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BEHAVIOURAL PLASTICITY AND FORAGING ECOLOGY OF INVASIVE
LIONFISH (*PTEROIS VOLITANS*)

by

Emma De Roy

A Thesis
Submitted to the Faculty of Graduate Studies
through the Great Lakes Institute for Environmental Research
in Partial Fulfillment of the Requirements for
the Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada

2019

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DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship

I hereby declare that this thesis incorporates material that is the result of joint research, as follows. Chapter 2 of this thesis was co-authored with and under the supervision of professors H.J. MacIsaac and N.E. Hussey. Both H.J. MacIsaac and N.E. Hussey provided feedback on the manuscript, helped refine the main themes of the manuscript, and assisted in assessing the broad implications of the results. In all cases, the key ideas, primary contributions, experimental designs, data analysis, interpretation, and writing were performed by the author, and the contribution of co-authors was primarily through intellectual guidance.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

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II. Previous Publication

This thesis includes one original papers that have been previously published/submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication title/full citation	Publication status
Chapter 2	De Roy EM, Hussey NE, MacIsaac HJ (2019) Memory retention and behavioural plasticity in an invasive marine fish. <i>Animal Behaviour</i>	Submitted

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ABSTRACT

Understanding the way in which species forage, both alone and relative to heterospecifics and conspecifics, has important implications for ecosystem functioning. This is especially true for invasive species for whom context-dependent impacts make comprehensive impact assessments challenging. This thesis examined the foraging ecology of a marine invasive species to better assess its spatially and temporally-mediated impact. The goal of this thesis was to quantify the impact of lionfish (*Pterois volitans*) in an understudied part of its invaded range, both alone and relative to native analogues. I conducted feeding experiments to evaluate the way in which lionfish move about their environment and to assess the relative roles of behavioural plasticity, foraging efficiency, and cooperative hunting in foraging success. Lionfish showed flexibility in their ability to adopt spatial navigation strategies to remember the locations of profitable prey patches, durations for which lasted up to 6 weeks. Relative to native predators, lionfish exhibited similar consumption rates and lower per capita impacts, though combining their per capita impact with field abundance bolstered their ability to impact native prey populations. Lionfish displayed a high degree of gregariousness and were most efficient when foraging in pairs, whose consumption rates were mediated by the degree of refuge afforded to prey. Overall, these results suggest that the success of lionfish in the eastern Gulf of Mexico lies in the efficiency with which they forage, their behavioural plasticity, and their high population abundance relative to co-occurring native competitors. Understanding the impacts of invasive species is pivotal to managing their threat, though these understandings are contingent upon the assessment of impacts throughout the invaded range of a species.

DEDICATION

For Paul.

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CHAPTER 1: GENERAL INTRODUCTION

Foraging sits at the cornerstone of ecology, motivating the directed movements of organisms (Pyke et al. 1977; Nathan et al. 2008), associations with conspecifics (Major 1978), and underscoring competitive dynamics between species (Schoener 1983). Predation is important for the structuring and functioning of communities (Sih et al. 1985) and a predator must learn to efficiently forage amongst the network of heterogeneous patches that comprise their environment (Jana et al. 2014). Consequently, animals should strive to maximize their foraging efficiency by aggregating to patches of high prey density (Hassell 1978; Stephens and Krebs 1986; Thompson et al. 2001). Attending to and remembering features of the environment that portend highly profitable prey patches are thus important for the fitness of an organism.

Animals use myriad strategies of increasing sophistication to spatially navigate (Rodriguez et al. 1994), often developing complex spatial representations of the environments in which they live and forage as a means by which to efficiently procure resources (Aronson 1971; O'Keefe and Nadel 1978; Odling-Smee et al. 2006). Fish are particularly adept at circumnavigating their landscape (reviewed in Dodson 1988). Fish use reference landmarks for direction (Reese 1989), to identify profitable food patches (Warburton 1990), and to develop cognitive maps with which to spatially orient (Rodriguez et al. 1994; Broglio et al. 2003). Doing so is essential given the need to compete with both conspecifics and heterospecifics for patchily distributed resources.

The foundation on which predator-prey interactions is built stems from an individual's functional response, that is, the way in which predator feeding rates change

across increasing prey abundance (Holling 1959; Murdoch and Oaten 1975). The functional response of an organism is one method used to measure a predator's foraging efficiency. A predator's functional response is often modified by changes in habitat (Lipcius and Hines 1986) and predator density (Long et al. 2012), each of which has implications for a predator's foraging efficiency (Kareiva 1987; Mansour and Lipcius 1991; Denno et al. 2002). For example, the per capita impacts of predators on prey populations scale non-linearly with increasing predator density (Abrams and Ginzburg 2000; McCoy et al. 2012), often leading to greater foraging efficiency and higher per capita consumption rates at higher predator densities (Eklöv 1992). However, the degree to which changing predator density affects prey populations across fluctuating prey densities is understudied (Soluk 1993; Kumar et al. 2014), prompting the need to better parse predator dependence in functional responses.

Structure-induced changes in predator behaviour, that is, aggregation of predators to patches containing high prey densities, drives density-dependent mortality (Hixon and Webster 2002; Toscano and Griffen 2013) and often promotes ecosystem stability (Hassell and May 1974). However, a predator's per capita consumption is contingent upon the influence of prey behaviour, given that predator-prey interactions are inherently dynamic (Lima 2002). Predators and their prey have conflicting interests which underlie the predator-prey 'space race' (Sih 1984; Schmitz 2005). While predators should aggregate to high-density prey patches, prey have a vested interest in avoiding such areas (Stephens and Krebs 1986; Sih 1998; Hammond et al. 2007). The behaviours of predators and their prey are seldom studied in tandem, though they contribute substantially to

predator-prey population dynamics (Brown et al. 1999; van Baalen and Sabelis 1999; Lima 2002).

Interactions between heterospecifics, conspecifics, and with the prey they consume can strongly influence ecosystem functioning, especially in the case of an invasive species. Invasive species act as a lens through which to better understand species' foraging ecology, for which conceptualizing predator-prey interactions is particularly important. To be termed invasive, non-indigenous species must overcome barriers to their uptake, transport, and establishment, and, in some cases, have ecological and economic impacts on recipient systems (Lockwood et al. 2013). Ecological and behavioural plasticity lend support during several of the aforementioned stages and may contribute to the successful establishment of non-indigenous species (Wright et al. 2010; Parker et al. 2013). Understanding predator-prey interactions and their variability in space and time is needed to comprehensively understand the impacts of invasive species.

This need is particularly evident for marine invasive species. While more plentiful than their freshwater counterparts (Vilà et al. 2010), impacts of marine invasive species are generally ambiguous, undocumented, or otherwise unknown owing to a dearth of research on marine systems relative to both terrestrial and freshwater systems (Grosholz 2002; Vilà et al. 2010; Ruiz et al. 2011; Tricarico et al. 2016). The data gaps that stem from this limited bank of knowledge may occlude an understanding of the true nature of these impacts and may restrict researchers' ability to develop broad models to mitigate such impacts (Parker et al. 1999; Davidson et al. 2017). Invasive species are often recognized for their greater maximum feeding rates, their disproportionate impacts, and their ability to outcompete co-occurring native species for shared resources (Salo et al.

2007; Dick et al. 2014). This is true both of invasive species generally and specifically of marine invasive species (DeGraaf and Tyrrell 2004). Thus, in a given community it is important to understand the degree to which the impacts of an invasive species differ relative to the native predators with which they co-occur, which provide a baseline measure of impact.

The primary goals of this thesis were to quantify the impacts of an invasive predator (in contrast to native mesopredators) in a newly invaded site and to ascribe mechanisms behind these impacts that relate to its foraging ecology and spatial navigation abilities. Specifically, I focused on the invasive lionfish. Lionfish (*Pterois volitans*) are native to the Indo-Pacific region and a hybrid species comprised of *Pterois miles* and *P. lunulata/russelii* individuals (Wilcox et al. 2017). Lionfish were introduced to the western Atlantic Ocean in the 1980s (Morris and Whitfield 2009). Their rapid spread, consumptive and non-consumptive effects on native reef fishes, and competition with native mesopredators (Albins and Hixon 2008; Schofield 2010; Kindinger 2015; Tamburello and Côté 2015; Ingeman 2016; Curtis et al. 2017) have heightened concern over the long-term implications of lionfish on coral reef communities (Albins and Hixon 2013). As a central place forager (Benkwitt 2016), lionfish have the potential to exert impacts across a range of habitats. However, whether their habitat preference is dictated by or irrespective of prey abundance has yet to be assessed. So too, similar habitat preferences and foraging ecology of lionfish relative to native predators may have implications for competition between the former and latter groups.

Lionfish are now prevalent throughout the Gulf of Mexico and along the West Florida Shelf since their introduction to the region in the late 2000s (Schofield 2010;

Fogg et al. 2013; Johnston et al. 2016). Numerous impact assessments have been conducted in the Bahamas and Caribbean (Albins 2013; Green et al. 2014), yet only a single field study has quantified the density-dependent impacts of lionfish (Benkwitt 2015). Further, few such studies have probed the degree to which cooperative hunting in lionfish influences their per capita impact (Rizzari and Lonnstedt 2014). Understanding their impacts over spatial and temporal scales and across increasing lionfish density is however important given the context-dependence of invasive species' impacts (Thiele et al. 2010; Ricciardi et al. 2013).

1.1 Thesis Objectives

This thesis sought to contextualize the ecological impacts of lionfish in the eastern Gulf of Mexico. I investigated learning proficiency, memory retention, and foraging efficiency of lionfish and their potential implications. In Chapter 2, I provide the first experimental account of learning speed and memory duration in lionfish using a spatial navigation experiment. I probed the ability of lionfish to remember the location of a profitable prey patch using navigational strategies singly and in conjunction and later tested their memory duration as it related to optimal foraging. I assessed per capita changes in predation using functional response methodology as a function of prey density and habitat heterogeneity relative to native analogues (Chapter 3) and to increasing lionfish density (Chapter 4). Finally in Chapter 5, I briefly summarize the novel contributions of this thesis to the fields of invasion biology, marine ecology, and predator-prey theory.

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CHAPTER 2: MEMORY RETENTION AND BEHAVIOURAL PLASTICITY IN AN INVASIVE MARINE FISH

2.1 Introduction

The way in which animals forage has been the subject of intense study (e.g. Schoener 1971), as have the relevant components of foraging (e.g. movement as dictated by patchily distributed resources). One of the factors influencing foraging behaviour is patch profitability (Mittelbach 1981). Numerous studies have explored how relative patch profitability informs foraging decisions in a variety of species, including monkeys (Boyer et al. 2012) and marine fishes (Warburton 2003). Determining and remembering the relative profitability of food patches is important to the success of fishes that live in inherently complex environments where resources are patchily distributed (Hughes et al. 1992; Bracis et al. 2015). Therefore, fishes must move within their environment in a way that best exploits these patchily distributed resources.

Foraging in fish is guided by spatial navigation, underpinned by different mechanisms of spatial orientation. Fish can spatially orient in several ways, including by creating a user-independent representation of their environment (i.e. a cognitive map), denoted as an allocentric spatial navigation strategy. Alternatively, fish may orient using body-centric cues, (an ‘egocentric’ strategy) or by employing a combination thereof (i.e. an ego-allocentric strategy), the latter strategy allowing navigation based on either or both body-centric cues and a cognitive map (O’Keefe and Nadel 1978; van Gerven et al. 2012). Often, species prefer to orient using the ego-allocentric strategy, as it is more flexible (Rodriguez et al. 1994). For simplicity, I will hereafter refer to each of allocentric and ego-allocentric strategies as place and response strategies, respectively.

How fishes learn and move about their environment is important to understanding predator-prey interactions, which have received considerable attention in behavioural ecology (e.g. Lima and Zollner 1996). However, memory duration in fishes *per se* is still poorly understood (Ingraham et al. 2016). This dearth of information underscores the need for more research, especially in light of myriad stressors that currently threaten marine systems. Invasive species, which are typically considered a leading threat to marine biodiversity, are a particularly potent stressor (McGeoch et al. 2010). Invasive species are organisms non-indigenous to a region that exert strong ecological, and/or economic effects, or spread rapidly (Lockwood et al. 2013). Non-indigenous organisms must traverse a series of stages with respect to transport, introduction, establishment, and spread, culminating in impact (Blackburn et al. 2011; Lockwood et al. 2013; Ricciardi et al. 2013). The breadth of invasive species impacts is tremendous, in both scale and scope, ranging from individual-level effects (e.g. alterations in foraging behaviour, Peck et al. 2014), to those at the population (e.g. extirpation of native species, Loppnow et al. 2013), community (e.g. pythons in the Florida Everglades, Willson 2017) or ecosystem (e.g. modified nutrient cycling, Simon and Townsend 2003) levels. A comprehensive understanding of the mechanisms underpinning such impacts is needed to efficiently manage these species (Pyšek and Richardson 2010).

While a great deal of research has been conducted on invasive species impacts, there has been a paucity of studies focused on those associated with marine invasive species (Grosholz 2002). This is concerning given that the impacts of marine invasive species may interact with those associated with overharvesting, habitat loss, and climate change (Dulvy et al. 2003). Consequently, understanding impacts and the underlying

mechanisms responsible for them is of utmost importance. Behavioural plasticity, of which learning and memory are part, may be one mechanism by which to better understand impacts of invaders. Although there has been limited research on the degree to which learning influences the success of invasive species (Weis 2010), some studies suggest superiority of invasive relative to native competitors (e.g. Roudez et al. 2008).

Understanding the way in which invasive species learn and move about their environment can better inform their impacts on native species and invaded ecosystems. Fish with better memories can develop frames of reference concerning prey availability and patch profitability (Warburton 2003), allowing them to forage more efficiently relative to conspecifics (Brown et al. 2011). In turn, superior efficiency can induce interference or exploitative competition with competing species (Petren and Case 1996), the intensity of which may be heightened because of introductions of invasive species (Rahel and Olden 2008). In the case of exploitative competition, an invasive species that can preferentially remember locations of highly profitable prey patches may deprive competitors of those resources by depleting the patches first (Schoener 1983; Roudez et al. 2008). This ability to exploit shared resources is particularly important when resource availability is low (Tilman et al. 1982; Murray et al. 2013), wherein the superior memory of an invasive species may allow it to preferentially deplete these resources. Such competition can subsequently precipitate decreased fitness in the native species and alter both biotic interactions and population dynamics (Kareiva 1990; McCoy and Bell 1991; Ruetz et al. 2003). How the impact of invasive species may be influenced by their behavioural plasticity is an evolving question and addressed in the present study. I conducted a suite of experiments to examine learning and memory retention using

lionfish as a model species. Lionfish (*Pterois volitans*) are demersal, marine organisms native to the Indo-Pacific region. Following their introduction to Florida's east coast in the 1980s, the species has become widespread throughout the western Atlantic Ocean, Caribbean Sea, and Gulf of Mexico (Schofield 2010).

Lionfish are reported to have high predation efficiency and very strong impacts (e.g. Albins and Hixon 2008; Albins 2013), the establishment and spread of which may have been facilitated by their behavioural plasticity and ability to exploit novel habitats and prey types (Cure et al. 2014). Given this, spatial memory is a particularly relevant metric to test in lionfish. The goal of my research was to assess behavioural plasticity as it relates to foraging behaviour in a highly invasive species - lionfish - specifically addressing issues of memory retention and duration. I explored these aspects and determined whether the speed with which lionfish learn is affected by the type of navigation strategy employed (place strategy vs. response strategy). I hypothesized that lionfish would learn more quickly when trained in the more flexible response strategy. I did not expect that memory duration or ability would differ between place and response groups given the relevance of a food reward, as food-based memories should be resistant to decay in both groups (Hughes et al. 1992).

2.2 Methods

All fieldwork was conducted at Florida Gulf Coast University's Vester Marine and Environmental Science Research Field Station in Bonita Springs, Florida. All fish were handled and collected under a Letter of Acknowledgement (LOA) from the National Oceanic and Atmospheric Administration (NOAA) and were handled under the approval

of University of Windsor's Animal Care Committee (AUPP #17-01) and in accordance with the Canadian Council on Animal Care.

Animals

Wilcox et al. (2017) revealed that western Atlantic Ocean lionfish (*Pterois volitans*) are a hybrid species comprised of several lineages, thus I will refer to the complex simply as lionfish. Lionfish (N=20, 216.53 ± 5.36 mm TL) were collected during summer and fall 2018 off the coast of Bonita Springs, Florida, USA. To eliminate the confounding influence of habitat type and complexity on learning speed and efficiency (White and Brown 2014), all fish were captured from a single sinkhole (26-28.900°, 82-84.190°) at an average of 30m depth. A team of three divers using SCUBA collected lionfish using short-handled hand nets, which were transferred to and brought to the surface in a large containment unit. Some fish displayed signs of barotrauma after surfacing and were vented accordingly using a 16g hypodermic needle. Briefly, the needle was inserted into the body wall at a 45-degree angle, plunged slightly into the flesh of the fish, and held in place until sufficient decompression was achieved. HexArmor SharpsMaster II® 9014 puncture-proof gloves were worn when handling lionfish. Following capture, all fish were held in either or both the boat's live well and in aerated coolers filled with seawater. All fishes were proactively treated with StressGuard™, a slime coat protection product. New water was added to the coolers periodically en route to the field station to maintain sufficient aeration. At the end of the experiment, all lionfish were kept alive for other research.

Experimental fish were acclimated for a minimum two weeks prior to being used in experimental trials and were housed with conspecifics throughout the experiment. All

animals were experimentally naïve. During acclimation, lionfish were fed *ab libitum* assorted live baitfish [silversides (*Atheriniformes spp.*) and pilchards (*Harengula jaguana*)], saltwater-acclimated live mosquitofish (*Gambusia affinis*), pink shrimp (*Penaeus duorarum*, both live and frozen), and frozen sardines *Sardinops sagax*).

