



12-8-2015


Feeding Ecology of the Invasive Lionfish (*Pterois* spp.) and Comparison with Two Native Species: Schoolmaster *Lutjanus apodus* (Walbaum, 1792) and Graysby *Cephalopholis cruentata* (Lacepède, 1802)

Jesse J. Secord

Nova Southeastern University, jesse.secord@gmail.com

This document is a product of extensive research conducted at the Nova Southeastern University [Halmos College of Natural Sciences and Oceanography](#). For more information on research and degree programs at the NSU Halmos College of Natural Sciences and Oceanography, please click [here](#).

Follow this and additional works at: http://nsuworks.nova.edu/occ_stuetd

 Part of the [Behavior and Ethology Commons](#), [Marine Biology Commons](#), [Oceanography and Atmospheric Sciences and Meteorology Commons](#), and the [Terrestrial and Aquatic Ecology Commons](#)

Share Feedback About This Item

NSUWorks Citation

Jesse J. Secord. 2015. *Feeding Ecology of the Invasive Lionfish (Pterois spp.) and Comparison with Two Native Species: Schoolmaster Lutjanus apodus (Walbaum, 1792) and Graysby Cephalopholis cruentata (Lacepède, 1802)*. Master's thesis. Nova Southeastern University. Retrieved from NSUWorks, . (396)
http://nsuworks.nova.edu/occ_stuetd/396.

This Thesis is brought to you by the HCNSO Student Work at NSUWorks. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of NSUWorks. For more information, please contact nsuworks@nova.edu.

Nova Southeastern University
Halmos College of Natural Sciences
and Oceanography

**Feeding ecology of the invasive lionfish (*Pterois* spp.) and comparison
with two native species: Schoolmaster *Lutjanus apodus* (Walbaum, 1792)
and Graysby *Cephalopholis cruentata* (Lacepède, 1802)**

By
Jesse J. Secord

Submitted to the Faculty of
Halmos College of Natural Sciences and Oceanography
in partial fulfillment for the requirements for
the degree of Master of Science with a specialty in:

Marine Biology

Nova Southeastern University

December 2015

Thesis of
Jesse J. Secord

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science:

Marine Biology

Nova Southeastern University
Halmos College of Natural Sciences and Oceanography

December 2015

Approved:
Thesis Committee

Major Professor: _____

David W. Kerstetter, Ph.D.

Committee Member: _____

Stephanie Green, Ph.D.

Department of Integrative Biology

Oregon State University

Committee Member: _____

Richard E. Spieler, Ph.D.

Acknowledgements

This project was funded in part with the MIASF scholarship. Thank you to my lab mates and friends, Joe Hornbeck, Ian Towne, Olmo Cinti, Noah Hansen, and Jessica Sutter for all their hard work collecting samples and assisting in set-up, maintenance, and monitoring of experiments. Thank you to my committee, Drs. David Kerstetter, Stephanie Green, and Richard Spieler for their guidance and assistance in the project. It has been a pleasurable experience.

Abstract

Predator satiation and prey-size preference were determined for locally caught lionfish, schoolmaster, and graysby, all co-occurring predatory fishes in the Florida coral reef ecosystem. Individuals were evaluated by exposing them to wild-caught killifish over a gradient of four size classes (20 mm to 60 mm, in 10 mm increments). Preference trials extended over a 2 hr time period and were filmed to determine the order in which each prey item was consumed. Satiation was evaluated by exposing the predators to an equal number of excess prey items for 24 hrs and evaluating consumed prey weight. Lionfish and schoolmaster showed a significant preference for the smallest size class (20-30 mm) while the graysby preferred the largest (50-60 mm) and smallest equally. Graysby were shown to consume the least amount of food per gram by biomass ($0.034\text{g}/\text{gram predator}^{-1}$) while lionfish consumed the most ($0.079\text{g}/\text{gram predator}^{-1}$). A significant difference was also found between consumption rates of lionfish and schoolmaster ($0.053\text{g}/\text{gram predator}^{-1}$). If consumption is compared by length between species, lionfish ($\bar{x} = 0.051\text{g}/\text{mm predator}^{-1}$) and schoolmaster ($\bar{x} = 0.061\text{g}/\text{mm predator}^{-1}$) consumption was not significantly different. These results suggest that lionfish success may stem from its ability to target prey sizes not preferred by the graysby while able to outcompete schoolmaster for the same prey size class. However, if the lionfish is compared on an individual level to schoolmaster by individual or length, consumption is similar, suggesting lionfish consumption is not significantly greater on the reef than the native species on an individual basis.

Key words: *Pterois volitans*, lionfish, *Lutjanus apodus*, schoolmaster, *Cephalopholis cruentata*, graysby, preference, satiation

Table of Contents

Acknowledgements	i
Abstract	ii
List of Tables	iv
List of Figures	v
Introduction	1
<i>Invasive Lionfish</i>	5
<i>Native Schoolmaster</i>	6
<i>Native Graysby</i>	7
<i>Prey Preference</i>	8
<i>Predator Satiation</i>	12
Materials and Methods	15
<i>Test Subject Collection and Husbandry</i>	15
<i>Prey Item Collection and Husbandry</i>	18
<i>Predator Preference</i>	22
<i>Predator Satiation</i>	23
Results	24
<i>Foraging Behavior</i>	24
<i>Preference Trials</i>	25
<i>Satiation Trials</i>	27
Discussion	45
<i>Prey Preference</i>	45
<i>Predator Satiation</i>	49
Conclusion	52
References	54

List of Tables

Table 1. Summary of beginning and ending time of predation by species.....	32
Table 2. Total number of the first consumed prey items by species.....	33
Table 3. Total number of the first three consumed prey items by species.....	35
Table 4. Total number of prey items consumed by species.....	36
Table 5. Average rank values of preference for each species.....	37
Table 6. Chi squared summary of rank data by species.....	38
Table 7. Paired t-test analysis of rank data for each species	39
Table 8. Tukey-Kramer HSD summary for total consumption by gram of predator.....	42

List of Figures

Figure 1. Photo of quarantine tanks.....	19
Figure 2. Photo of experimental tanks in testing mode.....	20
Figure 3. Photo interior layout of experimental tanks.....	21
Figure 4. One-way analysis of total length by species.....	29
Figure 5. One-way analysis of total weight by species.....	30
Figure 6. Graph of length-weight frequency of the three test species.....	31
Figure 7. Prey consumption of first three prey items by species.....	34
Figure 8. One-way analysis of prey length by predator length by species	40
Figure 9. One-way analysis of prey length by predator gape height by species.....	41
Figure 10. One-way analysis of weight consumed per millimeter by species.....	43
Figure 11. Fitted power functions of the change in prey consumption with weight.....	44

Introduction

Lionfish (*Pterois* sp.) in the western Atlantic are an invasive complex of marine fishes from the Indo-Pacific region that are currently colonizing temperate and tropical marine environments and outcompeting native fish species, leading to a reduction in overall reef health and a degradation of productivity. Lionfish in the western North Atlantic are known to consist of two species, the devil firefish and the red lionfish, *P. volitans* (Hamner et al. 2007). However, the respective percentages of these species in each location are variable spatially and temporally (Hamner et al. 2007), and recent molecular evidence has questioned the validity of these species within an Indo-Pacific continuum (Wilcox 2014). For the purposes of this thesis, the term “lionfish” will refer to this complex. The lionfish invasion originated from a small founding population, with the point of introduction likely being South Florida (Morris and Akins 2009). From this location, the introduced population has subsequently expanded north through Cape Hattaras, North Carolina and south throughout the Caribbean Sea to Brazil (Whitfield et al. 2002; Ferreira et al. 2015). Although the reasons for the rapid colonization and success of lionfish has been hypothesized, the mechanism(s) responsible have not been tested and confirmed.

The lionfish complex is the first confirmed example of a non-native marine fish colonizing the western North Atlantic (Meister et al. 2005). The rapid expansion has resulted in notable effects on native habitats. For example, Green et al. (2012) found that native predator biomass on continuous natural reef tracts in the Bahamas was reduced by 65%, while lionfish biomass expanded to 40% of predator biomass. Due to both its rapid and widespread range expansion, and its deleterious effects on populations of coral reef fishes, lionfish are considered an invasive species and a serious threat to reef health.

Lionfish not only affect the intrinsic ecological value of reefs, they also have several types of economic impacts. Predation by lionfish results in the loss of charismatic reef fauna as a whole, which affects attraction to recreational diving. This predation also decreases populations of herbivorous fishes, in turn reducing the health of associated corals due to the proliferation of algae (Albins and Hixon 2013). Finally, many of the predator species displaced or directly preyed upon by lionfish are important to fishing interests. These species include both the lutjanid snappers and serranid

groupers, two groups commonly targeted by both recreational and commercial fishermen (Morris and Akins 2009).

Understanding the reasons for the invasion success of lionfish can assist attempts to reduce biological advantages as part of a larger effort to control local populations. The ability to consume large amounts of prey (Albins 2008) as well as reduced predation due to venomous spines, may be a contributing factor to the Lionfish's success (Morris et al. 2009). Metabolic release from parasites has been also postulated to be a driver in the species' dominance. Due to the low parasitic loads within lionfish individuals, compared to native species, they may have increased energy that can be set aside for reproductive and somatic growth (Albins and Hixon 2013). The reproductive strategy of the invasive species may also influence population expansion due to females being asynchronous, indeterminate batch spawners who will spawn as long as conditions are favorable (Morris et al. 2011). This creates a situation where the population can spread rapidly. Further, food resource availability has a significant impact on the health and growth of a given population and lionfish may be taking advantage of an underutilized food resource or vacant feeding niche. Niche vacancy could be a product of natural causes or may in part be due to overfishing or climate change. Lionfish population expansion can also be influenced by increased ability to utilize prey when available in the form of greater predator consumption during times of excess forage. Both lionfish and native species have an effect on community structure through predation. Lionfish are shown to have a significant impact on native fish communities (Cerino et al. 2014). Laboratory experiments attempt to determine if the lionfish has the ability to consume more prey per gram than comparable native species, thereby making it more ecologically efficient. Efficiency in a biological setting can vary with the intended subject. Clutter and Theilacker (1971) defined gross ecological efficiency as simply the ratio of the energy yield in mortality to the energy of prey ingested. The definition of ecological efficiency in a feeding ecology context is a slight modification of the prior version, instead referring to the species' ability to readily take advantage of available resources such as food or habitat, and in turn utilize those resources for somatic and reproductive growth.

There are currently no effective means of eradication, or even control, of lionfish being utilized on an ecosystem scale, with the exception of removal by commercial and

recreational divers in local areas, as well as incidental by-catch in the existing spiny lobster (*Panulirus argus*) commercial fishery (Akins et al. 2012). Removal is especially difficult in depths greater than recreational dive limits, where divers cannot physically access lionfish and hook-and-line catchability is low due to their low affinity for consuming presented baits (Barbour et al. 2011).

Due to the lack of effective direct anthropogenic controls, i.e., physical removal, on the spread of lionfish, biological controls may be viable options. In traditional invasive species control, biological control involves importing mortality agents from the species' home range to naturally restrict populations in the introduced area (Messing and Wright 2006). The technique has been used in South Florida for freshwater finfish with the introduction of peacock bass (*Chichla ocellaris*) from South America to control invasive cichlid and tilapia species in the canals and Everglades (Trexler et al. 2000). However, this technique is very difficult to apply successfully to marine species due to the large range of these invasives, and problems associated with identifying species-specific predators and pathogens within their native range. Lionfish have colonized the western North Atlantic and will potentially extend into the subtropical western South Atlantic as well (Ferreira et al. 2015). Successful implication also requires years of testing required to evaluate the effect of introduced biological controls on native environments, and commonly fails due to unintended effects to native species (Messing and Wright 2006).

Since introduced biological control is likely not a valid option to control lionfish colonization, other avenues must be considered. Ecosystem based management (EBM) is a technique that manipulates communities or complexes of organisms, instead of individual species to maintain ecosystem health. Manipulating certain native species populations in order to create favorable competition matchups is a possible option. In order to optimize EBM control methods, a comprehensive understanding of the causes for lionfish proliferation is required. By studying potential factors, the most influential can be identified and quantified in order to create favorable conditions to mitigate the deleterious effects of invasive species. Possible causes include niche vacancy, ecological release, and lack of predation. Niche vacancy can manifest itself in the form of increased food availability resulting from an underutilized prey size or the outcompeting of other

species through increased ecological efficiency or direct antagonism. This ecological release, defined as the niche expansion due to the loss of interspecific competition (Wilson 1961; Bolnick et al. 2010), would allow for the greater availability of resources as well as an increased number of species or size of potential prey items due to lack of competition. Since lionfish are opportunistic feeders, feeding predominantly on small fishes and crustaceans (Fishelson 1997), there is a high potential for niche overlap with native species (Sano et al. 1984; Naughton and Saloman 1985; Matheson et al. 1986).

This project attempts to determine whether the aforementioned reasons (niche vacancy and ecological release) are contributing factors for lionfish success through evaluations of prey size preference and ecological efficiency for lionfish compared with two native predators. The objective of this study is to better understand the feeding ecology and predatory effect of invasive lionfish, and two native predators on small fish assemblages, with the intent to analyze prey size preference and differences in prey utilization. These results will provide insight on whether lionfish are in direct competition or potentially simply filling an underutilized ecological niche.

Two species native to the subtropical western North Atlantic Ocean and tropical Caribbean Sea, graysby grouper *Cephalopholis cruentata*, and schoolmaster snapper *Lutjanus apodus* like lionfish, are benthic, structure-oriented species that do not undergo any significant migration. All three species derive over 50% of their diet from fishes, making them dominant piscivores as mature adults (McCleery 2011; Nagelkerken 1979; Rooker 1995). Scientific divers in South Florida report observing individuals of all three species routinely residing on or within the same structure as these fishes prefer the same type of complex habitat. Occupation of the same habitat should expose the three species of interest to the same potential prey items. Any behavior or morphological variance between the three predators would result in prey preference differences. The variance in consumed prey will illustrate the individual feeding niche of the two native species and lionfish.

