5 grams, and most were under 2 grams. In addition, black sea bass ranked as the most important prey items in terms of %F, %N and IRI in the spring season (Table 2-3), which was the only season in which they were sampled. These two findings indicate lionfish are primarily feeding on juvenile black sea bass and that there are a large amount of juveniles present on offshore reefs in the spring. This relatively recent source of juvenile mortality may substantially affect M (the instantaneous rate of natural mortality) and should be considered in further stock assessments for this species. In addition, six barcodes of vermillion snapper (*Rhomboplites aurorubens*) were observed, indicating juvenile vermillion snapper are also a component of the lionfish diet, but not as highly important as other prey items. Nevertheless, even with a small importance in their diets, the high abundance of lionfish offshore in the South Atlantic Bight (Whitfield et al. 2007) could result in a noticeable effect on vermillion snapper recruitment and management may need to compensate for this change.

Although finding a commercially important species in such a high frequency and number is alarming, we also found a large proportion of empty stomachs throughout the study (49%). We suspect that this is due to the high consumption rates of this species (Cerino et al. 2015); generally, fish with high consumption rates will also have very fast evacuation rates (Bajkov 1935). We also think the observed pattern of empty stomachs is a result our sampling time; samples were taken in the morning or mid-day, instead of at dawn and dusk when lionfish are most actively feeding (Green et al. 2011). Sampling time may have also resulted in the prevalence of highly digested, unidentifiable prey items in our samples. We noticed a prominent pattern by site, where lionfish collected from the same site tended to have either a high occurrence of full stomachs or a high occurrence of empty stomachs. It is possible that lionfish have extirpated available prey in a certain area, making prey not as available as it is in other areas.

As generalist consumers, lionfish diets likely overlap with commercially important and protected species in this area and are likely having a negative competitive effect on native species, in addition to a predatory impact. The use of isotopic data in the Bahamas has corroborated that lionfish occupy a similar niche to that of native snappers (Layman and Allgeier 2012). In the South Atlantic, vermillion snapper (*R. aurorubens*) consume a large portion of serranids, labrids and carangids (Grimes 1979) and cohabitate with lionfish on natural and artificial reefs at intermediate depths (50-200m) (Grimes et al. 1982, Sedberry and Van Dolah 1984). Lionfish diets are also strikingly similar to small groupers in the region, such as scamp grouper (*Mycteroperca phenax*) which consume round scad (*D. punctatus*), tomtate (*H. aurolineatum*) and serranids in high frequencies (Matheson et al. 1986). The indirect effect of competition on native species can be difficult to measure (Leary et al. 2012). The first step is to more completely characterize, or publish existing data on, diets and trophic positions of important fishes in the South Atlantic.

CONCLUSION

Lionfish biology, ecology and impacts are understudied in many regions, including the South Atlantic Bight. This study provides high lionfish diet resolution in the South Atlantic Bight, using DNA barcoding. This study is the first to find black sea bass (*Centropristis striata*) ranking in the top of any dietary metric, and recommends the incorporation of lionfish predation on black sea bass juveniles into stock assessments in the South Atlantic. Our results indicate that lionfish are generalists on the population level, potentially impacting many teleost species. More research is needed into prey abundances and diets of large consumers in the South Atlantic region to accurately determine lionfish prey preferences and dietary competition with native species.

Table 2-1 A list of all species, separated by family, discovered in the guts of lionfish (*Pterois volitans*) from northeast Florida either by visual ID and DNA barcoding. *denotes species not found in Munoz et al. (2011).

