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Examining the Lionfish Invasion: How Growth and Recruitment Relates to Connectivity and Controls

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EXAMINING THE LIONFISH INVASION: HOW GROWTH AND RECRUITMENT
RELATES TO CONNECTIVITY AND CONTROLS

By

Dominique Lazarre

A DISSERTATION

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The global nature of travel and trade has increased the potential for the spread of invasive species around the world. These invasive alien species (IAS) have the potential to negatively influence the ecosystems they invade by preying upon, infecting or out-competing native species or altering their new habitat. The invasion of two Indo-Pacific lionfishes, *Pterois volitans* and *Pterois miles,* is having far reaching impacts on reef fish biodiversity and abundance throughout the Tropical Western Atlantic Ocean. The body of lionfish research from their native range is composed of studies related to their biology or behaviors in aquarium settings. These deficiencies have left researchers with knowledge gaps related to why lionfish spread so rapidly and the best methods to control lionfish abundance in the diverse habitats they occupy. Analysis of juvenile otolith birthdates verified that lionfish successfully spawn throughout the calendar year. This information was coupled with known early life history traits of lionfish to simulate dispersal of "lionfish" particles in the Caribbean. This dispersal identified Florida, the Bahamas and Cuba as major sinks for lionfish larvae throughout the region, and Jamaica as an important link between the Greater Antilles and southern portions of the Caribbean. Additionally, lionfish reproductive strategy facilitated their spread across a known geographic barrier between Florida and the Caribbean that creates a genetic break

between native reef fishes. Highly connected dispersal pathways, continuous spawning of lionfish, and the lack of natural predators help to make lionfish more successful in the invaded range, and prompt the development of management plans to counteract this growth. Lionfish are present in a host of diverse habitats, but monitoring and culling is restricted to shallow, diveable waters. A two-year observer study in the deeper water spiny lobster trap fishery (>20 m) was used to investigate the distribution of lionfish in an understudied environment. Lionfish have become a major component of the fishery's bycatch, and the trap fishery provides an additional mechanism for lionfish removals. This method can eliminate a larger number of individuals than the traditional methods used in shallower water culling derbies. The lack of lionfish caught in the shallower fishing areas precipitated a series of tank experiments to investigate the preference for benthic structures found in the different depth regimes within the fishery. Lionfish spent more time associating with coralline habitat structures than any other, but they were displaced from this habitat in the presence of lobster. The exclusionary behaviors of lobster indicate their potential to alter lionfish habitat choices. These insights provide marine managers with information to understand invasive species ecological dynamics, to better manage for future invasions, and to mitigate the impacts of lionfishes.

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Exotic and Invasive Species

Introductions of exotic species are becoming increasingly common, one of many ecosystem stressors that threaten biodiversity worldwide (Vinebrooke et al., 2004; Halpern et al., 2008). The global nature of travel and trade has increased the potential for the spread of exotic species around the world (Vitousek et al., 1996; Mack et al., 2000). A mismatch of resources, parasitism, predation or competition prevent the majority of introduced species from surviving after the transportation phase of an invasion (Lockwood et al., 2007). However, some newly introduced species can co-exist within their new environment and develop a self-sustaining population (Lockwood et al., 2007). New invaders that can live without demonstrating any measurable harm to native species or habitats are described by the terms "alien", "exotic", "introduced", or "non-native". Alternatively, when an exotic species creates quantifiable ecological or economic disruptions to their new ecosystem they are defined as "invasive" (Lockwood et al., 2007).

An increased emphasis was placed on investigating the dynamics that govern invasive species spread in the early 1990s, with a majority of field studies focused on plant species in terrestrial environments (Lowry et al., 2013). In addition to plants, mammals, reptiles, freshwater and marine species have successfully established breeding populations in new locales, and completed the transition from alien to invasive as they alter their new environment. Prominent examples from the southeast United States include: melaleuca trees (*Melaleuca quinquenervia*) that outcompete native grasses creating stands of trees in wetland areas (Turner et al., 1998); red imported fire ants

(*Solenopsis invicta*) that outcompete native keystone species and predate ground nest-laying species (Allen et al., 1994); burmese pythons (*Python bivittatus*) that prey upon large numbers of native mammal and reptiles in wetland and forested habitat (Dorcas et al., 2011); and Asian green mussels (*Perna viridis*) that outcompete native fouling organisms after settling on man-made underwater structures (Baker et al., 2011).