This laboratory study will evaluate size preference and satiation using a naïve prey species; the goldspotted killifish (*Floridichthys carpio*), a euryhaline species readily available in a broad range of sizes and in the quantities required for testing; and is not found in the same habitat as the adult experimental predator fishes and is thus a naïve

food source to all three experimental predators. The lack of previous interaction between predators and prey reduces any handling or preference bias gained by previous wild encounters; Killifish body morphology is also similar to the smaller labrid wrasses, apogonid cardinalfishes, and scarid parrotfishes that are commonly consumed by lionfish, graysby, and schoolmaster (Albins 2008; Rooker 1995; Randall 1967). Since the resulting data and analysis focuses strictly on prey size, this forage species will act as an effective proxy for reef-derived species.

Studying predator-prey interactions allows for evaluation within communities that can facilitate the management of species from an ecosystem perspective (Usmar 2012). The lionfish satiation threshold will be compared to the selected native species to determine if lionfish have the ability to ingest more prey by mass, resulting in a more efficient habitat utilization and greater ability to outcompete native species. Comparing consumption under the same conditions as two co-occurring native species (graysby and schoolmaster) further allows for determination of how the impact of lionfish of reef habitats relates to native predators. By focusing on the preference of the introduced species when exposed to varying prey size, with comparison to native species, prey size overlap can be determined and subsequently manipulated to optimize a particular management goal.

Invasive Lionfish

Due to *P. volitans* comprising 93% of the population, all data discussed here on will refer to this species (Hamner et al. 2007). Lionfish have a native home range that encompasses the western Pacific Ocean from the southern tip of Japan to Australia including Micronesia, Indonesia, and French Polynesia (Whitfield et al. 2002). They are commonly encountered in water depths from 0 to 50 m, where they can reach sizes of 380 mm (Schultz, 1986). In their introduced range, lionfish are established year round from North Carolina to throughout the Caribbean (Whitfield et al. 2007). Although there are no reports of an established population south of the Caribbean, Ferreira et al. (2015) has documented a single case of a lionfish found south of the Amazon River freshwater effluent. Individuals can attain over 450 mm total length and have been confirmed to survive in depths up to 100 m (Whitfield et al. 2007; Ruiz-Carus et al. 2006). Lionfish

are primarily piscivores over their non-native range, with teleosts comprising 78% of prey items by volume. In the Atlantic they are found in association with rocky outcroppings and vertical coral reef structure and artificial reefs (Morris et al. 2009; Whitfield et al. 2002). In the Indo-Pacific, they consume cardinalfish, damselfish, and anthias (Fishelson 1997). In their introduced range, Morris and Akins (2009) found they consume similar species, with pomacentrids and labrids comprising 13.9% of their diet in the Bahamas. Albins (2008) had parallel results in the same region with a total of 23 families of small-bodied reef fish preyed upon by lionfish. Lionfish exhibit a shift in diet with size, feeding more heavily on fish with increasing length (Morris and Akins 2009; McCleery 2011). McCleery (2011) found the shift occurred at roughly 180 mm with smaller individuals preferring crustaceans and individuals larger than 300 mm feeding almost exclusively on teleosts. Stomach weights of lionfish collected by Morris and Akins (2009) in the Bahamas were greatest at dawn and decreased during the day, suggesting that lionfish actively feed during the night. Green et al. (2011) observed that feeding was greatest during crepuscular hours combined with a higher level of overall activity and movement.

Lionfish feeding and consumption has been characterized using multiple methods. Using controlled experiments, Fishelson (1997) determined lionfish consumed 0.024g/g lionfish, While Cerino et al. (2013) modeled consumption over differing temperatures for bioenergetics purposes. Côté and Maljkovic (2010) and Green et al. (2011) used *in situ* observations and respectively determined feeding rates of 0.038g/g and 0.089g/g for lionfish.

Native Schoolmaster

The schoolmaster is a moderately-sized snapper species found from northern Florida south through Brazil (Carpenter 2002). They are commonly found in depths to 63 m (Cervigón and Los Roques 1991). Individuals attain maximum lengths of roughly 620 mm, but are commonly observed around 350 mm (Randall 1983). Schoolmaster prefer areas of complex bottoms and are often the most dominant snapper species on coral reefs in the Caribbean (Randall 1983). This species is one of the most highly reef-associated lutjanids and feeds heavily on the cryptic species found there (Randall and

Bishop 1967). The majority of the schoolmaster diet is comprised of scarid parrotfishes and acanthurid surgeonfishes, but they have been documented to also consume a variety of other reef-associated species (Rooker 1995; Randall and Bishop 1967). Schoolmaster are predominantly crepuscular and overnight feeders. Area utilized during daylight hours averaged $1,291 \pm 636 \text{m}^2$ while nighttime movement ranged $9,950 \pm 3,120 \text{m}^2$, suggesting distinct feeding and resting periods (Hitt et al. 2011). Although schoolmaster are documented to feed during the overnight hours, fresh prey items were found in the stomachs of individuals throughout the day (Randall and Bishop 1967). Rooker (1995) found a similar pattern and suggested that schoolmaster feed continuously throughout the day, with peaks during crepuscular and overnight hours.

The species experiences an ontogenetic shift from mangrove habitats to offshore coral reefs at around 70 mm total length (Rooker, 1995). Juvenile schoolmaster are found only in mangrove prop roots, feeding almost exclusively on invertebrates including crabs and amphipods. Rooker (1995) found that individuals larger than 70 mm can be found in both mangrove and coral reef habitats, but have undergone an ontogenetic shift to piscivory. By the time schoolmaster individuals reach 100 mm, 82.6% of prey items by occurrence are teleosts (Rooker, 1995).

Native Graysby

Graysby is a small (<415 mm) grouper species belonging to the family Serranidae (Nagelkerken 1979). The species is endemic to the western Atlantic Ocean and Caribbean Sea, extending from North Carolina to as far south as Brazil in depths from 3 to 40 m. Due to overfishing of the larger grouper species, graysby are now one of the most dominant grouper on many reefs including the Florida Keys, favoring reef habitats containing large quantities of coral with high rugosity and relief (Chiappone et al. 2000; Nagelkerken 1979).

Graysby have a distinct home range up to 4000m^2 and are more active at night than during the day, when they are most likely in search of food (Pople and Hunte 2005). Their territories are highly dependent on available structure, and hard-bottom reef was determined to have a greater correlation with graysby abundance than food availability (Parrish 1987; Chiappone et al. 2000). Approximately 50% of individuals

become sexually mature by 160 mm, at which point their diet shifts from dominantly crustacean to teleost prey (Nagelkerken 1979). Randall (1967) found similar results, with teleosts comprising 66.2% of graysby diet. Popple and Hunte (2005) noted increased movement and predation during crepuscular hours, peaking in the overnight hours. The species had a distinct territory averaging 2102m², and were highly associated with hard structure. Nagelkerken (1979) found the graysby to be crepuscular, with the fullest stomachs collected at 0630 and 1700, while Popple and Hunte (2005) found individual graysby to be most active during night hours.

Prey Preference

Predation is an important factor in influencing the ecological environment at a community and population level (Sih et al. 1985; Holmes and McCormick 2010). These influences can have significant impacts on the community structure (Hambright 1994; Juanes et al. 2002) and prey morphology (Brönmark and Miner 1992; Poleo et al. 1995). Predation is influenced by a variety of characteristics including prey size due to selectivity preferences (Ellis and Gibson 1995; Juanes 1994; Sogard 1997).

The definition of “feeding preference” differs slightly by author. For example, Underwood and Clarke (2006) apply an explanation of when confronted with choices of prey, a predator would exhibit a behavior that results in consumption not predicted when no choice is available. This definition is only sensitive to number or mass consumed and does not account for time. However, other authors (e.g., Manly 2006), view preference as predator consumption when confronted by prey choice, resulting in an individual behavior of choosing the most desirable size prey both earliest and most frequently. This distinction appears not to affect how preference is defined ecologically for a natural system, but more for the purpose of quantifying preference in a controlled experimental setting.

Prey-size preference is governed by a combination of behavioral and morphological characteristics. For example, feeding strategy, such as ambush predation or corralling, potentially affects size of prey taken. Orientation on the reef will also affect prey composition. Specifically, cryptic and sedentary predators will interact more often with apogonids and gobiids that reside on or within the reef while mobile species

that patrol above the reef will have a higher probability of interaction with active pomacentrid species such as sergeant majors (*Abedufduf saxatilis*) and various *Chromis* sp. damselfishes.

In a strict sense, any prey item that is smaller than the maximum jaw gape of a predator, that takes its food whole rather than in bites, is an available food source. However, indiscriminate feeding on any potential prey is uncommon, as large potential prey might not be able to be swallowed, while the smallest possible prey size is mechanically constrained by gill raker gap characteristics. Furthermore, the ability to consume a size range of prey items does not imply willingness to do so. “Optimal Forage Theory” (OFT) states that individuals will target prey items that will maximize the net rate of energy intake (Holmes and McCormick 2010). Optimal prey size is decided by numerous factors including nutrition, handling time, satiation level, and chance predation success determined by both the behavior and morphology of the predator and prey species (Holmes and McCormick 2010). Smaller prey are easier to capture, but do not contain the same amount of energy. Conversely, as individual prey become larger, they may be harder to capture, require longer handling times, and may have reduced capture success. However, large prey items represent more energy, thereby allowing the predator to feed less frequently. Under OFT, predators behaviorally select prey items that maximize energy intake while minimizing exposure to other predators, prey defense (spines), capture, and handling time. Plotting the prey size and frequency of predation results in a normal distribution with a maximum number of predations at the optimal-sized prey for a given species (or size class) of predator. A shortcoming of the OFT model is that it assumes a constant motivation across levels of stomach fullness, which is not the case in real-time experiments, as the level of stomach fullness may influence the targeted size class (Gill 2003).

Underwood and Clarke (2005) used a two-part experiment for a quantity of each variable (prey item) consumed in isolation and a quantity for each of the differing variables when exposed together. These values are then combined into ratios, and these ratios are used to evaluate preference. This method works for situations where the prey choices are quantified by weight, and time is not a controlling factor. However, it would be more appropriate in some situations to use number and order consumed, not weight of

prey items consumed at the termination of the experiment; for example, comparing consumption of a discrete number of fish or shrimp versus the weight of these prey items. In those discrete situations, one prey item may be more desirable than the others and thus be consumed first. Species may also skew their prey selection towards smaller or larger sized items depending on specific feeding strategies, taste, and experience.

Prey preference of commercially important species including multiple species of groupers has been analyzed in previous studies. The motivation to collect the resulting information has differed, from determining the effect of the introduced grouper species *roi* (*Cephalopholis argus*) on the Hawaiian reef ecosystem (Dierking et al. 2009), to defining competition and partitioning of food resources between three Red Sea grouper species within the genus *Cephalopholis* (Shpigel and Fishelson 1989).

To date, lionfish research has utilized stomach content analysis and underwater analyses (Shpigel and Fishelson 1989) with complementary data coming from controlled experimental trials. However, stomach content analysis can potentially provide biased results because prey must have been consumed within a short amount of time, as items with advanced digestion are difficult to identify (Bowen 1996). Identification is also potentially biased towards items of particular sizes and tissue types as differential digestion rates also result in some prey items being over- or under-represented (or absent entirely) (Bowen 1996).

Some preference studies have utilized controlled experimental designs in order to isolate predatory interactions to isolate factors relevant to prey preference. Predators have been exposed to a spectrum of prey sizes with the trial terminating after one item is consumed (Holmes and McCormick 2010). Stallings (2010) tested the preference of juvenile gag grouper for two different types of prey and determined whether prey density had an effect on satiation and selectivity. Preference data has also been used to examine the ecosystem effect of predators on fish assemblages (Scharf 2002).

Although data collected for prey preference and consumption in the natural environment has multiple confounding and highly variable factors such as temperature and salinity, it gives a snapshot representation of that specific habitat. Experimental trials do not have the ability to readily test variables including competition, predation, and available prey simultaneously. However, experimental tests allow for the most pertinent

variables to be tested such as prey preference according to size, while keeping all extraneous aforementioned factors constant. This experiment seeks to evaluate the role of predator and prey size class in consumption while maintaining equal prey distribution and physiological conditions.

There are currently limited ways to define and determine the prey selectivity or preference of a fish species. Data can be collected either *in situ* or through closed loop experimentation. Collecting prey preference data on the reef is difficult due to the cryptic nature of many small-bodied prey items, especially those species preyed upon by the three species of interest in this study (Bowen 1996). This cryptic nature makes determining the species and quantities present at any given location for use in proportion analysis difficult. The advent of DNA barcoding however, has allowed for the species to be identified but cannot quantify amount consumed (Côté et al. 2013). Determining prey items consumed by the predator is also difficult. Physical monitoring is often limited to daylight hours which poses a problem for detecting fishes that are suspected to be crepuscular and nocturnal. Techniques such as barcoding remove a considerable amount of this error from diet analysis and is sensitive enough to detect cryptic species not normally observed. However, barcoding cannot accurately determine the most consumed prey type by number or size which is the basis of preference data.

In situ analysis, however, allows real-time observations of prey species and sizes actually being consumed in a natural environment. Dierking et al. (2009) quantified the prey families present over a reef area and compared them to the prey items identified in the stomach of the invasive grouper species *roi* in Hawaii to develop Ivlev's electivity indices that, in turn, indicated either preference or avoidance of each family. Although controlled lab experiments do not allow for all variables within a natural system, they do hold all these variables constant to test for preference under fixed conditions. How to quantify the results varies with author, however. Underwood and Clarke (2005) utilized the weight of prey consumed in a two-stage experimental process: the first to set a baseline consumed for each prey item, and then a second to determine quantity consumed when exposed to all variables simultaneously. The resulting proportions were analyzed to determine preference. If the proportion of each prey type differs from expected, then that difference is considered the preference.