Live pink shrimp (\bar{x} = 30 mm) purchased from a local bait shop represented high-quality prey for lionfish and are readily consumed (South et al. 2017; Peake et al. 2018). Throughout the experiment, food was only provided during trials. Individual lionfish were identified with coloured tags (Floy® FTSL-73 streamer tags), each containing a unique three-digit number. Individual lionfish were identified with coloured tags (Floy® FTSL-73 polyethylene streamer tags, 4" length), each containing a unique three-digit number. Streamer tags are ideal given their negligible weight, ease with which they are applied, neutral buoyancy, and applicability across a wide range of fish sizes. Streamer tags have become widely used for tagging lionfish. The tagging method followed Akins et al. (2014). To increase ease of handling and to decrease stress incurred by both species during tagging, fish were anaesthetized in a bath of MS-222 dissolved in seawater. Fishes were captured from their communal tanks with shallow-bodied hand nets and transferred to a bath of well-aerated saltwater containing a concentration of 110 mg/ L MS-222, within the range used by Bacheler et al. (2015). Once sedated, fish were transferred to a shallow-bodied container housing a seawater bath with a maintenance dose of anesthetic. Briefly, a 16g hypodermic needle was inserted at the posterior end of the soft dorsal fin abutting the caudal peduncle. The hypodermic needle was used as a guide for the streamer tag-attached needle. Once the streamer tag was positioned with equal length on either side of the fish, the needle was detached from the tag. Over-the-counter antibiotic

cream was applied to the needle insertion location to promote wound healing. Once tagging was complete and before re-introduction to their home tanks, fish were placed in a 38L bath of aerated saltwater until normal swimming performance was achieved. No short or long-term adverse effects were noted for any of the fish. Normal swimming and feeding behaviours were observed shortly after fish were re-introduced to their communal tanks. All fish were tagged several weeks prior to the start of experimental trials to further reduce confounding effects of the anaesthetic on performance.

Lionfish were housed in several 1135 L recirculating tanks in a semi-enclosed aquaculture cage equipped with chemical, physical, and biological filtration systems and subject to a natural photoperiod. PVC pipe, concrete blocks, and scattered rocks were added to housing tanks to increase environmental complexity. Water was changed weekly using seawater pumped in from the surrounding bay, or by mixing Instant Ocean® artificial seawater. Temperature, pH, salinity, and dissolved oxygen were checked daily both in housing tanks and in the experimental arena using a YSI™ Professional Plus meter. In both housing and experimental tanks, water was maintained at 24°C, pH 8.2, 34 ppt salinity, and dissolved oxygen (DO) at or near saturation. Water in the experimental arena was aerated with submersible air stones between sessions. Ammonia, nitrate, and nitrite were monitored and never exceeded negligible levels. New water was added to the experimental system on a bi-weekly basis, or whenever water levels declined.

Apparatus

Trials were conducted in a four-arm plexiglass maze (arm dimensions 88.5 cm long x 12 cm wide x 46 cm high) (Figure 2.1). Two opaque guillotine doors (50 cm height x 32 cm width x 0.5 cm depth) were used to delineate the start box and to block off

the opposite arm, producing a T-maze. This apparatus is commonly used to assess spatial navigation in fishes (e.g. Rodriguez et al. 1994; White and Brown 2014). Three of the maze arms (including the start arm) were unrewarded. Doors were attached to a nylon filament and controlled by a pulley system used to raise and lower the doors as needed. The start box door was positioned 33 cm from the arm end. Four identical artificial plants (two in each of the possible goal arms) were placed 33 cm from arm ends. Each plant measured 15.5 cm tall. Four food holders made of white 1.5-inch PVC were installed in each of the four-maze arm ends and raised 25 cm off the bottom of the tank floor, such that the shrimp were introduced at eye level of the fish. Food holders were installed in all arms to preclude unintentional directional cues. Four sets of extramaze cues were positioned outside the maze but inside the tank arena. Selected cues were dissimilar in size, shape, colour, and height: 1) oyster shells; 2) small rocks; 3) large rectangular cinder blocks; and 4) stacked assorted cylindrical PVC pipe (Figure 2.1).

Navy-coloured electrical tape was placed 9 cm from the bottom of the maze arm along the interior of all arms and delineated maze boundaries. The maze was placed inside a circular tank of 2.13 m diameter and 0.72 m height. The tank interior was covered by a grey plastic tarp to provide contrast for video recording. The maze was filled with water to a height of 30 cm. The arm ends were aerated via single air stones. The entire arena was surrounded by a black plastic tarp to eliminate any global extramaze cues other than those positioned directly outside of the maze itself and to preclude a Hawthorne effect (James and Vo 2010). Six equidistant carabineers affixed the tarp to a black ceiling. No directional cues could be gleaned from the ceiling.

Trials were recorded via a Go-Pro Hero 5 camera (GoPro Inc., San Mateo, CA, USA) recording at high resolution (1080p, 30 frames per second). The maze arena was evenly illuminated by a single LED light (Husky: Model K40066). Both the GoPro and light were placed on a square platform suspended from a ceiling-mounted metal rod that was situated in the middle of the experimental arena, thus providing no relevant directional cues to fish. However, the platform was rotated on several occasions to further reduce this possibility. The Go-Pro was set up to allow trials to be viewed remotely via a paired mobile device.

Experimental Procedures

The experimental set-up was designed to discern whether lionfish could learn and remember the location of a food source using 1) a solely location-based spatial navigation strategy (the place strategy) or 2) a combined body-centric and location-based spatial navigation strategy (the response strategy). Fish were tested individually as opposed to in groups to eliminate the potentially confounding influence of social cues (Ingraham et al. 2016).

Lionfish were randomly assigned to either the place or response group (N=10 per group) using the RAND function in Excel. There were no significant size differences between groups (Mann-Whitney test: $W=26.5$, $P>0.10$). Given that there were no significant differences in size (and thus, age), I did not expect any confounding influences of age on learning ability. Each of the two experimental groups was broken down into two sub-groups to control for the influence of inherent directional or location biases (per Rodriguez et al. 1994) (Figure 2.2). In the response group, half of the fish were trained to turn left and the other half was trained to turn right, while in the place

group, half of the fish were trained to locate prey in G1 while the other half were trained to locate prey in G2 (Figure 2.2). These measures eschewed the need for a control group. The experiment was comprised of four phases: acclimatization, pre-training, training, and memory retention.

For all trial phases, fish were starved a minimum of 72 hours prior to experiments to ensure that they had completely evacuated their gut contents and increased the likelihood of responding to resource-related cues (Bell 1991). In addition, once satiated, lionfish become sessile and lack motivation to swim (Fishelson 1997).

Acclimatization

The acclimatization phase was used to familiarize lionfish with the maze. All maze arms were open during the acclimatization period and no extramaze cues were present. During each trial, fish were left undisturbed. Those unwilling to move about the maze could swim freely for up to three hours. No food was offered during this period. This phase was complete when all fish freely explored the maze.

Pre-training

Once fish freely explored the maze, several pre-training trials were conducted to establish an association between the food dispensers, the end of the maze arm, and the shrimp received. Fish were placed in the start box for two minutes before the door was raised after which fish could swim freely through the maze. The arm opposite the starting arm was blocked off via a guillotine door. The start box location was alternated between the start arm and the blocked off arm on every other trial (Figure 2.1). Fish were rewarded with shrimp at the end of both goal arms to spur an association between the arm ends and receiving food. Trials in which fish refused to swim were terminated after one

hour. This phase was deemed complete and training trials only commenced once fish made directed movements down one of the goal arms. The number of trials required varied minimally between fish (mean \pm SE= 9.81 \pm 0.31). All fish successfully completed the pre-training stage.

During pre-training trials, place and/or turn preferences were noted. For fish in the response group (Figure 2.2), those that displayed a turn preference (e.g. fish that consistently turned right irrespective of whether they started from S1 or S2) were assigned to the opposite sub-group (e.g. trained to turn left). For fish in the place group (Fig. 2), if fish displayed a place preference (that is, they preferred either G1 or G2), they were assigned to the sub-group whose goal location was in the opposite arm. Binomial tests were used to determine whether significant turn or place preferences existed for both place and response spatial navigation groups.

Training

Once all fish completed pre-training trials, training trials commenced. For lionfish in the place group, the goal position (G1 or G2) remained constant but the starting box alternated between start arm one (S1) and start arm two (S2) (Figure 2.2). Fish were not allowed to leave from same starting box more than twice in a row (but whether they were released from S1 or S2 was chosen at random). For lionfish in the response group, both the goal and start position both remained constant (Figure 2.2).

To initiate a trial, fish were netted from their home tanks, transferred to the start box of the maze and left for two minutes to allow them to acclimate. After two minutes, the guillotine door was raised, and the fish was allowed to swim freely until it correctly moved through the maze and ate the shrimp, or, if the fish was not motivated to swim,

until five minutes elapsed. An arm was recorded as chosen once the end of the caudal fin fully passed through the maze arm. Fish could visit other arms besides the goal arm, but a trial was only scored as correct if the goal arm was the first arm chosen. Shrimp were only introduced to the maze via the food holder when a lionfish reached the end of the goal arm. This lessened the degree to which unintentional olfactory, visual, and lateral line prey cues could motivate goal arm choice by lionfish. While I did not expect olfactory cues to play a significant role in navigation, given findings by past researchers (Saito and Watanabe 2005), the water within the maze was mixed between each trial as a precaution. At the end of a trial, a net was used to guide fish back to the start box where they underwent a two-minute inter-trial interval. Fish were not removed from the maze in between trials. To reduce the likelihood of a fish simply tracing their way from the start box back to the goal arm, fish were forced into other arms on intermittent trials before being guided back to the start box.

During the training phase, one session (comprised of five consecutive trials) was conducted once every several days. Fish were tested a maximum of twice in any seven-day period. The order in which fish were tested each day was randomized to eliminate order effects; trials for both fish in the place and response groups were interspersed on a given day. Food was only provided at the goal arm during training trials. During training trials, I measured the time lionfish took to emerge from both the start box and start arm ('shelter') and explore the maze. Willingness to emerge was interpreted as a measure of boldness (*sensu* Sneddon 2003). I then compared these measures of boldness with the learning performance of lionfish.

To reach criterion, fish had to make $\geq 70\%$ correct choices over three consecutive sessions. A choice was marked as correct if the lionfish exited the start box, navigated to the goal arm, and consumed the shrimp. The likelihood that this criterion could be obtained by chance is less than 0.05% (χ^2 test, $P < 0.05$). Fish were tested until they achieved criterion or, if they failed to do so, for a maximum of 15 sessions. Animals that did not reach criterion were excluded from further post-training trials. Four fish were removed from the experiment owing to an extended unwillingness to leave the start box or move through the maze. Their data were excluded from all analyses. Time constraints precluded longer training periods.

After lionfish reached criterion, several trials were conducted to ensure a learned location of the goal arm (denoted as overtraining trials). During these trials, a portion of lionfish in the response group that reached criterion were subject to probe trials. These were run to discern whether lionfish in the response group preferentially oriented using a place or response strategy. Probe trials were interspersed with regular overtraining trials (no more than one per session). These types of trials are frequently used to better discern the spatial navigation strategy employed (e.g. Rodriguez et al. 1994). In these trials, lionfish were displaced and required to navigate to the goal arm from a novel start position (S2) (Figure 2.3). No food was offered during probe trials.

Memory Retention

Once fish reached criterion, I tested the memory duration of lionfish across both spatial navigation groups. Fish were randomly selected to undergo training breaks from five up to a maximum of 42 days. Lionfish were assigned to one of three training break periods: short (up to seven days), medium (from eight to 14 days), or long (more than two

weeks). Of the fish that reached criterion, each fish was subject to at least one training break. At the end of each training break, fish were reintroduced to the maze and I evaluated their performance over a single session. The performance of each fish during the memory retention test was contrasted with the session that immediately preceded the training break. If warranted and if fish displayed remedial performances, additional sessions were conducted to determine the time required to regain the level of performance that preceded the training break. Time constraints precluded this segment of the experiment from implementing a full-factorial design.

Data analysis

Statistical analyses were conducted in R, version 3.5.2 (R Core Team 2018). Data are presented both for all fish that completed the experiment and the portion of fish that reached criterion. I measured and analyzed the following variables: average trial duration, latency to leave start box and start arm, and performance (i.e. percent correct choices). For measures of latency, an animal was considered to have left the start arm or start box once half its body moved outside of the arm and/or box. Animals that refused to swim were assigned a latency or trial duration time of 300 seconds (the total trial time). Correct choices were scored across five trials (one session) and denoted as a percentage. All choices were recorded but only marked as correct if the fish's first choice was the goal arm. Linear mixed models were used to determine the effects of session, group (place versus response), and whether fish reached criterion (yes/no) on performance (percent correct choices over time) (package *lme4*, Bates et al. 2014) with group and session as fixed effects and individual ID as a random effect, to account for repeated measures of the same individuals across trials. Normality of residuals was confirmed

visually by inspection of Q-Q plots. Due to non-normal residuals for both trial duration and measures of latency, these variables were rank transformed before being run in one-way repeated measures ANOVAs, with trial, group, and criterion status as fixed factors and individual ID as a random effect. Wilcoxon rank sum tests were used to compare latency values between lionfish that reached criterion and those that failed to surpass the criterion threshold.

To assess memory retention, I conducted comparisons between the performance (percent correct choices) of a fish after the training break with their performance directly before the training break using paired t-tests. A Shapiro-Wilk normality test confirmed that the differences of the pairs followed a normal distribution. If the performance of a lionfish during the memory retention test was significantly lower than their performance directly preceding the training break, I considered the training break to have exceeded their maximum memory duration. Fish that retained memory of the shrimp's location should have performed at par or better than before the training break.

2.3 Results

Although turn and/or place preferences were not significant across any of the fish (binomial tests, $P > 0.05$), those fish that displayed any biases (turn or directional) were placed in the opposite sub-group to avoid potential confounds. Eight of 16 fish that participated in the full course of the experiment reached criterion. An additional four fish were just below the criterion threshold (67- 69% correct choices over three consecutive sessions). There was no difference between fishes in the number of sessions required to reach criterion (Mean \pm SE= 10.63 \pm 1.39; Kruskal-Wallis test: $H_7 = 7$, $P > 0.10$). Performance (% correct choices) of all fish (irrespective of whether fish reached

criterion) was significantly better in the last session relative to the first session (Wilcoxon signed rank test, $P < 0.001$), demonstrating a learned location of the goal arm (Figure 2.4). In addition, lionfish made significantly more correct choices across sessions (estimate \pm SE = 3.82 ± 0.35 , $P < 0.0001$) but irrespective of spatial navigation group (4.25 ± 6.40 , $P > 0.10$) (Figure 2.4). While the performance of all fish improved across sessions, lionfish that reached criterion made more correct choices across sessions relative to those that did not, whose effect was marginally significant (14.05 ± 6.41 , $P = 0.05$).

During probe trials, two of the four lionfish tested consistently oriented using the response spatial navigation strategy in which they had been trained (Figure 2.2 and 2.3) more often than would be expected by chance (50%). However, the remaining two fish navigated to the previously rewarded goal arm (thus using a place strategy) equally as often as they used a response strategy (i.e. turning in the direction previously trained, Figure 2.2 and 2.3).

All eight fish that reached criterion were subject to training breaks. Lionfish were able to remember the location of the prey item for up to six weeks (the maximum tested period) in the absence of reinforcement. Irrespective of the length of training break, lionfish remembered and were able to successfully navigate to the goal arm. Performance (percent correct choices) during the memory retention test was better than performance directly preceding training breaks (Paired t-test: $t_{18} = 2.19$, $P < 0.05$), suggesting memory consolidation.

With respect to the time needed to seek and consume a single shrimp, trial duration decreased significantly across trials (ANOVA: $F_{1,967} = 275.37$, $P < 0.0001$) and varied significantly between lionfish that reached criterion and those that failed to do so

(ANOVA: $F_{1,12}=5.16$, $P<0.05$) (Figure 2.5). However, trial duration did not differ between place and response groups either across trials (ANOVA: $F_{1,12}=0.02$, $P>0.10$) or on average (Mann-Whitney: $W=120110$, $P>0.10$).

The latency with which lionfish left both the start box and start arm decreased significantly across repeated trials (start box: ANOVA: $F_{1,992}=35.05$, $P<0.0001$; start arm: ANOVA: $F_{1,967}=275.37$, $P<0.0001$). Lionfish that reached criterion exited the start arm much quicker than those that failed to do so (Wilcoxon Rank Sum: $W=141180$, $P<0.001$). Latencies were similar between fish that reached criterion and those that did not with respect to the start box (Wilcoxon Rank Sum: $W=128980$, $P>0.1$). Lionfish in the response group took longer relative to those in the place group to exit the start box, whose effect was marginally significant (ANOVA: $F_{1,13}=3.80$, $P=0.07$). There was no significant difference between spatial navigation groups with respect to the latency with which fish exited the start arm (ANOVA: $F_{1,13}=1.47$, $P>0.10$). Excluding the fish that failed to reach criterion, lionfish in the response group took longer to exit both the start box and start arm, whose effects were marginally significant (start box: ANOVA: $F_{1,6}=4.57$, $P=0.077$; start arm: ANOVA: $F_{1,6}=5.55$, $P=0.057$).

2.4 Discussion

To the best of my knowledge, this study is the first to investigate learning and memory in lionfish and, more broadly, in a marine invasive fish in an invaded habitat. My data suggest that lionfish preferentially use a response spatial navigation strategy to find and remember locations of profitable food patches and can retain such memories for periods up to six weeks (the maximum duration tested). These memory spans are

analogous to those demonstrated in other species (e.g. sharks, Schluessel and Bleckmann 2012). However, still other species have demonstrated longer-term memories in relation to feeding events (i.e. 3 months in cod; Nilsson et al. 2007), suggesting that memories in lionfish may be longer-lasting than reported here.

Fish acquire redundant spatial information as they move about their environment such that environmental conditions may dictate the type of spatial navigation strategy an animal employs (White and McDonald 2002). This tactic therefore allows an organism to choose a preferred spatial navigation strategy (place-based versus response-based) based on context, plasticity that may facilitate more reliable orientation relative to orienting using a single navigation strategy (Shettleworth 2005). Flexible foraging behaviour and behavioural plasticity may allow species to better exploit shared resources (Britton et al. 2009), both of which can be important determinants of success in invasive species (Sol and Maspons 2016). Probe trials interspersed during the experiment confirmed that lionfish in the response group were capable of orienting by both making specific turns and by using extramaze cues. Half of the fish in the response group tested in probe trials oriented using both single and combined strategies during different trials, navigating both to the location that had previously been rewarded as well as by using the directional response in which it had previously been trained (Figure 2.2 and 2.3). This phenomenon has also been demonstrated in other taxa, underscoring the context-dependence of spatial navigation strategies (Packard and McGaugh 1996; Gibson and Shettleworth 2005). For example, Rodriguez et al. (1994) demonstrated a similar preference for the combined use of spatial navigation strategies in goldfish (*Carassius auratus*) as opposed to the exclusive use of either a response or place strategy. My probe trials suggest that lionfish

can use spatial navigation strategies interchangeably under changing conditions, though given my limited sample size these findings require validation in subsequent studies.