Family	Scientific Name	Common name
Apogonidae	*Apogon affinis**	Bigtooth cardinalfish
	*Apogon maculatus**	Flame cardinalfish
	*Phaeoptyx pigmentaria**	Two spot cardinalfish
Blenniidae	*Hypleurochilus sp.*	Combtooth blennies
Carangidae	*Decapterus punctatus**	Round scad
Haemulidae	*Haemulon aurolineatum*	Tomtate grunt
Lutjanidae	*Rhomboplites aurorubens**	Vermillion snapper
Labridae	*Halichoeres bathyphilus**	Greenband wrasse
	*Halichoeres bivattatus**	Slippery dick
	*Halichoeres dispilus**	Chameleon wrasse
Labrisomidae	*Starksia ocellata**	Checkered blenny
Mullidae	*Mullus auratus**	Red goatfish
Pomacentridae	*Stegastes partitus**	Bicolor damselfish
	*Stegastes variabilis**	Cocoa damselfish
Sciaenidae	*Pareques umbrosus**	Cubbyu
Serranidae	*Centropristis ocyurus**	Bank sea bass
	*Centropristis striata**	Black sea bass
	Diplectrum formosum	Sand perch
	Serranus phoebe	Tattler bass
	Serranus subligarius	Belted sandfish
	Serranus tigrinus	Harlequin bass

Table 2-2 Prey items (n=109) consumed by lionfish (*Pterois volitans*) from northeast Florida and their frequency of importance, percent by number, percent be weight and Index of Relative Importance (IRI) score. Prey that are first, second or third in a category are denoted with a superscript.

Family	Scientific Name	Common Name	%F	%N	%W	IRI
Apogonidae	*Apogon affinis*	Bigtooth cardinalfish	0.88	1.34	0.38	0.87
	Apogon maculatus	Flame cardinalfish	6.19	6.04	4.88	5.70
	Phaeoptyx pigmentaria	Dusky cardinalfish	0.88	0.67	0.56	0.71
Blenniidae	*Hypleurochilus spp.*	Combtooth blennies	3.54	2.68	0.34	2.19
Carangidae	*Decapterus punctatus*	Round scad	26.55^{1}	22.82^{1}	24.85^{2}	24.74^{1}
Haemulidae	*Haemulon aurolineatum*	Tomtate grunt	8.85	9.40	4.76	7.67
Lutjanidae	*Rhomboplites aurorubens*	Vermilion snapper	1.77	1.34	1.20	1.44
Labridae	*Halichoeres bathyphilus*	Greenband wrasse	1.77	3.36	0.72	1.95
	Halichoeres bivattatus	Slippery dick	3.54	3.36	2.21	3.04
	Halichoeres dispilus	Chameleon wrasse	1.77	2.01	0.18	1.32
Labrisomidae	*Starksia ocellata*	Checkered blenny	4.42	4.03	4.42	4.29
Mullidae	*Mullus auratus*	Red goatfish	6.19	8.72	3.16	6.03
Pomacentridae	*Stegastes variabilis*	Cocoa damselfish	0.88	0.67	1.65	1.07
Sciaenidae	*Pareques umbrosus*	Cubbyu	0.88	0.67	0.14	0.56
Serranidae	*Centropristis ocyurus*	Bank sea bass	5.31	4.03	10.88^{3}	6.74
	Centropristis striata	Black sea bass	10.62^{3}	15.44^{2}	9.73	11.93^{3}
	Diplectrum formosum	Sand perch	13.27^{2}	11.41^{3}	28.65^{1}	17.78^{2}
	Serranus subligarius	Belted sandfish	2.65	2.01	1.29	1.98

Table 2-3 Prey items consumed by lionfish (*Pterois volitans*) from northeast Florida in the spring season (March-May) and their frequency of occurrence, percent composition by number, percent composition by weight and Index of Relative Importance (IRI) value. Prey that are first, second or third in each category are denoted with a superscript.

Family	Scientific Name	Common Name	%F	%N	%W	IRI
Apogonidae	*Apogon maculatus*	Flame cardinalfish	1.75	1.32	0.60	1.22
Blenniidae	*Hypleurochilus spp.*	Combtooth blennies	3.51	2.63	0.55	2.23
Carangidae	*Decapterus punctatus*	Round scad	21.05^{1}	17.11^{2}	6.42	14.86^{3}
Labridae	*Halichoeres bathyphilus*	Greenband wrasse	1.75	1.32	1.05	1.37
	Halichoeres bivatattus	Slippery dick	1.75	1.32	0.50	1.19
Labrisomidae	*Starksia ocellata*	Checkered blenny	3.51	3.95	0.40	2.62
Lutjanidae	*Rhomboplites aurorubens*	Vermilion snapper	5.26	3.95	1.86	3.69
Mullidae	*Mullus auratus*	Red goatfish	12.28	17.11^{2}	7.02	12.14
Sciaenidae	*Pareques umbrosus*	Cubbyu	1.75	1.32	0.30	1.12
Serranidae	*Centropristis ocyurus*	Bank sea bass	7.02	5.26	12.59^{3}	8.29
	Centropristis striata	Black sea bass	21.05^{1}	30.26^{1}	21.61^{2}	24.31^{1}
	Diplectrum formosum	Sand perch	14.03^{3}	10.53	44.23^{1}	22.93^{2}
	Serranus subligarius	Belted sandfish	5.26	3.95	2.86	4.02