This study will use the analytical method of Taplin (2007), which uses discrete data (e.g., counts of individual prey items) and assesses the order in which the prey are consumed, with those items consumed first being the preferred prey. Taplin (2007) uses a combination of chi squared and paired t-test techniques to determine if there are differences both within size classes of each species as well as between species as a whole. The methodology differs from traditional techniques including those of Underwood and Clarke (2005) that only take into account preference between one predator group. Taplin (2007) is also a better methodology for discrete count data and accounts for time. The chi squared analysis determines whether there is preference within the individuals within each species. Once preference is established, the paired t-test shows if the individuals within each species had an overall choice for a particular size class. The research conducted in this paper constructs prey preference distribution curves for two native species (graysby and schoolmaster) and one invasive species (lionfish), as well as determines the level of overlap and competition between species. The presence of a preference gap between the two native species could suggest additional resource partitioning and niche vacancy that the lionfish may be exploiting.

Predator Satiation

Multiple factors can influence how much food an individual will consume including temperature, feeding rate, food availability, and food quality (Cerino et al. 2013). In the case of lionfish, a change in temperature from 24°C- 28°C results in a 220% increase in consumption (Cerino et al. 2013). Côté and Green (2012) also projected an increase in consumption with temperature. Behavior has a significant effect on quantity of food eaten as well, with adult fish consuming 30%-50% of their physical maximum volume (Rice and Cochran 1984; Beauchamp et al. 1989; Kitchell et al. 1994; Harfman and Brandt 1995). Evaluation of the consumption by predators is important since it is an integral factor in determining ecological impact through bioenergetics models (Cerino et al. 2013). With these models, invasive species impacts on native species can be estimated, as shown by Kitchell (1990) in his assessment of sea lamprey (*Petromyzon marinus*) on the native fish community.

Utilizing comparison species in conjunction with the target invasive allows for relative values to be calculated. Though satiation values may not reflect actual *in situ* values, relative values allow us to make statements on how lionfish behave compared to native species under the same conditions. Consumption can be determined utilizing various methods but differs with the desired outcome, ranging from amount consumed over differing temperatures to starvation resilience (Cerino et al. 2013; Fishelson 1997). Satiation can be observed *in situ* or determined in controlled experimentation. Observation entails following the desired individual around the habitat on SCUBA or from the surface and assumes behavior is not influenced and the animal feeds normally. This method has the benefit of allowing the fish to feed on organisms present in their acclimatized environment in natural densities and with consideration to catchability differences due to structure. However, observation is constrained to daylight hours and is not conducive for long term study due to dive-air constraints and manpower. In experimental trials, satiation can be measured long term by feeding individuals over a period of months or years using a specific species of prey as in the work of Fishelson (1997). However, this method potentially results in the experimental fish becoming habituated to captivity, thus behaving in ways not observed in the natural environment. Specifically, captive trials typically hold influential variables such as temperature constant which has a significant impact on satiation. Also, since they are not exposed to predators, captive fishes are able to feed until completely full, an action that would normally limit mobility, leaving them more vulnerable to predation. Cerino et al. (2013) attempted to model satiation over differing temperatures using controlled experiments, circumventing the issue of constant temperature research such as stomach content analysis or direct observation which cannot account for the changing variable. They concluded lionfish consume the most prey by weight at 29.8°C. To allow for comparability across publications, consumption was also corrected for weight and length differences between individuals. A T-K HSD test was then run to determine if there was significant differences in prey consumed

As evident by a reduction approaching 80% in small reef-associated fishes on Bahamian reefs, lionfish are considered voracious predators on coral reef communities (Albins and Hixon 2008; Green et al. 2011). The reduction of reef-associated fishes has

also resulted in changes to the underlying community structure (Albins and Hixon 2008; Albins 2013; Green et al. 2012). Changes include overall reductions in adult reef fish abundance, a proliferation of algae, and a reduction in available prey for native species (Albins 2013). Such a decline suggests that individual lionfish are consuming considerably more forage than individual native predators. The ecological impact of lionfish could be effectively modeled if physiological response and restriction were sufficiently understood (Cooke and Hill 2010).

Fishelson (1997) calculated consumption and starvation rates of lionfish in long-term, controlled environmental settings using brackish water tilapia (Family Cichlidae) and mosquitofish (*Gambusia affinis*) as prey species. Mass loss due to starvation decreased with increasing body size, potentially due to a reduction in metabolic expenditure. Normal consumption for a 350 g lionfish was calculated to be 0.024 grams of prey weight per grams of lionfish weight (g/g predator^{-1}) at 25-26°C. Introduced individuals in the western Atlantic potentially have a different consumption rate than their native Indo-Pacific range due to the increased availability of resources. To expand on the work of Fishelson (1997), Cerino et al. (2013) created a bioenergetics model of invasive lionfish from the Western Atlantic over differing water temperatures in an effort to collect a more comprehensive outlook on their potential ecological impact. Prey for trials included mummichogs (*Fundulus heteroclitus*), and grass shrimp (*Palaemonetes* spp.) for satiation and metabolic rate respectively. Maximum consumption for lionfish was found to occur at 29.8°C. Data collected by field estimates support a higher consumption at warmer temperatures, a potential issue if current climate trends were to continue (Côté and Green 2012). Cerino et al. (2013) subsequently concluded that the determined Fishelson's value was within the calculated range for consumed prey weight at 25°C for the Western Atlantic stock, albeit higher than expected according to the bioenergetics model.

Lionfish satiation has also been calculated utilizing underwater observation. Green et al. (2011) and Côté and Maljkovic (2010) followed individual lionfish over the reef, recorded their behavior and prey consumed, and calculated consumption rates of 0.089 g/g predator^{-1} and 0.038 g/g predator^{-1} respectively from estimated lengths and

extrapolated weights. Similar studies on satiation have not been published for graysby or schoolmaster species, however.

Materials and Methods

Test Subject Collection and Husbandry

Live test subjects for lab experiments were collected off of the Ft. Lauderdale and Islamorada reef tracts from October 19, 2014 to July 4, 2015, using either hook-and-line or hand net. Lionfish were caught exclusively with plastic-sided or -coated nets to reduce fin entanglement that could result in physical damage or create additional stress on the animal. The majority of captures were carried out using two hand nets to prevent the lionfish from escaping back into the reef structure, which predominantly consisted of holes or caves. Most individuals were caught during daylight hours, with a few individuals caught at dusk.

Graysby and schoolmaster were collected using both hook-and-line and hand net methods. Non-offset 2/0 to 4/0 circle hooks were used due to the common ambush-and-swallow feeding strategy of the two native species. Two graysby were also inadvertently caught in nets while targeting schoolmaster. Schoolmaster were caught using two different methods that varied with time of day (or light level). Tickle sticks were used to coax schoolmaster individuals taking shelter within coral heads and cave structure into a precisely positioned hand net; roughly half of the tested schoolmaster were caught using this method. During crepuscular hours, with the diminishing light conditions, schoolmaster would strike baits with increased intensity. At this time, hook-and-line capture methods were used. A pair of snorkelers would enter the water with fishing rods and drop the live or dead shrimp bait down into the middle of the schools of schoolmaster and wait until an acceptable sized individual consumed the bait. Unfortunately, this period of concentrated feeding would last for only 30-45 minutes, at which point the school would dissipate over the reef and light levels would decrease to the point at which targeted bait presentation become impossible.

Once caught, individuals of all three species, were immediately brought to the boat, vented if necessary, and placed in a continuous flow livewell system. If the individual required venting, it was placed on a wet towel, had its eyes covered with a second wet towel, and vented with a hollow 22-gauge needle. Gentle pressure was applied to the abdomen to facilitate gas removal and ensure the air bladder was completely evacuated. Vented individuals were then monitored for signs of stress due to barotrauma including heavy operculum movement, floating, or loss of equilibrium. Those individuals that did not regain normal behavior after 3 hrs were removed and euthanized by severing the spinal column. The majority of graysby and all of the lionfish were vented, and both species seemed very susceptible to air bladder expansion. None of the schoolmaster required venting, with only one individual euthanized after the 3 hr observation period.

Collected schoolmaster and graysby were quickly measured and released alive if not between 40 mm to 240 mm total length. Any undersized or damaged lionfish were euthanized by severing the spinal cord immediately distal to the cranium. While Cerino et al. (2013) found lionfish around the 260 mm range to be the most commonly encountered size in the Bahamas, lionfish collected by Morris and Akins (2009) averaged 217 ± 7 mm. Lionfish caught in the targeted depths off Fort Lauderdale and Islamorada were closer in size range to lengths observed by Morris and Akins (2009), averaging 216 mm. By utilizing the size classes most commonly caught on shallow reefs for all three species, a representative portion of the natural population was characterized in ensuing experimental trials. The average size for lionfish was smaller than the corresponding length at which it is legal to retain schoolmaster, and smaller than the common size for graysby. Only individuals of the correct size and without damage to the jaw hinge or vital organs (e.g., gills) were retained to minimize experimental bias. All individual experimental fish were collected in waters shallower than 15 m depth to prevent excessive internal damage from barotrauma and ensure optimal condition.

Upon return from sampling trips, collected test subjects were placed in three monospecific quarantine tanks for a minimum of 14 days. From two days after capture until the experimental trials, individual experimental fish were fed a diet of Atlantic

silversides (*Menidia menidia*) *ad libitum* of varying sizes (15-40 mm) to reduce habituation to any particularly-sized prey item.

The three circular quarantine tanks were black and contained 60 gallons of saltwater (Figure 1). Each circular tank had a ten gallon sump containing filter media to facilitate mechanical and bio-filtration. Circulating pumps facilitated a turnover rate of 1893 liters l/h and contained a 40 watt ultraviolet filter to prevent the proliferation of pathogens. Within each tank were two 150 mm diameter by 300 mm long polyvinyl chloride (PVC) tubing pieces to mimic structure and reduce handling stress by giving the collected individuals a place to hide. Artificial lighting was set on a 12L:12D regimen with a daytime intensity of 400-1000 lux. Tanks were kept at ambient temperature of 24-25°C. Ionized copper (Cupramine[®]) was added in concentrations ranging from 0-0.25 ppm to further prevent the proliferation of pathogens in test animals that may have compromised immune systems due to handling stress. Twenty percent water changes were performed weekly to inhibit the buildup of organic nitrogen and other organic material. Water quality checks to measure levels of ammonia, nitrite, and nitrate were performed biweekly when fish were first introduced into a quarantine tank then reduced to weekly once levels stabilized.

Feeding occurred daily around 1800 hours. Food not consumed after 30 min was removed. The overall health of the test fish was assessed during this time, taking careful note of changes in behavior, coloration, fin condition, and eye clarity. Any individual fish that appeared to have declining health was removed and either placed in a separate tank or euthanized.

Experimental trials were run under constant artificial lighting conditions. To mimic crepuscular feeding and reduce light shock, light intensity was maintained at 400-1000 lux, levels similar to overcast and crepuscular conditions. Corresponding to the periods of simulated darkness, experimental trials began at 1800 and terminated at 2000 to maximize feeding behavior during maximum crepuscular activity and standardize across individual trials and species. Water temperature was maintained between 24-25°C since all three species reside in tropical to subtropical waters with comparable temperatures. Salinity was maintained between 33 and 35 ppt.

Human interaction and disturbance was minimized with the use of a wide angle camera, a 60 cm tan cardboard barrier above the lip of the tank, and a blue backdrop surrounding the experimental tanks to isolate them from ambient movement and visual distractions (Figure 2). The experimental room was a locked, minimum-access space, and “do not enter” signs were posted on the doors. All of the three test species are highly associated with the benthic environment, and plain brown cardboard was used to create a tank bottom that mimicked a hard-bottom or sand substrate.

To separate predator and prey, experimental tanks were equipped with a clear, perforated, Lexan[®] partition bisecting the space and creating equal sized chambers. A second, smaller perforated piece was placed across the corner containing the vertical drain pipe to prevent prey items from hiding from the predator or to allow the camera to confirm a successful strike (Figure 3).

Prey Item Collection and Husbandry

Goldspotted killifish (*Floridichthys carpio*), the prey item used for lab experimentation, were caught using a 15 m bag seine net with 6.35 mm mesh in the Whiskey Creek tidal waterway system, located immediately south of John U Lloyd State Park in Dania Beach, Florida. All killifish captured were immediately placed in a 5 gal bucket with an aeration pump. Fish were measured and retained if they matched one of the four size categories of 25, 35, 45, and 55 mm. Individuals that were not within 2 mm of these values were released back to the point of capture. Killifish larger than 60 mm were only seasonally present while individuals smaller than 20 mm experienced very high mortality in captivity, likely due to handling stress.

The killifish were fed chunks of de-shelled penaeid shrimp or silversides on a daily basis and kept for a maximum of two weeks in holding tanks to reduce autogenic differences in individual health and visual appearance. Freshly caught individuals were retained for a minimum of 48 hrs before being used in trials to allow for acclimation to tank conditions and prevent feeding bias towards individuals with differing coloration. Salinity and temperature was kept identical to the quarantine and experimental tanks to reduce shock during feeding trials.