The variety of habitats that invasive species are found in spans the world, as the global nature of trade and transport links communities. This necessitates continued research to understand invasive species spread, particularly in non-terrestrial enviornments.

Lionfish Invasion History and Distribution

Marine organisms are regularly transported to new regions via fouling on ship hulls and in ballast water used to stabilize large, ocean-going vessels (Ruiz et al., 1997; Bax et al., 2001). Low detection probability and lower densities in comparison to planktonic invertebrate species have lead to marine fish species being represented to a lesser extent than other marine taxa in the invasive species literature (Hare and Whitfield, 2003; Semmens et al., 2004; Ruiz-Carus et al., 2006;Lockwood et al., 2007; Lowry et al., 2013). In recent years, the Western Atlantic Ocean, Caribbean Sea and Gulf of Mexico have become home to two closely related species of Indo-Pacific lionfish, *Pterois volitans* (Linnaeus, 1758) and *Pterois miles* (Bennett, 1828). A host of Scorpaenidae species are commercially available in the United States (Table 1.1), but these species are the first marine fish invaders to develop an established population as a result of aquarium releases (Whitfield et al., 2002; Semmens et al., 2004; Ruiz-Carus et al., 2006; Morris and Whitfield, 2009). They have been designated the "*Pterois volitans/miles* complex"

due to inconsistencies in classifying them as two separate species, a single species, or closely related species with the ability to interbreed (Schultz, 1986; Kochzius et al., 2003). The term "lionfish" will be used to describe introduced individuals from both species, and/or hybrid offspring of the two species.

The first confirmed sightings of lionfish were recorded off the coast of Florida in the mid-1980s and 1990s (USGS Database 2014, Whitfield et al., 2002; Schofield, 2009). Lionfish extended their range northward along the coast of the United States until the early 2000s (Whitfield et al., 2002). In the 2000s, lionfishes continued to spread at a more rapid rate, extending their range through the Caribbean Sea and Gulf of Mexico (Schofield, 2009; Whitfield el al., 2002). These species can also be found at depths ranging from shallow near-shore environments less than 1 meter in depth to 300 meters or more (Morris, 2012; Nuttall et al., 2014). Lionfish have been observed in reef systems (Morris, 2012), hard-bottom structures (Whitfield et al., 2002; Morris, 2012), mangroves (Barbour et al., 2010, Morris, 2012), and estuarine and riverine habitats (Jud and Layman, 2012). Individuals are thriving in the Western Atlantic along the United States, found as far north as North Carolina year-round, with summer populations observed as far north as New England (Whitfield et al., 2002; Schofield, 2009).

Lionfish have a wide physiological tolerance for a nearshore marine fish species, which can allow them to impact a variety of habitats as their populations grow in size.

Lionfish Ecology and Life History Traits

Prior to the invasion in the Western Atlantic, studies on *Pterois volitans* and *Pterois miles* in their native range were limited in scope, with many based on analyses of captive specimens in their native range or observational studies. A detailed analysis of the

courtship and spawning behaviors of related lionfish species were carried out in the native range in the 1970s (Fishelson, 1975). Males from the species D*endrochirus brachypterus* (short fin turkey fish) and *Pterois volitans* lured females to the ocean surface during courtship where each female released two clear, buoyant egg masses that were then fertilized externally by males (Fishelson, 1975). Gonadal histological analyses of lionfish specimens showed that lionfish females possess the ability to reproduce throughout the year, with each female capable of releasing between 10,000 and 20,000 eggs every four days (Morris et al., 2009; Morris et al., 2011). The larval stage represents the mobile life phase connecting lionfish populations and allowing for their extensive spread.

The increase in lionfish abundance has encouraged investigations of the predatory habits of lionfish and to quantify their effect on local fish populations in the invaded range. Smaller lionfish have a diet consisting predominantly of crustaceans and small fishes, shifting to a piscivorous diet as they increase in size (Morris and Akins, 2009; Munoz et al., 2011). They prey upon species in relation to local abundance, consuming whatever is available (Munoz et al., 2011). Many prey species come from commercially and recreationally important fish families including: Apogonidae, Gobiidae, Labridae, Pomacentridae, Serranidae, and Synodontidae (Green et al., 2012). Multiple studies have demonstrated that lionfish significantly reduce the abundance and biomass of native species (Albins and Hixon, 2008; Morris and Akins, 2009; Green et al., 2011; Green et al., 2012), at rates nearly three times higher than some native conspecifics (Albins and Hixon, 2011, Albins, 2013).

