

AN ABSTRACT OF THE DISSERTATION OF

Timothy J. Pusack for the degree of Doctor of Philosophy in Zoology presented on May 24, 2013.

Title: Coral-Reef Fishes: Insights Into Larval Dispersal and Invasion Ecology

Abstract approved:

Mark A. Hixon

Because many coral-reef fishes are observable *in situ*, are amenable to transplantation, have small home ranges and short generation times, they provide a excellent system to investigate many topics within general ecology, fisheries biology, and conservation biology. The primary goal of this dissertation was to use the coral-reef fishes system to investigate two pressing sets of issues that face marine ecologists and managers of living marine resources. The first topic is the spatial and temporal patterns of larval dispersal and reproductive success in a marine metapopulation (Chapter 2). Because miniscule larvae are difficult to track in the vast pelagic environment, little is known about the patterns of larval dispersal. Yet, the more that is understood about the spatial and temporal variability in larval dispersal, the easier it will be to identify sites that are self-sustaining and exporting larvae to unprotect sites, a common goal of marine reserves. Incorporating this information into siting of marine reserves will improve their effectiveness. The second topic is fundamental in the ecology of biological invasions: species specific interactions between an invasive predator and native species. Specifically, I investigated the ability of an invasive predator to disrupt

natural population regulation of a native prey species (Chapter 3), and the ability of a native predator to provide biotic resistance against the invasive species (Chapter 4). Because management of the lionfish invasion is needed throughout the Caribbean and Atlantic waters, it is necessary to understand not only how lionfish can change the native system, but also potential ways to moderate the negative effects.

To address the first topic, we collected a total of 3,278 genetic tissue samples from bicolor damselfish (*Stegastes partitus*) over a four year period from reefs near four islands that encompass Exuma Sound, Bahamas (Chapter 2). Using a Bayesian parentage analysis, eight parent-offspring pairs were detected, which directly documented both connectivity between and self-recruitment on an ecological time scale. Remarkably, some larvae returned to the exact same reef where they were spawned, while others traveled to sites greater than 100 km distance. The only study island without a detected parent-offspring pair, Lee Stocking Island, was also the island that showed the most restrictive gene flow on evolutionary time scales. Additionally, variability was documented in the spatial and temporal signatures of sweepstakes reproduction and Wahlund effects. The variation we observed may be influenced by seasonal mesoscale gyres present in Exuma Sound, which play a prominent role in shaping local oceanographic patterns. Understanding how to identify pathways of larval dispersal is important to designing networks of marine reserves, because a common goal of reserves is to protect populations that are self-seeding and can export larvae. Thus, this research not only demonstrates that temporal

variability is a prominent characteristic of larval dispersal, but provides an example of how to identify these populations.

To address the second topic, two studies using both lab observations and manipulative field experiments were conducted to study the interaction between invasive Indo-Pacific red lionfish (*Pterois volitans*) and two different native species. In the first study (Chapter 3) three different data sets were used to document the effect of lionfish predation on their top prey species, the bridled goby (*Coryphopterus glaucofraenum*). The first data set was extracted from three previous studies to compare the change in abundance of bridled goby between patch reefs with lionfish and patch reefs that were predator-free. The second data set came from laboratory feeding trials to test for the presence of a size refuge for bridled goby caused by lionfish gape limitation. The third data set came from a manipulative field experiment using 22 nearshore reefs where the per capita mortality of bridled gobies was compared among four orthogonal predator treatments: (1) predator-free control, (2) a single native predator only -- the graysby grouper (*Cephalopholis cruentatus*) -- representing the pre-invasion system, (3) a single lionfish only, and (4) one native grouper and one lionfish, representing the invaded system. The combined results from these three data sets demonstrated that lionfish can consistently consume a significant amount and an extremely high proportion of bridled goby on small patch reefs. While small lionfish cannot eat bridled goby larger than 0.42 times their body size, large lionfish eat virtually the entire size range of bridled goby. These findings indicate that lionfish have the potential to extirpate local goby populations.

In the second experiment, lionfish were exposed to different abundances of a native grouper, the Nassau grouper (*Epinephelus striatus*), on 28 nearshore patch reefs in the Bahamas. Lionfish persistence and growth was monitored over 10 weeks, as well as the abundance of small, < 5 cm total length (TL), native reef fishes. Nassau grouper did not affect lionfish persistence or growth. Yet, reefs with a lionfish and many Nassau grouper saw a significant increase in the abundance of small reef fishes compared to reefs with only lionfish or lionfish with few grouper. Thus, it appears that high densities of Nassau grouper provide some biotic resistance on Bahamian patch reefs by potentially interfering with lionfish predation on native fishes, perhaps by interrupting stalking behavior.

This dissertation highlights the flexibility of the coral-reef fishes system for investigation of broadly different topics in basic and applied ecology. Coral-reef fishes are a tractable system for both large and small scale studies, as well as laboratory observations and manipulative field experiments. The findings of this dissertation advance the understanding of metapopulation dynamics and have implications for fisheries management and marine reserve design. This research also documents further evidence of the negative effects of lionfish, yet provides some promising findings that may indicate some native biotic resistance to this devastating of marine invasions.

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Coral-reef fishes: Insights Into Larval Dispersal and Invasion Ecology

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Timothy J. Pusack

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Timothy J. Pusack, Author

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Chapter 4 was co-authored with Mark Albins and Alex Davis. Mark Albins provided two of the three datasets from which data on bridled gobies was extracted. Alex Davis took the lead on collecting data for the laboratory experiment and worked closely with me to analyze the data.

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DEDICATION

Grandpa George your spirit continues to do good deeds through me.

Coral-reef fishes: Insights Into Larval Dispersal and Invasion Ecology

1 – General Introduction

Tropical coral-reef fishes are the most diverse assemblage of marine vertebrates, spread globally among more than 100 families (Allen and Adrim 2003) and some 8000 species (Bellwood et al. 2011). Because they are mostly small and sedentary, live in an ecosystem accessible to SCUBA divers, and occur at tractable spatial and temporal scales for scientific studies, coral-reef fishes represent an excellent system to study a broad variety of ecological and conservation topics (Sale 1991, Hixon 2011). Coral-reef fishes are both highly observable and amenable to manipulative field experiments. Thus, over the past 60 years, many studies of coral-reef fishes have advanced our knowledge of a broad variety of topics, including behavioral ecology, fisheries biology, community ecology, mating systems, species coexistence, population dynamics and regulation, larval dispersal, and marine reserve design (reviewed by Sale 1991, Sale 2006, Hixon 2011).

This dissertation draws on a variety of advantageous characteristics of coral-reef fishes to explore two distinct topics in marine ecology that both expand our understanding of ecological processes and inform conservation and management efforts. *Topic 1* is the spatial and temporal pattern of larval dispersal, knowledge of which is essential for understanding marine metapopulation dynamics, understanding the degree to which populations are open or closed and which may act as source or sink populations (Krizter & Sale 2004). From an applied perspective, this information can inform fisheries management (Gell & Roberts 2003, Fogarty & Botsford 2007), as

well as enhance marine reserve design (Palumbi 2003, McCook et al. 2009). *Topic 2* is the specific interactions between an invasive marine predator and native species. This work advances our understanding of the role species interactions play in facilitating or inhibiting marine invasions, which are increasing at a tremendous rate (Ruiz et al. 2000, Rilov and Crooks 2008). Moreover, marine fish invasions, in particular, are rare and poorly understood (Helfman 2007, Semmens et al. 2004).

Topic 1: Spatial and temporal patterns of larval dispersal

Most marine organisms have a biphasic life cycle with a pelagic larval stage followed by a demersal juvenile and adult phase. Because the juveniles and adults remain relatively site attached, most movement among local populations occurs during the larval phase. Pelagic larvae can disperse over both short and long distances (Shanks 2009) as a result of interactions among a variety of biotic and abiotic factors, including ocean currents (Gilg and Hilbish 2003, Cowen & Sponaugle 2009, White *et al.* 2010), larval behavior (Leis and McCormick 2002, Gerlach et al. 2007), pelagic larval duration (Shanks 2009, Weersing and Toonen 2009), and physiological condition (Sponaugle and Grorud-Colvert 2006, Sponaugle et al. 2006, Grorud-Colvert and Sponaugle 2011). The combination of site attached adults with dispersive larvae results in a marine metapopulation: a group of otherwise isolated local populations that are linked through the exchange of pelagic larvae (Kritzer and Sale 2004). The larval link between geographically separated local populations is called

"connectivity" (Cowen et al. 2007). In contrast, larvae that return to their natal population undergo "self-recruitment." Documenting the amount of larval connectivity among and self-recruitment within local populations is vital to understanding metapopulation dynamics, yet until recently it has been very difficult to accomplish.

Traditionally, indirect genetic methods are used to infer dispersal based of differences in allele frequencies among populations, for example using allozymes or mitochondrial DNA, as well as metrics such as F_{st} , G_{st} , and Φ_{st} (Hellberg 2007, Purcell et al. 2009, Hedgecock 2007). Populations with greater differences in allele frequencies between them were considered to exchange fewer larvae, while populations with fewer differences between them were considered to exchange larvae more frequently. These early studies found low differentiation among marine populations, which led to the conclusion that marine populations were panmictic with high levels of connectivity and low levels of self-recruitment (Shulman 1998, Warner and Cowen 2002). However these metrics usually documented dispersal on long-term, evolutionary scales, and did not have the temporal resolution needed to document patterns of dispersal on shorter, ecologically relevant time scales. Recent advances in a variety of techniques, such as otolith microchemistry (Swearer *et al.* 1999), direct tagging of individuals (Jones 1999, Jones *et al.* 2005) and parentage analysis (Hedgecock 2007, Jones 2005, Christie 2010), have advanced our ability to document patterns of larval dispersal. Studies using these methods have found both self-

recruitment (Jones *et al.* 2005, Almany *et al.* 2007, Christie *et al.* 2010a) and larval connectivity (Planes *et al.* 2009, Christie *et al.* 2010b, Harrison *et al.* 2012).

However, most studies have focused on one or few cohorts within a small area.

Chapter 2, “Spatial and temporal patterns of dispersal in a coral-reef fish metapopulation: Larval connectivity without panmixia,” describes a large scale study from 2005 to 2008 that collected and analyzed genetic samples from bicolor damselfish (*Stegastes partitus*) from four sites encircling Exuma Sound, Bahamas. The pattern of larval dispersal on an ecological time scale was directly documented using parentage analysis, while dispersal on the evolutionary time scale was inferred using an indirect population genetic measures (F_{st}). Genetic variation, genetic diversity, and inbreeding coefficients were used to examine patterns of reproductive success. We conclude that the high variance in spatial and temporal patterns of larval dispersal and reproductive success refute the traditionally accepted understanding of panmictic marine populations.

Topic 2: interactions between an invasive predator and native species

Biological invasions are a significant component of anthropogenic global change (Nelson 2005, Vitousek *et al.* 1997). They threaten native biodiversity (Wilcove *et al.* 1998, D’antonio *et al.* 2001, Bax 2001), alter community and ecosystem structure (Crooks 2002, Walker & Steffen 1997, Grosholz 2002), and disrupt food web dynamics (Semmens *et al.* 2004) of ecosystems. The cost of

damages and control efforts are considerable (Mack et al. 2000, Molnar et al. 2008), exceeding \$120 billion in the United States alone (Pimentel et al. 2005). Because the number of biological invasions is increasing rapidly (Lafferty & Kuris 1996, Ruiz 2000), the ecological effects and economic cost are likely to increase as well.

While not all invasions have equally damaging effects (Gurevitch and Padilla 2006), invasive predators usually have detrimental effects of native ecosystems (Salo et al. 2007, Cox and Lima 2006, Davis 2011). Examples include the brown tree snake (*Boiga irregularis*) causing extinctions of endemic bird species in Guam (Fritts and Rodda 1998), small rodents doing the same on other Pacific islands (Blackburn 2005), and the Nile perch (*Lates niloticus*) extirpating native fishes in Lake Victoria (Witte et al. 2000, Aloo 2003). However, some have questioned whether the observed effects caused by invasive species are actually driven by concurrent degradation to the native ecosystem, such as habitat loss (Gurevitch and Padilla 2004, Didham et al. 2005, MacDougall and Turkington 2005). While many observational studies have surmised that invasive species cause damage (Gurevitch and Padilla 2004), manipulative field experiments provide the best tool to document the actual effects caused by an invasive species (Davis 2011).

As research on biological invasions has increased, with most studies focusing on terrestrial systems, research on marine invasions is still in its infancy (Grosholz 2002, Ruiz 2000, Rilov and Crooks 2008). Specifically, marine fish invasions are extremely rare and poorly understood (Helfman 2007, Semmens *et al.* 2004). Few

invasive marine fishes have established viable populations (Randall 1987, Semmens *et al.* 2004), although there are a few examples from Hawaii (Randall 1987, Dierking 2009) and the Mediterranean (Goren & Galil 2005). Yet, only a few controlled experimental studies examined the effects on native species.

Recently, Indo-Pacific lionfishes (*Pterois volitans* and *P. miles*), hereafter referred to as lionfish, have invaded Atlantic and Caribbean waters. The invasion apparently started with a small number of individuals (Hamner *et al.* 2007) in the early 1980's off the coast of Florida. Subsequently, lionfish populations spread rapidly up the eastern seaboard of the United States, throughout the Caribbean and Gulf of Mexico, and currently extend southward along the South America coastline (Schofield 2010). Lionfish can inhabit a wide variety of tropical to warm-temperate coastal habitats including coral and rocky reefs (Whitfield *et al.* 2007), seagrass beds (Claydon 2012), mangrove forests (Barbour *et al.* 2010, Claydon 2012), estuaries (Claydon 2012; Jud and Layman 2012), and mesophotic reefs (Lesser and Slattery 2011). Local densities of invasive lionfish in reef ecosystems can reach up to 400-650 fish ha⁻¹ (Green and Côté 2009, Frazer *et al.* 2011), which is substantially higher than the 2-80 lionfish ha⁻¹ that occurs in the native range (Fishelson 1997, Grubich *et al.* 2009, Kulbicki *et al.* 2012). Invasive lionfish also can reach larger maximum sizes, with a maximum of 47cm total length (TL) in the Atlantic vs. 37cm TL in the Pacific (Morris *et al.* 2012, FishBase), and are likely more efficient hunters than lionfish in their native Pacific range (Cure *et al.* 2012).

The ecological impact of invasive lionfish can be very large. Lionfish can reduce the abundance of native prey fish by up to 90% on patch reefs (Albins and Hixon 2008, Albins 2012), which is greater than the effect of a native grouper, which reduced abundance by 36% (Albins 2012). This predation is spread across at least 41 species of coral-reef fish (Albins and Hixon 2008, Morris and Akins 2009), including ecologically important herbivorous parrotfishes and cleaner goby species. These negative effects may include large-scale reefs, where Green et al. (2012) observed reductions in biomass of prey species concurrent with increases in lionfish densities. Because lionfish can have devastating direct effects on their native prey, they may also cause cascading shifts in the coral-reef benthic community (Albins and Hixon 2011). Invasive lionfish have therefore been identified as a top concern for global conservation (Sutherland et al. 2010). While research to date has focused on how lionfish affect community metrics of diversity and abundance, no work has examined how lionfish affect the abundance and persistence of individual species (Chapter 3) or whether a native species can provide biotic resistance (Elton 1958, Sax et al. 2007) to the invasion (Chapter 4).

Chapter 3, “Invasive Indo-Pacific red lionfish (*Pterois volitans*) may extirpate local populations of a native coral reef fish,” describes the effect of lionfish predation on a common prey item in the Bahamas, the bridled goby (*Coryphopterus glaucofraenum*) using three different data sets. The first data set was extracted from three previous experiments (Albins and Hixon 2008, Albins 2012, T. Pusack

unpublished manuscript). The second data set was obtained from laboratory feeding trials in which lionfish (ranging in size from 4.0 cm – 23.1 cm TL) were fed bridled gobies (ranging in size from 1 cm – 6 cm TL) to investigate whether a gape limitation in lionfish provides a size refuge for this goby. The third data set came from a manipulative field experiment that subjected a natural range of goby densities (4-36 fish/reef) to four orthogonal predator treatments: (1) predator-free control, (2) one native predator present (the graysby grouper *Cephalopholis cruentatus*), representing the pre-invasion system, (3) one lionfish present, and (4) one native grouper and one lionfish present, representing the invaded system. The per capita mortality of bridled goby was recorded and then compared among predator treatments and across goby densities to determine whether lionfish disrupted the density-dependent mortality previously documented for bridled goby (Forrester and Steele 2000, Steele and Forrester 2004). We found that mortality was density independent and close to 100% on reefs with a lionfish present.

Chapter 4, “Biotic resistance in the sea: native Nassau grouper (*Epinephelus striatus*) moderate predatory effects of invasive Pacific red lionfish (*Pterois volitans*) on Atlantic coral-reef fishes,” examined the potential for a native grouper to control lionfish populations and reduce the negative effects of lionfish predation on small reef fishes. While management of lionfish by direct removal appears effective on a small scale (Morris and Whitefield 2009, Albins and Hixon 2011, Frazer et al. 2012),

identifying a native source of biotic resistant and protecting the species involved represents an enhanced lionfish management strategy. Anecdotal reports indicate that native grouper (Maljkovic and Van Leeuwen 2008, Mumby et al. 2011) and sharks (Albins and Hixon 2011) can consume invasive lionfish. However, no experiment had explicitly examined the interaction between lionfish and native predators. Chapter 3 describes a manipulative field experiment that exposed lionfish to a range of Nassau grouper abundances using 28 isolated translocated patch and artificial reefs in the Bahamas. The experiment ran for 10 weeks, and lionfish growth and survival, as well as the abundances of prey-sized (< 5 cm TL) native coral-reef fishes, were monitored. I conclude that grouper do not affect the persistence and growth of lionfish, but reduce the effect of lionfish on prey-sized fishes.

This dissertation presents a variety of ways that coral-reef fishes can be used to advance our understanding of marine ecology. These fishes were the focus of both a large-scale, long term observational study and small-scale, manipulative field experiments. This research has not only advanced our understanding of marine metapopulation dynamics and invasion ecology, but also can inform the placement and spacing of marine reserves to account for yearly fluctuations in larval supply, and provide managers of the lionfish invasion a potential tool to mitigate the numerous negative effects of this worst of marine invaders.

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**2 – Spatial and temporal patterns of dispersal in a coral-reef fish
metapopulation: larval connectivity without panmixia**

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Molecular Ecology
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ABSTRACT

Many marine organisms can be transported hundreds of kilometers during their pelagic larval stage, yet little is known about spatial and temporal patterns of larval dispersal. Although population-genetic tools can sometimes infer net movement of larvae on an evolutionary time scale, large effective population sizes and high rates of gene-flow present serious challenges to documenting dispersal patterns over shorter, ecologically-relevant, time scales. Here, we address these challenges by combining both direct parentage analysis and indirect genetic analyses over a four year period to document patterns of larval dispersal in a common coral-reef fish. At four locations in Exuma Sound, Bahamas, including a marine reserve, we genotyped 3,278 bicolor damselfish (*Stegastes partitus*) collected over a four year period. Using Bayesian parentage analysis, we identified eight parent-offspring pairs that directly documented both self-recruitment and connectivity up to a distance of 129 km between local populations. The dispersal pattern was highly variable among years and locations. Additionally, we detected spatial and temporal variation in signatures of sweepstakes and Wahlund effects. The variation we observed may be influenced by seasonal mesoscale gyres present in the Exuma Sound, which play a prominent role in shaping local oceanographic patterns. We conclude that there is high variance in the reproductive success of adult damselfish, both spatially and temporally, which erodes an expected signal of panmixia. Incorporating this variance into marine

metapopulation theory will improve the effectiveness of marine reserves by accounting for fluctuations in larval supply.