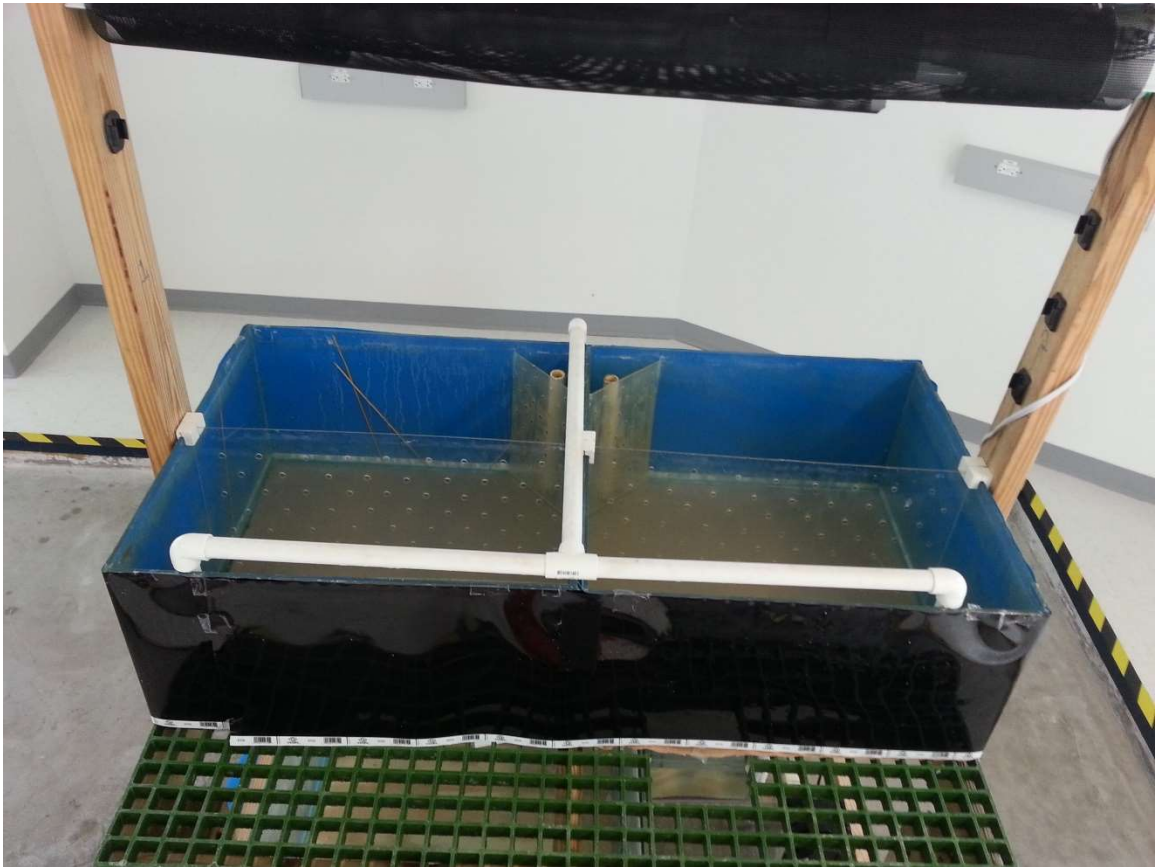
Figure 1. Photo of the quarantine tanks. Black tubes on tanks are UV filtration units. Blue bins are sumps containing biofiltration media and overflow back into the main tanks. All three tank systems are isolated from each other



Figure 2. Photo of the experimental tanks in testing mode. Image includes the restricted light bar, GoPro mounting brackets, and cardboard divider.



Figure 3. Photo of the interior of experimental tanks. Note the dividers to allow for prey item acclimation, blue backdrop and tan bottom, and drain isolator



Predator Preference

Individual predators to be tested were starved for 48 hrs to empty the digestive tract. Twelve prey items, three each from four size intervals (20 to 60 mm in 10 mm increments), were placed in the empty side of the experimental tank and allowed to acclimate for a minimum of 1 hr to a maximum of 2 hrs. At exactly 1800 hours, the partitions on both tanks were removed, and the ensuing interactions recorded using wide-angle video cameras oriented above and to the side of the tank in order to get an unobstructed view of prey item size and predation events. The video cameras used were 1080-pixel high-definition GoPro cameras (GoPro, Inc.; San Mateo, CA) with a viewing angle of 120°. Video recording continued for 2 hrs, the duration of the experiment with the termination signaled by the light timer shutting off. Upon completion, the overhead light was turned back on, and any remaining prey items were removed, recorded, and discarded.

A total of 12 control trials, excluding predators, were run to quantify any prey loss or other autogenic changes not attributed to predation, to allow triple the number of replicates as variables for use in parametric analysis (Williams and Titus, 1988). Twelve killifish were used for each trial in the same ratios used in experimental trials were allowed to acclimate for 2 hrs, at which point the partition was removed with interactions recorded using a wide-angle video camera mounted above the reservoir. Any mortalities or change in the killifish behavior were noted. All fish were accounted for and no significant change in behavior or appearance was noted.

Recorded videos were downloaded onto an external hard drive and given a unique identifier. Upon analysis, time when the divider was lifted was noted and subtracted from the following predation time to get an exact time of the strike. Time, size, and order of all feeding events were recorded. Total number of consumed prey items observed on the video was compared to the number recorded on the original data sheet to ensure an accounting of all prey items.

This study followed the analytical method of Taplin (2007), which used discrete data (e.g., counts of individual prey items) and assessed the order in which the prey were consumed, with those items consumed first being the preferred prey size. Each prey item was given a rank, with the first consumed being 1, the second given a value of 2, etc.,

continuing until all consumed prey items are given a value. The prey items not consumed are considered tied for last and are thus given an average of the remaining ranks. Then Kruskal-Wallis/Wilcoxon tests were run within each individual or species and summed to determine whether individuals of that particular species displayed a preference. Paired t-tests were used to determine whether there is a preferred size class between individuals of each species. The preferred size class between species was then compared to determine if there is size overlap. Significance for all statistical tests was assessed at $\alpha = 0.05$.

Predator Satiation

Test predators from the initial size preference experiment remained inside the partitioned test tank and were starved for 46 hrs. They were not starved for a full 48 hrs to allow for the experiments to begin at 1800 hours, the same time as the two hour experiment and quarantine feeding.) Twenty individual prey items, five from four size classes between 20 to 60 mm in 10 mm increments, were placed as a group in a bin lined with a paper towel and covered, and the total weight was recorded. Prey was placed in the empty side of the experimental tank and allowed to acclimate for 2 hrs to ambient water conditions. The empty bin and paper towel was weighed with the resulting value representing weight of the bin, paper, and water lost when the fish contacted the paper towel; this number was subtracted from the original total weight to give an actual prey weight.

Following the 2 hr acclimation of the prey, at 1800 hours, the partition was removed and the test predator allowed to interact with the prey. Individual trials lasted 24 hrs to allow for each predator to feed during the light phase they preferred in an attempt to mimic natural behavior. At the termination of the trial, the remaining prey were removed, weighed, counted, and identified by size. The test predators were removed from the tank, placed in an empty quarantine tank, starved for 48 hrs to evacuate their digestive system, and then removed and euthanized by severing the spinal column. Each fish was weighed and measured as total length, then a rudimentary necropsy was performed. The stomach and intestines were examined for residual food in the digestive tract in order to ensure an accurate predator weight that was not affected by differences in consumption between individuals. Viscera were also evaluated to ascertain the overall

health of the individual to ensure that data collected were from a healthy individual not otherwise hindered by illness or internal injury.

A total of 12 control trials were run to quantify any prey loss or other autogenic changes not attributed to predation. For example, mass loss or gain due to growth or starvation must be accounted for in order to get an accurate measure of mass lost by predation and not autogenic changes. Twenty individual prey items, five from each size class, were weighed and placed in the empty side of the experimental tank. Prey items were kept for 26 hrs, then removed and weighed again. Any mortalities and autogenic differences in mass loss were recorded. Mass loss over the 24 hr trial was deemed negligible and thus prey weight data did not have a correction applied.

Since the collected data points within each group did not exhibit normal distribution, a Welch's ANOVA was utilized to assess differences in mean consumption between the three species. The Tukey-Kramer HSD (T-K) test was then used to analyze differences between the groups due to the low sensitivity to groups with differing sample size between data sets. Significance for all statistical tests was again assessed at $\alpha = 0.05$.

Results

Foraging Behavior

Observations of feeding strategy for the tested predators noted a difference in behavior between all three species. Lionfish corralled their prey using flared pectoral fins, allowing ample time to inspect and select a particular prey item. Graysby used a combination of darting and ambush strategies that varied between individuals. When darting, the graysby would face the killifish roughly one body length away, then rush straight through the school and consume a single item. If the event was unsuccessful, the predator would not continue to chase the prey, but instead regain a peripheral position in preparation of another strike. In ambushing, the graysby would approach the killifish roughly a half a body length away then quickly strike the prey. Schoolmaster used a "frenzy" style of feeding, with the test fish repeatedly striking into the school of prey in rapid succession. Individuals would change directions and undertake erratic movements for 30 sec or longer.

Preference Trials

A total of 64 individuals were tested over both experimental trials: 22 graysby, 22 lionfish, and 20 schoolmaster. Test individuals ranged in length from 173 mm to 287 mm (\bar{x} length = 238.1 mm), with each species having a distinct size class (Figure 4). Schoolmaster were the largest (\bar{x} length = 256.2 mm) followed by graysby (\bar{x} = 238.3 mm) and lionfish (\bar{x} = 216.7 mm). Schoolmaster and graysby were the only group pair to have a significantly similar size distribution ($p=0.0497$).

Individual predator weights ranged from 79.3 g to 479.4 g (\bar{x} = 227.4 g), and varied significantly by species (Figure 5). Schoolmaster were the largest of the tested predators (\bar{x} weight = 227.4 g), followed by graysby (\bar{x} = 223.23 g), and lionfish (\bar{x} = 153.64 g). All three species followed a similar length-weight frequency growth curve, with schoolmaster exhibiting a slight increase in g/mm compared to the other two test species indicating a more robust body structure (Figure 6).

A total of 64 fish of the three predator species were subjected to preference testing. Of those, 61 yielded usable data: 20 graysby, 21 lionfish, and 20 schoolmaster. Three fish were excluded since they did not consume any prey items within the 2 hr experiment. Between all three species, a total of 346 successful predation events were documented. Graysby accounted for 87, lionfish 156, and schoolmaster 103 predation events. Time of feeding and time to first successful predation was similar between lionfish and graysby, with schoolmaster not feeding until over 30 mins had passed (Table 1). The overall feeding interval for all three species was 51 ± 1 min (Table 1).

When only the first predation is considered, graysby consumed most of their prey in the 50-60 mm size class (8 individuals, 38% of the total). Lionfish and schoolmaster consumed the most individuals in the 20-30 mm with 8 (38%) and 11 (55%) individuals respectively (Table 2). Both rank and count data analytical methods suggested that there is prey-size selectivity for all three species tested. Utilizing count data only from the first three combined predation events suggests that lionfish in the tested size range preferred the 20-30 mm size class (44%) with a decrease in number consumed as size increased. The 30-40 mm and 40-50 mm size classes had similar preference, making up 24% and 23% of consumption respectively, with a sharp decrease for the largest size at 10%

(Figure 7). This consumption pattern holds true when the second and third predation events are included as well. The 50-60 mm size class was the most consumed by graysby with 22 (39%). The 20-30 mm size class remained the most consumed by lionfish and schoolmaster with 27 (44%) and 26 (45%), respectively (Table 3).

The most consumed size class over the full 2 hr experiment for graysby was 50-60 mm with 31 with 20-30 mm close behind with 28 (Table 4). Two size classes, 20-30 mm and 30-40 mm, were the most consumed for lionfish, with 51 individuals each. Schoolmaster consumed the most prey in the 20-30 mm size class, with 37 individuals, followed closely behind by the 50-60 mm size class with 31 individuals (Table 4).

When all 12 potential prey items were ranked according to Taplin (2007), similar results were obtained. Graysby chose prey in the 50-60 mm size class earliest, with an average predation rank of 5.64. Lionfish and schoolmaster both preferred the 20-30 mm size class of prey, with ranks of 4.79 and 4.88 respectively (Table 5).

Using the Wilcoxon test methodology, the data indicated preference within each species, with all p-values being significant ($p \leq 0.05$). Graysby showed the weakest correlation ($p = 0.01$) while lionfish and schoolmaster had strong correlations ($p < 0.0001$, $df = 3$) (Table 6). Within each size class of each species, paired t-tests were utilized to determine significant difference. Graysby had significant difference ($p \leq 0.0098$) between all groups with the exception of 50-60 mm against 20-30 mm ($p = 0.4662$) and 40-50 mm against 30-40 mm ($p = 0.6948$). Lionfish showed significant differences ($p \leq 0.05$) between all groups. Schoolmaster had significant differences between all size class groups except 40-50 mm against 30-40 mm ($p = 0.0831$) and 50-60 mm against 40-50 mm ($p = 0.0862$) (Table 7).

When the first three consumed prey lengths are compared to the corresponding predator length, predator size adjusted preference coefficients are created (Figure 8). Lionfish exhibited no significant difference ($p > 0.1251$) between the two native species. Schoolmaster and graysby showed a significant difference ($p = 0.033$) between coefficients.

Gape height differences were analyzed using an ANOVA and T-K to determine whether the three test species had similar prey size constraints. Graysby were significantly different ($p < 0.001$) from both the lionfish and schoolmaster. Lionfish and

schoolmaster did not exhibit a significant difference ($p=0.4127$) in means. To account for the difference in gape height with prey size consumed, prey length by predator gape height coefficients were created (Figure 9). All three species were not significantly different ($p>0.0518$) from each other.

Satiation Trials

Average available prey biomass was 29.96 g with only one schoolmaster consuming all of the offered prey. The remaining fish in the trials consumed from 0.1 g to 27.1 g in prey weight, but averaged 11.03 g. Schoolmaster consumed the most on average (\bar{x} consumption = 15.73 g), followed by lionfish ($\bar{x} = 10.81$ g) and graysby ($\bar{x} = 6.99$ g). All three species were found to be significantly different from each other ($p\leq 0.0378$) using the (T-K) test.