Lionfish are fast growing, fecund, generalist predators, that can inhabit a wide range of habitat. Lionfish represent a new threat that can disrupt the trophic food webs in Tropical Western Atlantic Ocean ecosystems.

Lionfish Population Management Methods

The threat that lionfish pose to small-bodied and juvenile fish abundance and diversity prompted a review of potential predators that could effectively reduce the population size of these invasive predators. Observations of tiger and Nassau grouper consuming invasive lionfish have been documented in the literature (Maljkovic et al., 2008; Mumby et al., 2011), in addition to anecdotal accounts of moray eels, barracuda and sharks consuming live lionfish (personal communication Curt Slonim). Nevertheless, an assessment of the density and biomass of lionfish and native predators on various reefs throughout the Caribbean has shown no differences between the two groups, suggesting that native predators are not regulating the settlement of lionfish to Caribbean reefs (Hackerott et al., 2014).

Whether predators will adapt their long-term foraging habits to counteract lionfish population growth in years to come is yet unknown (Whitfield et al., 2002). Many potential predators are severely overfished (Ault et al 1998; Jackson et al., 2001; Pauly and Palomares, 2005), and may not be able to significantly contribute to controlling lionfish abundance. The negative effects of invasive lionfish can act synergistically with the many existing threats stressing marine ecosystems in the invaded region (Halpern et al., 2008): pollution (Shahidul and Tanaka, 2004; Diaz and Rosenberg, 2008), overfishing (Ault et al., 1998; Pauly and Palomares., 2005; Coleman et al., 2004), and global climate change (Edwards and Richardson, 2004; Hoegh-Guldberg et al., 2010).

Human intervention can compensate for the lack of native predators regulating lionfish populations; an accepted strategy to minimize the negative impacts of this invasive species on already degraded but important reef systems. Several key strategies have been employed to reduce lionfish populations: 1) physical removal, 2) limiting future introductions, and 3) creating incentives for targeted fishing efforts. The physical removal of lionfish with nets, spears, homemade capture devices or traps has been promoted for recreational divers and citizen scientists (Akins, 2012[b]). Lionfish removal has been shown to reduce localized abundance of the invasive pests on small scales as a result of organized derbies, repeated removals by managers, or by volunteers (Hare and Whitfield, 2003; Akins, 2012[b]; Green et al., 2014). Reef Environmental Education Foundation has been a leader in developing and conducting educational and outreach initiatives to raise awareness of lionfish impacts and the goal to remove lionfish from diving and fishing areas throughout the invaded range. Many countries within the Caribbean region have adopted and implemented lionfish control strategies, but these strategies require intense effort and cost that may not have been anticipated in long-term sustainable fishing plans before the introduction of lionfish (Akins, 2012[b]).

Lionfish abundance is not currently being controlled by native predator species, and has led to interventions from humans to regulate their population size. The majority of these efforts can only be carried out in shallow dive-able depths, which neglects the wide variety of habitats that lionfish can occupy.

Objectives

Lionfish represent the first marine invasive to become established in a new environment, and their ability to reduce the abundance of small-bodied and juvenile

fishes is well-documented in the literature. Effective management of lionfish populations hinges on our ability to understand how lionfish life history traits allow them to thrive, and refining capture methods to increase the yield of lionfish removed from the invaded region. The review of relevant lionfish studies presented above demonstrates the progress that has been made in understanding lionfish distribution, reproductive strategies, feeding ecology, and control methods. The goal of this dissertation is to build upon these findings to investigate the early life stages of lionfish that control lionfish dispersal between regions, and to investigate the distribution of lionfish in under-studied regions.

The initial goals of this research were to fill the knowledge gaps that remain in the lionfish life cycle, specifically regarding the dispersal of their larval phase. Age estimates ascertained from reading juvenile lionfish otoliths were used to determine a temporal trend in juvenile lionfish recruitment to reef habitat (Chapter 2). These results were used to parameterize a model simulation of lionfish dispersal, throughout the Caribbean basin, over the time period of rapid range extension within the invaded range. The simulation results were used to understand the aspects of lionfish biology enhance their dispersal and quantify their dispersal potential between regions (Chapter 3). Additionally, lionfish populations need to be described in areas outside of the shallow waters that can be easily reached. An observer study was conducted to define the distribution of lionfish bycatch in the fishery of the Florida Keys. This study identified catch rates of lionfish in comparison to the major species groups captured in the traps, over a two-year period (Chapter 4). The spatial patterns of lionfish revealed from the observer study were used to design a tank experiment to investigate lionfish bottom structure preferences. Proxy bottom structures were constructed to mirror the structure types available to lionfish at different depths

within the lobster trap fishery, and the time spent associating with each structure type was used to identify a preference for a particular structure type (Chapter 5). These analyses are used to synthesize strategies for enhancing the management of invasive lionfish populations in the invaded range (Chapter 6).