2.1 INTRODUCTION

The vast majority of marine animals have a biphasic life cycle that includes a pelagic larval stage followed by comparatively sedentary juvenile and adult stages. One consequence of this ubiquitous life history strategy is that minuscule larvae have the potential to be transported across hundreds of kilometers from where they are spawned via ocean currents. Many ocean species therefore exist within marine metapopulations, where local populations of demersal juveniles and adults are connected by the exchange of planktonic larvae (Kritzer and Sale 2006). However, the pattern and magnitude of larval dispersal depends on a complex interplay among ocean currents (Gilg and Hilbish 2003, Cowen and Sponaugle 2009, White et al. 2010), larval behavior (Leis and McCormick 2002, Gerlach et al. 2007), pelagic larval duration (PLD) (Shanks 2009, Weersing and Toonen 2009), and physiological condition (Booth and Hixon 1999, Sponaugle and Grorud-Colvert 2006, Sponaugle et al. 2006, Grorud-Colvert and Sponaugle 2011). Variation in one or more of these factors can substantially affect both the spatial and temporal patterns of larval dispersal. Thus, to gain a greater understanding of where marine populations lie along the continuum between demographically open or closed, it is necessary to scale-up field studies both in size (Mora and Sale 2002) and duration (Selkoe 2006, Siegel et

al. 2008, Watson et al. 2012). Results from large-scale studies are critical for informing the design of marine reserve networks (Palumbi 2003, McCook et al. 2009), and enhancing fisheries management (Gell and Roberts 2003, Fogarty and Botsford 2007).

Relatively little is known about the spatial and temporal patterns of dispersal in marine environments because marine larvae are difficult to track through the vast ocean environment (Warner and Cowen 2002, Sale et al. 2005, Cowen et al. 2006). Furthermore, large effective population sizes coupled with moderate to high rates of gene flow typically found in marine species present challenges to traditional genetic methods used to document dispersal in terrestrial systems (Manel et al. 2005; Hedgecock et al. 2007). Recently, researchers have directly documented larval dispersal by employing techniques such as otolith microchemistry (Swearer et al. 1999, Miller and Shanks 2004), chemical tagging of individuals (Jones et al. 1999, Jones et al. 2005), and genetic parentage analysis (Jones et al. 2005, Planes 2009, Christie et al. 2010ab, Harrison et al. 2012, Saenz-Agudelo et al. 2012). Studies using these methods have documented both self-recruitment (Jones et al. 2005, Almany et al. 2007, Christie et al. 2010a), and larval connectivity (Planes et al. 2009, Christie et al. 2010b, Harrison et al. 2012). However, most studies have focused on one or relatively few cohorts within a small area.

In this study, we coupled both direct parentage analysis and indirect genetic methods to characterize patterns of larval dispersal over a four-year period in Exuma

Sound, Bahamas. We define larval connectivity as the exchange of larvae between local marine populations, and self-recruitment, as the return of pelagic larvae to their natal population (Warner and Cowen 2002). Exuma Sound is a semi-enclosed basin characterized by a hydrodynamically complex environment that is punctuated with vigorous, semi-permanent, mesoscale gyres (Colin 1995, Stockhausen and Lipcius 2001). The gyres may facilitate connectivity between islands located on opposite sides or ends of the sound, self-recruitment within an island, or may simply create high mortality for larvae via entrainment and subsequent advection from suitable habitat. Computer simulation studies of spiny lobster (*Panulirus argus*) dispersal and records of adult and recruit abundances in Exuma Sound found variable levels of larval supply at locations throughout the region, and computer simulations indicated that the vigorous mesoscale gyres influenced the pattern of larval dispersal (Lipcius et al. 1997, Stockhausen and Lipcius 2001).

Our objective was to quantify temporal and spatial patterns of larval dispersal of bicolor damselfish (*Stegastes partitus*) in Exuma Sound over four years at four widely separated islands bordering the basin. We addressed three questions: (1) How variable are the spatial and temporal patterns of connectivity and self-recruitment in Exuma Sound? (2) Given the high rates of gene-flow previously documented in bicolor damselfish (Purcell et al. 2009, Christie et al. 2010a), is there evidence of panmixia within Exuma Sound? (3) Are directly documented patterns of larval dispersal congruent with those inferred with indirect genetic methods? To answer

these questions, we coupled parentage analyses with calculations of heterozygosity, genetic diversity, and genetic differentiation. By collectively considering the results of these analyses, we found that, although there is larval connectivity between adjacent populations, evidence of self-recruitment, Wahlund effects (Wahlund 1928, Hedrick 2005b), and sweepstakes reproductive success (Hedgcock 1994a, b) strongly reject the null hypothesis of panmictic larval dispersal within this metapopulation.

2.2 METHODS

2.2.1 *Study site and species*

Approximately 500 km of continuous and shallow patch reefs encircle Exuma Sound, a semi-enclosed, deep ocean basin (>1500m deep) bordered by the Grand Bahamas Bank (<5m deep) to the west and north and the Atlantic Ocean to the east and south (Fig. 2.1). Circulation is relatively self-contained and consists of predominantly northwesterly along-shore currents ($\sim 20 \text{ cm sec}^{-1}$) (Colin 1995) that are punctuated by mesoscale gyres that last from 10 to 30 days each, and can reach a depth of 200m (Lipcius et al. 1997, Stockhausen and Lipcius 2001).

The bicolor damselfish is a common inhabitant of coral reefs in the Western Atlantic and Caribbean. Both males and females are highly site-attached and tend to stay within small (1-3m) home ranges (Myrberg 1972, Schmale 1981). Males are territorial and guard demersal nests. Spawning activity follows the lunar cycle and overall reproductive effort peaks during the summer months. Eggs hatch after ~ 3.5

days of benthic development (Schmale 1981, Johnson et al. 2010). Larvae develop in the plankton for 24 to 40 days (mean \approx 30d, Wellington and Victor 1989) before settling to suitable coral-reef habitat and recruiting to the population (Sponaugle and Cowen 1996). In Exuma Sound, bicolor damselfish mature at 6-7cm and live an average of 0.88 years, with a maximum recorded life span of 3.6 years (Hixon et al. 2012). Previous work on bicolor damselfish dispersal in this system directly documented self-recruitment through parentage analysis at two islands (Christie et al. 2010a). This study also provided evidence for sweepstakes reproductive success, whereby the highly variable reproductive success of adults results in smaller genetic variation and diversity in each cohort (Hedgecock 1994a, b, Hedgecock and Pudovkin 2011).

2.2.2 Sample collection

From 2005 to 2008, we collected tissue samples from 3,278 bicolor damselfish. We focused our collection efforts on three reefs adjacent to each of four islands encompassing Exuma Sound: Cat Island, Eleuthera, Exuma Cays Land and Sea Park (a cluster of islands), and Lee Stocking Island (Fig. 2.1). In 2005, samples were collected during a single summer visit at each of the four islands (Christie et al. 2010a). From 2006 to 2008, we collected adult and recruit samples at each of the four islands in June, July, and August to correspond with the mean pelagic larval duration and subsequent lunar settlement period of this species. Because of the interval

between visits, and by restricting our recruit collections to within 20 mm of settlement size, it is reasonable to assume that all of the sampled recruits in each month belonged to the same cohort (Sponaugle and Cowen 1996). Adults were tagged with injections of colored elastomer (Northwest Marine Technologies) as part of a concurrent demographic study (Hixon et al. 2012) and to ensure that we did not collect their tissue twice.

While on SCUBA we anaesthetized damselfish with a solution of 10% quinaldine and 90% methanol mixed with equal amounts of seawater to facilitate collection with hand nets. We clipped a small portion of the pelvic fins from adults (> 5cm total length [TL], n = 1,348) before releasing them at the collection site. Recently settled recruits (<3 cm TL, n = 1,930) were euthanized and their caudal fins were preserved for subsequent analysis. All tissue samples were stored in a urea-based solution (10mM Tris, 125 mM NaCl, 10 mM EDTA, 1% SDS, 8 M urea, pH adjusted to 7.5 with HCL). The numbers of adults sampled varied by island and year based on the number of new adults that established territories in each of the monitored reefs. We haphazardly collected approximately 50 recruit tissue samples at each monthly visit to each island. Thus, we collected approximately 150 recruits from each island per summer (Table 2.1).

2.2.3 DNA extraction and microsatellite typing

DNA was extracted using a protocol optimized for the urea-based storage solution. Tissue samples were incubated in 300 μ l of an extraction buffer (75mM NaCl, 25 mM EDTA, 1% SDS) with Protienase K (2 μ L of 20 mg/mL) at 55°C for two hours. After the incubation period, 150 μ l of ammonium acetate (7.5 M) was added. The resulting solution was held at -25° C for 20 minutes, centrifuged at 13,000 rpm for 15 mins, and the supernatant was washed using isopropanol and ethanol washes to precipitate the genomic DNA (Sambrook and Russell 2001, see Christie et al. 2010a for details). Samples from 2005 (n=385) were previously extracted and genotyped at seven microsatellite loci (Christie et al. 2010), and because adults sampled in 2006 from Lee Stocking Island (n=104) were needed for quantitative genetic studies (Johnson et al. 2010, Johnson et al. 2011), they were also genotyped at the same seven microsatellite loci: SpAAC33, SpAAC 41, SpAAC42, SpAAC47, SpAAT40, SpGATA16, SpGATA40 (Williams et al. 2003). The remaining 2,839 samples were genotyped at the above seven loci and three additional microsatellite loci: SpTG10, SpTG16, and SpGGA7 (Thiessen and Heath 2007). PCR reactions were optimized and multiplexed with the Qiagen Multiplex PCR Kit and contained 5.0 μ L of Qiagen Multiplex mix, 1.0 μ L of each primer, and 2.0 μ L of approximately 100 ng/ μ L DNA template for a total volume of 10 μ L. The thermocycle profile consisted of an initial denaturing step at 95° C for 15 minutes, followed by 30 cycles of 30 sec at 94° C, 90 sec at 58° C, and 60 sec at 72° C. PCR products were screened on an ABI 3100 automated sequencer (Applied Biosystems) at the Center for Genome Research and

Biocomputing at Oregon State University. We used GENOTYPER 3.7 to determine allele sizes. We reprocessed 22.3% of the individuals (from extraction through scoring) to check for errors in genotyping and to check individuals that were difficult to score at the most polymorphic loci.

2.2.4 Parentage analyses

To directly document dispersal on an ecological time scale, we used a Bayesian parentage analysis (Christie 2010) to identify parent-offspring pairs. Briefly, this method calculates a prior probability from the data to determine the posterior probability of any given pair-wise comparison sharing alleles by chance. For each putative pair, the prior is combined with the frequencies of shared alleles to calculate the posterior probability of each putative pair occurring by chance. Pairs that were unlikely to occur through chance (i.e., posterior probability < 0.05) were considered to be parent-offspring pairs. All putative pairs were re-analyzed from DNA extraction through scoring at all loci to minimize the possibility of laboratory errors. Simulations, required for the calculation of the posterior probability, were conducted as recommended with 10,000 false pairs generated from over 1,000 null data sets (Christie 2010). None of the identified parent-offspring pairs had missing data. The possibility of pairs actually being some other first-order relative (i.e., full siblings) was eliminated by calculating the probability of simulated full sibs sharing an allele at all loci.

Dispersal distances between identified parents and offspring were determined using by both Euclidean distance and along-shore distance - the nearest along-shore path between the adult reef and the recruit reef following the fore-reef contour at a resolution of 1 km. Because bicolor damselfish do not move more than a few meters after settling to reef habitat (Hixon et al. 2012), the Euclidian distances between parents and offspring represent the minimum dispersal distances of planktonic larvae (i.e., the actual distances traveled by dispersing larvae may have been much greater if they did not follow a direct path).

2.2.5 Population genetic analyses

GENEPOP v. 4.0.11 (Raymond and Rousset 1995, Rousset 2008) was first employed to determine whether any locus-pairs were in linkage disequilibrium, and to test for departures from Hardy-Weinberg equilibrium (HWE) for adult and recruit samples collected each year at each island. For each measure, a total of 10,000 batches and 5,000 iterations per batch were used to reduce the standard errors below 0.01. We tested for large allele drop-out, null alleles, and stuttering that could explain deviations from HWE using MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004). We used ARLEQUIN v3.11 (Excoffier et al. 2005) to calculate observed and expected heterozygosities and FSTAT v2.9.3.2 (Goudet 2001) to calculate F_{is} values, mean number of alleles per locus, mean allelic richness, and gene diversity. To test for population differentiation, we used a variety of metrics based on allele frequencies in

each population. We first used FSTAT to calculate pairwise F_{st} , which infers gene flow, and thus larval dispersal, on an evolutionary time scale, and we adjusted the corresponding p-values for multiple comparisons with a sequential Bonferroni procedure ($\alpha = 0.05$). We also used randomization tests within FSTAT (5,000 permutations) to compare F_{st} values between recruits pooled by year. For comparisons between groups with ten and seven loci, only the matching seven loci were used. Because highly polymorphic loci can artificially reduce F_{st} values (Hedrick 2005a, Meirmans and Hedrick 2011), we used SMOGD v1.2.5 (Crawford 2010) to calculate pairwise D_{est} values (Jost 2008) and GENODRIVE (Meirmans and Van Tienderen 2004) to calculate standardized versions similar to F_{st} including multilocus G'_{st} (Hedrick 2005a) and pairwise population G''_{st} (Meirmans and Hedrick 2011).

Evidence for population structure can also be found by documenting the Wahlund effect, whereby an excess of homozygotes is produced by an admixture of genetically differentiated populations (Wahlund 1928, Hedrick 2005b). If this homozygous excess is due to the presence of a Wahlund effect, then the number of loci with a homozygous excess averaged across all cohorts will be lower than the pooled estimate for both groups of samples if the pooled sample includes recruits from different populations. To document evidence of a temporal Wahlund effect we compared the average number of loci that deviate from HWE for recruits collected by month at each island (i.e., each cohort of fish) to the pooled population of all recruits sampled at each island. To test for a spatial Wahlund effect in each month, we

compared the average number of loci that deviated from HWE per month at each island to the number of loci that deviated from HWE cohorts pooled across islands by month.

2.2.6 Sweepstakes reproductive success

Despite large population sizes and high fecundity of many marine species, genetic diversity is often reduced within a cohort, because only a small proportion of adults produce larvae that survive through their pelagic larval phase (Hedgecock and Pudovkin 2011). Hedgecock (1994a, b) called this phenomenon “sweepstakes reproductive success” and it has implications for population persistence as well as understanding patterns of larval dispersal. Hallmark characteristics of sweepstakes reproduction include genetically different cohorts, and decreased genetic variation and genetic diversity when recruits are compared to adult populations (Hedgecock 1994a, b, Hedgecock and Pudovkin 2011). To test for genetic differences among each cohort, we used a randomized test (5,000 permutations) in FSTAT to compare F_{st} among pooled recruit samples by islands and years. We also used randomized tests (5,000 permutations) in FSTAT to test for differences in genetic variation (observed heterozygosity), genetic diversity (expected heterozygosity and allelic richness [k]), and F_{is} between pooled recruit cohorts and pooled yearly adult populations.

2.3 RESULTS

2.3.1 Parentage analysis

We detected eight parent-offspring pairs, two during each year from 2005-2008. We documented five pairs that exhibited self-recruitment within an island (two of which were previously reported by Christie et al. 2010a) and three pairs that documented connectivity between islands (Fig. 2.2, Table 2.2). Remarkably, in three of the five self-recruitment events, the recruit fish returned to the exact same reef within the island from which it originated (Table 2.2). For the other two self-recruitment events, the recruits returned to a nearby reef at the same island (< 5 km away). The longest dispersal distance detected was 111.2 km in Euclidean distance, or 129.4 km in along-shore distance (Table 2.2). Only one of the eight offspring matched with a parent was detected in the early summer (June), four were detected in July, and three in August.

2.3.2 Population-level analyses

Across all adult and recruit populations grouped by yearly collection at each island, the mean number of alleles per population over all 10 loci ranged from 19 to 29. The mean allelic richness per population over all loci, calculated from a minimum sample size of 43 for adults and 37 for recruits, ranged from 17 to 19 alleles. Observed heterozygosity ranged from 0.758 to 0.916 (Table 2.1). Based on the MICRO-CHECKER analysis for deviations from HWE, homozygote excess appeared

to be the cause of the deviation. There was no evidence for large allele drop-out, and we detected low average numbers of null alleles in our 10 loci: 2.4 (0.22 standard error [SE]) in adult populations and 2.8 (0.18 SE) in recruit populations. We suspect that a large proportion of homozygous genotypes are from population admixture (i.e. a Wahlund effect). We found evidence for both a temporal and a spatial Wahlund effect (Fig. 2.3ab). To examine the evidence for a temporal Wahlund effect, we compared that average number of loci that deviated from HWE for each recruit cohort at each island to the pooled estimate across all years at that island. We found that the average number of loci for each recruit cohort that was out of HWE due to a homozygous excess was less than the pooled estimate of all recruits (Fig. 2.3a). Similarly, when we compared the average number of loci that deviated from HWE across all islands within months during each year to the pooled sample, we found that the averages of recruit cohorts across islands were lower than the pooled sample (Fig. 2.3b), indicating a spatial Wahlund effect.

Global F_{st} calculated when grouping by adults and recruits at each island for each year, global was low and not significantly different from zero (95% confidence interval: 0.000-0.001). Pairwise comparisons of F_{st} revealed low genetic differentiation across all years and islands for both adult and recruit populations, ranging from -0.0032 to 0.0046 (Table A.1, Appendices). The low F_{st} values most likely were not an artifact of the highly polymorphic loci we studied because pairwise comparisons using three other population differentiation metrics were also very low:

G'_{st} ranged from -0.001 to 0.005, G''_{st} ranged from -0.045 to 0.065, and D_{est} ranged from -0.016 to 0.057. Nevertheless, after correcting for multiple comparisons, there were 21 significant F_{st} pairwise comparisons of adult populations out of a possible of 120 (17.5%). The majority of those significant comparisons (86%) involved Lee Stocking Island (Table A1, Appendices). Comparisons of adult to recruit populations revealed 20.3% of the pairwise comparisons of F_{st} values to be significant (Table A.2, Appendices). Pairwise comparisons among all recruit samples found 22 out of 120 pairwise comparisons were significant (18.3%) at an alpha equal to 0.05 after a sequential Bonferroni correction and Lee Stocking Island was included in 68% of them (Table A1, Appendices). A randomization test of global F_{st} when recruits were pooled by years showed patterns of significant differentiation of allele frequencies for the following sets of recruits ($p=0.023$): 2005 vs. 2006 ($p=0.0056$) and 2008 ($p=0.0146$); 2006 vs. 2007 ($p=0.0034$); and 2007 vs. 2008 ($p=0.0252$).

2.3.3 Sweepstakes reproductive success

As predicted by the sweepstakes hypothesis, we detected evidence for lower genetic variance and diversity in recruit populations compared to adult populations. The randomization tests revealed lower observed heterozygosity ($p=0.016$) and expected heterozygosity ($p=0.019$) in recruits compared to adults (Fig. 2.4ab). Also the numbers of alleles present in recruit populations were lower than adult populations ($p=0.032$; Fig. 2.4c). We also documented a positive and significantly higher F_{is}

($p=0.034$) in recruit populations compared to adult populations. Samples containing large numbers of siblings from a single population often show negative F_{IS} (Rasmussen 1979, Balloux & Lugon-Moulin 2002). Thus, it is not clear why we observed higher F_{IS} in the recruit samples. Perhaps we are observing cohorts that are comprised of a small number of families, but that originate from different geographic sources, i.e. sweepstakes reproduction.

2.4 DISCUSSION

We documented direct evidence of both connectivity and self-recruitment in a metapopulation of bicolor damselfish. Given the lack of genetic differentiation previously documented in this system (Christie et al. 2010a), we expected that the pattern of dispersal observed from our parentage analysis would be consistent with a null hypothesis of panmixia. However, if bicolor damselfish within Exuma Sound were truly panmictic, we should observe two phenomena. First, the expected number of parent-offspring pairs would be quite low. The population size of bicolors within the sound is high and because we sampled a small amount of the available habitat, the number of adults we genotyped is comparatively low. Thus, we would expect that in a well-mixed population, the overwhelming majority of the recruits we sampled would have come from parents outside of our study areas (i.e., parents we did not genotype). Second, if the population were truly panmictic, then we expect that of the parent-offspring pairs we did detect, the probability of originating from each of the study

reefs would be equal. Under this hypothesis, we would expect populations at each island to contribute to a randomly mixed pool of larvae that would supply all islands with recruits. Because we sampled at four islands, we would expect to document roughly three times more instances of connectivity than self-recruitment (four self-recruitment pathways vs. twelve connectivity pathways). However, our parentage results rejected a null hypothesis of panmixia in that (1) there was more self-recruitment than expected, almost double the amount of connectivity, and (2) patterns of self-recruitment and connectivity were not evenly distributed. In particular, we detected no parent-offspring relationships involving samples from Lee Stocking Island, one of our four study sites. Relevant to management, we found that a long-established marine reserve, the Exuma Cays Land and Sea Park, not only was self-seeding (i.e., self-recruitment was documented twice at this island) but also generated larvae that seeded unprotected sites. While Lipcius et al. (2001) had inferred indirectly that this marine reserve is capable of both self-recruitment and exporting larvae to other islands, we provide the first direct evidence.