To account for differences in weight both within and between species, a coefficient of grams of prey consumed per gram of predator for each individual trial was calculated. The coefficient is calculated by dividing the total number of grams the predator consumed by either the total length or weight of the predator. This method of consumption comparison has been utilized in numerous previous studies (e.g., Côté and Maljkovic 2010; Fishelson 1997; Green et al. 2011). Coefficients ranged from 0.129 g/gram predator⁻¹ for a lionfish to 0.0003g/gram predator⁻¹ for a graysby with a mean of 0.056g/gram predator⁻¹ overall. Lionfish had the greatest average mass consumed ($\bar{x} = 0.079$ g/gram predator⁻¹) followed by schoolmaster ($\bar{x} = 0.053$ g/gram predator⁻¹) and graysby ($\bar{x} = 0.034$ g/gram predator⁻¹). Significant differences were found between the three species ($p<0.0001$) using Welch's ANOVA. The (T-K) test was used to analyze differences between individual species. All three species were confirmed to be significantly different from each other (Table 8).

Since length is a common measurement to determine maturity, fecundity, and catchability in commercial and recreational fisheries, a coefficient of grams of prey consumed per mm of predator was also calculated. Coefficients ranged from 0.0003 g/mm predator⁻¹ from graysby to 0.104 g/mm predator⁻¹ for a schoolmaster. Schoolmaster had the largest mean coefficient ($\bar{x} = 0.061$ g/mm predator⁻¹) followed by

lionfish ($\bar{x} = 0.051\text{g/mm predator}^{-1}$) and graysby ($\bar{x} = 0.029\text{g/mm predator}^{-1}$). Significant difference existed between lionfish and graysby ($p=0.0020$), and schoolmaster and graysby ($p<0.0001$) with no difference when comparing lionfish and schoolmaster ($p=0.1833$) using the (T-K) test (Figure 10).

To further address the issue of differences in predator weight between species, power functions using predator-prey weight coefficients plotted over predator weight were calculated (Figure 11). The resulting functions showed that schoolmaster and lionfish decrease consumption per pound with increasing weight. Graysby, however, exhibited an increase in consumption.

Figure 4. One-way analysis of total length by species. Circles provide visual representation of the Tukey-Kramer HSD analysis of degree of length overlap between species. Horizontal line represents the overall mean of all individual length values.

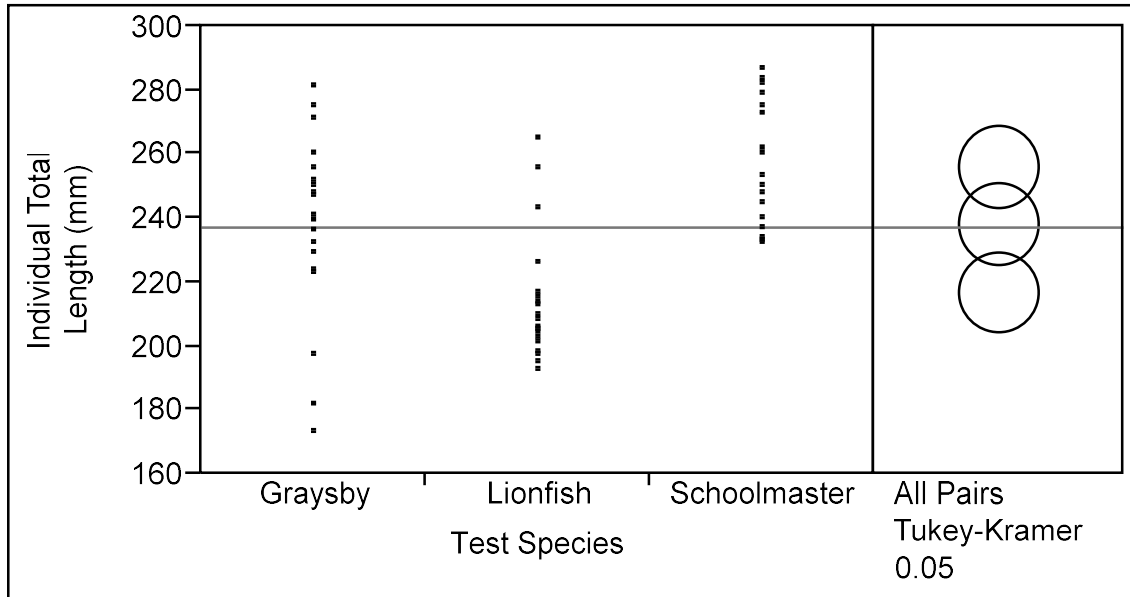


Figure 5. Summary of one-way analysis of total weight by species. Circles provide visual representation of the Tukey-Kramer HSD analysis of degree of weight overlap between species. Horizontal line represents the overall mean of all individual weight values.

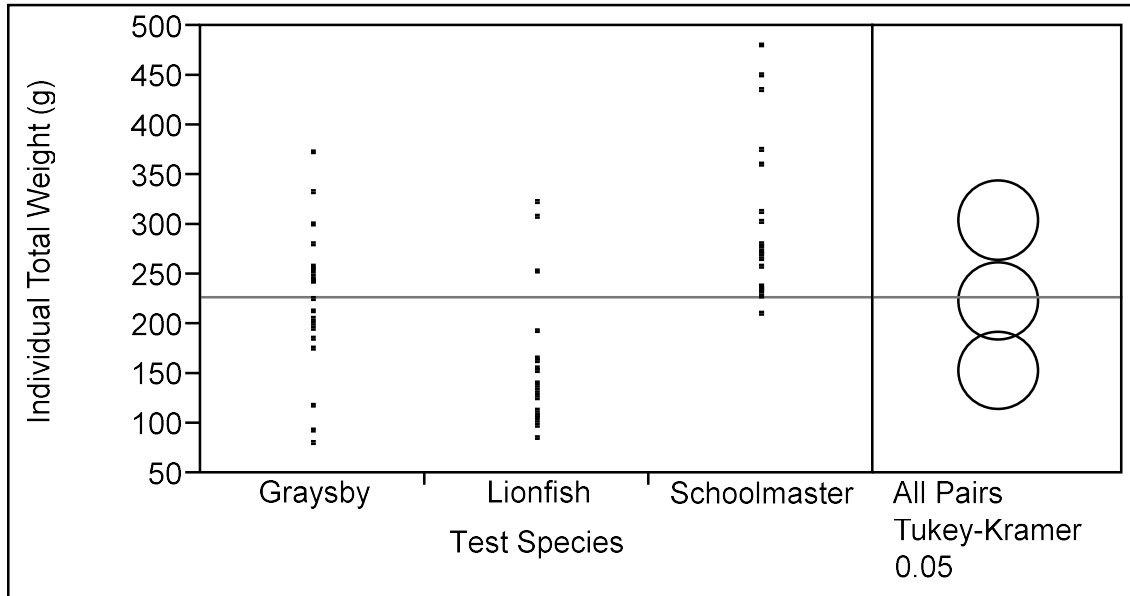


Figure 6. Graph of length-weight frequency of the three test species.

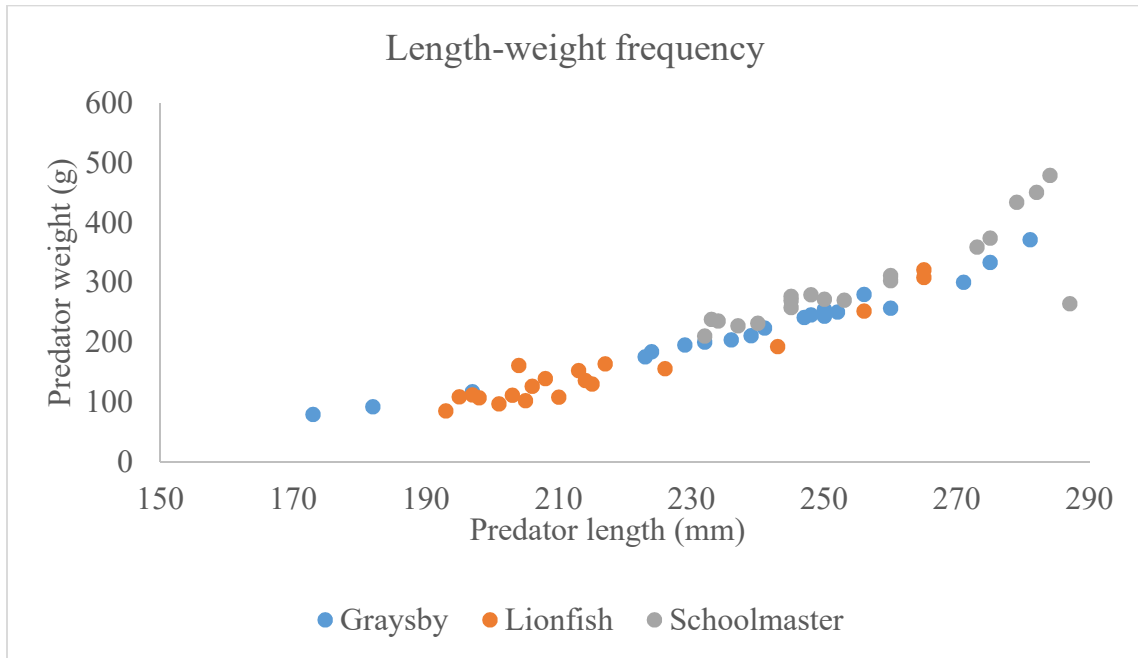


Table 1. Summary of beginning and ending time of predation for each species. Values in parentheses are standard deviations of corresponding time values (decimal minutes). All numbers are in minutes and decimal minutes.

Species	First Predation	Final Predation	Feeding Interval
Graysby	7.58 (9.00)	55.60 (36.81)	50.54 (37.63)
Lionfish	3.91 (3.53)	55.42 (29.10)	51.52 (29.40)
Schoolmaster	33.40 (25.38)	81.87 (31.27)	51.02 (25.83)

Table 2. Distribution table of the first consumed prey items by species and size class.
 Top row shows size classes of prey items in mm.

Species	20-30	30-40	40-50	50-60
Graysby	24%	29%	10%	38%
Lionfish	38%	29%	19%	14%
Schoolmaster	55%	10%	10%	25%

Figure 7. Prey consumption of first three prey items over available size classes by species.

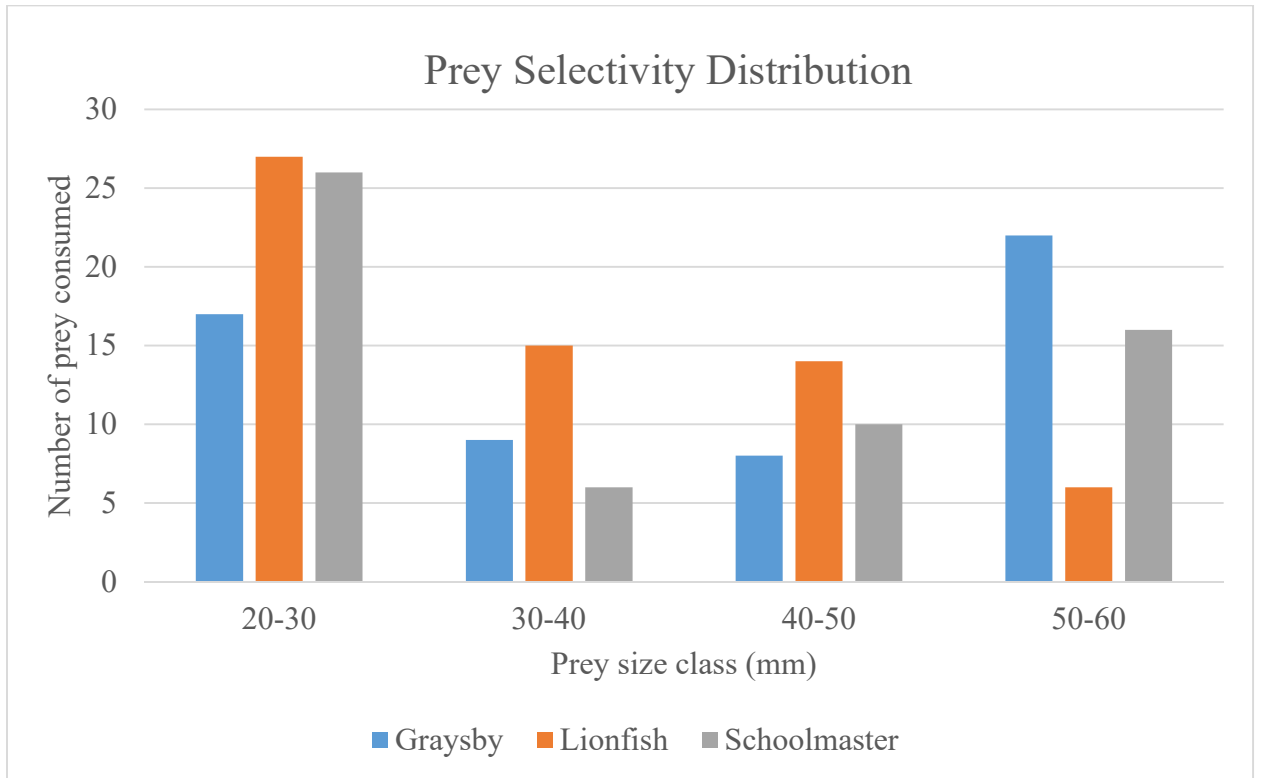


Table 3. Distribution of the first three consumed prey items by species and size class.

Top row shows size classes of prey items in mm.

Species	20-30	30-40	40-50	50-60
Graysby	30%	16%	14%	39%
Lionfish	44%	24%	23%	10%
Schoolmaster	45%	10%	17%	28%

Table 4. Total number of prey items consumed over the entire two hour duration of selectivity trials. Top row shows size classes of prey items in mm.