The performance of lionfish improved significantly across trials for all fish, including those that failed to reach the criterion threshold (Figure 2.4). There was no difference between place and response groups with respect to the number of sessions required to reach criterion, on par with findings by Alves et al. (2007) who similarly showed a negligible difference in the number of sessions cuttlefish required to reach criterion. However, lionfish that reached criterion in my study were almost exclusively of the response group; many lionfish in the place group required more time than that allotted by the experiment to reach criterion. Both in general and with respect to the fish that reached criterion, lionfish in the place group exhibited much greater variation in performance across sessions than fish in the response group (Figure 2.4 and 2.6). These findings contrast previous research that failed to show a demonstrable difference in learning rate between allocentric and ego-allocentric spatial navigation groups (e.g. in goldfish, Rodriguez et al. 1994). However, goldfish in the Rodriguez et al. (1994) study also exhibited greater variation in performance when trained in the place as opposed to the response navigation strategy, in accordance with my findings.

I operationally defined boldness as the latency with which fish exited both the start box and start arm. The latency with which fish emerge from a shelter is frequently used to assess boldness (e.g. Sneddon 2003). By this definition fish that reached criterion were bolder than those that did not, exiting the start arm more quickly. My findings conform to results of previous studies (e.g. Dugatkin and Alfieri 2003) that have similarly found a positive correlation between boldness and learning. Fish that reached criterion in

this study were also significantly faster to complete trials than those that failed to reach criterion (Figure 2.5) findings that are consistent with Boogert et al. (2006), who observed a positive correlation between the speed with which starlings (*Sturnus vulgaris*) solved a novel foraging task and their learning rate. Speed has commonly been used as a proxy for learning in studies comparing learning in invasive species and the natives with which they co-occur, though with contrasting results (Roudez et al. 2008; Bezzina et al. 2014).

Remembering food locations is important to fish, thus such memories should be long-lasting (Hughes et al. 1992; Valsecchi et al. 2000; Odling-Smee et al. 2008). Superior memory retention may predispose species to better food quality and greater quantities of food (Odling-Smee and Braithwaite 2003). Moreover, competition for a shared resource can influence the speed with which the location of a food reward is learned (Brown and Braithwaite 2004). Relative to the depauperate Caribbean Sea and western Atlantic Ocean where the fishes were introduced, the Indo-Pacific region from which lionfish originated has greater levels of biodiversity (Miller et al. 2018). Millennia of co-existing in these marine systems with myriad competitors may have precipitated the evolution of superior competitive abilities in lionfish, equipping these fishes with an exaptation that led to superior learning and memory. Indeed, the strong competitiveness of many invasive species has previously been ascribed to residing in regions of greater biodiversity in their native relative to their invaded ranges (Briggs 2010). Moreover, environmental complexity has been known to improve the cognitive capacity and spatial learning ability of many organisms, including fishes (Kihlslinger et al. 2006; Carbia and Brown 2019).

Competition between lionfish and native congeners for resources may have important ramifications for ecosystem-wide dynamics (Yamada et al. 2005). If lionfish are better able to remember and thus exploit high profitability prey patches relative to co-occurring native predators, they may outcompete them via exploitative competition (Côté et al. 2013). The ability to remember highly profitable prey patches is particularly important when resources are scarce, which can facilitate, or otherwise thwart, the viability of a species (Valsecchi et al. 2000). In instances where co-occurring species are limited by the same resource, the species with the lowest R^* , that is, the species able to thrive at under low resource conditions, should displace its competitors (Tilman et al. 1982). The spread and high abundance of lionfish in the western Atlantic Ocean is testament to their strong competitiveness relative to ecologically-analogous native fishes (e.g. Albins and Hixon 2008). However, whether superior learning and memory can serve as a means by which lionfish outcompete these natives is contingent on knowledge of learning speed and memory retention in native predators, information for which is currently lacking. Invasion biologists continue to seek to understand mechanisms and intensity of invasion impact. Behavioural plasticity may be one such mechanism that facilitates establishment success and high impact by invaders (Hazlett 2000; Kolar and Lodge 2001). This was the first study to assess learning and memory retention with lionfish in the context of foraging, and one of few such studies to test learning and memory retention in an invasive species. Future experiments should be conducted to refine learning abilities and memory capacity in lionfish and other invasive species relative to ecologically analogous native species.

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Figure 2.1. Maze set-up. S1 and S2 denote the two alternative arms from which lionfish started trials, while G1 and G2 denote goal arms (where shrimp were located). Extramaze cues were positioned directly outside the maze. PVC pipe food holders are shown in all four arm-ends. Guillotine doors denote both the start box and blocked-off arm.

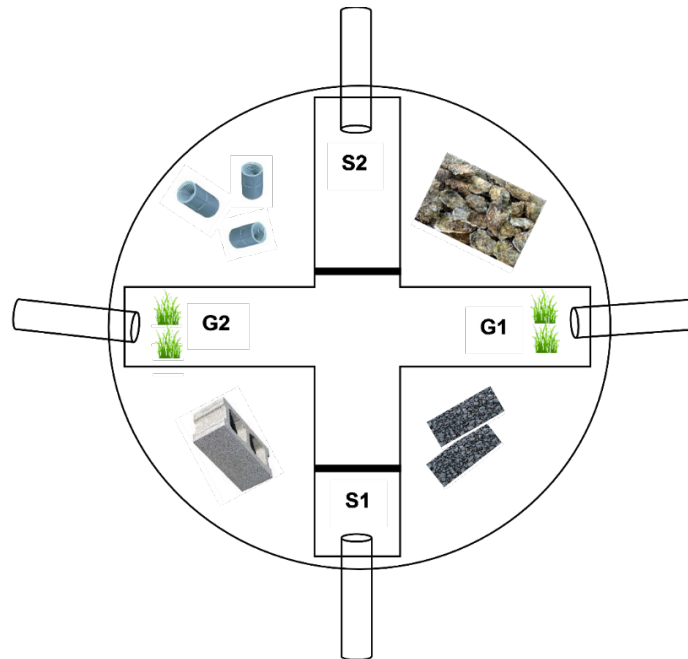


Figure 2.2. A-B Place strategy. Fish alternated at random from S1 and S2. Half of the fish in the place group were trained to locate the shrimp in G2 (A), while the other half of the fish were trained to locate the shrimp in G1 (B). **C-D Response strategy.** All fish consistently started from S1. Half of the fish in the group were trained to locate the shrimp in G2 (turn left) (C); the other half were trained to locate the shrimp in G1 (turn right) (D).

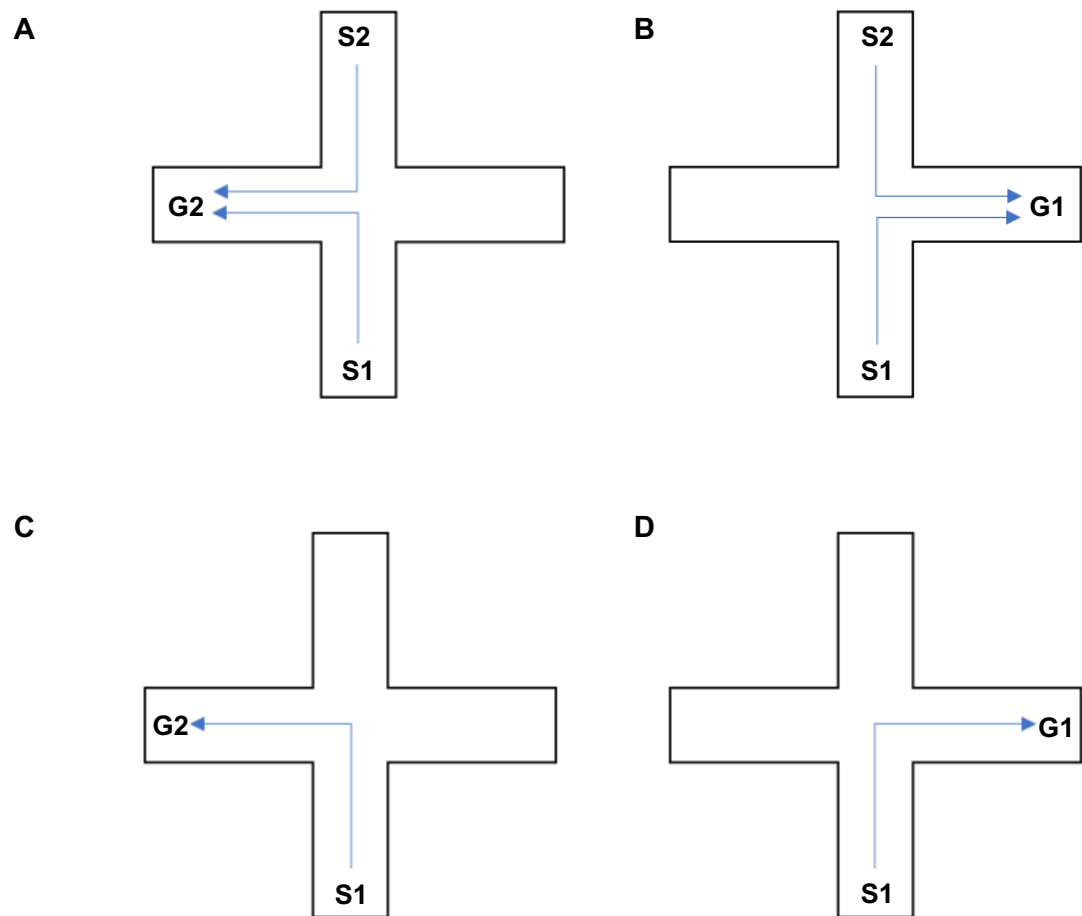


Figure 2.3. Probe trials during which lionfish were required to move about the maze after being displaced (i.e. required to navigate from a novel starting arm). Lionfish in the response group started from S2. A trial was marked as correct if lionfish behaved as they were trained. Lionfish that were previously trained to turn left (Fig. 2C) were required to make directed movements to G1 for a trial to be marked as correct (solid line). Those previously trained to turn right (Fig. 2D) were required to go to G2 for a trial to be marked as correct (dashed line).

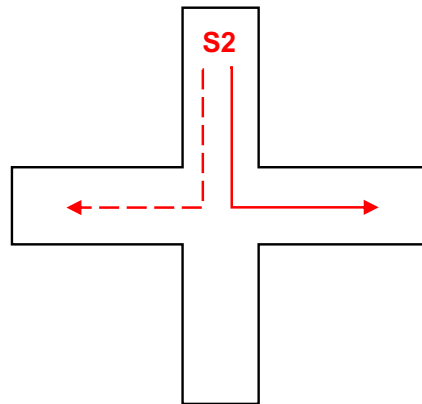


Figure 2.4. Mean (\pm SE) correct choices by lionfish as a function of number of repeated sessions. Both those fish that reached criterion and those that failed to do so are plotted. Performance is separated by spatial navigation group (place versus response).

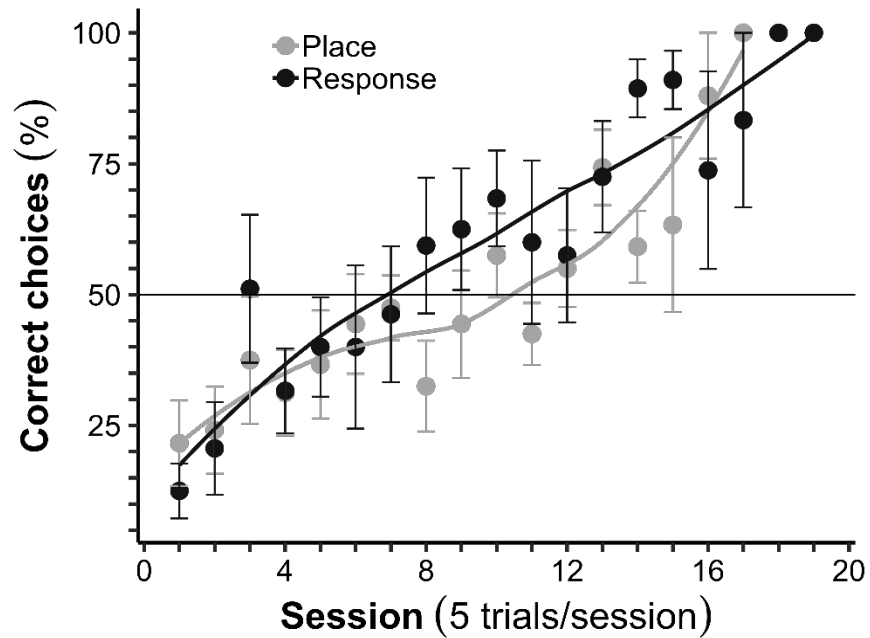


Figure 2.5. Mean (\pm SE) trial duration as a function of repeated trials, separated by whether lionfish reached criterion or failed to do so. Trial duration refers to the time each fish took to navigate from the start arm to the shrimp in the goal arm. Spatial navigation groups (place versus response) were collapsed given similar trial durations.

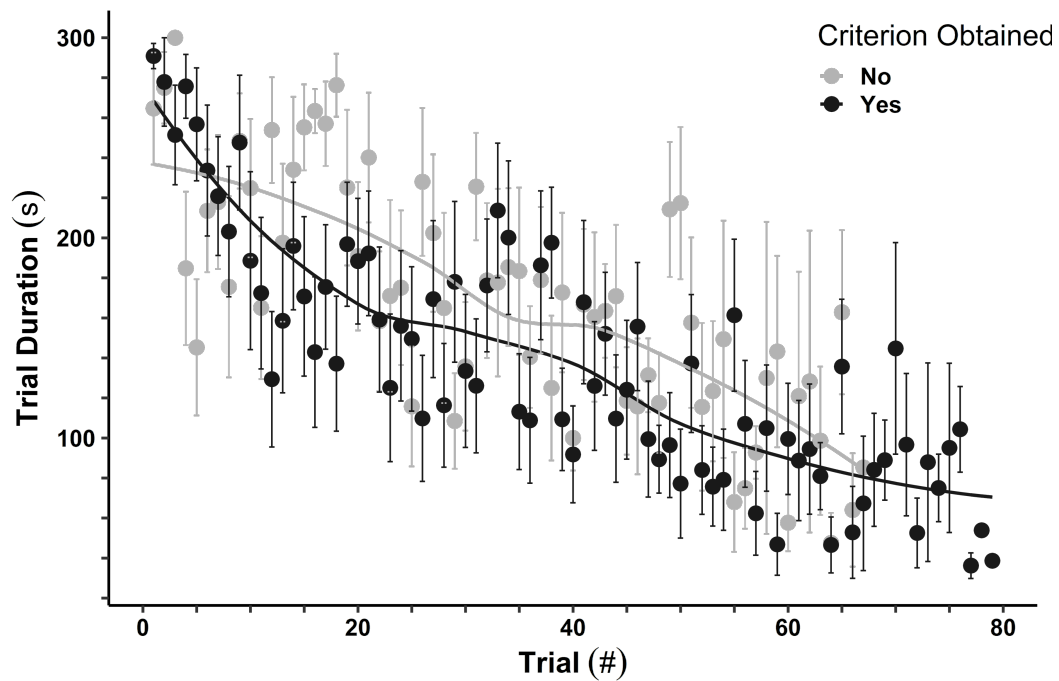
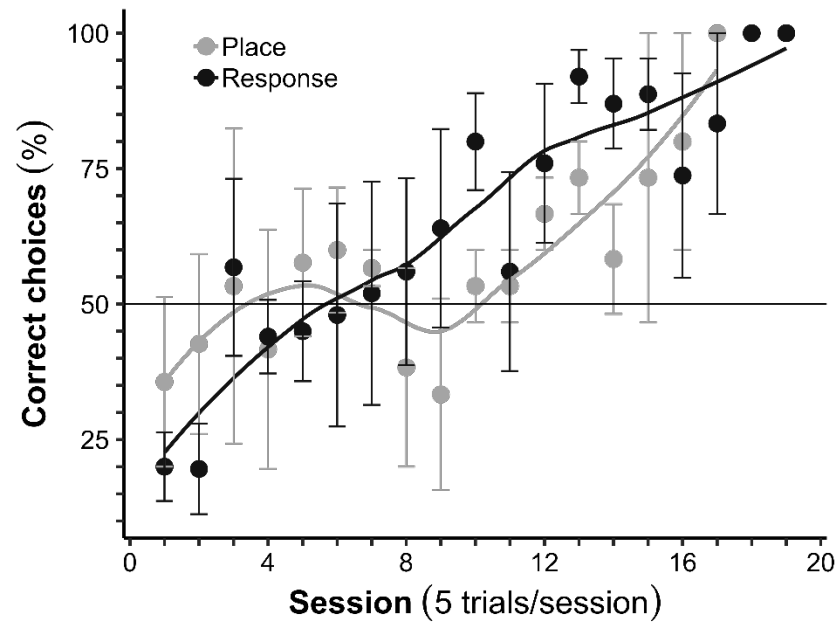


Figure 2.6. Mean (\pm SE) correct choices by lionfish as a function of repeated sessions, separated by spatial navigation group (ego-allocentric: response strategy, allocentric: place strategy). Only those fish that reached criterion are plotted.



CHAPTER 3: FORAGING ECOLOGY OF INVASIVE LIONFISH RELATIVE TO NATIVE GROUPER

3.1 Introduction

Predator-prey interactions underpin community and population dynamics, thus understanding their variability in both space and time is important, particularly in marine environments (Connell 1961; Hixon 1991). The need to understand predator-prey interactions has spawned numerous models of optimal foraging (e.g. Charnov 1976; Stephen and Krebs 1986). One such metric to assess predator impact is the functional response (FR) (Holling 1959), which describes the relationship between prey availability and prey consumption and can, at a mechanistic level, parse differences in predation rates between competing species. Thus, FRs can serve as a bridge between behavioural and community-level processes (Okuyama 2012) and provide insights into predator impact (Moustahfid et al. 2010).

Understanding the role of habitat in modifying predator-prey relationships is of particular value, as habitat complexity has been shown to promote co-existence between predators and their prey (Bartholomew et al. 2000). The density and heterogeneous distribution of prey may affect rates of encounter and predation (Lima and Dill 1990; Sutherland 1996; Boyd 1999). In turn, these variables may affect predator efficiency, particularly at low prey densities (Gause 1934; Schneider 1984; Lipcius and Hines 1986). Per Optimal Foraging Theory, predators ought to modify their time budget relative to changes in resource availability (Boyd 1999) and distribute themselves in such a manner that they occupy the most profitable prey patches (i.e. those with the highest prey concentrations) (Hassell and May 1974). This is in opposition to interests of the prey,

which should choose patches that afford protection from predators. These conflicting interests underlie the game-like nature of the predator-prey space race, the outcome of which has implications for patterns of spatial overlap between predator and prey populations (Sih 2005).