Table 1.1. List of Scorpaenidae species that are available for purchase in the aquarium trade in the United States.

Scientific Name	Common Name(s)
Dendrochirus barberi	Dwarf lionfish, Green lionfish, Hawaiian lionfish
Dendrochirus bellus	Japanese lionfish
Dendrochirus biocellatus	Fu manchu lionfish, Two-spot lionfish
Dendrochirus brachypterus	Fuzzy dwarf lionfish, Shortfin dwarf lionfish, Short-fin turkeyfish
Dendrochirus zebra	Dwarf lionfish, Dwarf zebra lionfish, Zebra lionfish
Pterois antennata	Antennata lionfish, Broadbarred firefish, Ragged finned lionfish, Spot-fin lionfish, Spot-finned lionfish
Pterois miles	Miles lionfish
Pterois mombasae	African lionfish, Deepwater firefish, Deepwater lionfish, Deepwater Mombassa lionfish, Devil's lionfish, Frillfin turkeyfish
Pterois radiata	Radiated Lionfish, Clearfin lionfish, Radial, Tailbar lionfish,Radial firefish, Whitefin lionfish, White-striped lionfish
Pterois russelli	Clearfin lionfish, Plaintail firefish, Plaintail turkeyfish, Red volitans, Russell's lionfish, Spotless butterfly cod, Spotless firefish, Zebrafish
Pterois volitans	Black lionfish, Black volitans, Butterfly cod, Caribbean lionfish, Common lionfish, Featherfins lionfish, Peacock lionfish, Red firefish, Red Lionfish, Scorpion cod, Turkeyfish, Volitan's lionfish, Zebrafish

Chapter 2 –Spawning Frequency and Larval Recruitment of Juvenile Lionfish to Coral Reef Habitat Based on Otolith Analyses

Background

The persistence of fish populations can be influenced by a host of human-mediated and natural factors that change the survivorship of each species. Fisheries managers can design regulations to diminish some of the negative anthropogenic influences on the marine environment, specifically those related to fishing pressure. Management regimes are employed to maximize yields of commercially important species by regulating the size of target species, implementing fishing seasons, restricting or modifying gears to deter harmful effects on target species and habitats, and controlling the quantity of landed catch. These measures are used to help increase the success of fish populations, but tend to most directly affect fish that are susceptible to fishing gears. Some of the natural fluctuations in population abundance over time are in large part due to the variation in juvenile recruitment from year to year (Hixon and Carr, 1997). Fish larvae must overcome the "wall of mouths" that glean zooplankton from the water column (Hamner et al., 1988), resist post-settlement mortality to reach adulthood, and finally reach sexual maturity to make reproductive contributions to sustain the survival of the species. During the transition from the juvenile life stage to the adult life stage some fishes become susceptible to fishing gears.

The lionfishes, *Pterois volitans* and *Pterois miles*, represent the first marine fishes to establish a breeding population in the Atlantic Ocean from the Pacific Ocean (Meister et al., 2005); and continue to extend their range and population sizes in the Western Atlantic Ocean, Caribbean Sea and Gulf of Mexico. They are also the first marine

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invasive species to be part of the complex of species targeted by fishers. It is important to determine the life history characteristics that make lionfish superior invaders. More specifically, the frequency and seasonality of spawning can drastically change the invasibiliry and ability of an organism to spread (Cowen et al., 2006; Cote et al., 2013). Lionfish spawning involves the release of two clear, gelatinous egg masses near the ocean's surface (Fishelson, 1975); but these events have not been witnessed in situ in the invaded range. Shortly after the lionfish invasion started taking hold in the Western Atlantic, the spawning capabilities of lionfish were investigated through histological examination of female lionfish ovaries. Morris et al showed that lionfish females collected in the Bahamas and North Carolina had ovaries at all stages of development, with mature females retaining the ability to spawn every 4-5 days (Morris et al., 2009). However, the incidence of vitellogenic and mature stage oocytes was variable seasonally (Morris et al., 2009). These findings indicate that lionfish have the potential to spawn year round; however, this does not guarantee the year-round settlement of successful larvae to adult habitat. Successful settlement depends on both the seasonal patterns of egg production and survival. If survival is strongly seasonal, animals that have year-round spawning will have strong seasonal settlement. One way to examine if survival is to examine successful settlers and their birth dates. This study will focus on determining the potential for larval lionfish to successfully survive to recruit to reef habitat throughout the calendar year.