We found similar patterns of larval dispersal in our indirect analysis compared to the parentage analysis: the greatest number of significantly different pairwise comparison of population structure was found at Lee Stocking Island, where no parent-offspring pairs were documented. The temporal stability of genetic differences involving Lee Stocking Island means that these differences were most likely not due to sampling error (Waples 1998). We also found a portion of low, yet significant,

pairwise F_{st} comparisons in our populations which had been documented in bicolor damselfish populations from other regions (e.g., Lacson 1992, Ospina-Guerrero et al. 2008, Hepburn et al. 2009, Hogan et al. 2010, Salas et al. 2010), and across the entire Caribbean region (Purcell et al. 2009). The fact that one site, Lee Stocking Island, had the majority of genetic difference compared to itself as well as other islands, indicates that dispersal is not equal among all sites, also rejects our null hypothesis of panmixia.

Additional evidence to reject our null hypothesis of panmixia includes evidence of the sweepstakes reproductive success, as well as the temporal and spatial Wahlund effects. Our findings concur with previous work by Christie et al. (2010a) that provided suggestive evidence of sweepstakes reproductive success. Given the vast size of the populations and the number of potential parents that may contribute to each cohort, the relative differences of genetic variation, gene diversity (measured as expected heterozygosity), and allelic richness provide further evidence that sweepstakes reproduction may occur in this system. Because evidence of the sweepstakes reproduction in bicolor damselfish was also found from studies in the Mesoamerican Barrier Reef System (Hepburn et al. 2009, Villegas-Sanchez et al. 2010), this effect may occur throughout the geographic range of this species.

Region-wide genetic homogeneity may be eroded by the predominant mesoscale gyres that commonly occur in Exuma Sound. Gyres may promote connectivity or self-recruitment, but they may also entrain larvae causing high levels of larval mortality. At islands where offshore gyres have been previously observed --

Exuma Cays Land and Sea Park, Eleuthera, and Cat Island -- we detected self-recruitment, whereas no parent-offspring pairs were observed at Lee Stocking Island, where gyres are less prevalent (Stockhausen and Lipcius 2001). Moreover, seasonal and annual fluctuations in location, duration, and strength of the gyres may further affect patterns of successful larval dispersal. Such variation may in turn affect the intensity of sweepstakes effects (Hedgecock and Pudovkin 2011). The other hydrodynamic feature of Exuma Sound, the prevailing northwesterly Antilles Current, could have transported larvae from Cat Island to Eleuthera, and when this current reverses direction, which can happen for short periods of time (Colin 1995), the opposite pattern may have occurred. This possibility was corroborated by the fact that we detected larval connectivity in both directions between these islands.

Our study documents the complex nature of larval dispersal in a coral reef fish both temporally and spatially, and highlights the importance of using multiyear data sets at multiple locations to examine the consistency of dispersal patterns. Despite observing high levels of gene flow on an evolutionary time scale and documenting connectivity on an ecological time scale, we did not observe panmixia among all of our sampled islands. The next steps towards understanding the details of marine metapopulation dynamics include quantifying larval retention and linking these genetic findings with local demographic and oceanographic data. Synthesis of these different fields of study will greatly advance our understanding of marine

metapopulation dynamics and enhance fisheries management and conservation efforts by identifying the spatial boundaries of population dynamic processes.

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Table 2.1 Summary statistics of local populations of bicolor damselfish. All adult and recruit populations from 2005, plus LSI adult populations from 2006 and 2007 were genotyped at 7 microsatellite loci, whereas the remaining individuals were genotyped at 10 microsatellite loci. ECLSP = Exuma Cays Land and Sea Park; LSI = Lee Stocking Island. N = sample size. k = mean allelic richness. Fis = Wright's inbreeding coefficient. Ho = observed heterozygosity. He = expected heterozygosity. HWE = the number of loci that deviate from Hardy-Weinberg Equilibrium/total number of loci. %LD = percentage of loci in linkage disequilibrium.

Adults									
Island	Year	N	Mean# alleles/locus	k	Fis	Ho	He	HWE	% LD
Cat	2005	46	23	19	0.102	0.834	0.928	3/7	0
Cat	2006	115	26	18	0.103	0.803	0.895	3/10	0
Cat	2007	123	24	18	0.088	0.814	0.893	3/10	0
Cat	2008	147	26	18	0.076	0.823	0.890	3/10	0
ECLSP	2005	47	22	19	0.060	0.874	0.928	2/7	0
ECLSP	2006	63	20	17	0.104	0.798	0.891	2/10	0
ECLSP	2007	59	21	18	0.097	0.811	0.897	2/10	0
ECLSP	2008	64	22	18	0.011	0.789	0.889	2/10	0
Eleuthera	2005	49	22	19	0.052	0.887	0.936	0/7	0
Eleuthera	2006	43	20	18	0.930	0.809	0.891	2/10	0
Eleuthera	2007	107	24	18	0.091	0.810	0.890	2/10	0
Eleuthera	2008	105	25	18	0.115	0.790	0.892	2/10	0
LSI	2005	43	20	18	0.036	0.899	0.932	0/7	0
LSI	2006	104	28	19	0.120	0.856	0.932	3/7	0
LSI	2007	108	19	18	0.091	0.817	0.893	2/10	0
LSI	2008	125	26	18	0.104	0.799	0.892	3/10	0
Recruits									
Island	Year	N	Mean# alleles/locus	Mean AR	Fis	Ho	He	HWE	% LD
Cat	2005	44	22	18	0.090	0.843	0.925	2/7	0
Cat	2006	152	29	18	0.104	0.801	0.894	5/10	0
Cat	2007	155	26	18	0.104	0.799	0.892	6/10	0
Cat	2008	147	26	18	0.110	0.794	0.893	3/10	0
ECLSP	2005	45	22	18	0.014	0.916	0.929	1/7	0
ECLSP	2006	108	25	18	0.091	0.812	0.894	3/10	0
ECLSP	2007	115	25	18	0.094	0.810	0.894	2/10	0
ECLSP	2008	129	24	18	0.107	0.796	0.890	2/10	0
Eleuthera	2005	37	21	19	0.049	0.883	0.927	1/7	4.4

Table 2.1 continued

Island	Year	N	Mean# alleles/locus	Mean AR	Fis	Ho	He	HWE	% LD
Eleuthera	2006	163	26	18	0.077	0.823	0.892	2/10	0
Eleuthera	2007	145	26	18	0.110	0.791	0.888	4/10	0
Eleuthera	2008	142	26	18	0.121	0.784	0.891	5/10	0
LSI	2005	74	25	19	0.081	0.858	0.933	3/7	0
LSI	2006	162	27	18	0.104	0.804	0.897	3/10	0
LSI	2007	162	28	18	0.124	0.781	0.891	5/10	0
LSI	2008	150	25	18	0.147	0.758	0.888	5/10	0

Table 2.2 Euclidean and along-shore distance between members of each parent-offspring pair of bicolor damselfish. Along-shore distance was measured by tracing the contour of the 20m isobaths with a resolution of 1km. ECLSP = Exuma Cays Land and Sea Park. The “p-value” is the posterior probability of the pair being false given the shared allele frequencies (Christie 2010). Notice that three offspring returned to precisely the same reef inhabited by their parents (distance = 0).

Adult Island (spawning island)	Offspring Island (recruitment island)	Distance (km)				
		Year	Month	Euclidean	Along Shore	p-value
Eleuthera	Eleuthera	2005	July	0	0	0.0360
ECLSP	ECLSP	2005	July	0	0	0.0110
Cat	Cat	2006	August	0	0	0.0116
ECLSP	Eleuthera	2006	August	61.1	129.4	0.0433
Cat	Cat	2007	June	3.1	3.2	0.0259
Eleuthera	Cat	2007	August	111.2	129.0	0.0377
Cat	Eleuthera	2008	July	111.2	129.0	0.0332
ECLSP	ECLSP	2008	July	4.4	4.7	0.0136

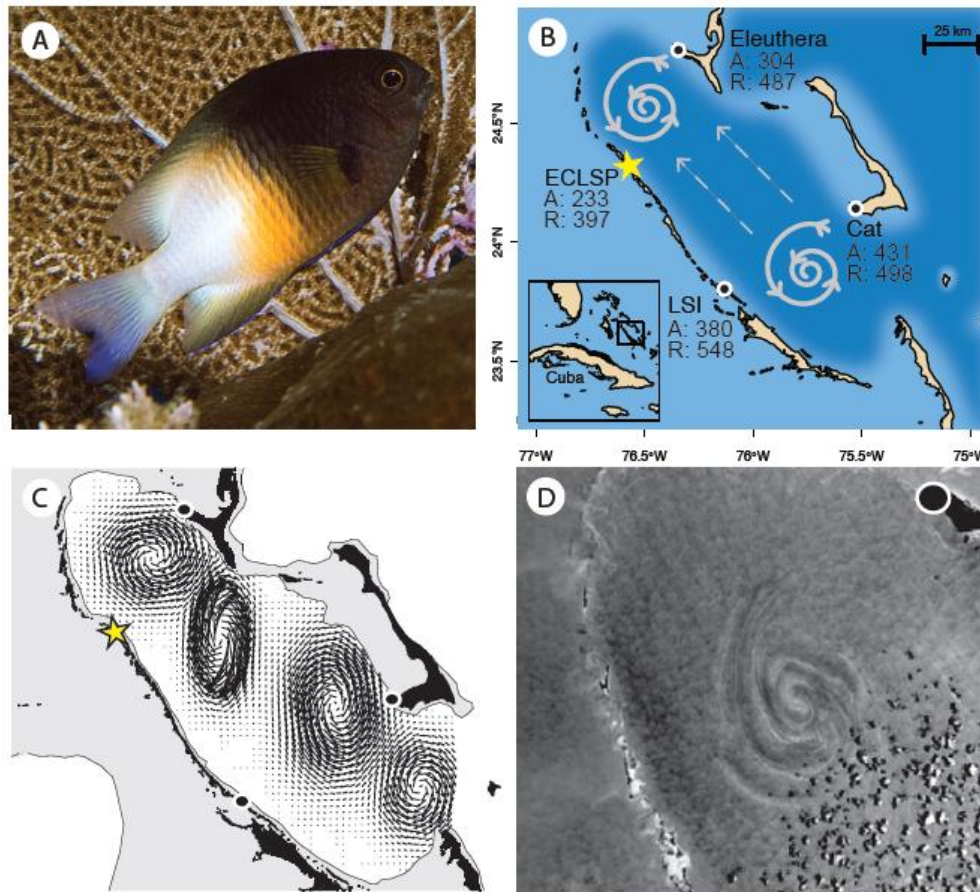


Figure 1.2 (A) Adult bicolor damselfish, *Stegastes partitus* (photo courtesy of David Nasser). (B) Map of Exuma Sound, with sampled islands and corresponding adult (A) and recruit (R) sample sizes. The arrows indicate the predominant currents in the Sound, including seasonal mesoscale gyres (thick, grey swirls) and the prevailing northwesterly Antilles Current (thin, grey, dashed arrows). The yellow star represents a marine reserve study area and black circles are currently unprotected study areas. ECLSP = Exuma Cays Land and Sea Park; LSI = Lee Stocking Island. (C) Near-surface geostrophic currents in Exuma Sound computed from conductivity, temperature, and depth (CTD) observations (Stockhausen and Lipcius 2001). Notice that the gyres are closer to ECLSP, Eleuthera and Cat Island than to LSI. (D) Satellite image of one of the northern seasonal gyres adjacent to Eleuthera (image courtesy of Larry Ammann, University of Texas).

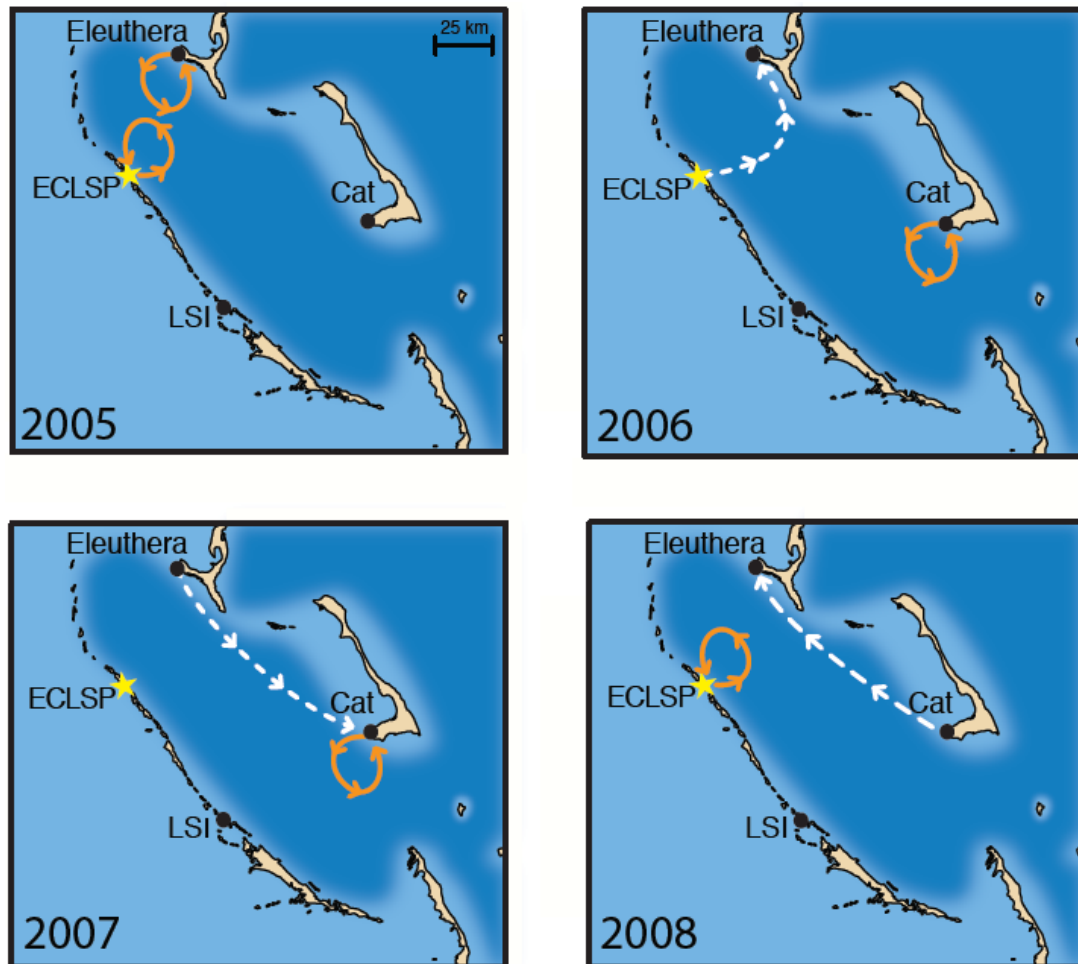


Figure 2.2 Parent-offspring pairs of bicolor damselfish documented in each of 4 years. White, dashed arrows indicate larval connectivity and orange solid arrows show self-recruitment. The yellow star is a marine reserve study area, and black circles are unprotected study areas. ECLSP = Exuma Cays Land and Sea Park; LSI = Lee Stocking Island. Notice that we detected two parent-offspring pairs in each year and documented self-recruitment events at ECLSP and Cat Island. No parents-offspring combinations were ever detected to originate from or arrive at LSI.

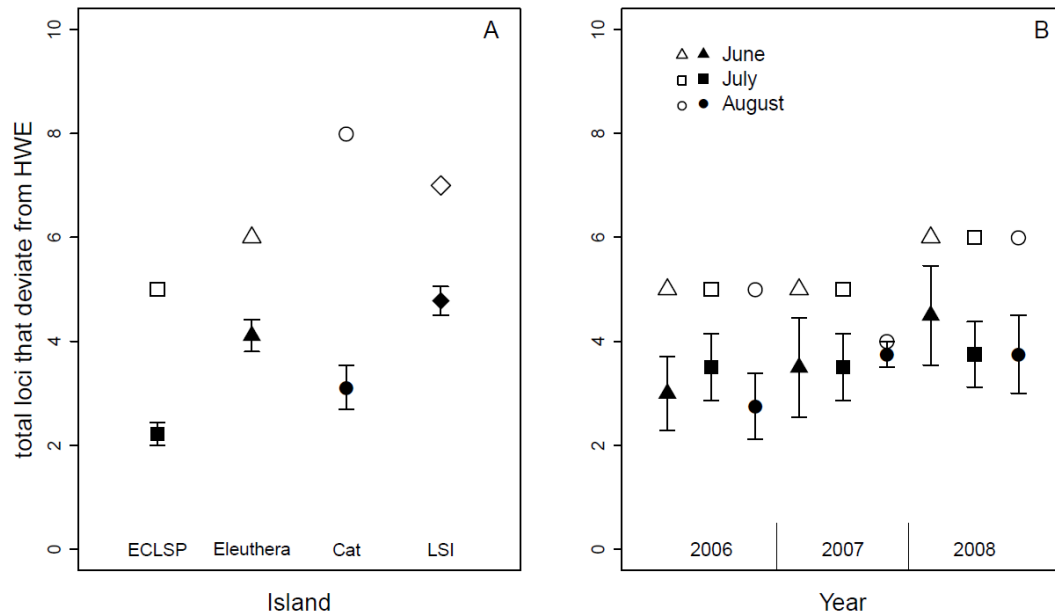


Figure 2.3. Temporal and spatial Wahlund effects. Each solid symbol is the average number of loci per cohort (i.e., monthly recruit collections) at each island that deviate from HWE at one island (± 1 SEM). Each open symbol is the number of loci that deviate from HWE when all cohorts are pooled. (A) Temporal Wahlund effect where open symbols are from pooled recruit populations and solid symbols are averages from each recruit cohort. Notice that the averages for all cohorts at each island do not overlap with the respective pooled estimates at each island. This pattern indicates that cohorts are coming from many different sources over time, and each cohort comes from fewer source “populations”. ECLSP = Exuma Cays Land and Sea Park; LSI = Lee Stocking Island. (B) Spatial Wahlund effect where open symbols are from pooled recruit populations and solid symbols are averages from each recruit cohort. Notice that all averages within each month across all years are lower than the pooled estimate for that particular month. This pattern indicates that during each monthly recruitment event, each island received larvae from different sources.

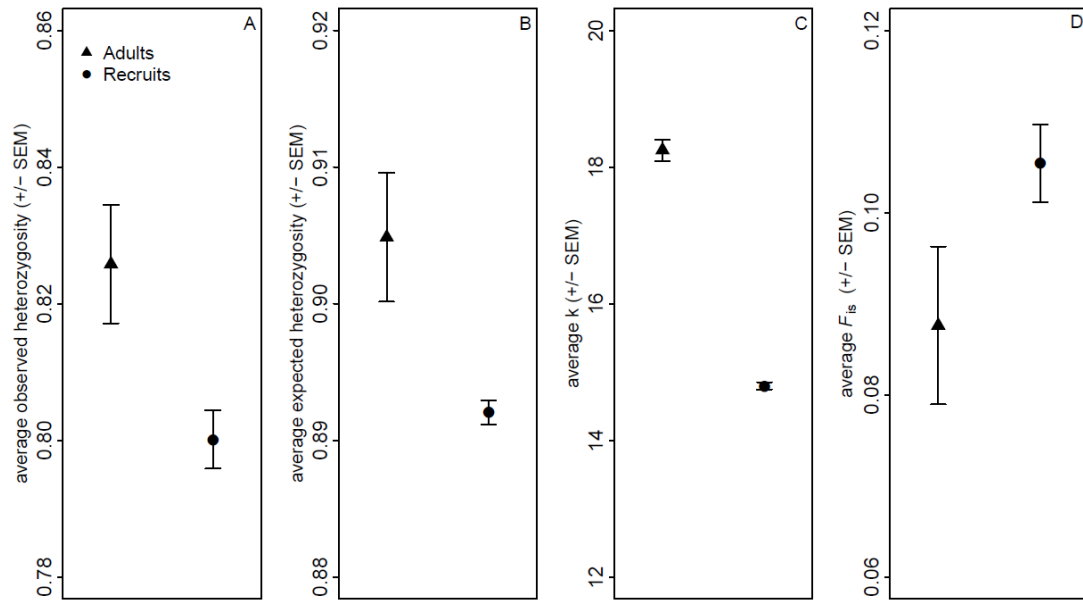


Figure 2.4 Comparisons of the average heterozygosity, gene diversity, and F_{is} between adult (triangles) and recruit (circles) bicolor damselfish. This pattern of lower heterozygosity and gene diversity and higher F_{is} in recruit compared to adult populations is suggestive of sweepstakes effects.