Table 2-4 Prey items consumed by lionfish (*Pterois volitans*) from northeast Florida in the summer season (June-August) and their frequency of occurrence, percent composition by number, percent composition by weight and Index of Relative Importance (IRI) value. Prey that are first, second or third in each category are denoted with a superscript.

Family	Scientific Name	Common Name	%F	%N	%W	IRI
Apogonidae	*Apogon affinis*	Bigtooth cardinalfish	4.44	3.85	1.15	3.15
	Apogon maculatus	Flame cardinalfish	11.11	13.46	12.62	12.40
	Phaeoptyx pigmentaria	Dusky cardinalfish	2.22	1.92	1.70	1.95
Blenniidae	*Hypleurochilus spp.*	Combtooth blennies	2.22	1.92	0.20	1.45
Carangidae	*Decapterus punctatus*	Round scad	17.78^3	15.38^2	25.31^1	19.49^2
Haemulidae	*Haemulon aurolineatum*	Tomtate grunt	22.22^1	25.00^1	12.55	19.92^1
Labridae	*Halichoeres bathyphilus*	Greenband wrasse	2.22	1.92	2.17	2.11
	Halichoeres bivattatus	Slippery dick	6.67	5.77	1.36	4.60
	Halichoeres dispilus	Chameleon wrasse	8.89	9.62	6.65	8.38
Lutjanidae	*Rhomboplites aurorubens*	Vermillion snapper	2.22	1.92	0.54	1.56
Serranidae	*Centropristis ocyurus*	Bank sea bass	4.44	3.85	13.03^3	7.11
	Diplectrum formosum	Sand perch	15.56^2	15.38^2	22.73^2	17.89^3

Table 2-5 Prey items consumed by lionfish (*Pterois volitans*) from northeast Florida in the fall and winter seasons (September-February) and their frequency of occurrence, percent composition by number, percent composition by weight and Index of Relative Importance (IRI) value. Prey that are first or second in each category are denoted with a superscript.

Family	Scientific Name	Common Name	%F	%N	%W	IRI
Apogonidae	*Apogon maculatus*	Flame cardinalfish	5.56	5.00	2.09	4.22
Carangidae	*Decapterus punctatus*	Round scad	61.11^1	65.00^1	49.77^1	58.63^1
Haemulidae	*Haemulon aurolineatum*	Tomtate grunt	5.56	5.00	14.65	8.40
Labridae	*Halichoeres bivattatus*	Slippery dick	5.56	5.00	1.16	3.91
Lutjanidae	*Rhomboplites aurobens*	Vermillion snapper	11.11^2	10.00^2	21.98^2	14.36^2
Serranidae	*Centropristis ocyurus*	Bank sea bass	5.56	5.00	4.30	4.95
Serranidae	*Diplectrum formosum*	Sand perch	5.56	5.00	6.05	5.53