Both habitat type and complexity have been implicated in modifying the shape of classic prey-dependent functional response curves (Vucic-Pestic et al. 2010). The same predator can exhibit a different FR in different habitats (Lipcius and Hines 1986) or in habitats of varying complexity (Nachman 2006; Alexander et al. 2012). For example, the FR of soft-shelled clams (*Mya arenaria*) changed from type III to type II when substrate was altered from sand to mud, respectively (Lipcius and Hines 1986). Changes to the FR relationship may be induced by several mechanisms, for example, varying attack and maximum feeding rates or handling times (Alexander et al. 2012). Together, these factors underline the importance of understanding the degree to which habitat type informs predation (Sandin and Pacala 2005).

Knowledge of predator-prey dynamics is important for invasive species that cause significant changes in ecological patterns or processes by preying on or competing with native species, with the extent of their impacts determined by the interplay between species' characteristics and the biotic environment (Thomsen et al. 2011; Ricciardi et al. 2013). Quantifying and predicting these impacts, however, remains an elusive task in invasion biology (Ricciardi et al. 2013; Blackburn et al. 2014). However, quantifying impacts is necessary to best manage both invasive species and co-occurring native species (Kumschick et al. 2015).

FR analyses have recently been used to quantify and predict impacts of invaders, both alone and relative to native analogues (e.g. Dick et al. 2014; Crookes et al. 2018). Analyses of FRs may be particularly useful for the study of marine invasive species impacts given that experimental assessments are relatively new in marine systems (Grosholz and Ruiz 1996) and invader impacts are comparatively less well-studied relative to both freshwater and terrestrial systems (Grosholz 2002). Taking FR metrics one step further, combining FRs with the field abundance of invasive species relative to native species can better contextualize their impacts at broader spatial scales. This notion underlies Dick et al.'s (2017) Relative Impact Potential (RIP) metric.

Lionfish are a detrimental marine invasive species whose rapid spread and high levels of predation have drawn concern about the long-term viability of invaded coral reef ecosystems (Albins and Hixon 2008). A hybrid species native to the Indo-Pacific region (Wilcox et al. 2017), lionfish (*Pterois volitans*) are reported to consume two to three times more prey than comparative native species (Albins 2013; Côté et al. 2013, unpublished reference data). Several experiments have previously examined the predation efficiency of lionfish relative to ecologically-analogous native predators, including various species of snapper and grouper (Pusack 2013; Raymond 2015; Secord 2015; Curtis et al. 2017; Marshak et al. 2018). Patch reef studies probing the impact of lionfish relative to native predators have revealed consistently stronger impacts by the former (e.g. Albins and Hixon 2008). Since their initial colonization of the Gulf of Mexico in the late 2000s, lionfish are now prevalent throughout the region and particularly along the Western Florida Shelf (Aguilar-Perera and Tuz-Sulub 2010; Fogg et al. 2013; Johnston et al. 2016). Given the strong impacts exerted in other parts of their invaded range, there

exists a need to assess the ecological consequences of lionfish in the eastern Gulf of Mexico, both alone and relative to native analogues.

Grouper are ideal native predators against which to establish a baseline measure of impact. Many species of grouper compete with lionfish for food and habitat and/or shelter (Raymond et al. 2015; Arredondo-Chávez et al. 2016) given similar piscivorous diets (Green et al. 2012) and ontogenetic shifts in both habitat and diet (Moe 1969; Bullock and Smith 1991; Curtis et al. 2017). The latter has been suggested as a means by which competition may transpire between lionfish and grouper (Curtis et al. 2017). I utilized both red and graysby grouper in this study, which are ecologically similar to and co-occur with lionfish.

Red grouper is a recreationally and commercially important reef fish in the eastern Gulf of Mexico (Bullock and Smith 1991; Lombardi-Carlson et al. 2008) that reside on shallow low-relief limestone reefs off the coast of Southwest Florida (Moe 1969; Bullock and Smith 1991). Lionfish have been documented in red grouper holes in this region (Reed and Hanisak 2016) and a recent study has forecasted that proliferating lionfish abundance will be particularly detrimental to red grouper populations in this region (Johnston et al. 2016). Graysby are a small-bodied, generalist mesopredator that grow to a similar maximum size as lionfish (Grace et al. 1994). They are prevalent on rocky reef ledges in the eastern Gulf of Mexico, though typically in deeper waters than those in which juvenile red grouper occupy, purportedly due to competition between the species (Bullock and Smith 1991). Graysby co-occur on reefs and experience a diet shift in areas in which they co-occur with lionfish (Sellers et al. 2015; Curtis et al. 2017). Under low resource conditions, competition between graysby and lionfish is thus likely.

Understanding the way in which lionfish forage amongst heterogeneous habitat patches relative to both aforementioned native species is essential to inform potential competitive dynamics and better understand interspecific differences in behaviour and foraging ecology. As no laboratory studies of comparative functional response have yet been conducted on lionfish, I sought to determine both the functional and aggregative responses of two native predators relative to an invasive one with which they co-occur. In addition, I investigated the degree to which heterogeneous structure influenced foraging behaviour and space use of predators relative to their prey. I hypothesized that lionfish would: 1) aggregate in high profitability prey patches to a greater degree than either native species; 2) have a greater consumption success rate relative to both native species; 3) exhibit greater consumption rates and a higher functional response than both native species, and take longer to satiate.

3.2 Methods

All fieldwork was conducted at Florida Gulf Coast University's Vester Marine and Environmental Science Research Field Station in Bonita Springs, Florida. All fish were handled and collected with permits from the National Oceanic and Atmospheric Administration (NOAA) and Florida Fish and Wildlife Conservation Commission (FWC) and were handled under the approval of University of Windsor's Animal Care Committee (AUPP #1701) and in accordance with the Canadian Council on Animal Care.

Lionfish (mean \pm SE = 227.98 mm \pm 10.64) were collected from a sinkhole (26-28.900°, 82-84.190°) off the coast of Bonita Springs, Florida using shallow-bodied hand nets by a team of three divers using SCUBA at an average depth of 30 m. Red grouper (mean \pm SE = 251.32 mm \pm 5.53) were caught mainly via hook and line at 12-18m depth

on artificial reef and hard bottom off the same coast. Dynasty Marine Associates provided us with graysby grouper (mean \pm SE = 202.52 mm \pm 3.22) and several lionfish and red grouper to supplement size classes due to a dearth of individuals available in the Gulf owing to a Ride Tide event in Southwest Florida that persisted throughout the sampling period. There were no significant size differences between lionfish and either red or graysby grouper (Kruskal-Wallis $P > 0.05$).

Given the great depths at which fish were captured, a subset experienced barotrauma and required venting. Fish (grouper and/or lionfish) were vented directly posterior to the base of the pectoral fin, by inserting a hypodermic needle (16g) into the body wall at a 45° angle and plunging it slightly into the flesh of the fish. The needle was held in place until sufficient decompression was achieved. Slight pressure was applied to the area while the needle was inserted to aid the escape of trapped gases. Personnel wore HexArmor SharpsMaster II® 9014 puncture-proof gloves when handling lionfish. Following capture, all fish were held in either or both the boat's live well and in aerated coolers filled with seawater. All fishes were proactively treated with StressGuard™, a slime coat protection product. New water was added to the coolers periodically en route to the field station to maintain sufficient aeration.

New fish were quarantined for a least one week following capture, and isolated from conspecifics in several 757 L fiberglass tanks (Red Ewald). All fish underwent a freshwater bath prior to being transferred to their quarantine tanks. ParaGuard™ and StressGuard™ were added to quarantine tanks daily to eliminate bacterial, parasital, or fungal infections and to restore the fishes' slimecoats, respectively. Large PVC pipe and several cinder blocks were added to increase environmental complexity. Species were

held separately both during quarantine and in regular housing tanks, though conspecifics were quarantined and housed together to reduce isolation-induced stress.

Fish were housed in several 1135 L recirculating tank systems equipped with chemical, physical, and biological filtration. Tanks contained several cinder blocks, PVC frames, large plastic pots, and rubber gaskets to provide hiding spaces and increase environmental complexity (Figure 3.1). Air stones kept dissolved oxygen levels at or near saturation. Water was maintained at 34 ppt, pH 8.2 and 24°C. During the initial nitrogen cycling process, several water conditioners (Safe™, Stability®, Prime®) and frequent water changes kept ammonia, nitrate, and nitrite at negligible levels. Water quality parameters were checked daily during the cycling process with a YSI™ Professional Plus meter and LaMotte Saltwater Fish Farm Test Kit (Part #: LMAQ4) and weekly once a sufficient population of nitrifying bacteria was established. Due to the semi-enclosed nature of the aquaculture cage in which fish were kept, all communal tanks were exposed to fluctuating temperature conditions and a natural photoperiod. Aqua Logic® titanium in-line water heaters maintained a stable water temperature (24°C) during winter months. Water changes were completed weekly using water that was pumped in from the surrounding bay, using artificial seawater mixed from Instant Ocean® sea salt, or using water pumped from an underground saltwater well. Prior to experiments, fish were acclimated for a minimum two weeks and fed *ab libitum* assorted baitfish [silversides (*Atheriniformes spp.*), pilchards (*Harengula jaguana*)], saltwater-acclimated mosquitofish (*Gambusia affinis*), pink shrimp (*Penaeus duorarum*, both live and frozen), and frozen sardines (*Sardinella aurita*). While both grouper species consumed all bait indiscriminately, lionfish showed a strong preference for live bait.

Lionfish were identified with coloured tags (Floy® FTSL-73 Streamer tags) each containing a unique three-digit number. As tag shedding was an issue with both graysby and red grouper, two coloured beads in unique colour combinations (one before and one after the 5th dorsal spine) identified fish from their conspecifics and were sutured just below the dorsal spine using 0.45mm nylon thread. For lionfish, tagging followed the procedure outlined in Chapter 2. Grouper were dosed at 125 mg/L, as per Neiffer and Stamper (2009) and Koch (2011). Sufficient anaesthetization was reached after one to three minutes of immersion, whereby fish became unresponsive to stimulation and display slowed gill movement. Once sedated, fish were transferred to a shallow-bodied container housing a seawater bath with a maintenance dose of anesthetic. The 16g hypodermic needle was inserted at the base of the dorsal spine, taking care to insert between pterygiophore bones, through which nylon thread and attached coloured beads was weaved. Several fishing knots were then secured with a small drop of Loctite® super glue. Once tagging was complete and before re-introduction to their home tanks, fish were placed in a bath of aerated saltwater until they regained normal swimming performance.

Live pink shrimp (*P. duorarum*) were used as the prey species and were procured from a local vendor shortly before trials and maintained on commercial fish food. They are abundant throughout the Gulf of Mexico, representing a high-quality prey source for fishes and one readily consumed by the size classes of all species included in this study (Randall 1965; Moe 1969; Harmelin-Vivien and Bouchon 1976; Nagelkerken 1979; Bielsa et al. 1983; Bullock and Smith 1991; Jasper et al. 2018). Shrimp (\bar{x} = 4cm, range 2-7 cm) size did not differ between species or treatments (Kruskal-Wallis $P > 0.05$). Prey

offered represented a size range readily consumed by all species, both in housing tanks and in past experiments (Morris and Akins 2009; Green and Côté 2014; Secord 2015) and in line with standard fish predator-prey size ratios, that is, the size of prey relative to TL of the fish (Scharf et al. 2000).

Experimental trials

Trials were conducted in a round tank of ~2500 L (2.13 m diameter x 0.91 m height, water filled to a depth of 0.71 m). The arena was divided into three equally sized patches with distinct habitat, each habitat chosen to represent differing degrees of refuge for the prey (Figure 3.2). Patch #1 (hereafter referred to as the medium refuge patch) was comprised of four clusters of open-ended PVC pipe stacked pyramidally (two of which measured 38 cm x 12.5 cm high x 12.5 cm wide, the remaining two 47 cm long x 19 cm high x 20 cm wide, arranged to mimic a typical artificial reef structure in the Gulf of Mexico formed of concrete culverts (Figure 3.3). It offered an intermediate level of refuge for prey. Patch #2 (hereafter referred to as the high refuge patch) was comprised of four artificial plants: 30.5 cm x 16.5 cm x 45.5 cm) and offered the highest degree of refuge for prey. Patch #3 (hereafter referred to as the low refuge patch) was comprised of three concrete blocks (23 cm x 12.5 cm x 40.5 cm: 19 cm x 19 cm x 40.5 cm: 22.8 cm x 22.8 cm x 22.8 cm) and several flat concrete pavers and offered the least refuge for prey (Figure 3.2). Predators and prey were able to transit freely between and among patches, deviating from what is typical in experiments of this nature (Hammond et al. 2007).

Trials were conducted at 24-26°C, 32 ppt salinity and pH 8.2, with dissolved oxygen at or near saturation. Trials were recorded using four LOREX® (4K Ultra HD LNR6100 series) security cameras; one security camera was mounted to the ceiling above

each individual patch, with a fourth mounted in the middle of the tank to provide an overview of the tank system. Trials were viewed remotely on a desktop computer. This precluded a potential observer influence on fish behaviour (i.e. Hawthorne Effect; James and Vo 2010). A single LED light (Husky: model K40066) mounted on a metal rod in the center of the tank provided consistent illumination to the tank.

Six prey densities (4, 7, 10, 13, 16, 19) were utilized. No individual fish from any species consumed more than 12 prey items in their communal tanks or in pilot trials. Four replicates of each were conducted over three hours, with the exception of red grouper, for which only three replicates were available at a prey density of 10 owing to availability of size classes. Fish were starved for a minimum 72 hours before experimental trials to ensure willingness to both feed and swim. Satiation dictates a predator's willingness to forage (Jeschke 2007), including lionfish that refrained from feeding after meals for at least one day (Fishelson 1997; personal observation). In instances where fish were recalcitrant, additional feeding trials were conducted. All replicates are presented here. In all cases, predators were randomly assigned to prey density treatments using the RAND function in Excel. Lionfish were only tested in a single trial. Given limited availability of red and graysby grouper, individuals were re-used but never at the same density or tested in contiguous trials to avoid pseudoreplication (*sensu* Hurlbert 1984). In cases where fish were assigned the same density treatment by chance, additional randomization tests were conducted until fish were assigned a density to which they had not previously been assigned. Species were alternated between trials. Prey items were replaced as they were consumed. Prey-replacement is an important component of FR trials and of any feeding trials and allows researchers to assess stochastic predation (Juliano 2001). Experiments in

which prey are not replaced produce significantly different parameter estimates and underestimates attack rates (Alexander et al. 2012). There was no difference in TL between density treatments for any species (Kruskal-Wallis: $P > 0.10$).

Shrimp were introduced to the center of the tank at least 30 minutes before a given trial and allowed to disperse freely to any given patch. Shrimp size was standardized across predator treatments and prey densities. Fish were introduced to the center of the trial tank via a plastic bucket and were able to freely choose a preferred patch, with a null expectation of 33% that they would select any given patch. I assessed the degree to which predators foraged optimally in patches containing the highest prey densities.

Data analysis

Holling's Type II, III and IV prey-dependent FR curves were fitted to prey consumption data for all three species via maximum likelihood estimation (package *bbmle*; Bolker and R Development Core Team 2016).

Holling's (1959) Type II FR was calculated using the disc equation:

$$f = \frac{aN}{d+N} \quad (1)$$

where f is the per capita feeding rate, a is maximum kill rate, N is prey abundance, and d is the half-saturation constant. Under this curve, increasing consumption rates relative to increasing prey abundance follow a hyperbolic curve (Bolker 2008).

Holling's Type III density-dependent FR was calculated as:

$$f = \frac{aN^2}{d^2 + N^2} \quad (2)$$

producing a sigmoid curve characterized by density-dependent consumption rates.

Holling's Type IV FR describes decreasing predation rates at high prey densities and was calculated as:

$$f = \frac{aN^2}{d + cN + N^2} \quad (3)$$

whereby $c > 0$ denotes positive attack rate with increasing prey density. If $c < 0$, attack rate is maximized at an intermediate prey density at the curve decreases producing a dome or roller-coaster shaped curve (Jeschke and Tollrian 2005; Bolker 2008).

I used AIC to find the most parsimonious model. Handling times were inferred from parameters of the model. The RIP metric (Dick et al. 2017) was subsequently derived to estimate the strength of impact exerted by lionfish in the eastern Gulf of Mexico relative to native species. Abundance data was procured for both lionfish and red grouper for the eastern Gulf of Mexico from trawl survey data collected by the Gulf States Marine Fisheries Commission (GSMFC) Southeast Area Monitoring and Assessment Program (SEAMAP). Graysby was found in low abundance under the SEAMAP survey and thus did not warrant use of the RIP metric. Abundance was quantified for 2017 to avoid anomalous estimates due to the Red Tide event that persisted throughout 2018.

The RIP metric combines the per capita FR of both invasive and native species with proxies of their numerical response (NR) (e.g. field abundance) to produce an overall score and indication of a species Total Response (TR):

$$RIP = \left(\frac{FR_{invader}}{FR_{native}} \right) \times \left(\frac{AB_{invader}}{AB_{native}} \right) \quad (4)$$

where FR is the estimated maximum feeding rate (that is, $1/h$, at which point the FR curve asymptotes) and AB is field abundance of the species. A RIP score > 1 indicates an invasive species with greater impact than the native species (Dick et al. 2017).

The number of successful attacks, unsuccessful attacks, stalking bouts, and failed pursuits were scored for each fish throughout each trial. As all predators are suction feeders (Bullock and Smith 1991; Morris and Akins 2009), prey were consumed whole. Success rate was calculated as the number of successful attacks divided by the number of total attacks made. Consumption rates were compared using raw values (successful attacks) and per unit biomass of prey for predators. Mass-specific predation rate is a common means by which to compare species' relative consumption rates (Crookes et al. 2018). To calculate mass-specific feeding rates, I subsampled a portion of trials across species, for which the lengths of shrimp consumed by predators were measured using the freeware *ImageJ* (Schneider et al. 2012). Shrimp weights were derived from published species-specific length-weight estimates (Diaz et al. 2001). I divided the total mass of prey consumed (g) by the weight of an individual predator (g) to derive consumption rates per unit biomass.

Movement and habitat use of predators were determined for each of the three habitat patches using BORIS, software version 7.4 (Behavioral Observation Research Interactive Software, Friard and Gamba 2016). To compare levels of activity across species, I scored random 30-minute trial segments for all three species across all prey densities. I quantified the proportion of time that species spent in each patch as well as the amount of time spent active in each patch. Pursuit distance (Novales-Flamarique and Hawryshun 1994) was taken as distance between a predator when it reacted to a given prey versus position of the prey itself, taken as the frame directly before the predator initiated movement. The distance between tip of the predator's head to the head of the prey was measured using *ImageJ* (Schneider et al. 2012).