**Chapter 3 – Invasive Indo-Pacific red lionfish (*Pterois volitans*) may cause local
extirpation of a native coral reef fish**

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Biological Invasions

In Prep

ABSTRACT

Invasive Indo-Pacific red lionfish (*Pterois volitans*) are highly effective predators that severely threaten coral-reef fish populations in the Caribbean and tropical to warm-temperate western Atlantic. Because lionfish have higher densities and feeding rates than ecologically similar native piscivores, they have the potential to elevate the per capita mortality rates of native prey. The bridled goby (*Coryphopterus glaucofraenum*) is a major prey of invasive lionfish, and local populations had been previously documented to be regulated by density-dependent mortality caused by native predators. While it was known that lionfish reduce the abundance of bridled goby, it was unclear whether or not mortality due to lionfish predation disrupts natural mechanisms of population regulation in this species, potentially leading to local extirpation. In the field, we investigated changes in the local abundance of bridled goby in (1) unmanipulated populations and (2) manipulated populations that started at different initial densities in the presence vs. the absence of lionfish. In the lab, we also tested for the presence of a size refuge in bridled goby caused by lionfish gape limitation. On Bahamian patch reefs measuring approximately 3.5 m^2 , we found that a single lionfish significantly reduced the abundance of bridled goby consistently across years by an average of 3 fish per m^2 of reef over five weeks and 11 fish per m^2 of reef over nine weeks. Lab feeding trials documented that juvenile lionfish less than 14 cm are restricted to a 0.42 ratio of prey size to lionfish size, but large lionfish can consume almost the full size range of bridled goby. We also found that lionfish in the

field elevate the per capita mortality of bridled goby in a density-independent way to nearly 100%, which is about double the effect size of an ecologically similar native predator, the graysby grouper (*Cephalopholis cruentatus*). Thus, lionfish not only cause large reductions in the abundance of native prey, but also cause sufficiently high mortality to possibly extirpate local populations.

3.1 INTRODUCTION

Biological invasions are considered one of the main drivers of anthropogenic global change (Nelson 2005, Vitousek et al. 1997), and can cause considerable ecological and economic damage (Mack et al. 2000, Pimentel 2005, Molnar et al. 2008). Invasive species can threaten native biodiversity (Wilcove et al. 1998, D'antonio et al. 2001, Bax 2001), alter community and ecosystem structure (Crooks 2002, Walker & Steffen 1997, Grosholz 2002), and affect food web dynamics (Semmens et al. 2004) of invaded ecosystems. While not all invasive species cause negative effects on the native community (Gurevitch and Padilla 2004, Davis 2011), invasive predators usually have devastating impacts. For example, the brown tree snake (*Boiga irregularis*) drove endemic birds extinct in Guam (Fritts and Rodda 1998), small rodents extirpated native birds on Pacific islands (Blackburn 2005), and the Nile perch (*Lates niloticus*) caused native fish extinctions in Lake Victoria (Witte et al. 2000, Aloo 2003)

For an invasive predator to extirpate a native species, they must disrupt the compensatory processes that contribute to population regulation. Necessary but not sufficient for population regulation is demographic density dependence, which is characterized by a negative relationship between population density and per capita gain rates (birth or immigration) or a positive relationship between population density and per capita loss rates (death or emigration) (Murdoch 1994, Turchin 1995). With the appropriate type and intensity of density dependence, population size will not grow unchecked or decline to zero, but rather will be maintained within some bounded range (Hixon et al. 2002). Conversely, disruption of density-dependent processes may lead to extinction of previously regulated local populations. While any demographic rate that affects population growth can be density-dependent, mortality due to predation is a common mechanism that causes density dependence in coral-reef and other fishes (Sinclair and Pech 1996, Hixon et al. 2002). Predators can potentially cause density-dependent mortality through an aggregative response (Hassell and May 1974), a type III functional response (Holling 1959, Murdoch 1969), a numerical response (Holling 1959), and a developmental response (Murdoch 1971). Because each predator may have different behavior and response to changes in prey density, adding a new predator to a system may change the strength or even the existence of density dependence.

It has been documented that invasive predators can have stronger negative effects than native predators on native prey (Salo et al. 2007), potentially due to prey

naïveté (Cox and Lima 2006, Sih et al. 2010). Consequently, invasives may alter or disrupt density-dependent mortality and threaten local prey population persistence.

Sinclair et al. (1997) outlined three lines of evidence necessary to predict the effects of predation by an invasive species on the persistence of native prey species: (1) Do the per capita rates of the prey species increase or decrease with declining densities? (2) Is predation depensatory or compensatory? (3) Is the overall magnitude of predation small or large? If the per capita demographic rates of the prey species are no longer compensatory, if predation is depensatory, and/or if the overall magnitude of predation is large, then the invasive predator may cause local extirpation of the native species.

One system where density dependence has been particularly well studied is in reef-associated fishes, where predation of early post-settlement individuals often causes density-dependent mortality that contributes to population regulation (Hixon and Webster 2002, Osenberg et al. 2002, White et al. 2010, Hixon et al. 2012). The effect of predation can be modified by multiple factors (review by Hixon and Jones 2005), including multiple predators (e.g., Hixon and Carr 1997), competitors (e.g., Carr et al. 2002), and availability of prey refuge space (e.g., Forester and Steele 2004). Because adding or removing native predator species can change the pattern or even the presence of density-dependent mortality (Hixon and Carr 1997), invasive predators are likely to have even stronger effects.

A particularly prolific and efficient invasive marine predator is the Indo-Pacific red lionfish (*Pterois volitans*) (reviewed by Morris and Whitfield 2009). The objective of this study was to investigate the potential of invasive lionfish to extirpate local populations of a common coral reef fish in the Bahamas. We considered the three lines of evidence outlined by Sinclair et al. (1997) to determine whether lionfish may threaten the local persistence of one of their primary native prey species, the bridled goby (*Coryphopterus glaucofraenum*) (Albins and Hixon 2008, Albins 2012). Because the bridled goby has a bipartite life cycle where demersal adults produce pelagic larvae, birth is locally decoupled from recruitment to the juvenile/adult population. Thus, it is unlikely that the birth rate would compensate for increased mortality. Therefore, the first line of evidence outlined by Sinclair et al. (1997) is irrelevant for this species, so we tested for the remaining two lines of evidence. Our two hypotheses were (1) the magnitude of lionfish predation is large relative to predator-free reefs, and (2) lionfish predation causes goby mortality to be depensatory. If the first hypothesis was true, then there would be a large decline in bridled goby abundance when lionfish are abundant. If the second hypothesis was true, then lionfish would cause inversely density-dependent mortality in bridled gobies. We used both manipulative field experiments and a laboratory experiment to test these two hypotheses.

3.2 METHODS

3.2.1. *Predator species*

Lionfish were first reported in the mid 1980's off the coast of Florida (Morris and Whitfield 2009), and in a few decades has spread throughout the Western Atlantic, Caribbean, Gulf of Mexico, and the northern coast of South America (Schofield 2010). Invasive lionfish grow can grow to a maximum size of 47.5 cm total length (TL) (Morris et al. 2012) and reach high local densities up to 400-650 fish ha⁻¹ (Green and Côté 2009, Frazer et al. 2011). Lionfish also have strong ecological effects, they have been found to reduce both the abundance and diversity of native species on small scales (Albins and Hixon 2008, Albins 2012), and observational evidence suggests the same effects at larger reef scales (Green et al. 2012), perhaps including mesophotic reefs (Lesser and Slattery 2011). Lionfish consume a wide variety of species, including ecologically important species, such as parrotfishes, and fishery species, such as grouper and snapper (Albins and Hixon 2008, Morris and Akins 2009, Valdez-Moreno et al. 2012).

3.2.2 *Prey Species*

The bridled goby is a common coral-reef fish in the Caribbean and the Bahamas (Randall 1996). After a pelagic larval duration of about 27 days, bridled goby settle to the reef at 6.5 - 8 mm standard length (SL) (Sponaugle and Cowen 1994). They mature around 25 mm SL and reach a maximum adult size around 60

mm SL (Cole and Shapire 1992, Forrester et al. 2006). Adults live on the sand in home ranges up to a few square meters in area, and use crevices at the reef/sand interface and coral rubble as refuge from predators. Adult densities in our Bahamas study area range from 6 to 15 gobies/m² (Forrester 1995, Forrester and Steele 2000). Predation on early post-settlement bridled goby causes density-dependent mortality, which likely leads to local population regulation on both small reefs (Forrester and Steele 2000, Steele and Forrester 2002) and large reefs (Steele and Forrester 2005). The intensity of density-dependent mortality is mediated by available refuge space; density-dependent mortality occurs with low amounts of refuge space but ample refuge leads to density-independent mortality (Forrester and Steele 2004).

3.2.3 Study Site

Four field experiments were conducted on a matrix of artificial 1-m³ concrete-block reefs and translocated 4-m² coral-patch reefs in a shallow (< 4m deep) sand-seagrass flat located near the Caribbean Marine Research Center (CMRC) on Lee Stocking Island, Bahamas (Fig. 3.1a). The reef matrix was translocated in the early 1990s (Carr and Hixon 1995, 1997, Hixon and Carr 1997) and have since become essentially natural reef habitat patches, completely covered by corals, sponges, and other benthos. Each reef is isolated from their nearest neighbor in the matrix by at least 200 m, and from the nearest natural reef by at least 1 km. CMRC was also the

site for laboratory experiments in 208 L outdoor aquaria fed by a flow-through, raw seawater system, described below.

3.2.4 Lionfish effects on goby mortality

To quantify the magnitude of invasive lionfish predation on local bridled goby populations, we extracted species-specific data from three separate manipulative field experiments conducted during the summers of 2007 (Albins and Hixon 2008), 2008 (Albins 2012), and 2010 (T. Pusack unpublished manuscript). Each of these experiments manipulated the presence of lionfish and monitored the abundance of prey-sized native coral-reef fishes (<5 cm total length [TL]) over the summer recruitment season from June to August. The predator treatments (lionfish present or predator-free control) were assigned using constrained randomization based on initial fish community structure (assessed from baseline surveys), reef location within the matrix, and reef type (artificial vs. translocated). At the beginning of each experiment, all resident predators were removed from each patch reef and one or two lionfish (ranging in size from 2.7cm – 28.5cm TL) were transplanted to the lionfish-only reefs. Predator treatments were maintained throughout each experiment, and small reef fishes were censused during weekly visits to each reef. The durations of these experiments varied: five weeks in 2007, eight weeks in 2008 and nine weeks in 2010. In each experiment, we examined change in abundance (ΔN) of bridled goby from

initial to final census between lionfish-only reefs (2007: n=8 reefs, 2008: n=5, 2010: n=4) and predator-free control reefs (2007: n=9 reefs, 2008: n=5, 2010: n=5).

We used a two-way analysis of variance (ANOVA) to compare changes in bridled goby abundances as a function of year and predator treatment, and included an interaction term between year and treatment to determine whether or not the effect of lionfish varied among years. Because experimental duration varied among years, we conducted separate analyses comparing 1) change in abundance over the entire duration of each experiment and 2) change in abundance through week five - the shortest experimental duration - to eliminate potential biases caused by experiment length. We used Tukey multiple comparisons of means tests to compare the change in abundance between treatments within each year.

To determine the effect of lionfish predation on the relationship between density and per capita mortality of adult bridled goby (2.5 – 4.5 cm TL), we subjected a gradient of goby densities within the observed natural range (4 – 36 fish/reef) (Forrester 1995, Forrester and Steele 2000) to four orthogonal predator treatments: (1) predator-free control (n = 6 patch reefs), (2) one native predator (the graysby grouper, *Cephalopholis cruentatus*), representing the pre-invasion system (n=5), (3) one lionfish (n=5), and (4) one native grouper and one lionfish, representing the invaded system (n=6) (Fig. 3.1b). Predator treatments and goby densities were randomly assigned to reefs within the constraints of similarity of the initial fish community assessed from baseline surveys, reef location within the matrix, and the available

refuge space per reef (calculated by summing all of the coral-head circumferences within each translocated patch reef). Because available refuge space can influence per-capita mortality of bridled goby (Forrester and Steele 2004), we used an ANOVA with refuge space as a function of predator treatment, and found no differences in refuge space among treatments ($F_{3, 18} = 1.885$, $p = 0.1683$).

Before establishing the above experimental treatments, SCUBA divers used hand nets and a weak solution of quinaldine to remove all resident predators of gobies, the competitively dominant goldspot goby (*Gnatholepis thompsoni*), and resident gobies. We also used removals to standardize the abundance of other strongly interacting species (i.e., territorial damselfishes and small Nassau grouper, which have not been reported to consume bridled goby [Randall 1967, Eklund 1999]). All experimental gobies were collected from a reef > 2 km from the matrix, and given a subcutaneous elastomer tag (Northwest Marine Technologies) to distinguish them from any new gobies appearing during the experiment. Although elastomer has been shown to have no detectable effects on this species (Malone et al. 1999), we also maintained 30 tagged and 30 untagged gobies in aquaria to test for any differential mortality based on the tagging procedure. At the end of the experimental period, 28 gobies remained alive in the tagged group and 26 remained alive in the untagged group, indicating that our tagging procedure did not artificially inflate mortality. Before we started each treatment, we performed a 48-hour, two-step acclimation period for experimental gobies. In the first step, we transplanted tagged experimental

gobies to a wire mesh cage (75cm x 20cm x 25cm) on their assigned reef and kept them in the cage for 24 hours to acclimate to the reef environment while protected from predation. During the second step, we released the gobies from the cage for a 24-hour acclimation period to account for any mortality due to the transplant process. The density of tagged gobies at the end of this two-step acclimation procedure was used as the starting density in our analysis. We visited each reef every ten days to maintain predator treatments, although all predators remained present on their assigned reefs, and to remove any non-tagged gobies that appeared on each reef. Each treatment ran for 20 days.

To test for differences in density-dependent mortality among treatments we used a multiple linear regression of per capita mortality as a function of predator treatment, initial goby density, and refuge space. Density-dependent mortality of gobies would be indicated by a significant effect of initial density on per capita mortality. We also tested for differences in patterns of per capita mortality among predator treatments by testing for two-way interaction terms between predator treatment and starting goby density. If the interaction term was not significant in the linear model, we then used an extra-sum-of-squares F-test and AIC scores to determine whether the full, non-additive model was the best model to use compared to the reduced, additive model. We also used hierarchical partitioning (Chevan and Sutherland 1991, Mac Nally 2002) to determine the amount of variation explained by each explanatory variable used in our final linear model.

Following the model selection procedure, we found that all interaction terms in the full linear model were non-significant (all $p \geq 0.20$). Using an extra-sum-of-squares F-test we found that the full, non-additive model was not different from the reduced, additive model ($F_{9, 16} = 0.82$, $p = 0.82$). Thus we used the more parsimonious, reduced model. Additionally, the reduced additive model had a lower AIC score (-75.7) compared to the non-additive model (-71.1). Therefore, we excluded the interaction term and used the final additive model of per capita mortality of bridled goby as a function of predator treatment, starting goby density, and refuge space ($F_{5, 16} = 18.92$, $p < 0.001$, $r^2 = 0.81$).

3.2.5 Goby size refuge

We performed feeding trials in aquaria to determine whether there is a size refuge for goby due to lionfish gape limits. Lionfish (ranging from 4.5 to 23.1 cm TL) were exposed to bridled goby of incrementally increasing sizes (approximately 1, 3, 5, and 6 cm TL representing the size range found at our study site). All experimental fish tested were collected from locations near CMRC using hand nets and stored in 208-L indoor aerated glass aquaria supplied with flow-through raw seawater. Individual lionfish were randomly selected for each trial, allowing at least a one-day fasting period between trials. Lionfish were exposed to individual gobies of 1 cm TL and then increased by 1 cm increments. Lionfish that responded to or ate the 3 cm TL goby but not the 5 cm TL goby were exposed to a 4 cm TL goby to determine their

upper limit of prey size. Similarly, those lionfish that responded to or ate the 1cm goby but not the 3cm were exposed to a 2cm goby.

For each trial, aquaria were divided into two sections by a shaded plexiglass partition, with lionfish on one side and the bridled goby on the other side. After a five-minute acclimation period, the partition was lifted and a 30 minute observation period ensued. During the first 10 minutes of continuous observation, we recorded the number of strikes, the size of the consumed goby, and time to ingestion (measured from the strike to when the goby was no longer seen extending out of the mouth). If after 10 minutes the lionfish had neither detected the goby nor commenced stalking, then continuous observation ceased. Thereafter, the tank was checked every 10 minutes for the presence of the goby for a total trial time of 30 minutes. If after 30 minutes the lionfish was actively stalking or engaged with the goby, then the trial continued until a successful attack ensued, otherwise the trial ended.

We used a simple linear regression of consumed bridled goby TL as a function of lionfish TL for the maximum size consumed and the minimum size consumed separately. We then compared the slopes using 95% confidence intervals (CI). We also used a simple linear regression to test for successful strike rate as a function of total lionfish size. Visual inspection of residuals was used for all analyses to ensure that the data met the assumptions of normality and homoscedasticity. All statistical analyses were conducted in the *R* software environment (*R* development Core Team

2012), including the packages *hier.part* (Walsh and Mac Nally 2013) and *pwr* (Champely 2009).

3.3 RESULTS

3.3.1 *Lionfish effects on goby mortality*

On predator-free reefs we observed a gradual increase in the abundance of gobies over the summer recruitment season, as expected. In contrast, on lionfish-only reefs we documented little change in the abundance of gobies. The effect of lionfish on the change in bridled goby abundance was similar among all three years (Fig. 3.2). Even though the 2007 experiment lasted only five weeks, the trend of goby abundance for both treatments followed that of longer experiments (Fig. 3.2).

The effect of the predation varied by treatment and year (two-way ANOVA; $F_{2, 29} = 3.8522$, $p = 0.033$). Although the change in abundance of bridled goby in 2007 was 70% less than that on predator-free reefs, this decline was not significant (Tukey HSD, $p = 0.76$). In contrast, in 2008, 90% fewer gobies accumulated on lionfish-only reefs compared to predator-free reefs ($p = 0.016$), and in 2010 lionfish-only reefs accumulated 100% fewer gobies ($p = 0.005$) than predator-free reefs (Fig. 3.3). Thus, in the longer experiments there were practically no additions of bridled goby to the reefs over the summer.

Because the treatment by year interaction was most likely due to the shortened duration of the 2007 experiment, we assessed the change in abundances at week five

for all years. In this analysis, the interaction term was no longer significant ($F_{2,30} = 0.269$, $p = 0.77$). Without the interaction term, goby abundance varied by predator treatment ($F_{1,30} = 15.60$, $p = 0.0004$) but not by year ($F_{2,30} = 0.2235$, $p = 0.80$). At the end of the five weeks across all years, 11 to 13 bridled goby accumulated. Average accumulation of gobies on lionfish-only reefs was 88% less than on predator-free reefs (Tukey HSD, $p = 0.004$). Because the trajectory of predator treatments in 2007 was similar to the trends from the other two years (Fig. 3.2), and the comparison among years of fifth week results showed that annually consistent predator effects, we conclude that lionfish had a temporally consistent negative effect on the abundance of bridled goby on small patch reefs.