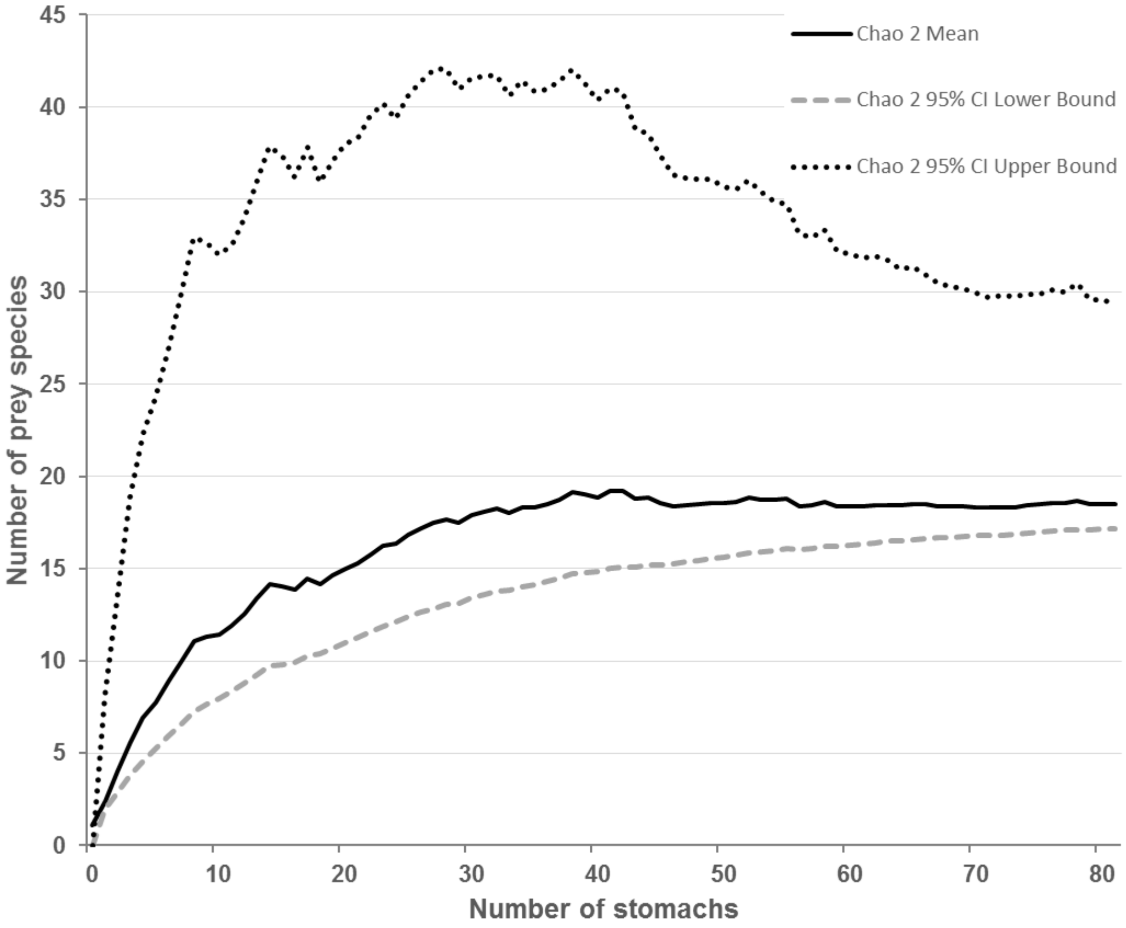

Figure 2-1. Cumulative prey curve for lionfish (*Pterois volitans*) diets in the South Atlantic Bight. The Chao 2 estimator of species diversity (solid line) and the Chao 2 upper 95% (dotted line) and lower 95% (dashed line) confidence intervals.