Species	20-30	30-40	40-50	50-60
Graysby	28	12	16	31
Lionfish	51	51	37	17
Schoolmaster	37	14	21	31
Total	116	77	74	79

Table 5. Average rank values of preference for each species by size. Rank was assigned by order consumed, with the first given a value of 1 etc. Top row shows size classes of prey items in mm. Rank was determined by the chronological order in which the prey items were consumed with the first prey item eaten given a value of 1, the second, 2, and so on.

Species	20-30	30-40	40-50	50-60
Graysby	6.03	7.23	7.09	5.64
Lionfish	4.79	5.56	6.97	8.68
Schoolmaster	4.88*	7.75	7.14	6.23

Table 6. Chi squared analysis of rank data by species.

Species	Chi Squared	P value
Graysby	12.68	0.01
Lionfish	54.01	< 0.0001
Schoolmaster	27.62	< 0.0001

Table 7. Paired t-test analysis of rank data (p-value) for each species between size classes. Top row shows size classes of prey items in mm.

Species	30-40 vs 20-30	40-50 vs 30-40	50-60 vs 40-50	40-50 vs 20-30	50-60 vs 20-30	50-60 vs 30-40
Graysby	0.0017	0.6948	0.0028	0.0098	0.4662	0.006
Lionfish	0.0469	0.0012	0.0002	0.0002	<0.0001	<0.0001
Schoolmaster	<0.0001	0.0831	0.0862	<0.0001	0.0236	0.0008

Figure 8. One-way analysis of prey length by predator length by species. Also shown are circles representing the Tukey-Kramer HSD analysis of degree of overlap between species. The horizontal line represents the average of all individual size coefficient values.

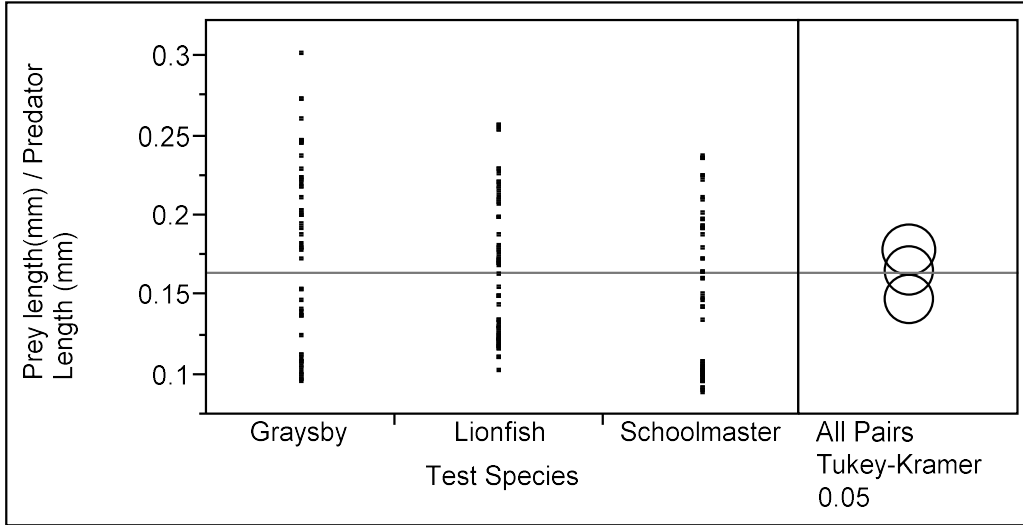


Figure 9. One-way analysis of prey length by predator gape height by species. Also shown are circles representing the Tukey-Kramer HSD analysis of degree of overlap between species. The horizontal line represents the average of all individual coefficient values.

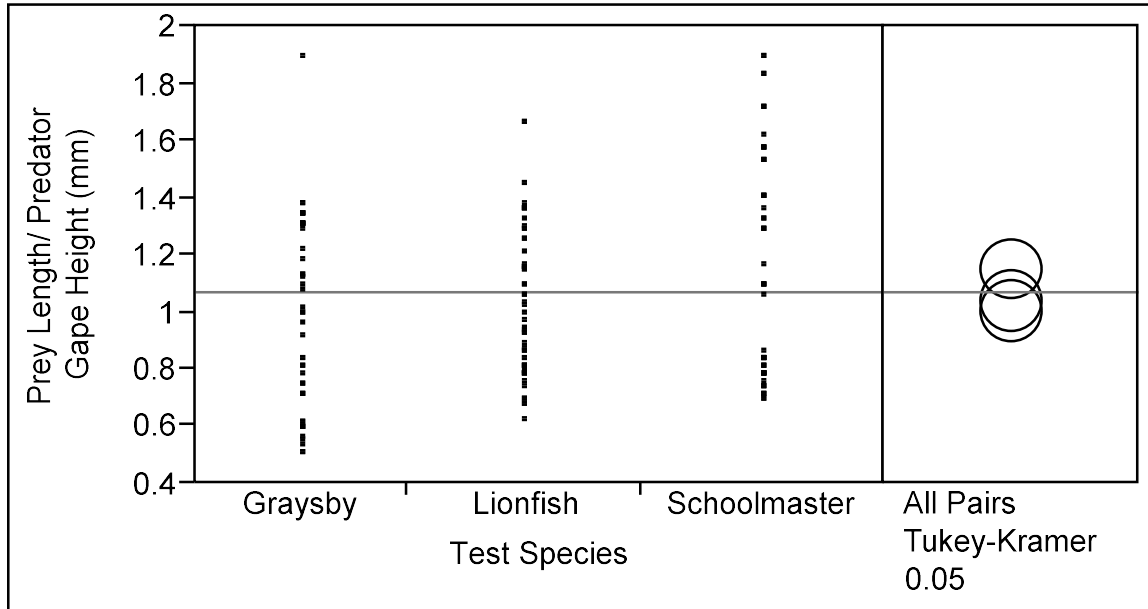


Table 8. Tukey-Kramer HSD summary for total consumption by gram of predator between species.

Species	p-value
Graysby vs Lionfish	<0.0001
Graysby vs Schoolmaster	0.0245
Schoolmaster vs Lionfish	0.0014

Figure 10. One-way analysis of weight consumed per millimeter by species. Also shown are circles representing the Tukey-Kramer HSD analysis of degree of $g/g \text{ predator}^{-1}$ overlap between species. The horizontal line represents the average of all individual consumption values.

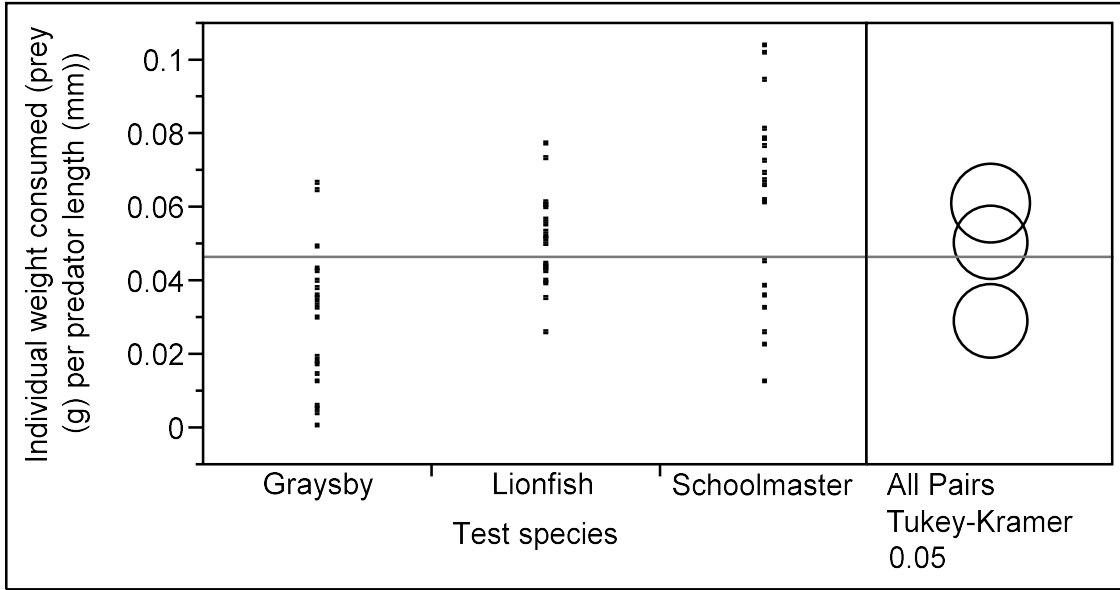
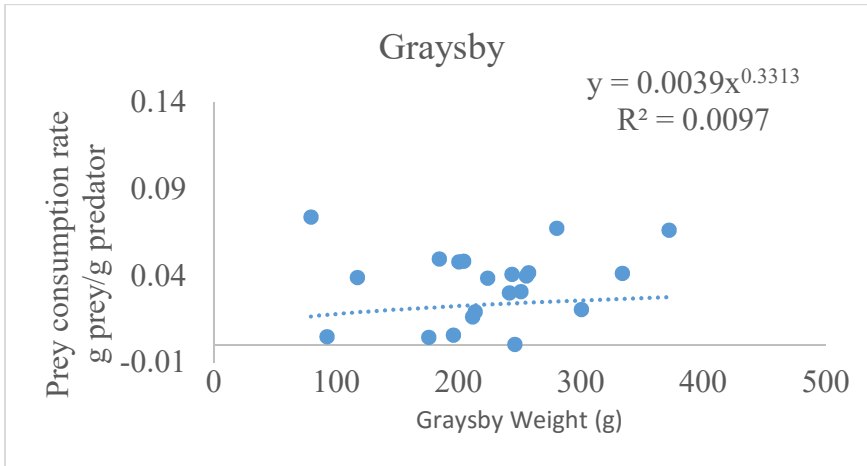
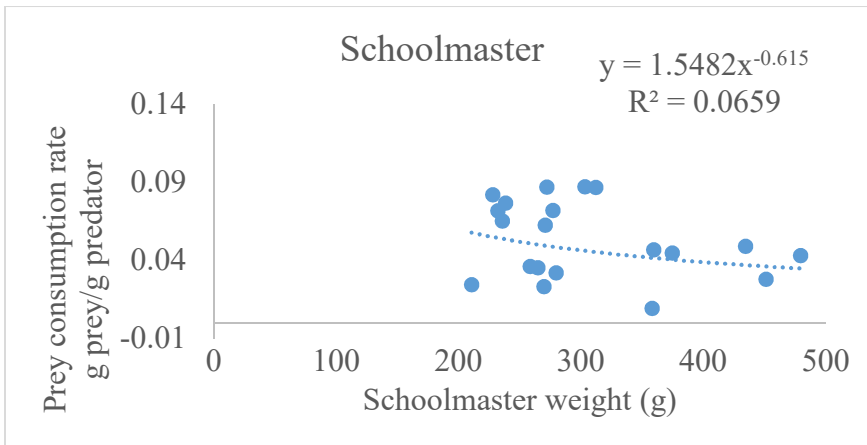


Figure 11, A, B, & C. Fitted power functions calculating the change in prey consumption (g) over predator size (mm). R^2 value represent the coefficient of determination and is a calculation of how well the calculated function represents the data.

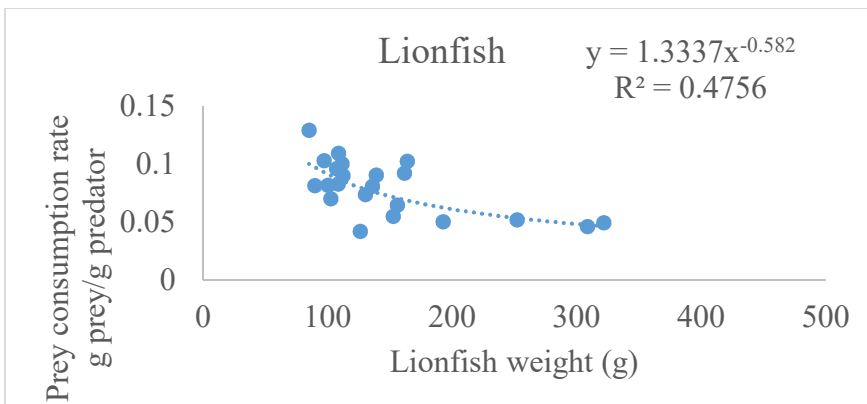
A.



B.



C.



Discussion

Physical removal is currently one of the most used control measures of lionfish in the Caribbean (Morris et al. 2009). However, in areas not frequently visited by divers, there are currently minimal population control measures, either ecological or anthropogenic (Valdivia et al. 2014). Determining what size class of prey fishes that both lionfish and native competitors target can allow managers to create favorable competitive population schemes including increased individual size of native species or the population as a whole. To facilitate the creation of these schemes, the reasons for proliferation must be determined, including through evaluations of prey size preference and ecological efficiency. The objective of this project was to better understand the feeding ecology and predatory effect of lionfish, graysby, and schoolmaster on small fish assemblages, with the intent to analyze prey size preference and differences in prey utilization. These results provide additional insight on whether lionfish are in direct competition or potentially simply filling an underutilized ecological niche.

Prey Preference

Time to first consumption and termination of feeding was highly variable within each species. Although lionfish and graysby appeared to begin and end feeding quicker than the schoolmaster, there were large standard deviations for each species and the times were not significantly different ($P > 0.05$). Differing behavior between the species could also have had an impact on time of first consumption. We observed that lionfish and graysby were not as disturbed by the presence of people and movement over the tank. Schoolmaster were very wary and easily startled, however, requiring several minutes to resume normal behavior. This startle reflex behavior for schoolmaster could explain the longer time required to begin feeding and is supported by the fact that feeding intervals were nearly the same for all three species when standard deviation is considered. Although schoolmaster had delayed feeding, they continued later into the 2 hr experiment, for a total amount of consumption time similar to lionfish and graysby.