We found that the presence of lionfish elevated per capita mortality close to 100%, which was higher than reefs with a native predator or predator-free reefs (Fig. 3.4). After accounting for predator treatment, the per capita mortality of bridled goby was unrelated to starting goby density (additive model, $t = -1.873$, $p = 0.079$), suggesting that per capita mortality was density-independent. However, a post-hoc test showed that we had very low power (0.055) to detect a significant slope. Because per capita mortality was independent across all goby densities, we compared the average percent mortality among predator treatments. The predator-free control reefs had a low average percent mortality of $16.9\% \pm 6.0\%$ SE (Fig. 3.4). Reefs with only a native grouper on them, representing the pre-invasion system, had higher mortality than predator-free control reefs ($t = 3.126$, $p = 0.007$) averaging $48\% \pm 14.3\%$ SE (Fig.

3.4). The reefs that represented the invaded system, with one native grouper and one lionfish, had $93.10\% \pm 4.7\%$ SE mortality, which was higher than the pre-invasion system ($t = -4.408$, $p < 0.001$) and the predator-free control reefs ($t = -7.926$, $p < 0.001$) (Fig. 3.4).

Notably, at the lowest goby densities, 4 – 13 fish/reef, mortality was 100% in the invaded treatment. Reefs with lionfish as the only predator also had high percent mortality at $81.3\% \pm 9.3\%$ SE, which was not different from the invaded system reefs ($t = 0.308$, $p = 0.76$), but did differ from both the pre-invasion reefs ($t = -4.264$, $p < 0.001$) and predator-free reefs ($t = -7.283$, $p < 0.001$) (Fig. 3.4). While we found an effect of refuge space ($t\text{-stat} = -2.518$, $p = 0.023$), predator treatment accounted 90.2% of the variance explained by the model, while starting goby density and refuge space accounted for 5.7% and 3.1%, respectively (hierarchical partitioning analysis).

3.3.2 Goby size refuge

When lionfish were smaller than 14cm TL, there was a strong, positive relationship between lionfish TL and the maximum size of consumed goby (slope = 0.42 with 95% CI 0.34 to 0.51; $t\text{-stat} = 10.867$, $p < 0.001$, $r^2 = 0.91$). When lionfish were larger than 14cm, they ate the largest bridled goby observed in the field (6 cm TL). In contrast, the minimum size of bridled goby consumed was only weakly related to lionfish size (slope = 0.08 with 95% CI -0.01 to 0.17; $t\text{-stat} = 2.093$, $p = 0.063$, $r^2 =$

0.23) (Fig. 3.5). Because the 95% confidence intervals did not overlap, the slope of the maximum curve was significantly greater than that of the minimum curve.

Lionfish strike rate increased with lionfish size (slope = 0.023 with 95% CI 0.012 to 0.033; t-stat = 4.560, $p \ll 0.001$, $r^2 = 0.46$). The smallest lionfish, 4 cm TL, had a successful strike rate of about 0.25 successful strikes per total attempts, which increased linearly to 0.70 successful strikes per total attempts for the largest lionfish (Fig. 3.6). These results indicate that larger lionfish were more successful hunters and consumed almost all sizes of bridled goby.

3.4 DISCUSSION

Our field experiments demonstrated that invasive lionfish can consume nearly 100% of a common native prey species, bridled goby, on Bahamian patch reefs. Further, reefs with resident lionfish have practically no net gain in bridled goby abundance over the summer recruitment season, thus substantially reducing their local population sizes. While there was a size refuge for bridled goby larger than about half the body length of small lionfish, practically the entire size range of this species was vulnerable to predation by lionfish larger than about 14 cm TL. The large reduction in bridled goby abundance and the high per capita mortality we observed is consistent with previous findings that lionfish have negative effects on native species. Albins and Hixon (2008) and Albins (2012) found up to 90% reductions in the overall abundances of small native coral-reef fishes on patch reefs, and Green et al. (2012)

observed similar reductions in prey biomass with increasing lionfish density. Additionally, we documented a maximum prey size to lionfish ratio of 0.42, which is similar to previously documented ratios of 0.44 (Albins and Hixon 2008) and 0.48 (Morris and Akins 2009). While previous manipulative (Albins 2012) and observational (Lesser and Slattery 2011) studies documented reductions in species richness when lionfish are present, the mechanisms behind these community changes remained unclear. By demonstrating that lionfish elevate predation close to 100% at low densities, and practically eliminate the recruitment of new fish to reefs, this study provides evidence that lionfish may be able to extirpate local populations by simply consuming them.

In the context of Sinclair et al.'s (1997) hypotheses, the magnitude of lionfish predation was large and caused mortality to be depensatory. Immigration of gobies is limited between the isolated reefs used in our study (Steele and Forrester 2005), and spawning is decoupled from recruitment to these small reefs. Thus, it is unlikely that the per capita birth and immigration rates could increase to counteract lionfish predation and maintain the pre-invasion population levels at the local population scale.

The relationship between per capita mortality and goby density was density-independent for all treatments, a finding that differs from previous research that found density-dependent mortality for bridled goby due to either parasitism (Forrester and Finley 2006) or predation (Forrester and Steele 2000), particularly at low refuge availability (Steele and Forrester 2004). There are several possible explanations for

this difference. The most likely explanation is low statistical power to detect a significant slope due to small sample sizes (White et al. 2010). It is also possible that we did not elevate the densities high enough to observe density-dependent mortality (White et al. 2010). In a meta-analysis of reef fish studies testing for density dependence, Osenberg et al. (2002) found that authors were more likely to conclude that per capita mortality was density-dependent when the density gradient employed was high; in contrast, low density gradients often led to results supporting density-independence. Finally, our study reefs may have provided high levels of refuge space, relative to goby density across all treatments, which have been shown to result in density-independent mortality in bridled goby (Steele and Forrester 2004). Because Steele and Forrester (2004) used visual assessments of refuge space, we could not directly compare the amount of refuge space in our study to theirs. Based on these limitations, we are cautious about drawing conclusions about density dependence in this study. Nonetheless, we have demonstrated that goby mortality was high across all densities in the presence of lionfish, which could disrupt any density-dependent population regulation. The percent mortality we documented for bridled goby at low densities is higher than found in previous studies (Forrester and Steele 2000, Steele and Forrester 2004), suggesting that lionfish have reduced this prey resource to lower density levels than native predators. We therefore conclude that population persistence may be threatened at the local level.

The effect size of lionfish on per capita mortality of bridled goby was 2 times larger than a native grouper. Albins (2012) found a similar difference in relative effect size, where lionfish had an approximately 2.5 times stronger effect size on the total abundance of native prey fishes than the same native grouper. These findings are consistent with the hypothesis that invasive predators have a stronger effect than native predators (Salo et al. 2007), potentially due to naïve prey not recognizing an invasive predator as a threat (Cox and Lima 2006, Sih et al. 2010). Lionfish may represent a novel predator archetype on Bahamian coral reefs. The cryptic coloration and ornate, elongated fins of lionfish are unique within the invaded range and may obscure recognition of lionfish as a threat. When foraging, lionfish slowly stalk their prey before a rapid attack, unlike ecologically similar native predators that ambush their prey. Lionfish also employ a unique hunting strategy, blowing directed jets of water at prey fish, which may potentially confuse the prey or orient them for head-first swallowing (Albins and Lyons 2012). All of these factors combined may reduce the ability of prey fish to recognize lionfish as a threat and take evasive action. In the only study to date of the ability of native prey to recognize lionfish as a threat, Marsh-Hunkin et al. (2013) found that bridled goby had a reduced response to lionfish compared to a native predator, supporting the hypothesis that the bridled goby are naïve to lionfish.

In our study region, the bridled goby is the top prey species of lionfish (Albins and Hixon 2008, Albins 2012) and may be at risk of local extirpation. Because

lionfish consume at least 40 species across 21 families of fish and invertebrates (Albins and Hixon 2008, Morris and Akins 2009, Valdez-Moreno et al. 2012) and have been found in a broad variety of coastal habitats (Whitfield et al. 2007, Barbour et al. 2010, Lesser and Slattery 2011, Claydon 2012, Jud and Layman 2012), future research should examine whether lionfish alter and/or elevate per capita mortality of other native species. In particular, severe community effects can result if lionfish predation elevates mortality of ecologically important species, such as herbivorous parrotfishes and cleaner species. The possibility has been suggested that lionfish may be the final stressor that pushes many already degraded reef systems from coral-dominated to algal-dominated (Albins and Hixon 2011). For this to occur, lionfish would force herbivorous parrotfishes, a top grazer of algae in coral reef systems (Mumby et al. 2006), to functional extirpation (Parker et al 1999). While not all invasive species cause damage to native systems (Gurevitch and Padilla 2006, Davis 2011), our study documents that lionfish can elevate native prey mortality close to 100%, potentially leading to local extirpation and disrupting regional population dynamics. To fully understand whether lionfish can cause extirpations and change Atlantic and Caribbean coral reef communities, further studies at multiple spatial and temporal scales are needed.

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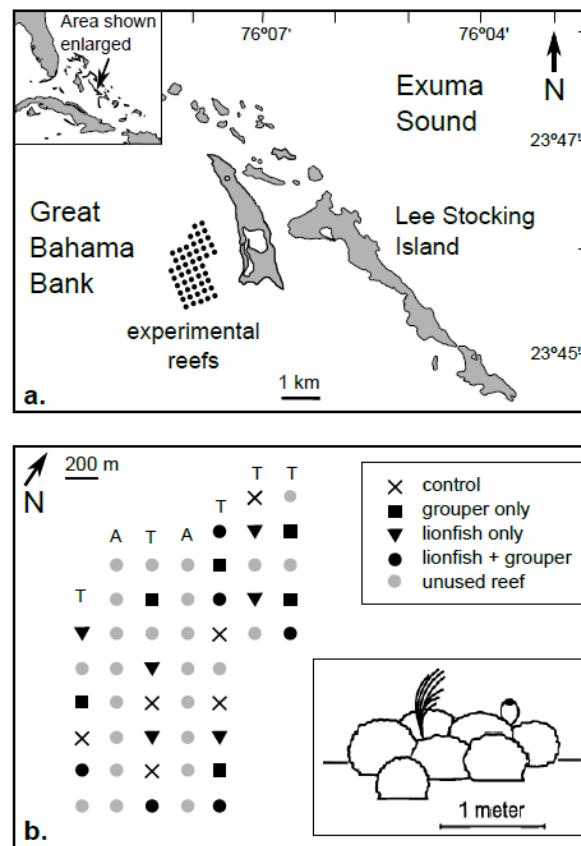


Figure 3.1 a. Map showing the location of experimental patch reefs near the Caribbean Marine Research Center on Lee Stocking Island, Bahamas (modified from Albins 2012). b. Location of predator treatments within the reef matrix. Reef type, A = artificial and T = translocated, is indicated at the top of each column and designates the reef type for all reefs in that column. Only translocated reefs were used to study the effect of lionfish on the per capita mortality of bridled goby. The drawing shows the general structure of translocated experimental reefs (modified from Carr and Hixon 1997).

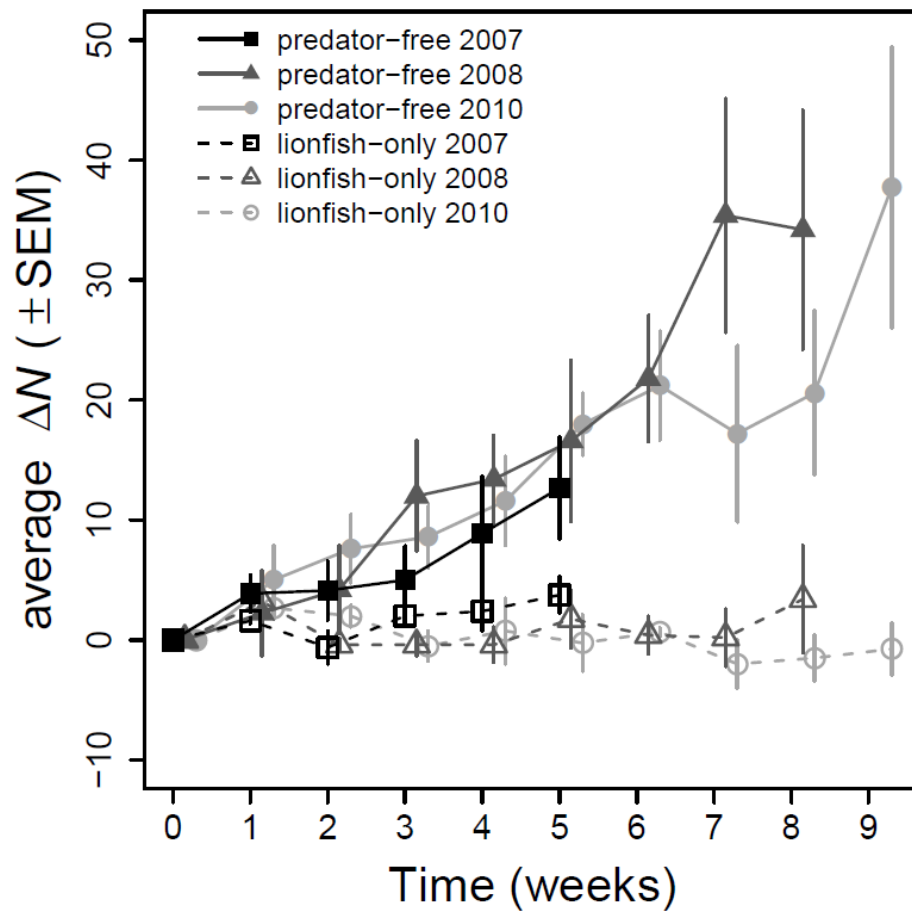


Figure 3.2 The change in abundance (ΔN mean \pm SEM) of small reef fish (≤ 50 mm TL) over time on lionfish-only reefs (open symbols) and predator-free reefs (closed symbols) for three different experiments: 2007 (squares), 2008 (triangles), and 2010 (circles). Each point gives the mean (\pm SEM) change in abundance between the given week and the initial survey for each treatment. Symbols are offset to aid view.

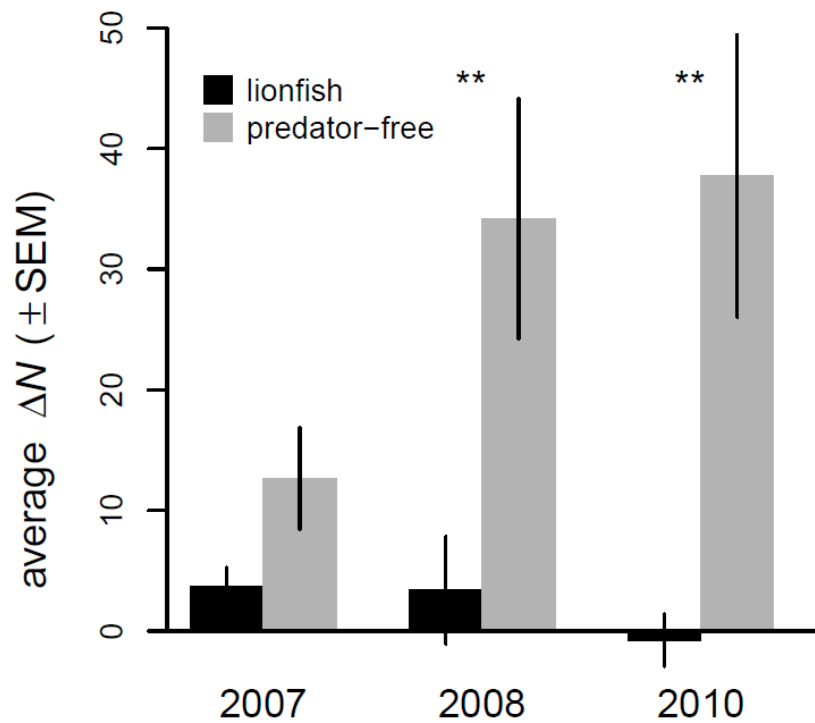


Figure 3.3 The change in abundance (ΔN mean \pm SEM) of small reef fish (≤ 50 mm TL) between lionfish-only (black bars) and predator free (gray bars) for the final week of all experiments. The years on the x-axis denote the year in which each experiment was conducted. ** indicates significant differences with a p-value < 0.01 from a Tukey multiple comparisons of means test.

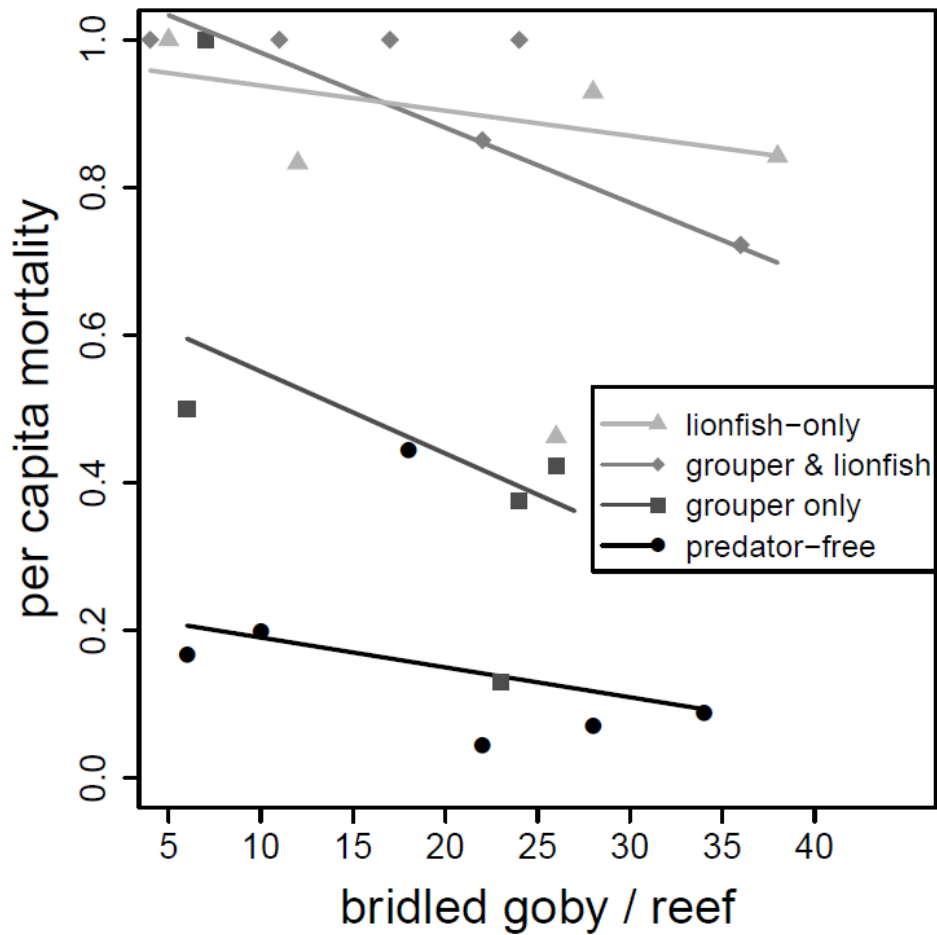


Figure 3.4 The per capita mortality of bridled goby as a function of predator treatment, refuge index, and starting goby density. Predator treatments included predator-free control (black circles), native grouper only (lighter gray squares), invasive lionfish with native grouper (lighter gray diamonds), and invasive lionfish only (lightest gray triangles). While the slopes appear to be negative, they were not significantly different from zero ($t = -1.873$, $p = 0.079$). Also there was no evidence that the slopes were different among predator treatments, and despite the fact that some curves cross, there was no significant interaction among predator treatments across goby densities (all $p \geq 0.20$).