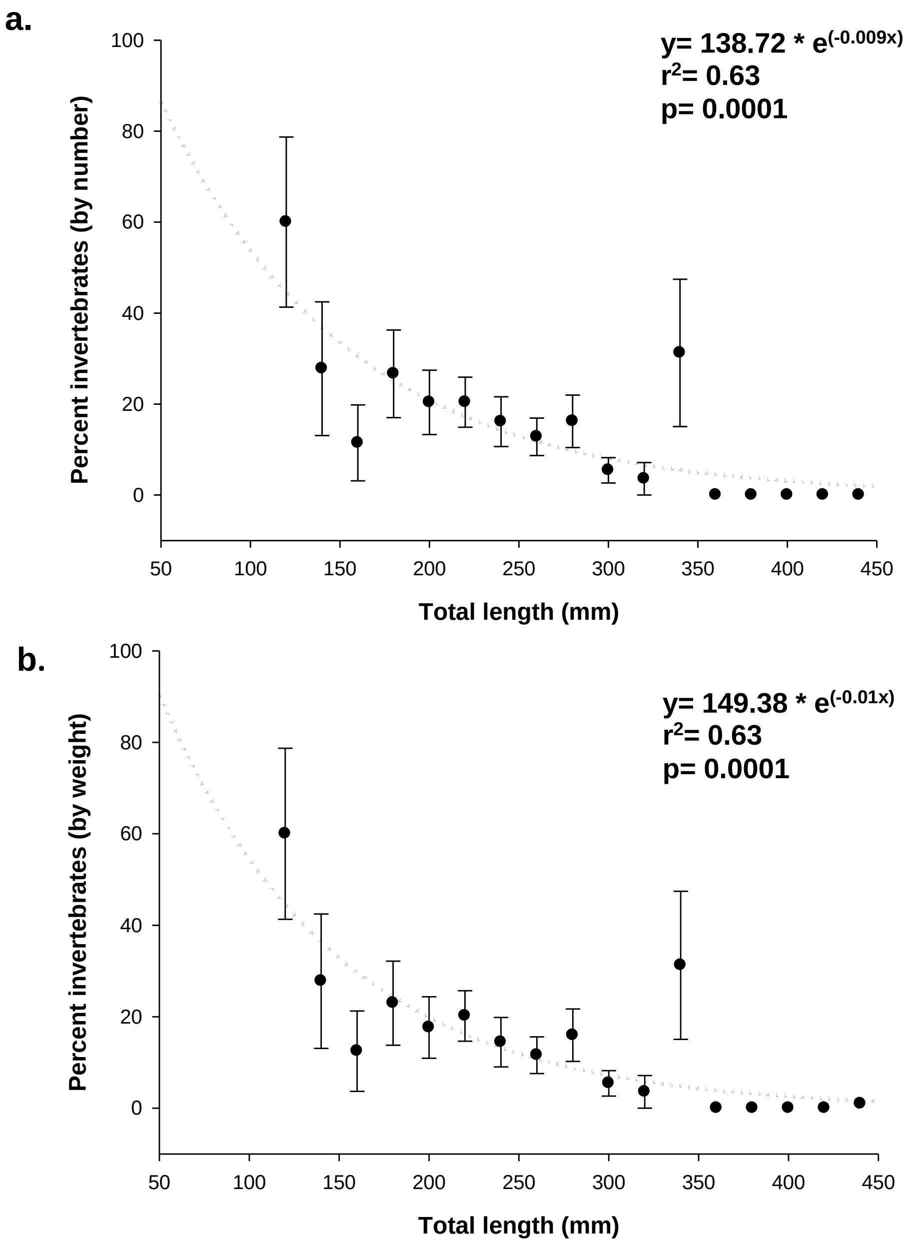

Figure 2-2. Percentage of the stomach contents that were invertebrates (a.) by number and (b.) by weight for lionfish (n=294) caught off the coast of northeast Florida. Lionfish were separated into 20mm bins and the average proportion of invertebrates/ vertebrates is plotted.

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VITA

MaryKate became interested in marine science research and the lionfish invasion after participating in a field studies program in South Caicos in 2010. Throughout the rest of her time as an undergraduate, she studied marine mammal population ecology at MOTE Marine Lab, settlement of Alaskan flatfish at Hatfield Marine Science Center, the distribution and salinity tolerances of freshwater invasive species at the Sanibel-Captiva Conservation Foundation and worked in the lab of Olaf Jensen at Rutgers University on various fisheries ecology projects. She graduated with a degree in Ecology and Natural Resources Management from Rutgers University in 2012. During her time at the University of North Florida, she volunteered at numerous lionfish derbies around the state, presented at various dive club meetings and scientific conferences and was selected to participate in a National Marine Fisheries Service workshop evaluating lionfish management strategies in Florida. She was awarded scholarships from the Coastal Biology program and Environmental Center at UNF, as well as the Guy Harvey Scholarship from Florida Sea Grant. She is currently working on publishing her thesis research and preparing for a field season in Alaska, where she will research climate change impacts on fish populations.