All analyses were conducted in R, version 3.5.3 (R Core Team 2018). Data exploration was carried out following the protocols described in Zuur et al. (2010). Raw consumption data were analysed using generalized linear mixed models (R package *glmmTMB*, Brooks et al. 2017) assuming a negative binomial distribution. To select the appropriate error distribution, I fit negative binomial and Poisson probability distributions to individual response variables (*fitdistrplus* package in R; Delignette-Muller and Dutang 2015). Goodness-of-fit was compared graphically and by comparing AIC values. Species, prey density, and habitat patch were included as fixed effects. Total length (TL) was scaled and included as a covariate initially. However, TL impact was negligible and thus was not included in any final model. Animal ID was included as a random effect. Models were compared via Akaike's Information Criterion (AIC) values. The best model blended a low AIC, and thus parsimony and optimal fit, with biological relevance (Bolker 2008, Chapter 6.6.2 and references therein).

Results of the generalized linear mixed models are denoted as estimates \pm SE. Tukey-adjusted multiple comparisons were conducted *post hoc* (package *emmeans*, Lenth 2019). Model assumptions were verified by plotting residuals versus fitted values, versus each covariate in the model and, where possible, versus each covariate not in the model. In addition, I assessed the residuals for temporal and spatial dependency (package *DHARMA*, Floridan 2019).

Proportion data were analyzed with beta regressions assuming a binomial family with a log-log link function (package *betareg*, Cribari-Neto and Zeileis 2010). I verified goodness of fit visually by inspecting diagnostic plots of standardized weighted residuals, predictions, and leverages. Finally, I assessed joint spatial distributions by examining predator and prey patch use across replicates (*sensu* Sih 2005; Hammond et al. 2007).

3.3 Results

From the GLMM, red grouper had the highest consumption rate of all three predators, across all prey densities, and had significantly higher consumption rates relative to lionfish (-0.55 ± 0.23 , $P < 0.05$). Consumption rates were similar between lionfish and graysby ($P > 0.10$) and marginally significant between red grouper and graysby (-0.66 ± 0.30 , $P = 0.07$). When comparing consumption rate alone as a function of species, there were significant differences between red grouper and both lionfish and graysby grouper (Kruskal-Wallis₂: $\chi^2 = 9.53$, $P < 0.01$) (Figure 3.4). Consumption rates in lionfish were best fit by a Type IV FR (Figure 3.5) differentiated by its dome-shaped (Crawley 1992); or roller coaster curve (Jeschke and Tollrian 2005). Type II FRs are characterized by constant search rates. The addition of habitat structure provides refuge

for prey, the addition of which may increase search rate and decrease success rate at high prey densities, inducing the characteristic shape of a Type IV FR curve (Vucic-Pestic et al. 2010; Jeschke and Tollrian 2005).

A Type II FR provided the best fit for consumption rates in both graysby and red grouper (Figure 3.5). Graysby grouper had both higher attack rates and greater handling times relative to red grouper (Table 3.1), whose consumption rates remained relatively constant across increasing prey density. These consumption rates mimicked those reported by Stallings (2010) for gag grouper (*Mycteroperca microlepis*). The Type II FR displayed by both grouper species is commonly observed in reef fishes (Murdoch and Oaten 1975; Murdoch and Bence 1987), though both Type II and Type IV FR curves have been previously reported for graysby (Harborne 2012). Consumption rates in lionfish were intermediate to those of red and graysby grouper, which demonstrated higher and lower consumption rates, respectively (Figure 3.4, 3.5). In addition to the potential implications for competing heterospecifics, red grouper's ability to exploit prey populations at low prey densities has the potential to exert destabilizing effects on prey populations (Oaten and Murdoch 1975; Neutel and Thorne 2015).

The RIP metric for lionfish relative to red grouper was 3.83. The TR of lionfish was bolstered by its high abundance along the eastern Gulf of Mexico during the period surveyed and compensated for modest per capita impacts. The magnitude of the response exceeding the threshold value of 1 is indicative of strong ecological impacts of lionfish relative to red grouper in this region (Dick et al. 2017).

Consumption rates were mediated by both the degree of habitat refuge afforded to prey and prey density. At low prey densities, consumption rates were highest in the high

refuge patch (relative to the medium refuge patch: -1.06 ± 0.44 , $P < 0.05$; low refuge patch: 0.86 ± 0.41 , $P = 0.09$). Consumption rates increased with increasing prey density for both the low and medium refuge habitats, but decreased significantly in the high refuge patch. Conversely, at high prey densities, consumption rates were greatest in the low refuge patch and marginally higher relative to the high refuge patch (-0.83 ± 0.36 , $P = 0.06$). Feeding rates (gram of prey consumed per gram predator) did not differ between species (Kruskal-Wallis₂: $\chi^2 = 0.97$, $p > 0.10$) (Figure 3.6) nor did attack distance, either between species ($p > 0.10$) or across prey densities (-0.02 ± 0.17 , $p > 0.10$).

There were significant interactive effects between prey density and both species and habitat on success rate. Lionfish maintained high success rates across prey densities and had the highest success rate at high prey densities. Their robust success rates particularly at lower densities suggests efficiency at low resources levels, a characteristic of some invasive species (i.e. invasive plants; Funk 2013). The success rate of red grouper improved with increasing prey density. At low prey densities, red grouper had marginally lower success rates than both lionfish and graysby grouper (lionfish: 1.37 ± 0.60 , $P = 0.06$; graysby: 1.43 ± 0.64 , $P = 0.07$). Success rates between lionfish and graysby were similarly high at low prey densities, though consumption rates of both species at low densities were lower relative to red grouper. Both lionfish and red grouper had significantly higher success rates relative to graysby grouper at high prey densities (lionfish: -2.39 ± 0.61 , $P < 0.001$; red grouper: -1.92 ± 0.55 , $P < 0.01$). At low prey densities, success rates were highest in the high refuge patch and significantly higher relative to the medium refuge patch, in which success rates were lowest (-1.61 ± 0.60 , $P < 0.05$). Predators experienced similar success rates across habitat patches at high prey

densities. Success rates in the low refuge patch were intermediate to either alternative patch across increasing prey densities.

There was a significant interactive effect between species and prey density on attack rate. Red grouper had the highest attack rate at low prey densities and significantly higher than both other species (graysby: -1.03 ± 0.38 , $P < 0.05$; lionfish: -0.87 ± 0.37 , $P = 0.05$). Attack rates were similarly low between lionfish and graysby grouper at low prey densities. At high prey densities, graysby grouper had a significantly higher attack rate relative to either competitor (lionfish: 1.66 ± 0.34 , $P < 0.0001$; red grouper: 0.83 ± 0.27 , $P < 0.01$). Red grouper had an intermediate attack rate at high prey densities, which was significantly higher than that of lionfish (-0.84 ± 0.32 , $P < 0.05$).

There were significant species-level differences in the percentage of time predators spent active (Kruskal-Wallis₂: $\chi^2 = 39.54$, $P < 0.0001$). Red grouper spent significantly more time active relative to both lionfish and graysby grouper (Wilcoxon rank sum test with Bonferroni adjustment, $P < 0.01$). Lionfish were also significantly more active than graysby grouper ($P < 0.01$).

The proportion of prey consumed was similar between species and across prey densities in both the low and high refuge patches ($P > 0.10$). Attack rate had a significant non-linear effect on the proportion of prey consumed (0.77 ± 0.09 , $P < 0.0001$). The proportion of prey consumed decreased significantly with increasing prey density for all species in the high refuge patch ($P < 0.01$). For both lionfish and graysby grouper, the proportion of prey consumed decreased with increasing prey density across habitat patches. With respect to red grouper, the proportion of prey increased with increasing prey density, but only in the medium refuge patch. Both lionfish and graysby grouper

consumed a significantly greater proportion of prey relative to red grouper at low prey densities in the medium refuge patch (lionfish: 2.36 ± 0.92 , $P < 0.05$; graysby: 2.46 ± 1.00 , $P < 0.05$), though consumption rates were similar relative to one another. At high prey densities, graysby grouper consumed a significantly lower proportion of prey in the medium refuge patch relative to both lionfish and red grouper (lionfish: -3.86 ± 1.44 , $P < 0.05$; red grouper: -3.34 ± 1.36 , $P < 0.05$).

3.4 Discussion

In my study, both prey density and the degree of prey refuge impacted foraging behaviour of predators. Many invasive species exert stronger impacts on prey populations and are better competitors than native analogues (Salo et al. 2007; Paolucci et al. 2013, but see Simberloff and Gibbons 2004). In my study, however, red grouper took the longest to satiate and had the highest FR (Figure 3.5). Though previous research has reported consumption rates of lionfish twice that of graysby grouper (Côté et al. 2013, unpublished reference data), the species' consumption rates were not significantly different in my trials (Figure 3.4). Lionfish exerted neither stronger per capita impacts nor did they have higher feeding rates. Rather, lionfish displayed a FR intermediate to that of either grouper species (Figure 3.5).

Red grouper spent more time active and were more transient relative to both lionfish and graysby grouper. High levels of activity may have enhanced their consumption rate by increasing the probability of encountering prey. However, active predators expend more energy searching for, pursuing, and capturing prey (Griffiths 1980; Mitchell and Biro 2017), thus red grouper may have incurred higher movement

costs. Therefore, higher consumption rates may have been offset by greater energy expenditure relative to lionfish.

These findings are largely contrary to previous studies of per capita impact of lionfish relative to native predators (Albins 2013; Pusack 2013; Secord 2015), as the former out-consumed and were more active than the latter in previous experiments (Albins 2013; Marshak 2018). While lionfish did not exert greater per capita impacts relative to competing native predators, they did maintain higher relative success rates across increasing prey densities than either native predator. The comparatively high success rates of lionfish relative to red grouper support the notion that lionfish may be more efficient predators, a characteristic shared by numerous invasive species (Paolucci et al. 2013; Dick et al. 2014). The hunting model of lionfish may have played a role in their success rate, the novelty of which has been well-studied (Morris and Akins 2009; Green et al. 2011). Lionfish actively stalk their prey. Predator cues from actively stalking predators are more diffuse and provide less reliable information on local predation risk (Pressier et al. 2007). Grouper may have been more conspicuous to prey as a consequence of their slow stalking behaviour and more concentrated scent relative to lionfish. The proposed difference in hunting mode between lionfish and native grouper has also been used to explain the absence of anti-predator behaviour by bicolor damselfish towards lionfish (Davis 2018). Vitousek (1990) similarly proposed that differences in both resource acquisition and efficiency contribute to the large impacts of invasive relative to native species. Whether predatory efficiency as opposed to greater rates of consumption *per se* is responsible for the detrimental impacts of lionfish is a question in need of further study.

A single functional response study provides a baseline measure of lionfish impact in its native range (South et al. 2017). Lionfish in the South et al. (2017) study were characterized by greater maximum feeding and attack rates than those observed here, where FR was unchanged by introduction of habitat complexity. However, I refrain from making direct comparisons of consumption rate given the lack of prey replacement in their study, which can significantly alter parameter estimates (Alexander et al. 2012). Further, trials were conducted in an experimental arena $\sim 1/70^{\text{th}}$ the size of that used here, which can further alter parameter estimates (Englund and Cooper 2003).

Exploitation of patches by predators is informed by both prey patchiness and prey density (Sutherland 1996). Habitat heterogeneity can modify a predator's FR, given the difference between prey density and density available to the predator (Gorini et al. 2011). In my study, consumption rates were mediated by both habitat and prey density. All predators experienced a decrease in consumption at a relatively high prey density (16) (Figure 3.5), suggesting that this was the point at which habitat patches saturated and when prey were most occluded from predators. At a prey density of 19, consumption rates rebounded (Figure 3.5). Prey preferentially aggregated in the patch offering the highest degree of refuge. Prey aggregation in turn induced an aggregative response by predators (*sensu* Hassell and May 1974), bolstering consumption rates at low prey densities, where consumption rates were highest in the high refuge patch. Both the medium and high refuge patches offered regions of absolute refuge from predators, that is, areas physically inaccessible to predators (Murdoch and Oaten 1975). The number of hiding spots for prey took longer to saturate in the high refuge patch relative to those offering either a medium or low degree of refuge. At higher prey densities, absolute prey

refuges saturated, and it proved costlier for predators to hunt in the high refuge patch when “cheaper” meals could be found elsewhere, leading to lower consumption rates in the high refuge patch relative to the alternative patches. The low refuge patch provided little refuge for prey, leading to greater conspicuousness to predators and disproportionally high consumption rates at higher prey densities. These findings conform to results reported by Sih (2005), who suggested that predators should aggregate in patches of high success irrespective of prey density.

Our feeding rate estimates for lionfish ($\bar{x} = 0.037$ g/gram predator) (Figure 3.6) are intermediate to previously published ones. My lab-derived estimates are analogous to visual field observations made by Côté and Maljković (2010) in the Bahamas (0.038 g/gram predator) and slightly higher than feeding rates reported by Fishelson (1997) (0.024 g/gram predator), the latter of which provides a baseline feeding rate for lionfish in their native range. Conversely, my feeding rates are lower than both visual field observations made by Green et al. (2011) (0.089 g/gram predator) in the Bahamas and a lab study from Southeast Florida (0.079 g/gram predator; Secord 2015).

In my study, lionfish displayed a FR intermediate to that of both red and graysby grouper. However, their high field abundance combined with their moderate maximum feeding rates produced a high Relative Impact Potential (RIP) and a greater forecasted impact in the eastern Gulf of Mexico than red grouper. Given similar feeding rates of lionfish in my study relative to those in the Green et al. (2011) study, the degree of impact of lionfish on ecosystems throughout the eastern Gulf of Mexico seems contingent on their abundance distributions. Indeed, patterns of impact are often contingent upon the abundance distribution of an invader (Latzka et al. 2016). Lionfish no doubt have the

potential to cause profound impacts on recipient systems (Ballew et al. 2016). However, whether this potential is fully realized throughout their invaded range is only slowly becoming clear. Red grouper is one of the most abundant and important species in the Gulf of Mexico. However, the abundance of red grouper in the eastern Gulf of Mexico pales in comparison to that reported for lionfish, which are ~ 7.5 times more abundant. It is thus unclear the degree to which native predators will be able to co-exist with lionfish given the latter's current pattern of abundance. Moreover, the abundance of lionfish has been exponentially increasing in this region (Switzer et al. 2015), with an 80% increase over a 4-year period relative to 2013.

Successful predators must efficiently search for, encounter, kill, and consume prey (Endler 1986). Understanding predator-prey interactions is contingent upon an understanding of their behaviour, both relative to one another and to the heterogeneous environments in which they live. Effectively quantifying the impact of an invasive species is the first step towards management prioritization (Sofaer et al. 2018). The aim of my study was to assess foraging behaviour and efficiency of lionfish, across habitat types and relative to native analogues to gain a measure of ecological impact for lionfish in the eastern Gulf of Mexico. In my study red grouper were stronger predators and exerted greater per capita impacts relative to invasive lionfish. Feeding rates reported here represent context-specific estimates for lionfish feeding in the eastern Gulf of Mexico. These consumption estimates should be corroborated by *in situ* feeding observations (Hunsicker et al. 2011), which would fill a knowledge gap for field FR estimates (Abrams and Ginzburg 2000). It is imperative that researchers continue to document the impacts of lionfish in the eastern Gulf of Mexico to determine the interplay

between their actual and potential impacts. Risk assessments on lionfish should not be confined to and extrapolated from impact assessments conducted at a single spatial scale or location. Continued monitoring of lionfish populations will help determine their impact relative to both native species and under different prey density scenarios.

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Table 3.1. Table of coefficients for all functional response curves fitted across the three species. a= *maximum killing rate*; b= *handling time*. For Holling Type IV curve, d=*half saturation constant*, c= *prey interference with predation*. For all curves, Log= *log likelihood*, AIC= *Akaike Information Criterion*.

	Holling II				Holling III				Holling IV				
Species	a	b	AIC	Log	a	b	AIC	Log	d	a	c	AIC	Log
Lionfish	1.38	0.32	183.14	179.14	0.29	0.35	182.28	178.28	109	17.27	-16.04	177.58	171.58
Red grouper	1.21	0.14	130.65	126.65	0.37	0.19	131.14	127.14	372.09	355.64	30.32	133.19	127.19
Graysby	1.28	0.36	116.71	112.71	0.51	0.41	116.95	112.95	291.11	280.83	80.9	119.01	113.01

Figure 3.1. Environmental complexity present in housing tanks. PVC pipe, concrete boulders, plastic pots, and rubber gaskets were added to communal tanks to provide shelter and structure for predators



Figure 3.2. Experimental arena in which trials were conducted: a) Patch #1 was comprised of PVC pipe to mimic a Gulf of Mexico artificial reef and offered an intermediate degree of prey refuge; b) Patch #2 was comprised of several artificial plants and offered a high degree of prey refuge; c) Patch #3 was comprised of concrete boulders and offered a low degree of prey refuge.