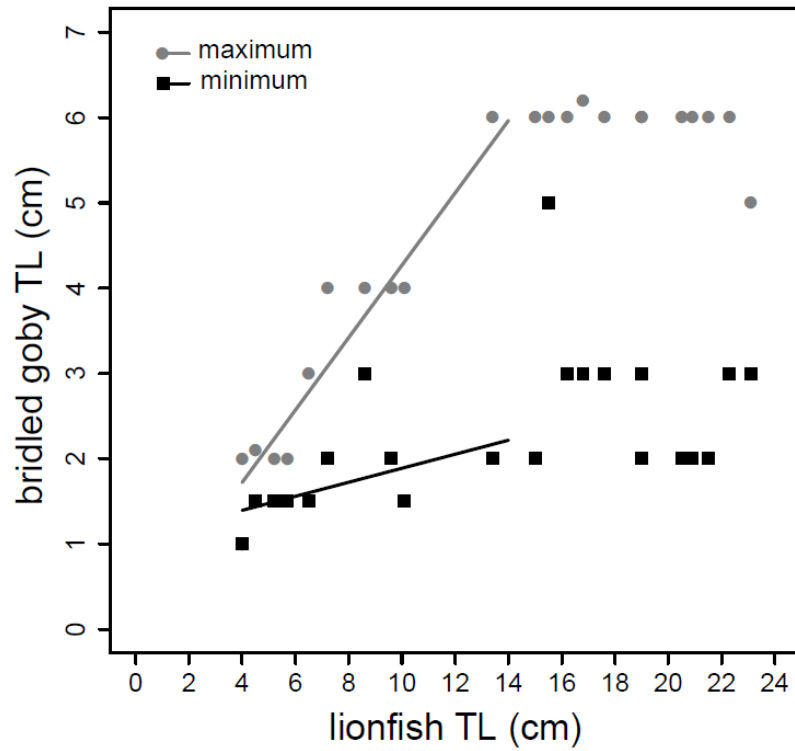


Figure 3.5 The relationship between the maximum (gray circles) and minimum (black squares) total length (TL) of consumed bridled goby as a function of lionfish TL. The simple linear regression for the maximum-sized consumed goby as a function of lionfish TL is described by the model: $\text{goby TL} = 0.42 \times \text{lionfish TL} + 0.024$. The simple linear regression for the minimum-sized consumed bridled goby as a function of lionfish TL is described by the model: $\text{goby TL} = 0.08 \times \text{lionfish TL} + 1.07$.

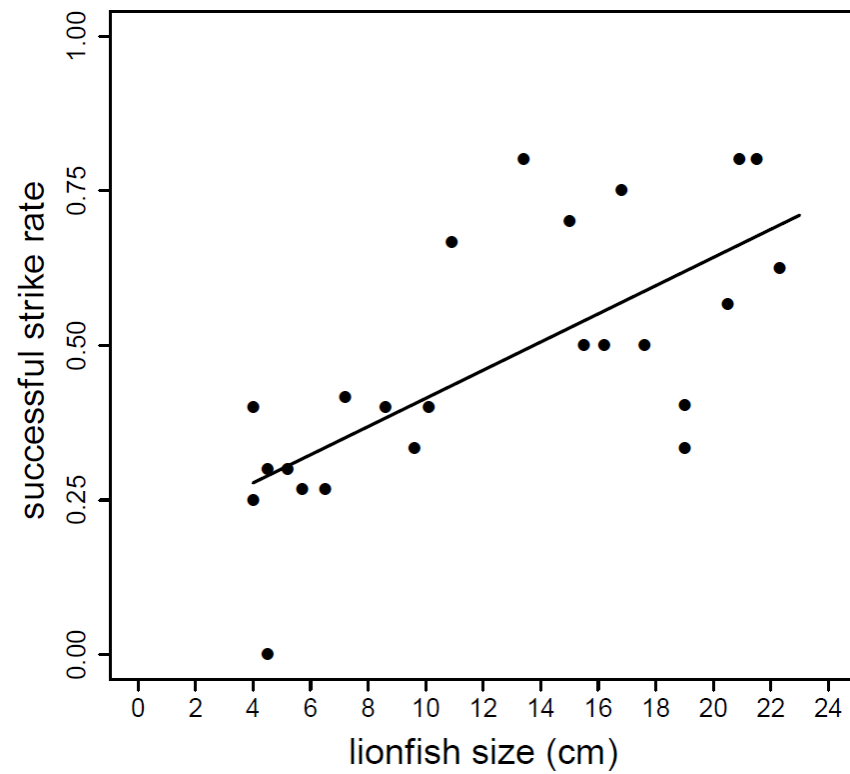


Figure 3.6. Successful strike rate (successful strikes/total attempts) of lionfish as a function of lionfish TL (cm). The linear relationship is describe by the model: $\text{success rate} = 0.005 \times \text{lionfish size} + 0.19$.

Chapter 4 – Biotic resistance in the sea: native Nassau grouper (*Epinephelus striatus*) moderate predatory effects of invasive Pacific red lionfish (*Pterois volitans*) on Atlantic coral-reef fishes

Timothy J. Pusack

Biological Invasions

In Prep

ABSTRACT

Invasive Indo-Pacific lionfish (*Pterois volitans*) can cause extreme reductions in the densities of small native reef fishes. Management strategies are desperately needed to control this invasion. Manual removal efforts show only local success and the current level of biotic resistance is low, perhaps due to overfishing of potential predators and competitors of lionfish. If a native species can be identified that negatively affects lionfish populations, then policies to protect this species may increase biotic resistance to the invasion. The Nassau grouper (*Epinephelus striatus*) has been suggested as perhaps the most important potential natural enemy of lionfish because lionfish have occasionally been found in the stomachs of large adults (>500 mm total length). However, large adult grouper live offshore, whereas most lionfish inhabit inshore reefs. Therefore, I investigated the effects of large juvenile (150 - 400 mm) Nassau grouper on the growth and survival of juvenile (20-140 mm) lionfish by manipulating the abundance of grouper on 28 nearshore patch reefs in the Bahamas. I monitored the individual growth and persistence of lionfish, and censused the community of small reef fishes (< 50 mm) on each reef over 10 weeks during summer 2010. While there were no differences in lionfish persistence or growth rates with increasing grouper abundance, the abundance of small reef fishes on reefs with lionfish and a high abundance of Nassau grouper were not different than control reefs with no predators. Thus, Nassau grouper provide some biotic resistance on Bahamian patch reefs by interfering with lionfish predation on native fishes, perhaps by

interrupting stalking behavior. Protecting Nassau grouper, and possibly other large-bodied groupers, is likely to help to reduce the negative effects of invasive lionfish.

4.1 INTRODUCTION

Invasive species are one of the key drivers of anthropogenic environmental change (Vitousek et al. 1997). They threaten biodiversity (D'antonio et al. 2001, Bax 2001), community and ecosystem structure (Crooks 2002, Walker and Steffen 1997, Grosholz 2002), food web dynamics (Semmens et al. 2004), and ecosystem services (Pejchar and Mooney 2009). Invasive predators in particular are a major extinction or extirpation threat to native species (Davis 2011). The predatory Indo-Pacific red lionfish (*Pterois volitans*) is one such devastating invasion. Lionfish threaten already degraded Atlantic and Caribbean reefs (Albins and Hixon 2011), and are a top conservation issue (Sutherland 2010).

Lionfish were first sighted off the coast of Florida in the mid 1980s (Morris and Whitfield 2009), and subsequently spread throughout the Western Atlantic, Caribbean, and Gulf of Mexico (Schofield 2010). Throughout the invaded range, lionfish can reach high local densities -- up to 650 fish/hectare (Frazer et al. 2012) -- and reside in a variety of habitats, including coral and rocky reefs (Whitfield et al. 2007), seagrass beds (Claydon 2012), mangrove forests (Barbour et al. 2010, Claydon 2012), estuaries (Claydon 2012; Jud and Layman 2012), and mesophotic reefs (Lesser and Slattery 2011). Invasive lionfish are voracious predators (Albins and Hixon 2008, Côté and Maljkovic 2009, Green et al. 2011), and field experiments have documented

that a single lionfish can reduce the abundance of small coral-reef fishes on a small patch reef by up to 90% (Albins and Hixon 2008, Albins 2012). Observational studies further indicate that these negative effects may scale-up to the larger reefs (Green et al. 2011, 2012). Lionfish consume at least 40 species in 21 families of fish and invertebrates (Albins and Hixon 2008, Morris and Akins 2009, Valdez-Moreno et al 2012). Because the lionfish invasion occurs around many island nations that depend on the marine ecosystem for sustenance, protection, and their livelihood (Moberg and Folke 1999, Cesar et al. 2003, Burke and Maidens 2004), it is imperative to find an effective and inexpensive approach to reduce lionfish populations.

The current management approach to control invasive lionfish is through manual removals by divers. Local, targeted removal efforts in the Caymans (Frazer et al. 2012) and lionfish derbies throughout the Florida Keys (Morris and Whitfield 2009, Albins and Hixon 2011) have achieved some success on a local scale. However, management on a large scale is not yet in place and would require considerable effort (Barbour et al. 2011). For example, Morris et al. (2011) estimated that average monthly removals of about 30% of the lionfish across the entire invaded region would be needed to reduce their populations substantially. The amount of effort necessary to meet this target is likely to be prohibitively expensive at SCUBA depths, and logistically impossible in deeper habitats because lionfish have been observed in water up to ~300m deep (Albins and Hixon 2011). Another option is to develop a lionfish fishery (Ferguson and Akins 2010, Morris et al. 2011), yet reports of ciguatoxin from

St. Martin (McFadden 2011), the Florida Keys (Cearnal 2012), and elsewhere may inhibit the success of a targeted fisheries.

An additional or alternative control strategy to manual removals is to promote biotic resistance of the native system (*sensu* Elton 1958, Levine et al. 2004, Fridley et al. 2007), which occurs where natural enemies in the invaded range control or limit populations of invasive species and their effects. While the original idea of biotic resistance put forth by Elton (1958) focused on competition between native and invasive species, predation is now viewed as a stronger interaction to provide biotic resistance (Sax et al. 2007). For example, many invertebrates (deRivera et al. 2005, Jensen et al. 2007) and vertebrates (King et al. 2006), including freshwater fishes (Baltz and Moyle 1993), can provide biotic resistance against invasive species. Examining interactions between native Atlantic predatory fishes and lionfish may reveal native species that could provide effective biotic resistance.

In Atlantic and Caribbean systems, many of the top predators have been drastically overfished (Paddack et al. 2009, Stallings 2009), leaving few native species with populations large enough to have a substantial effect on lionfish. Yet, if an effective predator of lionfish can be identified, then policies to protect populations of that species could potentially mitigate the lionfish invasion. Currently there is little information on lionfish predators in both their native Pacific and invaded Atlantic range. In the Pacific the only published report of lionfish predation describes a cornetfish (*Fistularia commersonii*) consuming a lionfish (Bernadsky and Goulet

1991). In the invaded range, anecdotal evidence suggests that large groupers may occasionally eat lionfish. Maljkovic and Van Leeuwen (2008) documented lionfish in the guts of Nassau grouper (*Epinephelus straitus*) and tiger grouper (*Mycteroperca tigris*), and divers in the Cayman Islands have trained Nassau grouper to consume speared lionfish (Albins and Hixon 2011). The only published research to date on the effect of large-bodied groupers on invasive lionfish found a negative correlation between lionfish biomass and grouper biomass comparing sites inside and outside a large marine reserve in the Bahamas (Mumby et al. 2011). However, because this observational study did not account for manual removals of lionfish within the marine reserve by managers, this correlation may have been confounded by local removal efforts. It remains unclear whether and how effective large-bodied grouper are at controlling invasive lionfish.

The objective of this study was to test the hypothesis that Nassau grouper provide detectable biotic resistance against invasive lionfish. Specifically, I predicted that increasing Nassau grouper abundance would (1) decrease lionfish survival (lethal effect), (2) decrease lionfish growth rates (nonlethal effect), and (3) reduce the negative effects that lionfish have on the community structure of native reef fishes. To test these predictions, I conducted a manipulative field experiment in the Bahamas that examined the responses of invasive lionfish and small native reef fishes along a gradient of Nassau grouper abundance.

4.2 METHODS

4.2.1 Study Site

I conducted this study during summer 2010 near the Caribbean Marine Research Center on Lee Stocking Island, Bahamas (Fig. 4.1a). I used a matrix (Fig. 4.1b) of artificial 1 m³ concrete-block reefs and translocated 3.5 m² coral patch reefs (Fig. 4.1c) in a shallow (< 4m deep) sand-seagrass flat. The reef matrix was constructed/translocated in the early 1990s (Carr and Hixon 1995, 1997, Hixon and Carr 1997), and all are now natural reef habitat patches completely covered by corals, sponges, and other benthos. Each isolated reef is at least 200 m from the nearest other reef in the matrix and at least 1 km from the nearest natural reef.

4.2.2 Experimental design

I studied all 16 artificial reefs and selected 12 translocated reefs based on the abundance of Nassau grouper, reef fish community (species composition, relative abundance, and size structure), and location in the matrix. I transplanted one or two juvenile lionfish (27 mm – 110 mm total length [TL]) to each reef, except for predator-free control reefs, which had neither grouper nor lionfish (5 reefs). I used hand nets to collect lionfish, which I held overnight in 208 L aquaria before release on experimental reefs. Before releases, I measured their TL and mass (g) and gave each lionfish a unique subcutaneous Elastomer tag (Northwest Marine Technologies), which have been shown to have no detectable effects on a variety of reef fishes (Willis

and Babcock 1998; Malone et al. 1999). I then manipulated the abundance of large juvenile Nassau grouper (150 mm – 500 mm TL) by removing grouper from experimental reefs to establish one of six different treatment levels: 5 grouper per reef (3 reefs), 4 grouper (3 reefs), 3 grouper (4 reefs), 2 grouper (4 reefs), 1 grouper (5 reefs), and zero grouper (4 reefs). All experimental grouper abundances were within naturally occurring ranges (e.g., Stallings 2008). Nassau grouper in this size range are mainly piscivorous, and because of the large size differences between the lionfish and grouper, were unlikely to compete with juvenile lionfish for food (Sadovy and Ekland 1999). Treatments were assigned to reefs randomly within the constraints of distributing treatments evenly based on the community of reef fishes on each reef (species composition, relative abundance, and size structure), reef type (artificial vs. translocated), reef structure (artificial reefs: number of open holes, translocated reefs: number of coral heads), and location in the matrix to ensure that none of the treatments would be biased by these factors (Fig. 4.1b.) At the start of the experiment all resident predators (e.g., small groupers, moray eels, and soapfish) and strong interactors (territorial damselfish > 40 mm TL) were removed from the reefs and transplanted > 3.5 km from the experimental reef array to reduce the probability of removed fish returning.

Following the above manipulations, the experiment ran for 71 days with treatments being maintained during weekly visits to each reef. I maintained Nassau grouper treatments through manual removals of immigrants with nets (26 in total).

There was no emigration of grouper. I also removed any new lionfish that appeared on my experimental reefs (4 in total). All resident predators and strong interactors that appeared on the reefs were also removed throughout the experiment; across all reefs, I removed a total of 9 graysby grouper (*Cephalopholis cruentatus*), 9 freckled soapfish (*Rypticus bistrispinus*), 13 harlequin bass (*Serranus tigrinus*), and 9 adult beaugregory damselfish (*Stegastes leucostictus*).

To monitor growth of lionfish, I measured the TL of each individual biweekly, and individual mass at the beginning and end of the experiment. Direct effects of Nassau grouper abundance on lionfish were measured as relative patterns of survival (estimated as persistence because mortality could not be separated from emigration with certainty) and lionfish growth. Each week, I censused prey-sized reef fishes (< 50 mm TL) on each reef. I recorded small-reef fish abundance to evaluate any positive indirect effect that Nassau grouper had on small reef fish, mediated through direct negative effects on native mesopredators, as Stallings (2008) had documented for the effects of larger Nassau grouper on smaller native grouper.

4.2.3 Statistical analysis: Invasive lionfish persistence and growth

Persistence was analyzed using a Cox proportional-hazards regression model. This procedure analyzes the time it takes for a specific event to occur, in this study the disappearance of a lionfish from an experimental reef, and then compares the survival distribution to a number of predictors to determine whether any have an effect on the

survival distribution. I modeled lionfish survival as a function of Nassau grouper abundance (0-5 per reef), lionfish abundance (1-2), last measured lionfish size (TL), and reef type (artificial vs. translocated). Mean individual growth rates (mm /day, g/day) were calculated by dividing the total change in lionfish TL or grams by the total time. Both growth measures were analyzed as a function of Nassau grouper abundance (0-5 per reef), lionfish abundance (1-2 per reef), and lionfish initial size on each reef using linear regression.

4.2.4 Statistical analysis: Native reef-fish abundance

I examined the change in the combined abundance ($\Delta N = \text{final } N - \text{initial } N$) of all small reef fishes (≤ 50 mm TL)). Nassau grouper treatment levels were pooled into low-abundance reefs (1-3 per reef, $n = 13$) and high-abundance reefs (4-5 per reef, $n = 6$) based on similar results within categories. I used a linear model to analyze ΔN at week 10 as a function of predator treatment (lionfish only, lionfish + low grouper abundance, lionfish + high grouper abundance, predator-free control), lionfish abundance (1 or 2), and reef type (artificial or transplant). I used AIC scores to select the most parsimonious model, which was ΔN as a function of predator treatment and lionfish abundance.

4.2.5 Statistical analysis: Multivariate community response

To further investigate the effects of Nassau grouper abundance on the effects of lionfish on small native reef fishes, I ran a Non-Metric Multidimensional Scaling (NMDS, PC-ORD v.6 McCune and Grace 2002) to ordinate patch reefs in species space. In conjunction with NMDS, I used the Canonical Analysis of Principal Coordinates (CAP) (Anderson and Willis 2003) to describe the ordination of reefs in species space. In the CAP analysis, I used the Canonical Correlation Analysis (CCorA) to estimate the correlation of quantitative variables with the ordination. The two matrices used for the CCorA were a reef-by-species abundance matrix and an environmental matrix with four quantitative environmental variables: reef type (transplant or artificial), reef complexity (1-4, based on the number of open holes on artificial reefs or the number of coral heads on translocated patch reefs), predator level (lionfish only, low grouper abundance + lionfish, high grouper abundance + lionfish, predator-free control), and lionfish abundance (0-2). I also used the Discriminant Analysis (DA) in the CAP analysis to test for differences between groups; the DA calculates a p-value ($\alpha = 0.05$) from a permutation test (9,999 permutations) to determine the probability of differentiating reefs based on a priori groups. I defined groups by either reef type (artificial reefs, transplant reefs) or predator treatment (lionfish only, low grouper abundance + lionfish, high grouper abundance + lionfish, predator-free control). I used the Bray-Curtis distance (Bray and Curtis 1957) for the CCorA and DA, which is mathematically the same as the Sørensen distance that was

used in the NMDS. Because the abundances of species showed a typical lognormal distribution, data were $\log(x+1)$ transformed. I then ran an NMDS ordination and CAP analyses on both the initial and final surveys on all patch reefs. An outlier analysis in PC-ORD found only peripheral outliers (A-09 initial: 2.713 SDM [standard deviations from the mean]; A-14 final: 2.28 SDM; A-06 initial 2.28 SDM). Because these reefs were not extreme outliers, and the log transformation created a more linear relationship, no other manipulations of relativization were used. All statistical analyses, unless otherwise specified, were conducted using the R statistical environment (R Core Team 2012), with the associated packages survival (Therneau 2011), lattice (Sarkar 2012), and vegan (Oksanen 2011).

4.3 RESULTS

4.3.1 *Invasive lionfish persistence and growth*

Persistence of juvenile lionfish over 10 weeks ranged from 70% to 100% (Fig. 4.2a). A total of 16 out of 44 lionfish did not persist on the experimental reefs. The majority that did not remain (56%) disappeared within the first five days. Of the lionfish that remained longer than one week, all but one (9.6cm TL) was > 10 cm TL. The last measured length had an effect on lionfish persistence (Cox proportional-hazards model, $z = -4.302$, $p < 0.0001$), while lionfish persistence did not vary among Nassau grouper treatment abundance (Cox proportional-hazards model, $z = 0.782$, $p = 0.434$). Lionfish growth rate (mm/day) was also unrelated to grouper abundance ($t =$

0.157, $p = 0.876$); lionfish across all treatments grew about 1 mm/day (Fig. 4.2b).

Additionally, after accounting for the final length of lionfish, lionfish growth rate in mass was unrelated to grouper abundance ($t = 1.444$, $p=0.158$); growth in mass across all treatments was about 0.25 g/day (Fig. 4.2c).