When all consumed prey items over the 2 hr duration of the experiment are compared to the distribution of the first three prey items, the significance of the most

preferred size class decreases from 39 to 36%, 44 to 33%, and 45 to 36% for graysby, lionfish, and schoolmaster respectively. This suggests collecting data of consumed prey items over a time interval results in a dilution of the actual preferred prey as the chance of equal exposure across all prey sizes decreases. If all three individuals of a size class are consumed, an exclusion of the preferred prey can occur. The lack of preferred prey will cause an artificial shift in diet to the less preferred sizes that would not have occurred if the number of available prey was unlimited. For this reason, data was not considered past the third consumed prey item in this discussion.

Lionfish exhibited a consumed prey distribution that resembles the traditional OFT curve. The two native species, however, displayed a bimodal distribution that does not follow traditional models. The consumed prey distribution of lionfish followed the OFT curve which theoretically peaks at the most preferred prey and decreases as prey get farther away from optimal size (Figure 3). Lionfish feeding mimicked this trend with the smallest size class being the optimum prey and consumption occurrence decreasing as the size deviated from ideal. However, due to available size class constraints in the experimental design, the left side of the OFT curve is not evident. These constraints were caused by the inability to collect and maintain prey smaller than 25 mm. As previously noted, prey items of that size range had a high post-capture mortality rate and were difficult to contain within the experimental tanks. Future research should shift prey size focus to sizes smaller than 25 mm to confirm the left hand tail of the preference curve.

Schoolmaster preferred the smallest size class (45%) at the same rate as lionfish. However, the 50-60 mm size class was the next most consumed (28%). Graysby preferred to consume the largest size class available (39%), with the smallest size being the second most preferred (30%). The preference distributions of the native species had two peaks in size preference, occurring at both ends of the spectrum, resulting in a bimodal distribution which does not follow the theoretical OFT curve. The distribution is more pronounced in graysby feeding which has relatively equal nodes while schoolmaster has a definitive peak at the smaller end of the spectrum followed by a less pronounced preference at the largest size class.

Using the ranking system described by Taplin (2007) for all prey consumed within the 2 hr experimental window resulted in a similar preference outcome. The 50-

60 mm size class had the lowest rank (5.64) for graysby, followed by 20-30 mm class (6.03). Schoolmaster preferred the 20 to 30 mm size class (4.88) with 50-60 mm second (6.23).

A chi squared analysis of individual lionfish displayed a significant overall preference, which allowed us to test which size class was preferred for the species as a whole. Lionfish demonstrated significant difference in rank between all size classes, thereby supporting evidence of a preference for particular size classes and not random feeding (T-K, $P < 0.05$). Rank comparison of 30-40 mm against 20-30 mm size classes showed an extension of the optimal prey curve with 30-40 being closest in rank to the most preferred size class. Lionfish exhibited an increase in rank compared to size, with the 20-30 mm size class having the lowest rank (4.79) and 50-60 mm having the highest (8.68) mimicking the distribution curve using the count data above. Stomach content analysis of lionfish in previous studies have shown that they prefer small prey relative to their body length. For example, Morris and Akins (2009) found the average size of consumed teleost prey was 25 mm for lionfish averaging 201 mm in length which supports the findings of this study. Furthermore, their data showed that selected prey size remained small with the largest lionfish, 424 mm in length, consuming prey with an average size less than 30mm. The Morris and Akins (2009) finding of small sized prey preference for larger lionfish also supports the validity of our experimentally derived prey curves, as their data from the natural environment was equivalent.

Graysby showed a co-preference for the largest (50-60 mm) size class. However, the difference in rank between the most preferred size class and the 20-30 mm size class was insignificant ($P > 0.05$) and the species exhibited the same bimodal distribution seen above with count data. The inability to show a difference between the two top consumed classes suggests that both are preferred by graysby and not a display of minimum vulnerable size, due to the reduced selection of the middle size classes. The lack of difference in rank between the middle size classes can be attributed to the low numbers consumed of both classes, showing they were equally selected in low numbers. If only a minimum and maximum vulnerable predation scheme existed, preference would be equal across all classes. Although there are no published data for graysby on prey size from stomach content analysis, *in situ* observations by Albins (2013) on patch reef

communities using lionfish and a grouper species similar to graysby, coney (*Cephalopholis fulva*), found that coney consumed relatively larger prey items, while lionfish had a significant impact on juvenile reef fish recruitment.

Schoolmaster had a bimodal preference rank as well, with the 20-30 mm size class being the most preferred followed by the 50-60 mm size class. This result differs from the graysby, however, due to the significant difference in rank between the classes. Rooker (1995) found that schoolmaster regularly consumed prey in the 20-30 mm size range and preyed on fish much smaller than their gape size, although he found no significant correlation of predator size with prey size.

Both native species did not exhibit an expected predation curve; the lowest ranked (i.e. preferred) classes were on either end of the size spectrum. This distribution scheme does not follow the theoretical optimal foraging curve (Holmes and McCormick 2010) with a normal distribution with a single preferred prey item. Instead, it suggests a bimodal distribution with preference for small individuals with potentially high catchability and large individuals with high energy input. The discrepancy could also be due to differences in catchability between the different size classes. However, this seems unlikely given the naïve nature of both the prey and predator (resulting in little to no avoidance), the tendency of the predator to lock into a single prey item before striking, and the agility of the smallest size class. Alternatively, selectivity could be influenced by hunting style with both native species using an ambush style of feeding, while lionfish would corral their prey. Schoolmaster would also routinely use a “frenzy” style of feeding, with the test fish repeatedly striking into the school of fish in rapid succession, possibly in an attempt to disorient and separate the school. The outcome was usually a low overall strike success rate but consumption of a few individuals in roughly the same amount of time as the other two predator species. However, all methods resulted in preference for the small size class and lionfish had repeated opportunities to consume the largest size class but chose to pursue the smaller size classes. The data may support the theory that while small, recruiting juvenile reef species may not be the most optimal prey, schoolmaster and graysby may inherently utilize the resource as a way to augment their predominantly large-bodied prey.

When predator body size and gape height is accounted for in the influence of preference, the overall trend differs. Lionfish exhibited a preference for prey items by length that suggests that they prefer the same sized prey as both of the native species. Schoolmaster and graysby exhibited a separation of consumed prey size suggesting a version of niche partitioning. The weight correction is beneficial for modeling purposes as it allows for comparison between species. However, there are issues with applying these results to a population level as the three species exhibit a natural difference in size resulting in different prey sizes selected on the reef. Gape height by prey length coefficients by species showed that all three species consume roughly the same sized prey when corrected for gape suggesting that gape may play a role in influencing preference. Coefficient data also shows that lionfish, graysby, and schoolmaster may be consuming prey at the lower end of their potential prey sizes.

Evidence from the present work allows speculation that lionfish may not be indiscriminately consuming all available prey on the reef, but rather feeding selectively upon certain size-classes. Observational data from Green and Côté (2014) supports this conclusion. Small-bodied reef fish are heavily fed upon naturally as adults by the native predatory fishes. Lionfish may not be consuming all available reef fishes, but primarily impacting the recruitment of the juvenile small-bodied reef species. With recruitment diminished, consumption by the native predators of the adult reef fish may lead to an overall collapse of the population. Albins (2013) noted this phenomenon on patch reef communities between lionfish and coney, a grouper species similar to graysby. He found that coney consumed relatively larger prey items, while lionfish had a significant impact on juvenile reef fish recruitment.

Predator Satiation

In terms of absolute weight consumed, schoolmaster ate 4.9 g more per individual predator fish than lionfish. However, prey weight consumed could not be directly compared because the three species did exhibit significant length or weight overlap between groups. Ideally, maintaining all three species to a small length or weight range in order to compare the species on an individual to individual basis would have been preferred. However, size-specific targeting for all three species was not possible due to

the limited size range and abundance of individuals available at the collection sites. Specifically, lionfish in both the Islamorada and Fort Lauderdale collection locations were significantly smaller both in size and weight compared to all available schoolmaster and most graysby. The reason for the absence of lionfish greater than 225 mm is not specifically known, although the high levels of recreational dive effort in those locations suggest that there is also a high rate of lionfish removal by individual divers.

Significant difference did not exist between the coefficient of mean consumed mass of prey per mm for schoolmaster and lionfish leading to the conclusion that schoolmaster and lionfish consume comparable amounts of prey at the same lengths. Tested individuals of both species were representative of the most commonly available size classes on the reef. Schoolmaster were found to be significantly larger than available lionfish in this habitat, suggesting that in the community from which these fish were collected, schoolmaster are consuming more prey biomass per fish than lionfish.

Lionfish consumed significantly more prey than both schoolmaster and graysby by gram unit of body weight. Graysby and schoolmaster consumed prey at levels of 43% and 67% respectively compared to lionfish. This could be an artifact of the disparity in robustness (Increased weight/length coefficient) between schoolmaster and lionfish within this size range. Fishelson (1997) calculated weight per unit length for his well-fed experimental lionfish with values (0.75 g/mm^{-1}) marginally higher than calculated in this study (0.68 g/mm^{-1}) for comparable size classes. The discrepancy may be caused in part by habituation to artificial conditions and an abundance of food used in this study that was not experienced by wild caught test subjects. However, the slight increase in the coefficient is not enough to significantly affect the results found here as schoolmaster (1.18 g/mm^{-1}) weigh significantly more per mm than both graysby and lionfish. Since schoolmaster weigh almost twice as much per mm, an individual of both species of identical length, consuming identical amounts of prey, would result in a g/g predator^{-1} rate half that of lionfish. Although models take differences in the length-weight frequency into consideration, assessments using count data or mass data independently will result in a skewed overall calculated consumed biomass if proper estimators are not used as correction factors.

The lionfish consumption rate of $0.079 \text{ g/g predator}^{-1}$ is more than double the rate found by Fishelson (1997) of $0.024 \text{ g/g predator}^{-1}$ at a temperature 1°C greater. Côté and Maljkovic (2010) published similar results with a coefficient of $0.038 \text{ g/g predator}^{-1}$ at an identical temperature regime. Although consumption is sensitive to temperature fluctuations, especially for ectothermic fishes, it alone cannot account for such a large difference. Green et al. (2011) calculated a rate of $0.089 \text{ g/g predator}^{-1}$, which is similar to results found in this study, considering that the ambient water temperature was 1°C warmer. The increased rate of consumption due to the 48 hr starvation performed in this experiment is also likely not the cause for the discrepancy, since Fishelson (1997) noted that many of the lionfish in his experiment would choose not to feed for periods of 24 hr or longer after a big meal.

The prior diet studies calculated consumption of lionfish using grams of prey consumed per gram predator⁻¹, making it difficult to compare across species due to the inherent differences in body morphology. Both schoolmaster and graysby displayed consumption rates closer to the calculated lionfish values in comparison studies, though they were significantly lower than lionfish values derived from this study. Although the feeding rate of lionfish among studies differ and indicate require further research in this regard is warranted the feeding rate calculated from this study can be directly compared to the two native species tested under identical conditions, something previous studies cannot accomplish due to differences in experimental design and physiological conditions.

When differences in weight is taken into account in conjunction with consumption coefficients, trends showing how feeding changes with size are realized. Schoolmaster and lionfish exhibited similar functions with decreasing rates of mass-specific prey consumption with size. Graysby, however, increased their consumption as body mass increased. Feeding strategy may be influencing these differences as all three species displayed very different feeding techniques.

With enough information on the most influential drivers of lionfish success, the invasive species can potentially be limited using EBM controls. Techniques would include increasing size limits or reducing bag limits of potential key competitors such as schoolmaster and graysby. Altering the population structure of these native species

would increase their overall impact on the reef community, preventing niche vacancy and increasing competition with lionfish populations, by reducing available prey and limiting growth (Usmar 2015). Ecological impacts (prey preference and consumption) of the two native species could also contribute to the quantification of variables relating to physiological responses and limitations for use as species-specific parameters in ecological models (Cooke and Hill 2010).

Conclusion

Research has done little to solve the question of how and why the western Atlantic invasive lionfish is so prolific and detrimental. Using this controlled experimental design, lionfish clearly prefer a small size class of prey when exposed to a spectrum of available targets. Graysby, popularly assumed to utilize the same niche, co-preferred the largest and smallest size classes available and thus appear to partially compete for prey with lionfish of similar size. Schoolmaster feeding trials suggest that they may potentially compete with lionfish, as the preferred size classes of prey were not significantly different. The results highlight the need for further studies in the natural environment on the behavior and interaction of schoolmaster with lionfish in a similar fashion to those currently being undertaken for graysby and lionfish

Values derived here show that schoolmaster have comparable consumption rates at the same size (length) as lionfish, making it a potential candidate as a biocontrol tool to naturally restrict the proliferation of local lionfish populations. Fisheries managers could utilize this finding in ecosystem-based fishery management schemes. By modifying size and bag limits for the schoolmaster, managers could optimize the species' biomass in an effort to increase competition and reduce prey resources available to lionfish. If performed successfully, the effects of lionfish could be minimized, thus mitigating some of the damage done to the small-bodied reef species and overall reef health.

Per unit of biomass, lionfish are more effective at consuming available prey from a population biomass standpoint than two ecologically important reef species, thereby likely contributing to their proliferation. This study suggests that lionfish success stems

from its ability to target prey sizes not preferred by the graysby. The invasive species may also be able to outcompete schoolmaster for the same prey size class. This may be due to a depletion of commercially and recreationally targeted schoolmaster populations, the lionfish's ability to locate and feed more rapidly, or a combination of unaccounted factors.