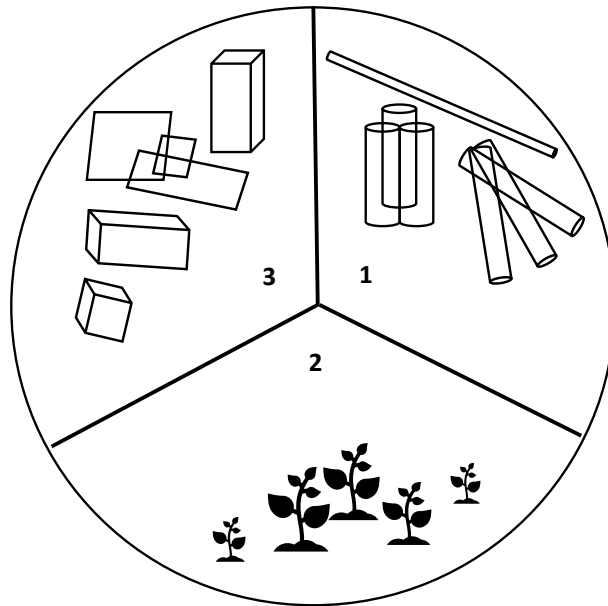


Figure 3.3. A typical artificial reef deployed in the Gulf of Mexico, comprised of haphazardly strewn concrete culverts (Photo: Florida Fish and Wildlife County Commission)

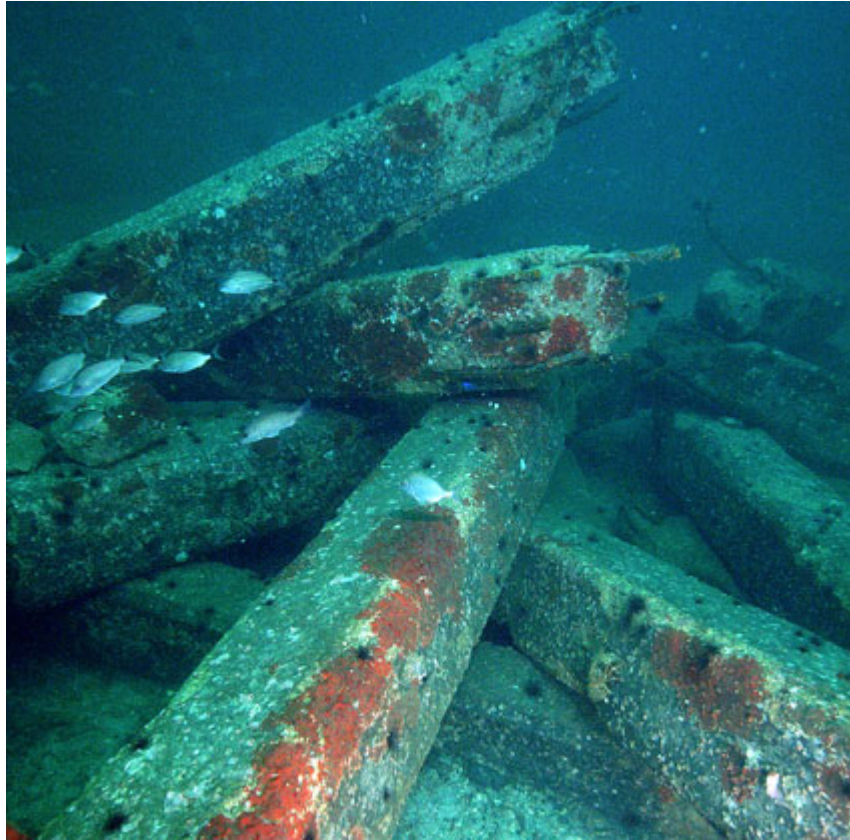


Figure 3.4. Mean (\pm SE) consumption rate for all three species. Outliers are represented by closed circles.

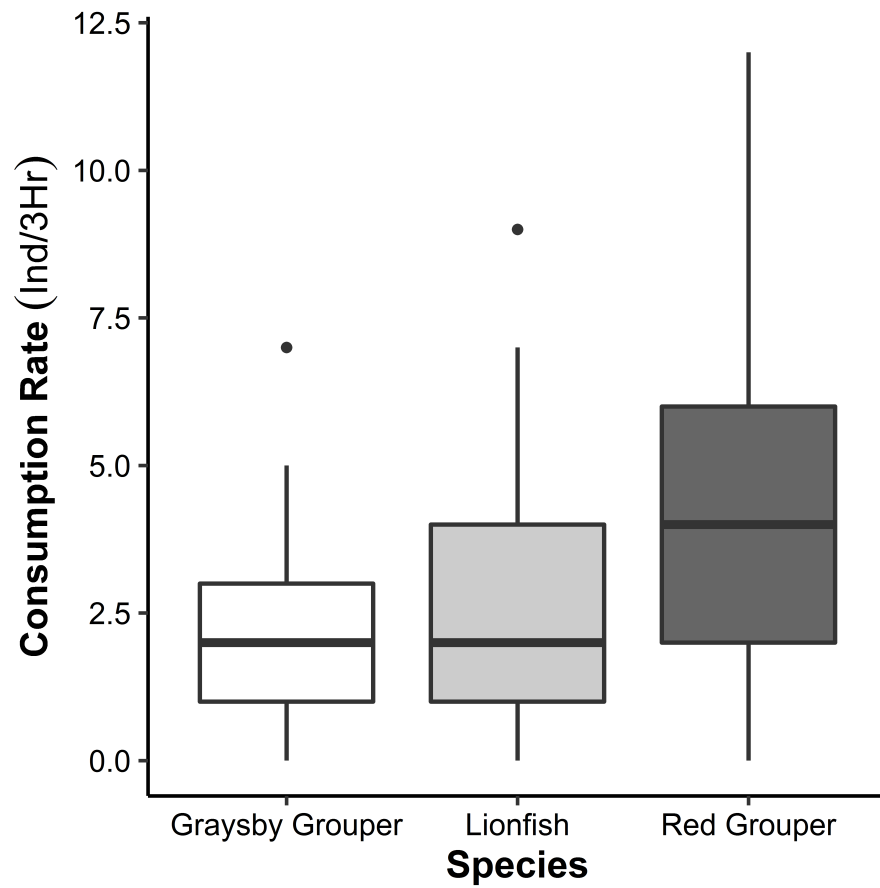


Figure 3.5. Mean (\pm SE) consumption rate as a function of increasing prey density, plotted for all three species.

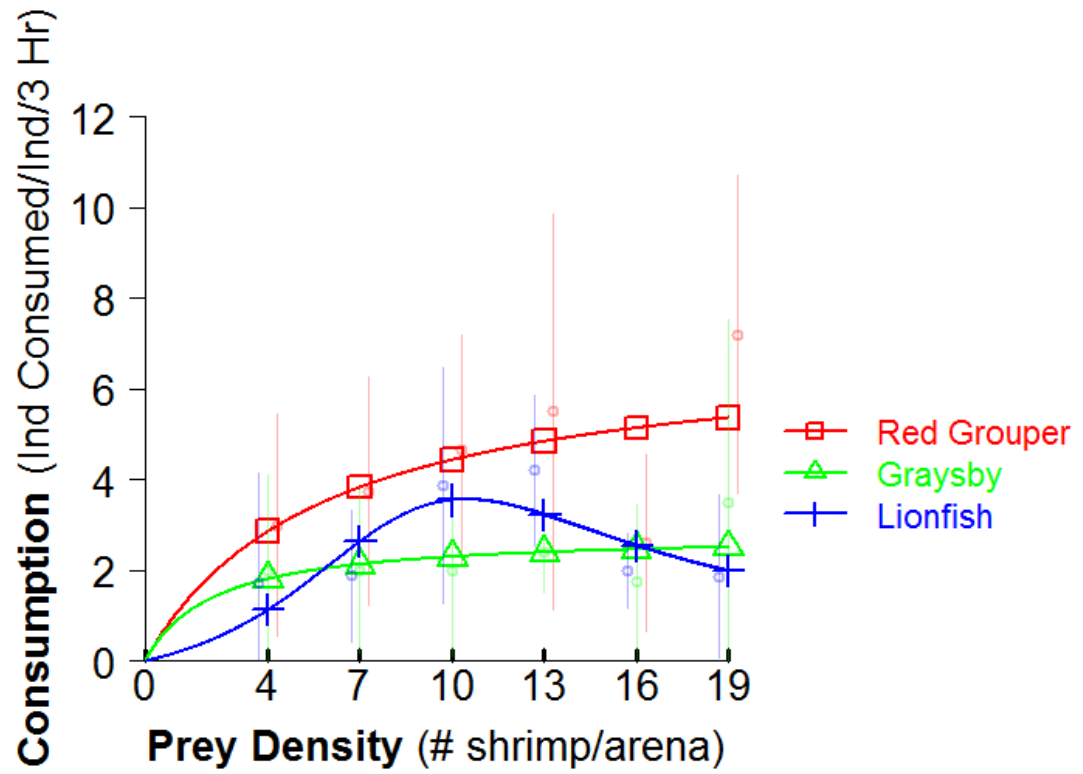
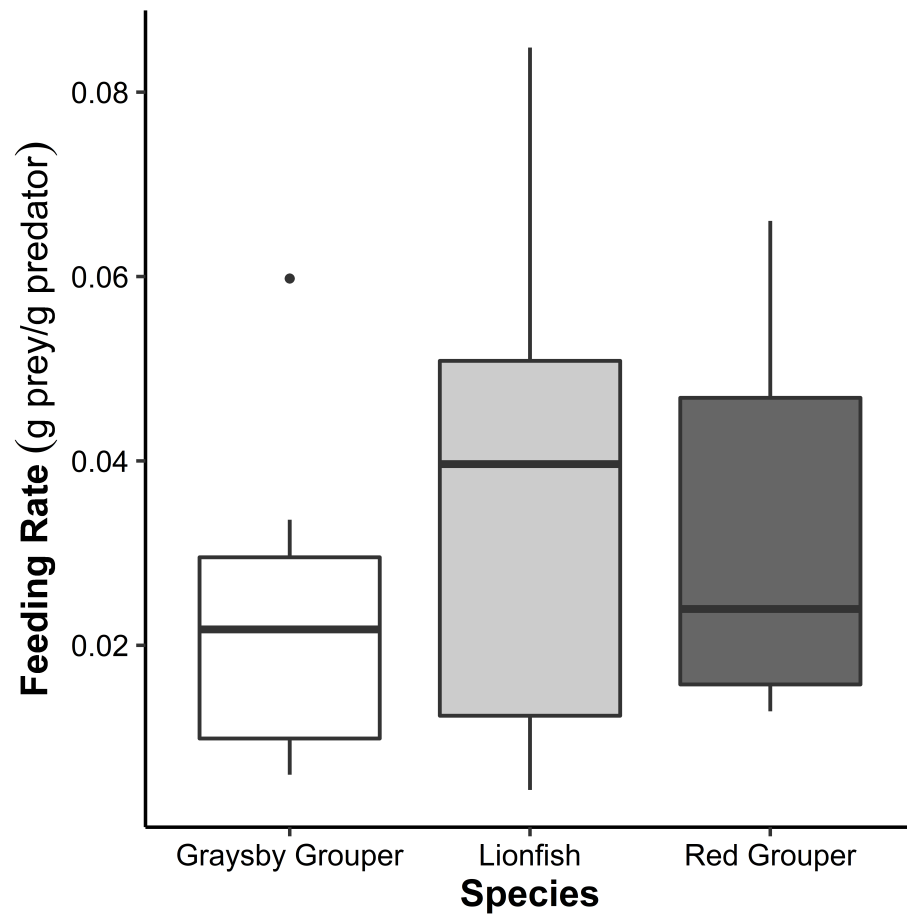


Figure 3.6. Mean (\pm SE) feeding rate per unit biomass estimates (gram of prey consumed per gram predator) for all three species. Outliers are represented by closed circles.



CHAPTER 4: DENSITY-DEPENDENT PREDATION AND GROUP FORAGING ECOLOGY IN LIONFISH

4.1 Introduction

Predator-prey dynamics influence food web and ecosystem functioning (Frank et al. 2005). How predators move in response to both prey movement and prey aggregation has received considerable attention (e.g. Bell 1991). Holling's (1959) functional response approach-which models prey consumption as a function of prey density-has strongly influenced ecological theory pertaining to predator-prey relationships. Predator density offers additional insight into predation in functional responses (Griffen and Williamson 2008). Behaviour of predators and their prey relative to each other, their conspecifics, and the heterogeneous habitats in which they live may drive prey aggregation, which can influence predator-dependent functional responses (Beddington 1975; Cosner et al. 1999). Quantifying the impact of predators across increasing densities of both conspecifics and their prey is important given that prey density can influence the effect of predation by multiple predators (Soluk 1993). Conspecifics can facilitate or hinder consumption rates of individual foragers through cooperative hunting and competition, respectively (Beddington 1975; Abrams and Ginzburg 2000). In the former case, increasing predator density may facilitate greater collective as well as per capita predation rates relative to individual predators (e.g. McCoy et al. 2012).

However, despite the increased biological relevance offered by predator-dependent functional response models (Mech 1977) and the ubiquity of predator dependence in functional response relationships (e.g. Skalski and Gilliam 2001), the effect of predator density on consumption remains an understudied facet of functional

response research (Sih 1998), especially for marine predators (Stier and White 2014). This is exemplified by the numerous foraging theories on which classical models have been built (Charnov 1976; Stephens and Krebs 1986), which operate at the individual level and differ fundamentally from both group foraging theory and real-world dynamics that these theories were designed to model. In addition, the behavioural dynamics underlying predator-prey interactions have seldom been parsed or incorporated into theory (Lima 2002; Sih 2005).

Spatial heterogeneity can significantly influence predator-prey dynamics in natural environments (Li and Reynolds 1994). Habitat structure and the prey refuges it creates can decrease predator search rate success and foraging efficiency at both low and high prey densities (Savino and Stein 1982; Vucic-Pestic et al. 2010). Greater levels of complexity may also increase the strength of density-dependent mortality (Johnson 2006) or modify the form of functional response (e.g. from Type II to III) (Li et al. 2017). Patchily distributed prey drives predator aggregation, facilitating an area-restricted search (Hassell and May 1974) and prolonging the time spent by predators in a patch (Fauchald and Tveraa 2003). This behaviour contrasts with early assumptions, including those incorporated into traditional functional response models, that predator search is random (Hassell and Varley 1969). The type of habitat in which predators reside can also lead to changes in predatory behaviour (Auster et al. 2005) and interaction strength (Vucic-Pestic et al. 2010).

Given the importance of impact and the influences of both predator density and habitat complexity, I probed the impact of group foraging on area-restricted search and foraging efficiency in lionfish, an invasive species in the western Atlantic Ocean.

Lionfish (*Pterois volitans*) are a hybrid species comprised of *P. miles* and *Pterois miles* and *P. lunulata/russelii* individuals (Wilcox et al. 2017). Native to the Indo-Pacific region, lionfish were introduced off the east coast of Florida in the 1980s, following which the species spread very widely across the western Atlantic Ocean (Schofield 2010) and has exerted profound ecological and economic impacts across much of the area where they have spread (Lesser and Slattery 2011; Albins 2013). Lionfish are habitat and prey generalists (Claydon et al. 2012; Muñoz et al. 2011), with broad physiological tolerances (Jud et al. 2015), diets (Green et al. 2014), and a wide depth distribution (Lesser and Slattery 2011). While local studies on patch reef systems indicate strong impacts (Albins 2013), whether these impacts apply across their invaded range remains unclear. The eastern Gulf of Mexico is a region in which lionfish have only been present since the late 2000s (Schofield 2010) and is a relatively unstudied region, thus providing an opportunity to contrast their impacts across temporal and spatial scales.

Lionfish are often observed in groups throughout their invaded range (Jordan 2016) and appear to alter their hunting behaviour based upon conspecific density (Benkwitt 2016). Per capita consumption in fishes often benefits from group hunting (Major 1978) and while lionfish are purported to hunt cooperatively (Lönnerstedt et al. 2014), no lab experiment has addressed cooperative hunting in the species in a heterogeneous environment. To my knowledge, only Benkwitt (2015) assessed the effect of lionfish density on native prey populations. However, the interactive effect of both increasing predator and prey density on predation rate remains unexplored.

Understanding the degree to which habitat complexity and differing densities of both

prey and predators affect lionfish predation rates is important to inform management efforts to reduce the species' impacts.

I sought to determine the effects of prey refuge, predator density, and prey density on foraging behaviour and activity. I hypothesized that: 1) per capita consumption rates would increase with increasing predator density; 2) predators would preferentially spend time in the most profitable prey patches; and 3) predation success rate would be greatest at the highest predator densities.

4.2 Methods

All fieldwork was conducted at Florida Gulf Coast University's Vester Marine and Environmental Science Research Field Station in Bonita Springs, Florida. All fish were handled and collected with permits from the National Oceanic and Atmospheric Administration (NOAA) and Florida Fish and Wildlife Conservation Commission (FWC) and were handled under the approval of University of Windsor's Animal Care Committee (AUPP #1701) and in accordance with the Canadian Council on Animal Care.

Animals

Wilcox et al. (2017) revealed that western Atlantic Ocean lionfish (*Pterois volitans*) are a hybrid species comprised of several lineages, thus I will refer to the complex simply as lionfish. Lionfish (N=39, 215.74 ± 9.16 mm TL) were collected via a team of divers using SCUBA. Divers operated in pairs and were equipped with two short-handled shallow-bodied nets. Lionfish were transferred to and brought to the surface in a large containment unit. Field collections took place off the coast of Bonita Springs between summer and winter 2018 at an average depth of 30m. Several lionfish were also procured through Dynasty Marine Associates under a FWC Special Activities License

(SAL) to supplement size classes. While all efforts were made to prevent barotrauma, a condition to which lionfish are particularly susceptible (Akins et al. 2014), many individuals required venting immediately upon surfacing. A hypodermic needle (16g) was inserted into the body wall at a 45-degree angle, plunged slightly into the flesh of the fish, and held in place until sufficient decompression was achieved. HexArmor SharpsMaster II® 9014 puncture-proof gloves were worn when handling lionfish. Fish were transported back to the field station in the live well onboard the research vessel or in coolers. 50% water changes were periodically conducted en route to maintain sufficient aeration.

New fish were quarantined for a least one week following capture, and isolated from conspecifics in several 757 L fiberglass tanks. 5 and 7 cm diameter PVC pipe and several cinder blocks were added to increase environmental complexity. Fish were housed with conspecifics to reduce isolation-induced stress. All fishes were proactively treated with StressGuard™, a slime coat protection product. Following quarantine, lionfish were housed communally in several 1135L recirculating fiberglass tanks in a semi-enclosed aquaculture cage equipped with chemical, physical, and biological filtration systems and subject to a natural photoperiod. Water was maintained at 34 ppt salinity, 24°C and pH 8.2. Air stones kept dissolved oxygen (DO) at or near saturation. Aqua Logic® in-line titanium water heaters (Model #TIL5) were used as needed during winter months. PVC pipe, concrete blocks, and scattered rocks were added to increase environmental complexity. Water changes were conducted weekly using seawater pumped in from the surrounding bay, pumped from an underground saltwater well, or by mixing Instant Ocean® artificial seawater. Water quality parameters were checked weekly

with a YSI™ Professional Plus meter and LaMotte Saltwater Fish Farm Test Kit (Part #: LMAQ4). Lionfish were identified with coloured tags (Floy® FTSL-73 Streamer tags) and identified via a unique three-digit number, following the methods outlined in Chapter 2. Prior to experiments, fish were acclimated for a minimum two weeks and fed *ad libitum* assorted baitfish [silversides (*Atheriniformes spp.*), pilchards (*Harengula jaguana*)], saltwater-acclimated mosquitofish (*Gambusia affinis*), pink shrimp (*Penaeus duorarum*, both live and frozen), and frozen sardines (*Sardinops sagax*).

Identification

Lionfish were identified with coloured tags (Floy® FTSL-73 Streamer tags) and identified via a unique three-digit number. To increase ease of handling and to decrease stress incurred by both species during tagging, fish were anaesthetized in a bath of MS222 dissolved in seawater. Fishes were captured from their communal tanks with shallow-bodied hand nets and transferred to a bath of well-aerated saltwater containing a concentration of 110 mg/ L MS-222, as per Bacheler et al. (2015). Once sedated, fish were transferred to a shallow-bodied container housing a seawater bath with a maintenance dose of anesthetic. Tagging method followed that described in Chapter 2.