4.3.2 *Native reef-fish abundance*

During the 10-week experiment, the change in abundance (ΔN) of prey-sized native reef fishes (≤ 50 mm TL) increased by 52.6 ± 19.4 fish per reef (mean \pm SEM) on the predator-free control reefs (Fig. 4.3). The change in abundance on lionfish-only reefs decreased by 6.5 ± 5.4 fish per reef and was smaller ($t = 3.621$, $p = 0.001$) than on the predator-free control reefs (Fig. 4.3). The difference between these two treatments indicated a proportional reduction of small reef fish of essentially 100% (112% calculated) caused by lionfish. Similarly, on lionfish + low-grouper reefs, the abundance of small reef fish decreased by 9.6 ± 6.4 fish, and was less than predator-free control reefs ($t = -4.56$, $p < 0.001$) (Fig. 4.3). However, on lionfish + high-grouper reefs, abundance of small reef fishes increased by 35.7 ± 16.4 fish per reef. This change was larger than both lionfish-only reefs ($t = -2.721$, $p = 0.012$), and lionfish + low- grouper reefs ($t = -3.490$, $p = 0.002$). Importantly the change in abundance of small reef fishes on predator-free control reefs did not differ from that on lionfish + high-grouper reefs ($t = 1.406$, $p = 0.17$) (Fig. 4.3). The difference

between these two treatments was only a 32% reduction in abundance of small reef fishes.

The overall changes on predator-free control reefs were primarily due to increases in two goby species: the bridled goby (*Coryphopterus glaucofraenum*) and the goldspot goby (*Gnatholepis thompsoni*) (Table 4.1). Eighteen other species in nine families also contributed to the increase on control reefs. Of the twenty species that increased in abundance on the predator-free reefs, nineteen species had a smaller change in abundance on lionfish-only reefs, most notably the bridled goby and the goldspot goby. Other ecologically important species that suffered reduced abundance on lionfish-only reefs were a facultative cleaner -- the Spanish hogfish (*Bodianus rufus*) -- and three herbivorous parrotfishes: the redband parrotfish (*Sparisoma aurofrenatum*), the stoplight parrotfish (*S. viride*), and the greenblotch parrotfish (*S. atomarium*) (Table 4.1). By comparison to lionfish-only reefs, nineteen species on lionfish + high-grouper reefs had a smaller reduction in abundance, and six species that experienced a net loss on lionfish only reefs increased in abundance: the goldspot goby, juvenile grunt species (*Haemulon* sp.), the flamefish (*Apogon maculatus*), the green razorfish (*Xyrichtys splendens*) and two herbivorous parrotfishes -- the redband parrotfish and the stoplight parrotfish.

4.3.3 Multivariate community response

The NMDS ordination of experimental reefs showed segregation based on reef type at the start of the experiment (Fig. 4.4a), but by the end of the experiment, the two groups of reefs partially overlapped (Fig. 4.4b). In contrast, groups of reefs clustered by Nassau grouper abundance showed a high degree of overlap at the start of the experiment (Fig. 4.4c), yet by the end of the experiment, were partially segregated (Fig. 4.4d).

At the start of the experiment, the NMDS ordination of reefs (final stress = 13.073, $r^2 = 0.86$, final instability = 0, iterations = 67, Monte-Carlo $p = 0.020$) revealed that experimental reefs separated out by reef type with translocated reefs to the left and artificial reefs to the right (CAP CCorA, $r^2 = 0.92$, Fig. 4.4a). Additionally, the DA procedure in the CAP analysis of initial communities successfully differentiated reefs by reef type (p-value = 0.0001, mis-classification error = 0.00%), but could not differentiate by grouper treatment (p-value 0.213, mis-classification error = 85.71%). These three analyses indicate that reef type was the dominant factor influencing the communities of small reef fishes at the beginning of the experiment.

At the end of the experiment, the NMDS ordination of the reefs (final stress = 13.75, $r^2 = 0.84$, final instability = 0, iterations = 87, Monte-Carlo $p = 0.020$) showed that both Nassau grouper abundance (CAP CCorA, $r^2 = 0.730$) and lionfish abundance (CAP CCorA, $r^2 = -0.467$) had high correlations with NMDS1. From the CCorA procedure, the species that showed the strongest positive correlation with NMDS1,

meaning they were more likely found on predator-free reefs or reefs with many Nassau grouper, were the bridled goby ($r^2 = 0.83$), the rosy blenny (*Malacoctenus macropus*) ($r^2 = 0.70$), the goldspot goby ($r^2 = 0.62$), and the beaugregory damselfish ($r^2 = 0.58$). The species with a negative correlation with NMDS1, and thus found more predominantly on reefs with lionfish and few to no Nassau grouper, were the reef squirrelfish (*Sargocentron coruscum*) ($r^2 = -0.60$), the blue tang (*Acanthurus coeruleus*) ($r^2 = -0.55$), and the foureye butterflyfish (*Chaetodon capistratus*) ($r^2 = -0.49$). Additionally, the DA procedure now differentiated reefs among Nassau grouper treatments (p-value = 0.002) with a lower, albeit moderate, miss-classification error of 53.57% than at the beginning of the experiment. While this procedure still differentiated reefs by reef type ($p = 0.03$), there was a higher mis-classification error of 28.57% than at the beginning of the experiment. These results indicate that Nassau grouper and lionfish abundances explained more variation in fish communities at the end of the experiment compared to the beginning, while reef type explained less variation.

4.4 DISCUSSION

The goal of this study was to determine whether native Atlantic grouper provide biotic resistance to invasive Pacific lionfish on shallow patch reefs. While Nassau grouper did not affect lionfish growth or persistence, high abundances of grouper prevented large declines in the local abundance of small reef fishes normally

caused by lionfish. Reef fish abundance was highest on reefs with no lionfish as well as reefs with lionfish and many grouper, and lowest on reefs with lionfish and few or no grouper. These findings indicate that Nassau grouper, in adequate abundances, may mitigate the negative effects of lionfish on small reef fish communities on patch reefs, and that managers may be able to limit the invasion by promoting policies to protect Nassau grouper.

4.4.1 No effects of grouper on lionfish persistence and growth

There was no evidence of biotic resistance as measured by persistence or growth of lionfish. Persistence on patch reefs was roughly equal and high across all treatments. Only a few lionfish disappeared during the experiment, and the majority that disappeared did so within the first week, perhaps due to transplantation effects. The remaining lionfish that disappeared had approached 150 mm TL, which is the size where lionfish appear to undergo an ontological shift and move towards larger reefs (T. Pusack, *unpublished data*). Moreover, I did not observe any negative effects of increasing grouper abundance on lionfish growth. Lionfish grew similarly across all treatments, and the growth rate was comparable to previous findings (Albins 2012, Pusack et al. *in review*).

Other studies indicate that larger groupers, including Nassau grouper, may occasionally affect lionfish negatively. Maljkovic and Van Leeuwen (2008) documented lionfish in the guts of grouper caught by fisherman. Mumby et al. (2011)

observed a negative relationship between grouper biomass and lionfish biomass by comparing sites inside and outside a large marine reserve in the Bahamas. There are two key differences between my study and Mumby et al. (2011) that may have produced the different findings: (1) my study was manipulative, whereas Mumby et al. (2011) was observational, and (2) I investigated the effects of one species of large-bodied grouper, while Mumby et al. (2011) pooled five species of grouper: Nassau grouper, the tiger grouper, the black grouper (*M. bonaci*), the yellowfin grouper (*M. venenosa*), and the yellowmouth grouper (*M. interstitialis*). Although it still remains possible that other grouper species or a diverse population of large-bodied groupers may reduce lionfish abundance (via diffuse predation, Hixon 1991), the results of this study suggest that Nassau grouper alone do not reduce lionfish abundance.

It may be that the venomous spines of lionfish (Nair et al. 1985, Cohen and Olek 1989) deter predation (Morris and Whitfield 2009) and/or that Nassau grouper have not yet learned that lionfish are suitable prey. Indeed, although divers in the Cayman Islands and elsewhere have trained wild Nassau grouper to eat speared lionfish, the same grouper species have not been observed attacking live lionfish (M.A. Hixon *personal communication*). Many fishes can learn behaviors through social interactions (reviewed by Brown and Laland 2003, Bshary et al. 2002). Many groupers, including Nassau grouper, may learn to take advantage of new food sources, such as following octopuses and foraging for disturbed food items (Diamant and Shpigel 1985, Roberts et al. 1995), as well as learn migratory routes to spawning

aggregations (Bolden 2000). However, there may be a lag time between the beginning of an invasion and a period when native predators begin to prey on an invasive species (reviewed by Carlsson et al. 2009, e.g. López et al. 2010). Because the lionfish invasion in the Bahamas is less than 10 years old, Nassau grouper and other native predators may not have had enough time to learn to consume lionfish.

4.4.2 Grouper inhibit negative effects of lionfish on native reef fishes

Evidence of biotic resistance to the lionfish invasion came from comparing the change in local abundance of small native reef fishes among different abundances of Nassau grouper in the presence and absence of invasive lionfish. In the absence of grouper, lionfish often extirpated many small reef fishes. In contrast, when many Nassau grouper were present on reefs with lionfish, the abundance of small reef fishes increased. While the accumulation of small reef fishes on these reefs was 32% less than predator free reefs, this reduction was not significantly less. Hence, a high abundance of Nassau grouper reduced the negative effect of lionfish predation, resulting in a positive indirect effect between Nassau grouper and small reef fishes. Notably, two ecologically important parrotfishes that had decreased in abundance on reefs inhabited only by lionfish (and no grouper), increased in abundance on reefs with lionfish and many grouper. Herbivorous parrotfishes are vital to maintaining coral reefs because they can control macroalgae, which can hinder coral recruitment and growth (reviewed by Mumby 2009).

One question that still remains is to identify the behavioral mechanism that caused the observed indirect positive effect of native grouper on small reef fishes. Stallings (2008) conducted a study on same Bahamian patch reefs before the lionfish invasion, and found that Nassau grouper had a positive indirect effect on small reef fishes. The mechanism underlying this positive indirect effect was inhibition of predation by smaller mesopredators (Stallings 2008). Given that lionfish did not experience reduced persistence or growth in the presence of Nassau grouper, predation was not the interaction that caused the observed indirect positive effect in my study. One potential mechanism is amensalism between Nassau grouper and lionfish, where typical frequent movement of many grouper around a reef interfered with the slow stalking behavior of lionfish, causing the lionfish to forage off the reef. This interaction would explain the increase in abundance of small reef fishes on reefs with many grouper, even though lionfish growth remained consistent across treatments. Anecdotally, lionfish on reefs with many grouper were often observed foraging > 2m from the patch reef, while lionfish on reefs with few or no grouper foraged within the reef structure or < 1m from the reef. It appeared that lionfish on grouper-laden reefs foraged in the surrounding seagrass where they were uninterrupted by grouper, returning to the reef when entering shelter. While this behavioral shift may displaced lionfish predation onto seagrass communities, spreading lionfish predation across multiple habitats could reduce overwhelmingly negative effects on any one habitat.

4.4.3 Multivariate community response

Community-level multivariate analysis provided further evidence that many Nassau grouper can confer some biotic resistance against invasive lionfish on patch reefs. By the end of the experiment, the ordination of reef-fish assemblages predominantly segregated by Nassau grouper treatment and lionfish abundance. Reef-fish assemblages with a high abundance of Nassau grouper were closer in the ordination to predator-free control assemblages than were assemblages with lionfish only (and no grouper), indicating that invaded communities with many grouper were similar to a pre-invasion system. The small reef fishes that drove this pattern were the same ones that experienced the largest negative effects of lionfish predation: bridled goby, the rosy blenny, the goldspot goby, and the beaugregory damselfish. These species experienced the largest reduction caused by lionfish, as previously documented by other field experiments (Albins and Hixon 2008, Albins 2012).

4.4.4 Conclusions

This is the first study to experimentally investigate how a native predatory species affects invasive lionfish. The results show that Nassau grouper have the potential to provide some biotic resistance against lionfish. While Nassau grouper did not limit either the persistence or growth of lionfish, they did mitigate the negative effects of lionfish predation on the local abundance of small reef fishes, likely by behaviorally displacing lionfish from feeding on the reefs. These findings suggest that

managers should promote policies to protect large-bodied groupers. Because this study examined only one native grouper and was conducted at the scale of small patch reefs, interactions at larger scales warrant investigation. Nevertheless, these promising findings indicate that there may be sources of biotic resistance to the lionfish invasion on Atlantic reefs, justifying further studies to investigate the effects of other native species on lionfish.

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Table 4.1 Mean change in abundance per species of small native coral-reef fishes on predator free control reefs (ΔN control) from the beginning to the end of the 11 week experiment. The effect size per species of each grouper and lionfish treatment was calculated by subtracting the mean change in abundance of each treatment from the mean change in abundance of control reefs. The table is sorted by decreasing change in abundance on control reefs.

Family	Species	Control ($\Delta N_{\text{control}}$)	Lionfish effect ($\Delta N_{\text{lionfish}} - \Delta N_{\text{control}}$)	Low grouper effect ($\Delta N_{\text{low grouper}} - \Delta N_{\text{control}}$)	High grouper effect ($\Delta N_{\text{high grouper}} - \Delta N_{\text{control}}$)
Gobiidae	<i>Coryphopterus glaucofraenum</i>	34.80	-35.30	-34.80	-21.47
Gobiidae	<i>Gnatholepis thompsoni</i>	9.00	-6.75	-5.85	6.33
Labrisomidae	<i>Malacoctenus macropus</i>	2.80	-3.80	-7.57	-2.80
Haemulidae	<i>Haemulon</i> sp. (juvenile)	2.40	-3.90	-0.63	3.93
Labridae	<i>Bodianus rufus</i>	2.20	-2.20	-2.28	-1.87
Pomacentridae	<i>Stegastes leucostictus</i>	1.60	-3.35	-3.52	-0.77
Scaridae	<i>Sparisoma viride</i>	1.60	-2.10	-1.06	0.90
Pomacentridae	<i>Stegastes dictus</i>	1.40	-1.40	-1.32	-1.23
Pomacanthidae	<i>Holocanthus tricolor</i>	0.80	-0.80	-0.72	-0.80
Labrisomidae	<i>Malacoctenus triangulatus</i>	0.60	-0.60	-0.60	-0.60
Pomacentridae	<i>Stegastes variabilis</i>	0.60	-0.60	-0.52	-0.60
Scaridae	<i>Cryptotomus roseus</i>	0.40	-0.65	-1.40	-0.23
Apogonidae	<i>Apogon maculatus</i>	0.40	-0.65	-0.25	0.10
Pomacentridae	<i>Stegastes partitus</i>	0.20	-0.95	-0.51	-0.87
Scaridae	<i>Sparisoma aurofrenatum</i>	0.20	-0.70	-0.89	0.30
Scaridae	<i>Sparisoma atomarium</i>	0.20	-0.45	-0.12	-0.37
Pomacanthidae	<i>Pomacanthus arcuatus</i>	0.20	-0.20	-0.20	-0.20
Serranidae	<i>Cephalopholis cruentatus</i>	0.20	-0.20	-0.12	-0.20
Apogonidae	<i>Apogon townsendi</i>	0.20	-0.20	-0.28	-0.53
Chaetodontidae	<i>Chaetodon ocellatus</i>	0.20	0.05	-0.20	-0.20
Labridae	<i>Thalassoma bifasciatum</i>	0.00	-2.00	-2.23	-2.17
Gobiidae	<i>Gobiosoma genie</i>	0.00	-0.25	0.00	0.00
Scaridae	<i>Sparisoma radians</i>	0.00	-0.25	0.15	0.00
Callionymidae	<i>Paradiplogrammus bairdi</i>	0.00	0.00	-0.31	0.00
Acanthuridae	<i>Acanthurus chirurgus</i>	0.00	0.00	-0.15	0.00
Labridae	<i>Halichoeres poeyi</i>	0.00	0.00	-0.08	0.00
Synodontidae	<i>Synodontidae</i> sp.	0.00	0.00	-0.08	0.00
Mullidae	<i>Mulloidichthys martinicus</i>	0.00	0.00	-0.08	0.00
Labridae	<i>Halichoeres pictus</i>	0.00	0.00	0.00	0.17
Apogonidae	<i>Apogon aurolineatus</i>	0.00	0.00	-0.08	0.33
Serranidae	<i>Serranus tigrinus</i>	0.00	0.00	0.00	0.33
Acanthuridae	<i>Acanthurus bahianus</i>	0.00	0.00	0.23	0.33
Lutjanidae	<i>Ocyurus chrysurus</i>	0.00	0.00	0.08	0.50
Pomacentridae	<i>Chromis cyanea</i>	0.00	0.00	0.00	-0.50
Labridae	<i>Halichoeres garnoti</i>	0.00	0.00	-0.62	-0.33
Apogonidae	<i>Apogon binotatus</i>	0.00	0.25	0.15	0.50
Tetraodontidae	<i>Canthigaster rostrata</i>	0.00	0.25	0.08	-0.17
Haemulidae	<i>Haemulon album</i>	0.00	0.50	0.00	0.00
Acanthuridae	<i>Acanthurus coeruleus</i>	0.00	0.75	0.38	0.50
Labridae	<i>Xyrichtys splendens</i>	-0.20	-0.05	0.20	0.03
Chaetodontidae	<i>Chaetodon capistratus</i>	-0.20	0.45	0.28	0.20
Labridae	<i>Halichoeres radiatus</i>	-0.40	-0.10	-0.06	-0.10

Table 4.1 continued

Scaridae	<i>Scarus taeniopterus</i>	-0.40	-0.10	-1.37	-2.43
Gobiidae	<i>Priolepis hipoliti</i>	-0.40	0.40	0.25	0.07
Holocentridae	<i>Sargocentron coruscum</i>	-0.40	1.90	-0.14	-0.10
Labridae	<i>Halichoeres maculipinna</i>	-1.60	0.10	0.06	-0.07

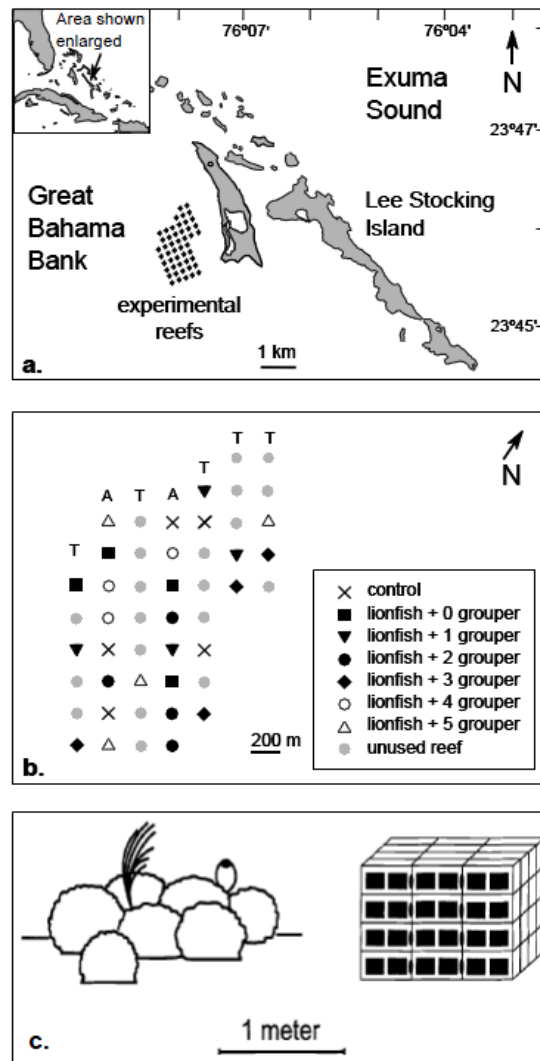


Figure 4.1 a. Map of the location of the experimental patch reefs in proximity to the Caribbean Marine Research Center on Lee Stocking Island, Bahamas (modified from Albins 2012). b. Experimental design showing the treatment assignments. Reef type, A = artificial and T = translocated, is indicated at the top of each column and designates the reef type for all reefs in that column. c. Drawings of the general structure of translocated (left) and artificial (right) experimental reefs (from Carr and Hixon 1997).