References

- Akins L, Lazarre D, Die D, Morris J (2012) *Lionfish Bycatch in The Florida Lobster Fishery: First Evidence of Occurrence and Impacts*. Proceedings of the 65th Gulf and Caribbean Fisheries Institute.
- Albins MA (2013). Effects of invasive Pacific red lionfish *Pterois volitans* versus a native predator on Bahamian coral-reef fish communities. *Biological Invasions*, 15(1), 29-43.
- Albins MA, Hixon MA (2008). Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. *Marine Ecology Progress Series*, 367, 233-238.
- Albins MA, Hixon MA (2013). Worst case scenario: potential long-term effects of invasive predatory lionfish (*Pterois volitans*) on Atlantic and Caribbean coral-reef communities. *Environmental Biology of Fishes*, 96(10-11), 1151-1157.
- Barbour AB, Allen MS, Frazer T K, Sherman KD (2011). Evaluating the potential efficacy of invasive lionfish (*Pterois volitans*) removals. *PloS one*, 6(5), e19666.
- Beauchamp DA, Stewart DJ, Thomas GL (1989). Corroboration of a bioenergetics model for sockeye salmon. *Transactions of the American Fisheries Society*, 118(6), 597-607.
- Bolnick DI, Ingram T, Stutz WE, Snowberg LK, Lau OL, Paull JS (2010). Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B: Biological Sciences*, rspb20100018.
- Bowen SH (1996). "Quantitative description of the diet." *Fisheries techniques, 2nd edition*, American Fisheries Society, Maryland. 513-532.
- Brönmark C, Miner JG (1992). Predator-induced phenotypical change in body morphology in crucian carp. *SCIENCE-NEW YORK THEN WASHINGTON-*, 258, 1348-1348.
- Carpenter KE, Niem VH (2002). FAO species identification guide for fishery purposes. The living marine resources of the Western Central Atlantic. Volume 3. Bony fishes part 2 (Opistognathidae to Molidae) sea turtles and marine mammals. *FAO*

species identification guide for fishery purposes. The living marine resources of the Western Central Atlantic. Volume 3. Bony fishes part 2 (Opistognathidae to Molidae) sea turtles and marine mammals.

- Cerino D, Overton AS, Rice JA, Morris Jr JA (2013). Bioenergetics and Trophic Impacts of the Invasive Indo-Pacific Lionfish. *Transactions of the American Fisheries Society*, 142(6), 1522-1534.
- Cervigón F, Los Roques FC (1991). *Marine fish of Venezuela*. Los Roques Scientific Foundation.
- Chiappone M, Sluka R, Sealey KS (2000). Groupers (Pisces: Serranidae) in fished and protected areas of the Florida Keys, Bahamas and northern Caribbean. *Marine Ecology Progress Series*, 198, 261-272.
- Clutter RI, Theilacker GH (1971). Ecological efficiency of a pelagic mysid shrimp; estimates from growth, energy budget, and mortality studies. *Fish. Bull*, 69(1), 93-115.
- Cooke SL, Hill WR (2010). Can filter-feeding Asian carp invade the Laurentian Great Lakes? A bioenergetic modelling exercise. *Freshwater Biology*, 55(10), 2138-2152.
- Côté IM, Maljkovic A (2010). Predation rates of Indo-Pacific lionfish on Bahamian coral reefs. *Mar Ecol Prog Ser*, 404, 219-225.
- Côté IM, Green SJ (2012). Potential effects of climate change on a marine invasion: the importance of current context. *Current Zoology*, 58(1), 1-8.
- Côté IM, Green SJ, Morris Jr JA, Akins JL, Steinke D (2013). Diet richness of invasive Indo-Pacific lionfish revealed by DNA barcoding. *Marine Ecology, Progress Series*, 472, 249-256.
- Dierking J, Williams ID, Walsh WJ (2009). Diet composition and prey selection of the introduced grouper species peacock hind (*Cephalopholis argus*) in Hawaii. *Fishery Bulletin*, 107(4), 464-476.
- Ellis T, Gibson RN (1995). Size-selective predation of 0-group flatfishes on a Scottish coastal nursery ground. *Marine ecology progress series. Oldendorf*, 127(1), 27-37.

- Fishelson L (1997). Experiments and observations on food consumption, growth and starvation in *Dendrochirus brachypterus* and *Pterois volitans* (Pteroinae, Scorpaenidae). *Environmental Biology of Fishes*, 50(4), 391-403.
- Gill AB (2003). The dynamics of prey choice in fish: the importance of prey size and satiation. *Journal of Fish Biology*, 63(s1), 105-116.
- Green SJ, Akins JL, Côté IM (2011). Foraging behaviour and prey consumption in the Indo-Pacific lionfish on Bahamian coral reefs. *Marine Ecology Progress Series*, 433, 159-167.
- Green SJ, Akins JL, Maljkovic A, Côté IM (2012). Invasive Lionfish Drive Atlantic Coral Reef Fish Declines. *PLoS One*, 7(3).
- Green SJ, Côté IM (2014). Trait-based diet selection: prey behaviour and morphology predict vulnerability to predation in reef fish communities. *Journal of Animal Ecology*, 83(6), 1451-1460.
- Hambright KD (1994). Morphological constraints in the piscivore-planktivore interaction: implications for the trophic cascade hypothesis. *Limnology and Oceanography*, 39(4), 897-912.
- Hamner RM, Freshwater DW, Whitfield PE (2007). Mitochondrial cytochrome b analysis reveals two invasive lionfish species with strong founder effects in the western Atlantic. *Journal of Fish Biology*, 71(sb), 214-222.
- Harfmar KJ, Brandt SB (1995). Predatory demand and impact of striped bass, bluefish, and weakfish in the Chesapeake Bay: applications of bioenergetics models. *Canadian Journal of Fisheries and Aquatic Sciences*, 52 (8), 1667-1687.
- Hitt S, Pittman SJ, Brown KA (2011). Tracking and mapping sun-synchronous migrations and diel space use patterns of *Haemulon sciurus* and *Lutjanus apodus* in the US Virgin Islands. *Environmental biology of fishes*, 92(4), 525-538.
- Holmes TH, McCormick MI (2010). Size-selectivity of predatory reef fish on juvenile prey. *Marine Ecology Progress Series*, 399, 273-283.
- Juanes F (1994) What determines prey size selectivity in piscivorous fishes? In: Stouder DJ, Fresh KL, Feller RJ (eds) Theory and application in fish feeding ecology. S Carolina University Press, Columbia, 79–100 (Belle W. Baruch Library in Marine Sciences No. 18)

- Juanes F, Buckel JA, Scharf FS (2002). Feeding ecology of piscivorous fishes. *Handbook of fish biology and fisheries, volume 1: Fish biology*, 267-283.
- Kitchell JF (1990). The scope for mortality caused by sea lamprey. *Transactions of the American Fisheries Society*, 119(4), 642-648.
- Kitchell JF, Eby LA, He X, Schindler DE, Wright RA (1994). Predator-prey dynamics in an ecosystem context. *Journal of Fish Biology*, 45 (SA), 209-226.
- Manly BF (2006). On a proposed method for analysing experiments on food choice. *Journal of experimental marine biology and ecology*, 335(1), 154-155.
- Matheson RH, Huntsman GR, Manooch CS (1986). Age, growth, mortality, food and reproduction of the scamp, *Mycteroperca phenax*, collected off North Carolina and South Carolina. *Bulletin of Marine Science*, 38(2), 300-312.
- McCleery C (2011). A comparative study of the feeding ecology of invasive lionfish (*Pterois volitans*) in the Caribbean. *Physis: CIEE Research Station Bonaire*, 9, 38-43.
- Meister HS, Wyanski DM, Loefer JK, Ross SW, Quattrini AM, Sulak KJ (2005). Further evidence for the invasion and establishment of *Pterois volitans* (Teleostei: Scorpaenidae) along the Atlantic coast of the United States. *Southeastern Naturalist*, 4(2), 193-206.
- Messing RH, Wright MG (2006). Biological control of invasive species: solution or pollution? *Frontiers in Ecology and the Environment*, 4(3), 132-140.
- Morris Jr JA, Akins JL (2009). Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago. *Environmental Biology of Fishes*, 86(3), 389-398.
- Morris Jr JA, Akins JL, Barse A, Cerino D, Freshwater DW, Green SJ et al. (2009). Biology and ecology of the invasive lionfishes, *Pterois miles* and *Pterois volitans*. In *Proceedings of the Gulf and Caribbean Fisheries Institute* (Vol. 61, pp. 409-414).
- Morris Jr JA, Sullivan CV, Govoni JJ (2011). Oogenesis and spawn formation in the invasive lionfish, *Pterois miles* and *Pterois volitans*. *Scientia Marina*, 75(1), 147-154.

- Nagelkerken WP (1979). *Biology of the graysby, Epinephelus cruentatus, of the coral reef of Curaçao*. Foundation for Scientific Research in Surinam and the Netherlands Antilles.
- Naughton SP, Saloman CH (1985). *Food of gag (Mycteroperca microlepis) from North Carolina and three areas of Florida*. NMFS-SEFSC. NMFS-SEFC-160, Panama City.
- Parrish JD (1987). The trophic biology of snappers and groupers. In 'Tropical Snappers and Groupers: Biology and Fisheries Management'. (Eds JJ Polovina and S. Ralston.). 405–463.
- Popple ID, Hunte W (2005). Movement patterns of *Cephalopholis cruentata* in a marine reserve in St Lucia, WI, obtained from ultrasonic telemetry. *Journal of fish biology*, 67(4), 981-992.
- Poléo A, Osxnevad SA, Össtbye K, Heibo E, Andersen RA, Vøllestad LA (1995). Body morphology of crucian carp *Carassius carassius* in lakes with or without piscivorous fish. *Ecography*, 18(3), 225-229.
- Randall JE (1983). *Caribbean reef fishes*, 2nd ed. T. E H. Publ. Inc., Jersey City, New Jersey. 318.
- Randall JE, Bishop B (1967). Food habits of reef fishes in the West Indies. *Stud. Trop. Oceanography. Miami* 5:655-847.
- Rice JA, Cochran PA (1984). Independent evaluation of a bioenergetics model for largemouth bass. *Ecology*, 732-739.
- Rooker JR (1995). Feeding ecology of the schoolmaster snapper, *Lutjanus apodus* (Walbaum), from southwestern Puerto Rico. *Bulletin of Marine Science*, 56(3), 881-894.
- Ruiz-Carus R, Matheson Jr RE, Roberts Jr DE, Whitfield PE (2006). The western Pacific red lionfish, *Pterois volitans* (Scorpaenidae), in Florida: Evidence for reproduction and parasitism in the first exotic marine fish established in state waters. *Biological conservation*, 128(3), 384-390.
- Sano M (1984). Food habits of teleostean reef fishes in Okinawa Island, southern Japan. *Univ. Mus., Univ. Tokyo, Bull.*, 25, 1-128.

- Scharf FS, Buckel JA, Juanes F (2002). Size-dependent vulnerability of juvenile bay anchovy *Anchoa mitchilli* to bluefish predation: Does large body size always provide a refuge? *Marine Ecology Progress Series*, 233, 241-252.
- Schultz, E.T. (1986). *Pterois volitans* and *Pterois miles*: two valid species. *Copeia*, 1986 (3), 686–690.
- Sih A, Crowley P, McPeck M, Petranka J, Strohmeier K (1985). Predation, competition, and prey communities: a review of field experiments. *Annual Review of Ecology and Systematics*, 269-311.
- Shpigel M, Fishelson L (1989). Food habits and prey selection of three species of groupers from the genus *Cephalopholis* (Serranidae: Teleostei). *Environmental Biology of Fishes*, 24(1), 67-73.
- Sogard SM (1997). Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science*, 60(3), 1129-1157.
- Stallings CD (2010). Experimental test of preference by a predatory fish for prey at different densities. *Journal of Experimental Marine Biology and Ecology*, 389(1), 1-5.
- Taplin RH (2007). Experimental design and analysis to investigate predator preferences for prey. *Journal of experimental marine biology and ecology*, 344(1), 116-122.
- Trexler JC, Loftus WF, Jordan F, Lorenz JJ, Chick JH, Kobza RM (2000). Empirical assessment of fish introductions in a subtropical wetland: an evaluation of contrasting views. *Biological Invasions*, 2(4), 265-277.
- Underwood AJ, Clarke KR (2005). Solving some statistical problems in analyses of experiments on choices of food and on associations with habitat. *Journal of Experimental Marine Biology and Ecology*, 318(2), 227-237.
- Underwood AJ, Clarke KR (2006). Response on a proposed method for analysing experiments on food choice. *Journal of experimental marine biology and ecology*, 335(1), 151-153.
- Usmar NR (2012). Ontogenetic diet shifts in snapper (*Pagrus auratus*: Sparidae) within a New Zealand estuary. *New Zealand Journal of Marine and Freshwater Research*, 46(1), 31-46.

- Valdivia A, Bruno JF, Cox CE, Hackerott S, Green SJ (2014). Re-examining the relationship between invasive lionfish and native grouper in the Caribbean. *PeerJ*, 2, e348.
- Whitfield PE, Gardner T, Vives SP, Gilligan MR, Courtenay Jr WR, Ray GC, Hare JA (2002). Biological invasion of the Indo-Pacific lionfish *Pterois volitans* along the Atlantic coast of North America. *Marine Ecology Progress Series*, 235, 289-297.
- Whitfield PE, Hare JA, David AW, Harter SL, Munoz RC, Addison CM (2007). Abundance estimates of the Indo-Pacific lionfish *Pterois volitans/miles* complex in the Western North Atlantic. *Biological Invasions*, 9(1), 53-64.
- Williams BK, Titus K (1988). Assessment of sampling stability in ecological applications of discriminant analysis. *Ecology*, 1275-1285.
- Wilson EO (1961). The nature of the taxon cycle in the Melanesian ant fauna. *American Naturalist*, 169-193.
- Wilcox CL (2014). *Molecular investigations of the Pteroinae: Insights into the invasive lionfishes from the native range*. Dissertation, University of Hawaii at Manoa