Experiment

Live pink shrimp (\bar{x} = 4 cm, range 2-7 cm) served as the prey for all trials given their ubiquity throughout the Gulf of Mexico. Shrimp are consumed by all size classes of lionfish, with the diets of juvenile and immature lionfish comprised mainly of invertebrates (i.e. shrimps, crustaceans). While the proportion of crustaceans in the diet of lionfish decreases with ontogenetic stage (Arredondo-Chávez et al. 2016), they still maintain high relative importance.

Trials were conducted in a round tank of approximately 2500 L (2.13 m diameter x 0.91 m height, filled to a depth of 0.71 m). The arena was divided into three patches of equal area but distinct habitat (Figure 4.1). Habitats were selected given the degree of refuge they afforded prey. Following McNair (1987), I operationally defined refuge as any physical shelter that provides temporary or permanent shelter from predation pressure. Patch #1 (hereafter referred to as the medium refuge patch) was composed of PVC pipe, arranged to mimic the typical artificial reef structure in the Gulf of Mexico formed of concrete culverts. Patch #2 (hereafter referred to as the high refuge patch) was comprised of four artificial aquarium plants, with heights ranging from 15 to 61 cm. Patch #3 (hereafter referred to as the low refuge patch) was composed of concrete blocks and rocks. Both predators and prey were able to transit freely within and among patches.

Trials were conducted at 24-26°C, salinity 32 ppt and pH 8.2, with DO at or near saturation. Water temperature was held constant using single-tube straight heavy-duty immersion heaters (Pentair Aquatics Part #H18T). Water in the experimental arena was aerated with submersible air stones between trials. Ammonia, nitrate, and nitrite were all maintained at negligible levels. New water was added to the experimental system on a bi-weekly basis, or whenever water levels declined. Trials were recorded using four LOREX security cameras (4K Ultra HD LNR6100 series). One security camera was mounted to the ceiling above each individual patch with a fourth mounted in the middle of the tank to provide an overview of the tank system. Trials were viewed remotely on a desktop computer to eliminate a potential Hawthorne Effect, that is, observer-induced change in lionfish behaviour (James and Vo 2010). A single LED light (Husky: Model K40066) mounted on a metal rod in the center of the tank provided constant light.

Trials were conducted across three predator (1, 2, 4) and seven prey densities (4, 7, 10, 13, 16, 19, 22), with at least three replicates of each. Limited field availability constrained replicate numbers at the highest predator density. Each trial was conducted over a span of three hours. Pilot trials did not reveal longer trial durations to be useful. Predator densities corresponded to group sizes in which lionfish have been commonly found (Lönnstedt 2014; Jordan 2016; Jimenez et al. 2016). Fish were starved for a minimum 72 hours before experimental trials to ensure willingness to both feed and swim. Satiation dictates a predator's willingness to forage (Jeschke 2007) which has been tested specifically in lionfish (Fishelson 1997). Shrimp were introduced to the center of the tank at least 30 minutes before a given trial and allowed to disperse freely. Shrimp movements were monitored by the experimenter to determine their proclivity and settlement in each patch, and the relative density in each patch. Fish were introduced to the center of the tank via a plastic bucket and were able to freely choose patches with a null expectation of 33% for each patch.

At the highest predator density, limited available size classes made it necessary to re-use lionfish. Fish were not tested in concurrent trials or in trials with the same conspecifics at the same predator and prey densities. Density treatments conducted on a given day were randomly selected. Fish were randomly assigned to prey density treatments using the RAND function in Excel. Given that individuals in predator density treatments of two and four were size-matched, several iterations of the random number generator were often required to ensure limited to no re-use of individuals. There were no significant size differences between predator density classes (Kruskal-Wallis₂: $\chi^2=0.11$, $P>0.10$) or between prey density treatments within each predator density class (Kruskal-

Wallis₆: $P > 0.10$). Prey were replaced as they were consumed throughout the trial. Prey-replacement is an important component of FR trials and of any feeding trial because it allows researchers to assess stochastic predation (Juliano 2001). For each patch and for each individual fish, I recorded the time and location of both successful and unsuccessful attacks, stalking bouts, and failed pursuits. Successful attacks were those that ended in consumption of prey. Given that lionfish are suction feeders, all prey were consumed whole. I calculated per capita estimates for trials with more than one predator by dividing the total number of prey consumed by the number of lionfish in the trial. I calculated success rate as the number of successful attacks made as a function of attack rate. All lionfish were euthanized at the end of the study period in a bath of eugenol (45mL clove oil and 450mL isopropanol per L seawater). Lionfish spines were removed with tin snips and contained in puncture-proof sealed glass jars. Carcasses were double-bagged and discarded as per Florida Gulf Coast University's biological waste disposal protocols. Total length (TL), standard length (SL), and weight (g) were recorded from all fish post-euthanasia.

Data analysis

I fitted Beddington-DeAngelis (Beddington 1975; DeAngelis et al. 1975) and Crowley-Martin (Crowley and Martin 1989) predator-dependent functional response models to the empirical data inclusive of all predator densities. In addition to attack rates computed manually, attack rates and handling times were inferred from parameters of the FR model. As with other reef fishes, lionfish consumed prey whole thus handling time was more a measure of pursuit and digestion (Stier et al. 2013).

The Beddington-DeAngelis FR extends upon Holling's disc equation which models a Type II FR (Holling 1959):

$$f = \frac{aN}{1+abN} \quad (1)$$

where a refers to attack rate, N the number of prey, and b handling time.

Beddington (1975) and DeAngelis et al. (1975) extended upon (1) to include the implications of multiple predators in an arena:

$$f = \frac{aN}{1+bN+c(P-1)} \quad (2)$$

where P denotes predator abundance and c describes the magnitude of interference between predators.

The Crowley-Martin FR (Crowley and Martin 1989) allows for simultaneous handling of prey and interference between predators:

$$f = \frac{aN}{(1+bN)(1+c(P-1))} \quad (3)$$

I assessed movement and habitat use of predators for each of the three habitat patches using BORIS, version 7.4 (Behavioral Observation Research Interactive Software, Friard and Gamba 2016). I scored random 30-minute trial segments for all lionfish present in a trial across both predator and prey densities. I quantified the proportion of time that lionfish spent in each patch as well as the time spent active in each patch. I also scored instances of cooperative hunting and gregarious behaviour for trials with more than one predator.

All statistical analyses were carried out in R, version 3.5.3 (R Core Team 2018). Data exploration was carried out following the protocols described in Zuur et al. (2010). I analysed raw consumption data using generalized linear mixed models (GLMM) (package *glmmTMB*, Brooks et al. 2017). To select the appropriate error distribution, I fit negative binomial and Poisson probability distributions to individual response variables (package *fitdistrplus*, Delignette-Muller and Dutang 2015). Goodness-of-fit was compared graphically and by comparing Akaike's Information Criterion (AIC) values. Predator density, prey density, and habitat patch were included as fixed effects. Animal ID was included as a random effect. Across all models, I used a dispersion formula to account for heteroskedasticity in *prey density*. The best model blended a low AIC, and thus parsimony and optimal fit, with biological relevance (Bolker 2008, Chapter 6.6.2 and references therein). Tukey-adjusted multiple comparisons were conducted *post hoc* (package *emmeans*, Lenth 2019). Model assumptions were verified by plotting residuals versus fitted values, versus each covariate in the model and, where possible, versus each covariate not in the model. In addition, I assessed the residuals for temporal and spatial dependency (package *DHARMA*, Floridan 2019). Proportion data were analyzed using beta regressions assuming a binomial family with a log-log link function (package *betareg*, Cribari-Neto and Zeileis 2010). I verified goodness of fit visually by inspecting diagnostic plots of standardized weighted residuals, predictions, and leverages. Unless otherwise noted, results are presented as estimates \pm SE.

4.3 Results

Model validation indicated no problems across any of the final models. Cumulative consumption rates increased significantly with increasing predator density (Kruskal-Wallis₂: $\chi^2=69.61$, $P<0.0001$), and were higher in multiple versus single predator density treatments (Figure 4.3, 4.4). Per capita consumption rates also varied significantly across predator density treatments (Figure 4.3, Figure 4.5, Table 4.1). Lionfish foraging in pairs displayed the highest maximum per capita feeding rates across all prey densities and were significantly lower when four predators were present (Figure 4.5, Table 4.1). The Crowley-Martin FR provided the best fit for consumption rates overall, across predator densities (Table 4.1). Beddington-DeAngelis and Crowley-Martin FR models produced analogous fits of the empirical data, with the exception of consumption rates for lionfish foraging in pairs, for which the Crowley-Martin model provided a better fit (Table 4.1).

Lionfish in pairs had significantly higher per capita consumption rates relative to both individuals foraging alone and those in groups of four (alone: -0.40 ± 0.16 , $P<0.05$; four: 0.66 ± 0.14 , $P<0.0001$) (Figure 4.5). Lionfish foraging in pairs also had the lowest FR-derived interference coefficient of all three predator density treatments (Table 4.1). The interference coefficient was significantly greater when lionfish foraged in groups of four relative to in pairs (Table 4.1). Per capita attack rates were also higher when lionfish foraged in pairs relative to lionfish foraging alone or in groups of four (alone: -0.38 ± 0.14 , $P<0.05$; four: 0.64 ± 0.12 , $P<0.0001$). Unsuccessful attack rates were lowest when lionfish foraged in groups of four, and significantly lower than when they foraged alone or in pairs (alone: 0.65 ± 0.26 , $P<0.05$; pairs: 0.66 ± 0.23 , $P=0.01$). Both per capita

consumption and attack rates were similar between the highest and lowest predator density treatments (Figure 4.5). Model-derived attack rates of lionfish foraging in groups of four were higher than when they hunted singly but significantly lower relative to lionfish hunting in pairs (Table 4.1).

Across predator density treatments, per capita consumption rates were bolstered by increasing activity levels and by greater numbers of failed pursuits and stalking bouts. The latter two covariates had non-linear effects on consumption rate. Per capita attack rate also increased non-linearly with a greater number of stalking bouts (0.55 ± 0.12 , $P < 0.0001$), for which there was a concave relationship between stalking bouts and attack rate. Neither attack rates (total and unsuccessful) or consumption rates were significantly modified by increasing prey density nor habitat patch.

Success rates were mediated by habitat, predator density, and lionfish activity. Success rates increased for all predator density groups with increasing levels of activity in the low refuge patch. With higher levels of activity, success rates decreased significantly for lionfish in groups of four in the high refuge patch (9.89 ± 3.15 , $P < 0.01$) and in contrast increased for both single lionfish and pairs of lionfish. In the medium refuge patch, all predator density treatments suffered a decline in success rate with increasing activity.

When alone, lionfish spent more time active with increasing prey density, relative to lionfish in pairs, whose activity precipitously declined with increasing prey density. In groups of four, the proportion of time lionfish spent active remained constant across increasing prey density. Lionfish foraging alone were the least active at low prey densities, whose effect was marginally significant relative to lionfish foraging in pairs

(-0.27 ± 0.11 , $P=0.05$). At high prey densities, lionfish foraging alone were most active and significantly more active than lionfish foraging in pairs (0.35 ± 0.12 , $P=0.01$). Lionfish in pairs were also marginally more sessile than lionfish in groups of four at high prey densities (-0.33 ± 0.15 , $P=0.08$). With respect to multiple predator densities, lionfish became more sessile as gregariousness increased, the effect of which was more pronounced in the low refuge patch relative to either alternative patch (medium refuge patch: -0.31 ± 0.14 , $P=0.07$; high refuge patch: -0.37 ± 0.14 , $P<0.05$).

Lionfish made significantly more stalking bouts in the low refuge patch relative to the medium or high refuge patch (medium: -0.38 ± 0.13 , $P=0.01$; high: -0.37 ± 0.13 , $P=0.01$). At low prey densities, lionfish in groups of four made the fewest stalking bouts and made marginally fewer stalking bouts relative to lionfish foraging alone (1.18 ± 0.53 , $P=0.06$). Stalking rate was similar amongst predator density treatments at high prey densities. There was a significant difference in the amount of time lionfish allocated to each patch (Kruskal-Wallis₂: $\chi^2 = 66.93$, $P<0.0001$), lionfish spent significantly more time in the low refuge patch relative to either alternative patch, a trend which was consistent across predator densities.

4.4 Discussion

In my study, per capita consumption and attack rates were highest when lionfish foraged in pairs (Figure 4.5) and cumulative consumption rates increased with increasing predator density (Figure 4.4). However, while both overall and per capita consumption rates benefited significantly when lionfish foraged in pairs relative to as singletons, doubling the number of predators from two to four led to a negligible increase in cumulative consumption rate (Figure 4.4). Rather, both per capita consumption and attack

rates were analogous between lionfish hunting singly and in groups of four. My findings are consistent with Benkwitt's (2015) manipulative field experiment in which she observed that addition of multiple lionfish to a patch reef system bolstered their cumulative negative effect on prey fish communities, the addition of each additional predator exerted a negligible impact on the system.

Previous predator-dependent functional response studies have reported a similarly negative effect of increasing predator density on foraging efficiency (i.e. on attack rate) (Stier and White 2014). Interactive effects of prey refuge, prey density, and predator density impacted both consumption and success rates in my study and led to reduced per capita attack rates of lionfish foraging in groups of four relative to both lionfish foraging in pairs and alone. Several mechanisms have been proposed to explain reduced per capita predation rates at increasing predator densities. The presence of conspecifics has been shown to both induce competition between consumers for common resources and precipitate changes in prey behaviour (Schoener 1983; Kratina et al. 2009). Structural complexity has also been shown to affect consumption rates through reduced foraging efficiency, especially at low prey densities (Auster et al. 2005; Kauffman et al. 2007; Vucic-Pestic et al. 2010). In my study, concomitant effects of increasing predator density and refuge drove changes in prey behaviour. However, I did not observe significant impacts of prey density *per se* on success rates; rather, the relative prey density available in each habitat relative to the overall prey density had a greater impact on success rates.

Intraspecific competition often limits availability of prime hunting spots (Kauffman et al. 2007) and is generally quite strong amongst invasive species (Connell 1983). Competition between conspecifics may lead to antagonistic interactions and is one

mechanism by which per capita consumption rates are lowered, evidence of which was detected in the functional response models (Table 4.1). With respect to lionfish, intraspecific competition has been attributed to both non-linear impacts on prey populations and as a mechanism by which slower growth occurs with increasing lionfish density (Benkwitt 2013; 2015). While I did not observe overtly antagonistic behaviours in lionfish, the non-random search for prey characteristic of exploitative competition can lead to the appearance of predator interference (Free et al. 1977). By nature of its negative effect on resource acquisition by conspecifics, exploitative competition manifested unequal consumption rates amongst conspecifics. I also observed instances of shadow competition, wherein predators often enjoyed higher consumption rates relative to conspecifics simply by positioning themselves closer to a given prey source (Wilson 1974). In the context of this experiment I refer to ‘source’ as the refuges within a given patch in which prey spent much of their time and from which they periodically emerged.

Encounter rate of prey by predators depends both on the presence of co-occurring predators and on prey density (Mols et al. 2004). Foragers also make ample use of public information, that is, information about a resource gleaned by attending to the way in which conspecifics exploit that resource (Danchin et al. 2004). Fish learn to recognize the foraging behaviour of individuals around them as cues and to inform patch profitability (Johnson 2006). Predation by conspecifics can substantially influence capture rate of co-occurring predators competing for the same resource (Mols et al. 2004). For example, Bocaccio rockfish were more effective attacking prey already being pursued by conspecifics (Johnson 2006). Indeed, pursuit of prey by one lionfish would ‘tip off’ conspecifics to its location, which would initiate a chase by all predators in proximity.

However, this often had the unintended consequence of indirectly facilitating consumption of a trailing fish at the expense of the lionfish that initiated the pursuit. While behavioural syndromes *per se* were not assessed in this study, aggression is one such trait that has been correlated with both foraging efficiency and dominance (Sih et al. 2004). Thus, I allow for the possibility that aggression underlined the voraciousness of the most successful predators in this study and bolstered the consumption rates of aggressive lionfish relative to conspecifics.

Predators often decrease time spent foraging at low prey densities (Lipcius and Hines 1986), though increased levels of activity can manifest at higher predator densities (Overli et al. 1998; Benkwitt 2015). My data support this notion, whereby levels of activity at the highest predator density were unchanged by prey density. Larger group sizes lessen the time needed to find prey and allows for quicker assessments of relative patch profitability (Pitcher and Magurran 1983; Benkwitt 2016). When predators forage in multiples, the costs of foraging are dispersed among conspecifics, which may provide one explanation as to why higher activity levels in lionfish manifested at higher predator densities when resource levels were low. While activity levels at the highest predator density were unchanged by prey density, gregariousness had a significant negative effect on activity, which was most pronounced in the low refuge patch. The negative influence of lionfish group size on activity level was similarly reported by Garcia-Rivas et al. (2018) in the Mexican Caribbean.

Proliferating lionfish abundance is purported to drive strong ecological impacts in the eastern Gulf of Mexico (Chapter 3), thus reducing abundance of lionfish may best mitigate the impacts of this species and their relative impact potential (Hoag 2014). The

gregarious nature of lionfish combined with their low activity levels at high predator densities may allow for more efficient culling of these invaders. Effective management efforts must be guided by knowledge pertaining to the species being managed. My study suggests that these management efforts not be specifically allocated to artificial reefs offering the greater structural complexity, as lionfish did not aggregate in the most complex habitat in my study. Rather, those regions in which prey are most vulnerable are likely to house the greatest lionfish densities. However, field studies investigating the synergistic effects of habitat, prey density, and lionfish density are presently lacking for the eastern Gulf of Mexico. Complimentary field studies are needed to verify whether the relationships shown here will translate at greater spatial scales in nature.

I observed similar per capita consumption rates across habitat patches, suggesting that lionfish consumed prey opportunistically. Prey preferentially aggregated in the high and intermediate refuge patches, respectively, and under the stipulations of the aggregative response, prey density should have dictated predator abundance (Hassell 1978). That is, higher densities of prey in the high refuge patch should have driven aggregation of predators in that patch. However, lionfish neither spent more time nor consumed significantly more prey in the high refuge patch relative to either alternative patch. Conversely, they spent the greatest amount of time in the low refuge patch. The presence of conspecifics and prey vulnerability, not prey abundance, dictated patch residency. The low refuge patch also boasted the highest attack rates, the most unsuccessful attacks, stalking bouts, and failed pursuits. While prey density was proportionally low in the low refuge patch, any resident or transient prey were more conspicuous to predators in this relative to other patches. Similar phenomena have been

reported in flour beetles (*Tribolium confusum*) and salamander larvae (*Ambystoma tigrinum*) (Korona 1990; Sih 2005). Lionfish in my study behaved in accordance with predictions by Sih (2005) by concentrating their time in the habitat patch of high prey risk despite low prey density. By doing so, they selected the habitat patch in which energy intake was maximized, consistent with classical foraging theory (Stephens and Krebs 1986).