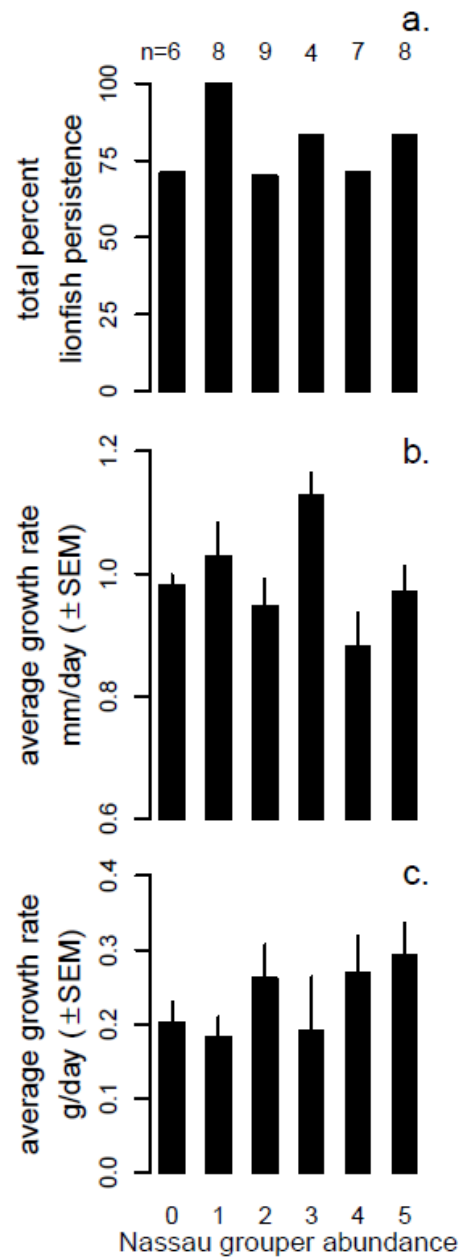


Figure 4.2 a. Total persistence (100% - mortality - emigration) of lionfish across Nassau grouper treatment abundances. n = total abundance of lionfish at each grouper abundance pooled among reefs. b. Lionfish growth rate in length across Nassau grouper abundances. c. Lionfish growth rate in mass across Nassau grouper abundances.

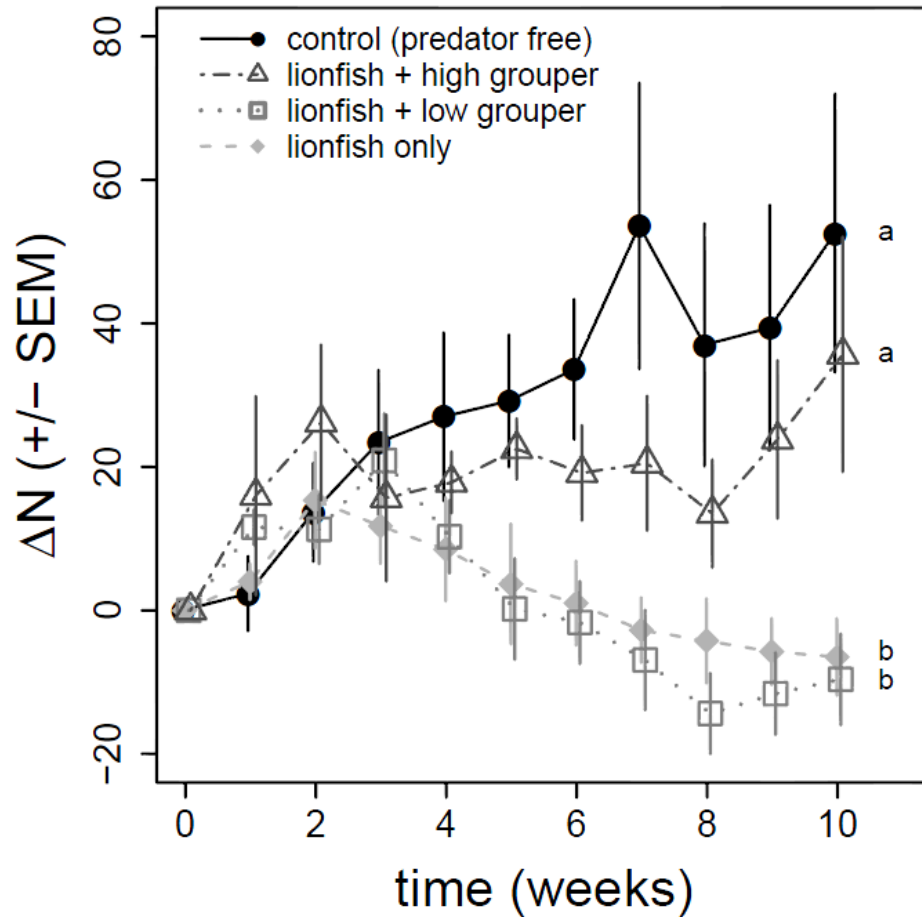


Figure 4.3 The change in abundance (ΔN mean \pm sem) of small reef fish (≤ 50 mm TL) on predator-free control reefs (solid black circles), lionfish + high-grouper reefs (4-5 grouper, open grey triangles), lionfish + low-grouper reefs (1-3 grouper, open grey squares), and lionfish-only reefs (solid grey diamonds). Each point gives the mean (\pm SEM) change in abundance between the given week and the initial survey for each treatment. Letters on the right side of the plot indicate pairwise t-test comparisons, where the same letters indicate a non-significant difference between treatments ($\alpha = 0.05$). Symbols are offset to aid view.

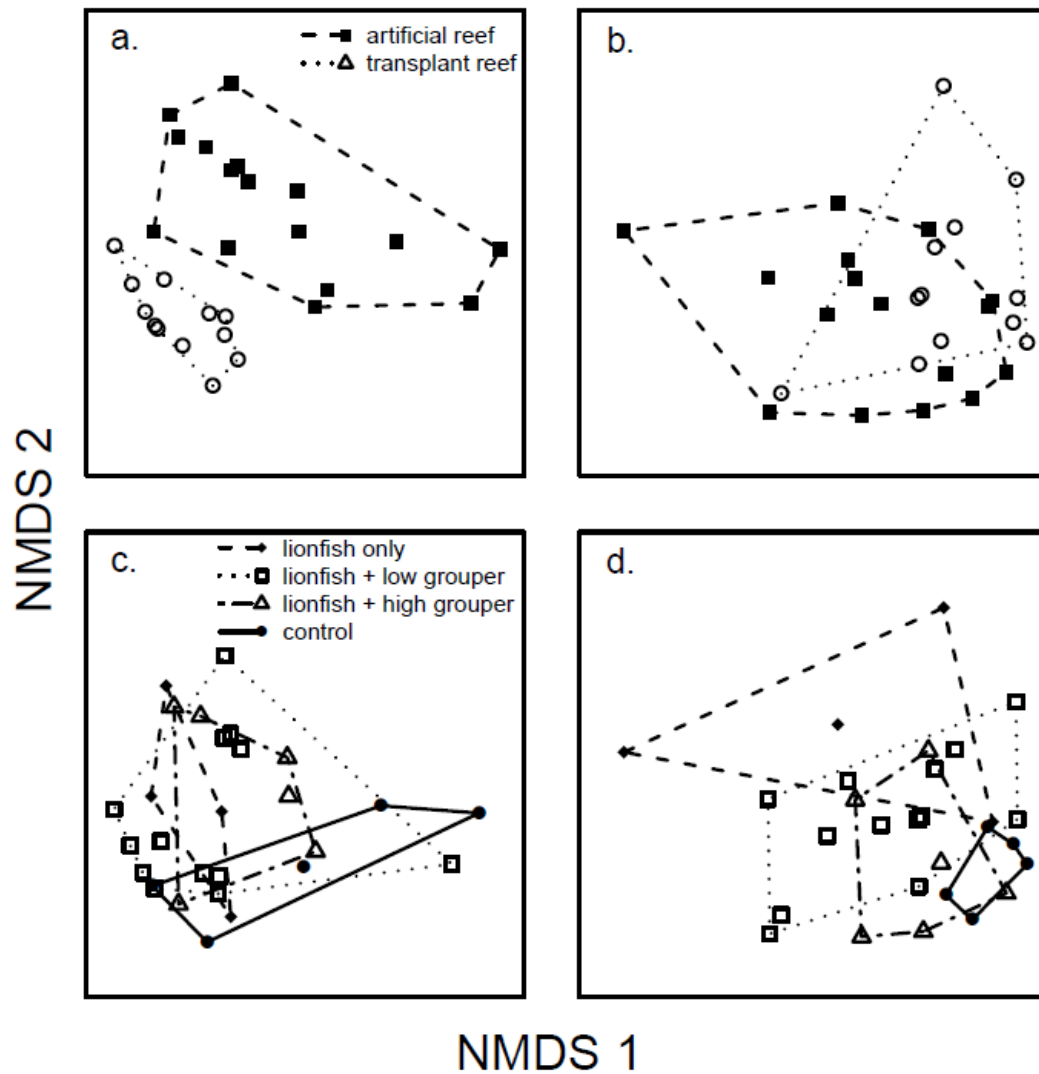


Figure 4.4 Non-metric Multidimensional Scaling (NMDS) plots where points closer to each other represent more similar sample units (reefs) in multidimensional species space. The top two plots show reefs designated by reef type, artificial (solid squares and dashed lines) and translocated (open circles dotted lines) at the (a) start and (b) end of the experiment. The bottom two plots show reefs designated by lionfish and Nassau grouper abundance: lionfish-only reefs (open diamonds, dotted lines), lionfish + low-grouper reefs (solid circles, dashed line), lionfish + high-grouper reefs (open triangles, dot-dash line), and predator-free control reefs (solid circles, solid line). To aid in view plots c and d have been rotated for maximum correlation with Nassau grouper treatment.

Chapter 5 – General Conclusions

The research described in this dissertation exemplifies the versatility of coral-reef fishes in facilitating field and lab studies relevant to both general ecology and conservation biology of marine systems. I studied five different species of coral-reef fish in a variety of experiments that investigated various questions. Bicolor damselfish (*Stegastes partitus*) were studied in a large scale, multi-generational study of larval dispersal and reproductive success within a marine metapopulation.

Interactions between invasive lionfish (*Pterois volitans*) and three native coral-reef fishes -- Nassau grouper (*Epinephelus striatus*), graysby grouper (*Cephalopholis cruentatus*), and bridled goby (*Coryphopterus glaucofraenum*) -- were studied in both manipulative field experiments and a laboratory experiment.

Chapter 2 describes a broad-scale study of temporal variability of larval dispersal of a common coral-reef fish over multiple cohorts. On the ecological time scale, eight parent-offspring pairs were identified, which directly documented both connectivity between and self-recruitment within local populations. The longest dispersal distance was 129 km between reefs, and remarkably, we found three juveniles from three different islands that had returned to their natal reef. Additionally both self-recruitment and connectivity were documented, at a marine reserve, indicating that the reserve was both self-seeding and exporting larvae to an unprotected area. The signatures of sweepstakes reproduction and Wahlund effect provided further evidence of temporal variability in larval dispersal patterns. While an

increasing number of recent studies have documented the spatial patterns of larval dispersal, most have not been conducted over multiple generations. Accounting for temporal variability in larval dispersal patterns is necessary to more accurately describe the range of metapopulation dynamics, specifically, to understand the degree to which populations are open or closed and which local populations may act as source or sink populations. Including this information in marine reserve design will enhance the ability of networks of marine reserves to account for interannual fluctuations in larval supply.

The experiments reported in Chapters 3 and 4 explored interactions between invasive lionfish and native species. While a growing number of studies have documented the ability of lionfish to decrease the abundance of small reef fishes, no study to date had examined how lionfish may affect processes that lead to population regulation. The experiments in Chapter 3 provide the first published evidence that invasive lionfish may be able to extirpate local populations of a native prey species. The average percent mortality of bridled goby on invaded reefs reached 93%, and at the lowest goby densities, mortality was 100%. This average percent mortality was higher than the 48% average on experimental reefs manipulated to recreate the pre-invasion system. Combined with the fact that lionfish consistently reduced the abundance of bridled goby among three different studies over three years, and that large lionfish consume almost the entire size range of bridled goby (i.e., the absence of a prey size refuge), it appears that lionfish have the potential to extirpate local

populations. These findings have grave implications for biodiversity on invaded western Atlantic and Caribbean reefs, and provide new evidence that lionfish may disrupt coral-reef population and community dynamics.

There have been many studies that document the negative ecological effects of invasive lionfish. The field experiment described in Chapter 4 is the first to determine whether a native predator may be a source of biotic resistance to invasive lionfish. There was no evidence that Nassau grouper affect survival or growth of juvenile lionfish. Across a broad range of Nassau grouper densities, lionfish had a high survival rate, above 70% over 10 weeks, and a nearly constant individual growth rate, approximately 1 mm/day and 0.25 g/day. However, the non-predatory behavior of Nassau grouper may nonetheless be able to moderate the negative effect of lionfish predation on small reef fishes. At the end of the 10-week experiment, the average net abundance of small reef fishes on reefs with a lionfish and many grouper was not different from the average on predator-free reefs. However, these average abundances were higher than the average on both lionfish-only reefs and reefs with one lionfish and few Nassau grouper. Behavioral observations suggested that Nassau grouper activity interfered with the slow stalking behavior of lionfish, forcing them to hunt off the reef where they would not be interrupted. This result indicates that adult Nassau grouper may have an amensalistic interaction with lionfish: grouper negatively affecting lionfish, yet lionfish having no effect on large grouper. Thus, if managers

protect Nassau grouper populations, then the increase in their abundances may reduce some of the negative effects of lionfish predation.

In conclusion, the research described in this dissertation demonstrates how coral-reef fishes can be effectively studied to answer a variety of basic scientific questions and provide findings relevant to marine conservation. These species can be the focus of both large-scale, multi-generation observational studies, as well as small-scale, manipulative experiments. From the perspective of basic ecology, the findings of this dissertation advance our understanding of larval dispersal and marine metapopulation ecology, as well as the population ecology of rarely studied marine fish invasions. From an applied perspective, the results also inform the placement and spacing of marine reserves by documenting temporal changes in larval dispersal, and provide evidence that there may be potential sources of native biotic resistance to the lionfish invasion.

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APPENDICES

Appendix A Chapter 2

Table A.1 Pairwise F_{st} values for both adult vs. adult (above diagonal) and recruit vs. recruit (below diagonal) bicolor damselfish populations. White text in black boxes indicates significant F_{st} values based on a sequential Bonferroni correction. Black text in grey boxes indicates pairwise F_{st} values with a p-value <0.05. Negative F_{st} values can be considered to be zero. ECLSP = Exuma Cays Land and Sea Park, LSI= Lee Stocking Island. Notice F_{st} values are low, ranging from -0.0032 to 0.0046.

		Cat				ECLSP				Eleuthera				LSI			
		2005	2006	2007	2008	2005	2006	2007	2008	2005	2006	2007	2008	2005	2006	2007	2008
Cat	2005		-0.001	0.000	0.000	-0.002	0.000	0.001	0.001	0.000	-0.003	0.000	0.001	0.000	0.002	0.001	0.001
	2006	0.001		0.000	0.000	-0.001	-0.001	0.000	-0.001	-0.001	-0.002	0.000	-0.001	0.001	0.001	0.000	0.000
	2007	0.002	0.000		0.001	0.000	0.000	0.000	0.001	-0.001	-0.001	0.000	0.000	0.004	0.001	0.001	0.000
	2008	0.002	0.000	0.000		0.000	0.000	0.000	0.001	-0.002	-0.001	0.001	-0.001	0.003	0.001	0.000	0.001
ECLSP	2005	0.004	0.002	0.001	0.003		-0.001	0.000	0.002	-0.002	0.000	-0.001	0.001	0.001	0.000	-0.001	-0.001
	2006	0.001	-0.001	0.000	0.000	0.001		0.001	0.001	-0.001	-0.002	0.000	0.000	0.001	0.001	0.001	0.000
	2007	0.002	0.000	0.001	0.000	0.001	0.000		-0.001	-0.002	0.000	0.000	-0.001	0.001	0.002	0.002	0.000
	2008	0.001	-0.001	0.000	0.000	0.002	0.000	0.000		-0.001	-0.001	0.001	0.000	0.001	0.004	0.000	0.001
Eleuthera	2005	0.000	0.001	0.000	0.001	0.005	0.000	0.000	0.000		-0.001	-0.001	-0.002	0.001	0.001	0.000	-0.001
	2006	0.001	0.000	0.001	0.001	0.002	0.000	0.000	0.000	0.001		-0.001	-0.001	0.001	0.001	-0.002	-0.001
	2007	0.003	0.000	0.001	0.001	0.004	0.000	0.000	0.000	0.001	0.000		0.000	0.002	0.001	0.001	0.000
	2008	-0.001	0.000	0.000	0.000	0.002	0.000	0.000	-0.001	0.000	0.000	0.000		0.001	0.003	0.000	0.000
LSI	2005	0.001	0.001	0.002	0.002	0.002	0.001	0.002	0.002	0.001	0.002	0.003	0.002		0.002	0.003	0.003
	2006	0.000	0.000	0.000	0.000	0.001	0.000	-0.001	0.000	0.000	0.000	0.000	0.000	0.001		0.001	0.003
	2007	0.001	0.001	0.001	0.000	0.003	0.001	0.001	-0.001	0.000	0.000	0.000	0.000	0.002	0.001		0.001
	2008	0.002	0.000	0.000	0.000	0.001	0.000	-0.001	-0.001	0.001	0.000	0.000	0.000	0.002	0.000	0.000	

Table A.2 Pairwise F_{st} values among all adult vs. recruit populations. Black boxes with white text indicate significant F_{st} values based on a sequential bonferroni correction. The dark grey boxes with black text indicate addition significant pairwise F_{st} values with a p-value <0.05. Negative F_{st} values can be considered zero.

		RECRUITS																
		Cat				ECLSP				Eleuthera				LSI				
		2005	2006	2007	2008	2005	2006	2007	2008	2005	2006	2007	2008	2005	2006	2007	2008	
ADULTS	Cat	2005	0.000	0.000	0.000	0.001	0.001	-0.001	-0.001	0.001	-0.003	0.000	0.001	-0.001	0.000	-0.001	0.000	-0.001
		2006	0.000	-0.001	-0.001	-0.001	0.001	-0.002	-0.001	-0.001	0.000	-0.001	-0.001	-0.001	0.000	-0.001	0.000	-0.001
		2007	0.002	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.002	0.001	0.001	0.001	0.002	0.000	0.001	0.000
		2008	0.001	0.000	0.000	-0.001	0.003	0.000	0.000	0.000	0.000	-0.001	0.000	0.000	0.002	-0.001	-0.001	0.000
	ECLSP	2005	0.000	0.000	0.000	0.002	0.001	-0.002	-0.002	0.000	-0.001	-0.001	-0.001	-0.001	0.000	-0.002	0.001	-0.001
		2006	0.000	0.000	0.001	0.001	0.000	0.000	0.000	-0.001	0.002	0.000	0.001	-0.001	0.001	-0.001	0.000	-0.001
		2007	0.002	0.000	0.001	0.001	0.003	-0.001	-0.001	0.000	0.002	0.000	0.000	0.000	0.001	0.000	0.001	-0.001
		2008	0.002	0.001	0.001	0.000	0.003	0.001	0.000	0.000	0.001	0.001	0.000	0.001	0.003	0.001	0.000	0.000
	Eleuthera	2005	0.000	-0.001	-0.001	-0.001	0.001	-0.001	-0.002	-0.001	-0.002	-0.001	0.000	0.000	0.001	-0.002	0.000	-0.002
		2006	0.001	-0.001	-0.001	-0.001	0.002	-0.001	-0.001	0.000	-0.003	0.000	-0.001	-0.001	0.000	-0.002	0.001	-0.001
		2007	0.002	0.000	0.001	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.001	0.001	0.000	0.001	0.000
		2008	0.003	-0.001	0.001	-0.001	0.004	0.001	-0.001	-0.001	0.000	-0.001	0.000	0.000	0.003	0.001	0.001	-0.001
	LSI	2005	0.002	0.002	0.003	0.004	0.003	0.001	0.002	0.003	0.003	0.001	0.004	0.002	0.002	0.001	0.003	0.002
		2006	0.004	0.001	0.001	0.002	0.004	0.000	0.003	0.001	0.002	0.001	0.002	0.001	0.002	0.001	0.002	0.002
		2007	0.001	0.001	0.001	0.000	0.003	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.002	0.000	0.001	0.001
		2008	0.003	0.001	0.001	0.001	0.000	0.001	-0.001	0.001	0.001	0.000	0.000	0.001	0.002	0.000	0.001	-0.001