Functional responses provide meaningful insights into predator-prey dynamics (Soluk 1993) and provide an efficient way by which to assess invasive species impacts. The per capita impact of an invasive species is contingent upon their abundance, both of which are modified by biotic and abiotic conditions (Thomsen et al. 2011; Sofaer et al. 2018). Impacts of invasive species are often density-dependent (Griffen and Byers 2009), underscoring the need to mechanistically test the effects of increasing predator density on resultant impact, as done in this study. As the abundance and per capita effect of invasive species are known to vary both spatially and temporally, this study underscores the need for regionally tailored estimates of impact and the importance of discerning an invasive species' relative impact on communities under varying habitat conditions (Hansen et al. 2013; Sofaer et al. 2018).

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Table 4.1. Table of coefficients for both Crowley-Martin and Beddington-DeAngelis predator-dependent functional responses. *a*= attack rate; *b*= handling time; *c*= magnitude of interference between predators; σ = sigma, standard deviation for the normal distribution assumed; Log= log likelihood; AIC= Akaike's Information Criterion.

	Crowley-Martin						Beddington- DeAngelis					
Predator density	a	b	c	σ	Log	AIC	a	b	c	σ	Log	AIC
1	1.44	0.46	2.00	2.01	186.10	194.10	1.44	0.46	1.00	2.01	186.10	194.10
2	23.01	5.02	0.17	3.15	189.98	197.98	15.09	3.72	3.95	28.96	317.52	325.52
4	9.51	0.28	4.23	1.57	89.73	97.73	4.95	1.96	2.06	1.57	89.73	97.73

Figure 4.1. Experimental arena in which trials were conducted: a) Patch #1 was comprised of PVC pipe to mimic the typical Gulf of Mexico artificial reef and offered an intermediate degree of prey refuge; b) Patch #2 was comprised of several artificial plants and offered a high degree of prey refuge; c) Patch #3 was comprised of concrete boulders and offered a low degree of prey refuge.

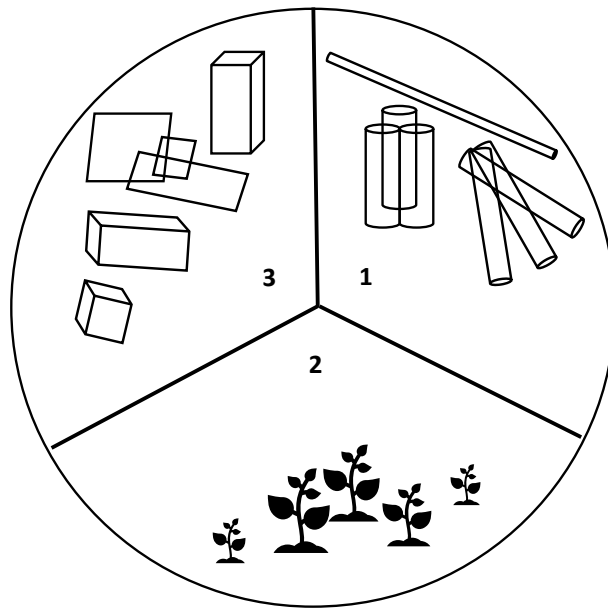


Figure 4.2. A typical artificial reef deployed in the Gulf of Mexico comprised of haphazardly strewn concrete culverts (Photo: Florida Fish and Wildlife County Commission).

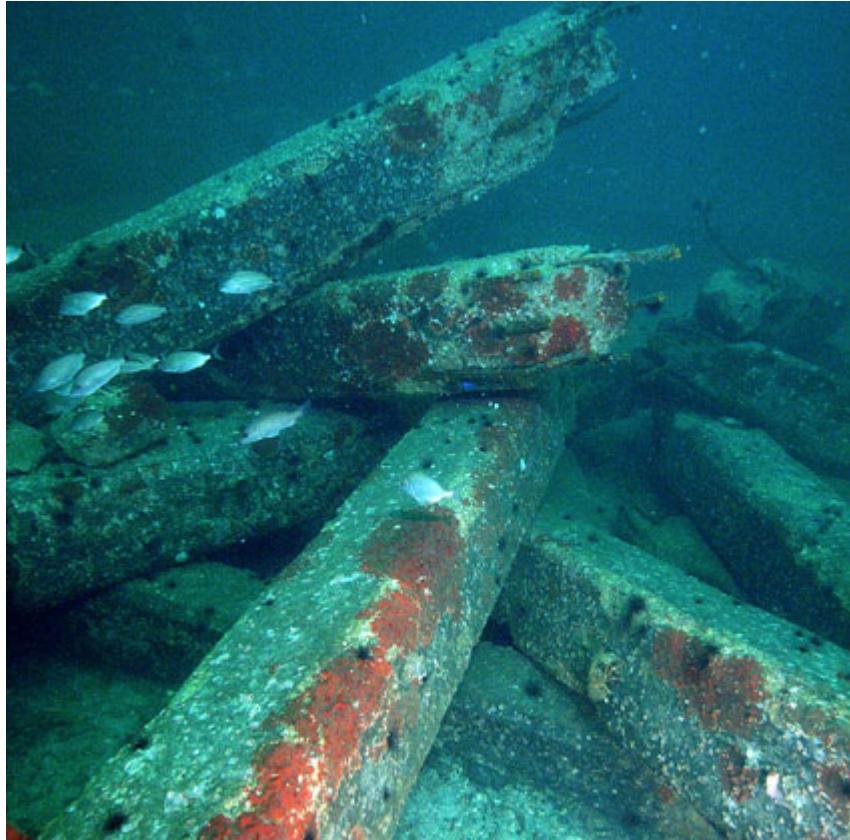


Figure 4.3. Crowley-Martin predator-dependent functional response showing mean (\pm SE) per capita lionfish consumption rates as a function of increasing prey density. Lines for each of one, two, and four lionfish are plotted. Per capita consumption rates represent total consumption rates divided by the number of predators in the system.

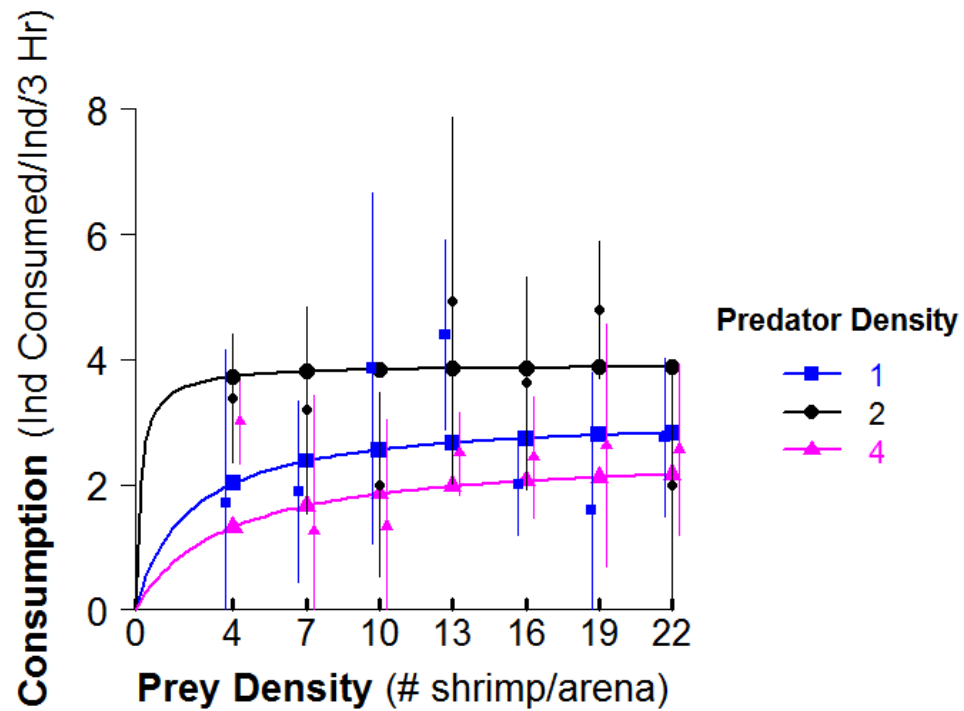


Figure 4.4. Mean (\pm SE) lionfish consumption rate across predator density treatments.

Outliers are represented by closed circles.

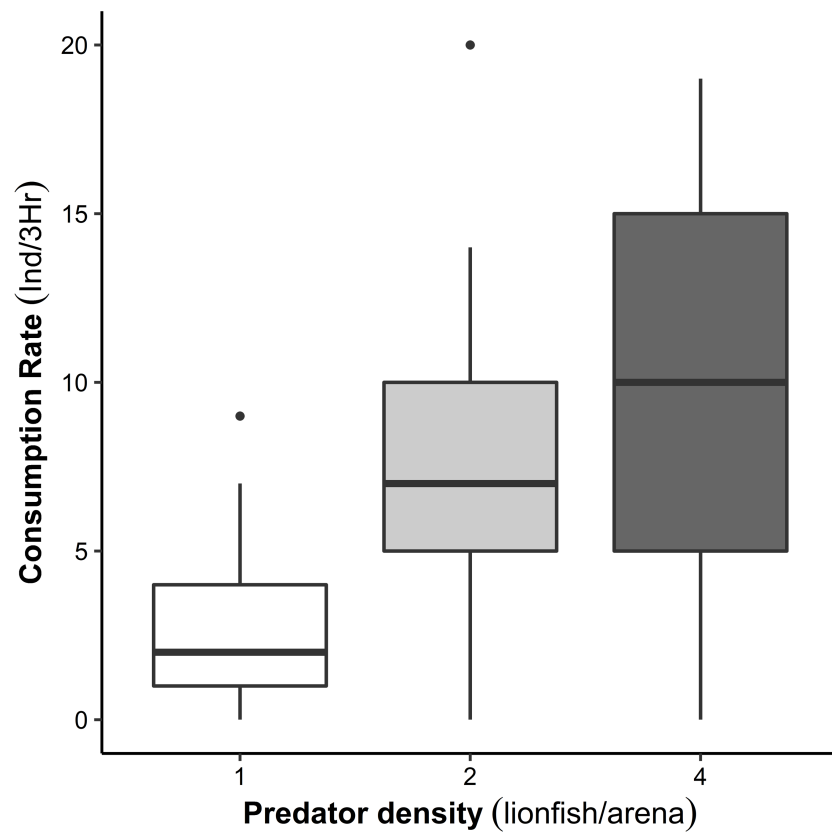
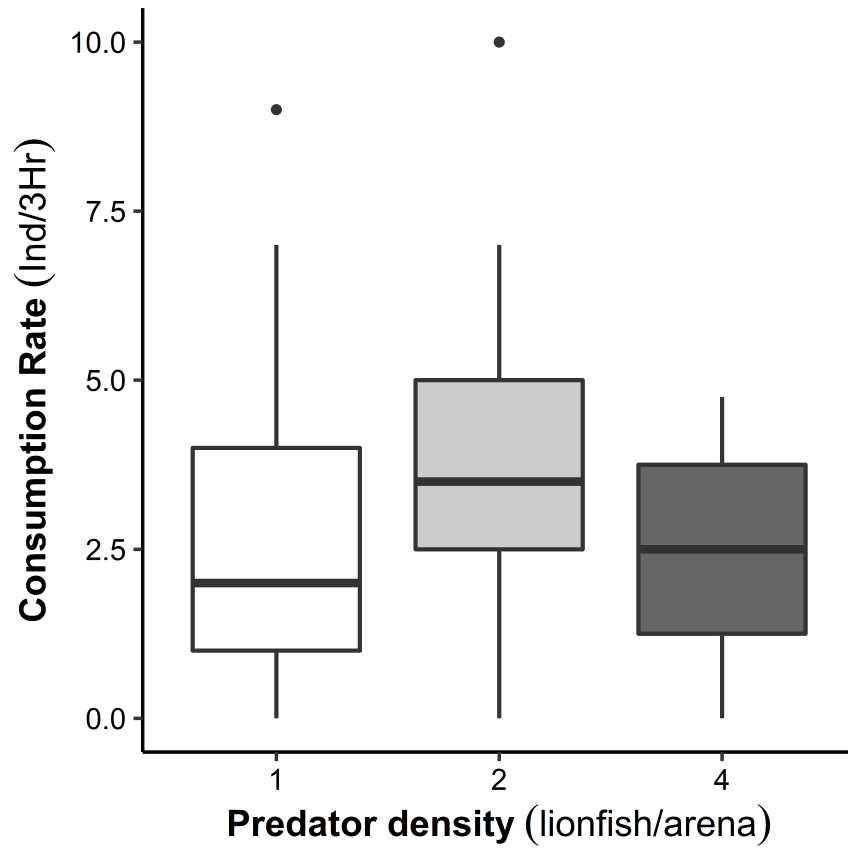


Figure 4.5. Mean (\pm SE) lionfish per capita consumption rate across predator density treatments. Outliers are represented by closed circles.



CHAPTER 5: GENERAL DISCUSSION

Marine invasive species substantially alter habitats (Graham et al. 2014) and often outcompete native species through higher feeding rates (DeGraaf and Tyrrell 2004). However, defining the mechanisms underlying invasive species impacts has proven elusive given that these impacts are highly context-dependent (Ricciardi et al. 2013). This has precipitated calls for studies to investigate the factors determining impact (e.g. Gallardo et al. 2016). This thesis sought to better quantify the impacts of lionfish by probing their behavioural plasticity and foraging behavior, alone and relative to ecologically analogous native species with which they co-occur. An understanding of both of the aforementioned are purported to be important in understanding the impacts of invasive species (Weis 2010; Braga et al. 2012).

In this thesis, I explored impact assessments for lionfish and provided estimates of impact for a relatively unstudied portion of their invaded range. While lionfish populations have been subject to rapidly increasing densities along the West Florida Shelf since 2014 (Switzer et al. 2015; Chagaris et al. 2017) studies of impact in this region have lagged behind those in other parts of their invaded range. Understanding the extent of lionfish impacts throughout their invaded range is important, especially in light of climate change-induced range expansions of both native and invasive species (Invasive Species Advisory Committee 2010; Feary et al. 2013; Grieve et al. 2016).

In Chapter 2, lionfish learned to locate prey items using navigation strategies of varying sophistication, for which their memories proved particularly robust. Understanding the role of interspecific competition in structuring communities has long-standing roots in ecological literature (Gause 1934; Schoener 1983), particularly for

marine fishes (McCormick and Weaver 2012), and is an oft-discussed consequence of invasions in marine systems (Ruiz et al. 1999). Moreover, superior learning and behavioural plasticity has been catalogued for several invasive species relative to native analogs (Hazlett 2000; Hazlett et al. 2002). Though many researchers have expressed concern over exploitative competition between lionfish and native predators, no study to-date has examined the way in which lionfish spatially navigate, nor their ability to remember and navigate to highly profitable prey patches. This understanding may provide insight into the mechanisms underlying their impacts. On the West Florida Shelf, lionfish are prevalent in hard-bottomed non-reef habitat (Switzer et al. 2015). The seascape in this region is peppered with artificial reefs, in between which is barren substrate. Given that resources are extremely patchy, the ability to retain information about relative patch profitability in this region is pertinent. If lionfish are able to find and remember the locations of reefs that contain the most prey, this may provide a competitive edge over native species lacking such persistent memories.

Given the long-standing implications of habitat structure for both predators and their prey (Heck and Crowder 1991), in Chapter 3 I quantified the impacts of lionfish relative to two native mesopredators foraging in a heterogeneous environment. Lionfish consumed similar quantities or fewer numbers of prey relative to native grouper, providing a contrast to the competitive advantages generally afforded invasive species relative to the native species with which they co-exist (Parker et al. 1999; Lord and Williams 2017). Invaders with greater per capita effects relative to native species are generally both more abundant and have greater community-level impacts (Dick et al. 2017). In this study, lionfish did not exert significantly greater per capita effects relative

to native grouper. However, lionfish are found at several times the abundance of red grouper in the eastern Gulf of Mexico. Their ubiquity throughout the eastern Gulf of Mexico thus appears to compensate for their moderate per capita impact- as suggested by their high Relative Impact Potential- and bolsters their potential to exert strong ecological impacts in this region. To my knowledge, this is the first comparative functional response study to be conducted with lionfish.

Chapter 4 extended these results to assess the density-dependent impacts of lionfish, given that an accurate understanding of a species' abundance-impact relationship is essential to determine localized impacts and best direct management efforts of a species (Yokomizo et al. 2009; Latzka et al. 2016). I assessed movement and foraging in lionfish relative to increasing prey density, increasing predator group size, and across three heterogeneous habitat patches to probe their interactive effects on density-dependent mortality. Consumption rates in lionfish were subject to the law of diminishing returns, whereby increases in predator density did not produce proportional increases in predation efficiency or success. Rather, lionfish were afforded the highest consumption rates at intermediate predator densities. In contrast to expectations guided by Optimal Foraging Theory, lionfish did not aggregate in habitat patches offering the most prey. Instead, they preferred to hunt in habitat patches of low relative complexity that provided easier meals. Understanding the non-linearity inherent in the impacts of lionfish is important to better refine estimates of impact as lionfish densities increase throughout the eastern Gulf of Mexico, especially in light of the context-dependence of abundance-impact relationships (Thiele et al. 2010).

The way in which knowledge of patch profitability informs movement and subsequently foraging success has generated a great deal of interest (Bell 1991; Turchin 1998). Although predator-prey movements relative to one another were first probed in the 1980s (Iwasa 1982), only a handful of studies examining the influence of structural heterogeneity on reef fish predator-prey interactions have been conducted (Catano et al. 2015). Specifically, per capita consumption estimates are essential but lacking (Morales et al. 2010). This thesis addressed both of these shortcomings. As a whole, the experiments conducted in this thesis help to better ascribe mechanisms behind the impacts of lionfish, by elucidating the way in which lionfish learn and the implications of habitat complexity and prey refuge on invasive species impacts. While lionfish have the potential to exert profound impacts, their ability to do so in the eastern Gulf of Mexico has yet to be determined. This thesis thus provides information on lionfish ecological impacts forecasted for this region.

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