Studies of otoliths can be used to determine birth dates of fish. Otoliths provide sensory support for hearing and balance and are located in the semi-circular canals in the cranial cavity. Lionfish have three pairs of otoliths, however, the sagittae are the largest

and are used most often in age and growth analyses due to their size (Morales-Nin, 1992l Campana and Neilson, 1985). The alternating pattern of light and dark calcium carbonate deposits on the otolith provides a record of an individual's growth, in either days or years (Pannella, 1971; Pannella, 1974; Morales-Nin, 1992).

As a fish grows it deposits a series of circular rings of calcium carbonate, in a regular interval, usually 24-hours during the early stages of life. A daily increment is made up of a pair of bands, one translucent and the second a slightly narrower darker band (Victor, 1982; Pannella, 1971; Pannella,1974). These daily increments can be identified and counted within the first year of life (Pannella, 1974; Brothers et al., 1976), as accretion occurs at a regularly spaced interval. Usually larval and juvenile otoliths do not require sectioning to expose the microstructural patterns of growth, but in general sectioning the otolith removes obscurity caused by overlying calcium carbonate deposition (Campana and Neilson 1985).

As age increases towards the first annulus, or first yearly age band, daily increment marks become narrower and less distinct. After one year most daily increments can no longer be individually distinguished, at which point a seasonal pattern with a darker "annulus" laid down during the major growth segment of each year is easily detected (Brothers et al., 1976; Morales-Nin, 1992).

Previous lionfish aging studies have focused on identifying the age of individual lionfish older than one year to estimate yearly growth rate of these species within their invaded range. This analysis, however, focused on the aging and growth of juveniles in their first year of life to improve our understanding of juvenile growth rates and to assess the occurrence of successful lionfish spawning throughout the year. An index of juvenile

lionfish hatch or "birth" dates was created by aging juvenile lionfish by counting daily increment marks, and using estimated ages and sampling dates to create a distribution of lionfish settlement days throughout the calendar year.

Methods

A collection of 72 juvenile lionfish otoliths were sourced from the National Oceanic and Atmospheric Administration (NOAA) Beaufort Laboratory collection. These otoliths were extracted from the cranial cavity of lionfish collected in the coastal waters of The Commonwealth of the Bahamas between June 2007 and September 2008 (Figure 2.1). The otoliths were cleaned, dried, embedded in resin, transversally sectioned and mounted. Each otolith was given a unique identifier to prevent the reader from associating any data with an individual lionfish while analyzing each otolith. The sectioned otoliths were examined and photographed under an Olympus (BH2) compound microscope and attached Leica DEFC290 HD digital camera, allowing otoliths to be viewed on a large LCD monitor. ImagePro was used to stitch together a single photo of the entire otolith at 40x magnification (Rasband, 1997-2014). Each otolith section was photographed, enhanced with the "Best Fit" ImagePro filter to improve the contrast of the image, and saved as a high resolution tiff file for further analysis.

ImageJ was used to view the daily increment marks on each otolith along two reading axes (Rasband, 1997-2014): the narrow axis from the core to the edge on the dorsal side of each otolith and from the core to the broad top axis of the ventral side of each otolith (Figure 2.2). Methods for aging juvenile lionfish has not be described previously, so this analysis aims to determine the best axis for determining the age of juvenile lionfish. Each otolith was blind read by a single reviewer four times. A "read"

consisted of marking and tallying each daily increment from the otolith core to edge, with

an annotation tool. Enumeration of increment marks was completed on a separate

occasion to avoid reader bias when performing subsequent reads. All otoliths were

marked on both reading axes before re-analyzing the set for successive reads. Four reads

were completed for each otolith, on both the narrow and broad reading axes. An age

estimate was calculated for each of the two reading axes. Additionally, the precision

between the two reads closest in value were calculated with the following coefficient of

variation equation:

Equation 2.1:

$$CV = 100 * \left(1 + \frac{1}{4n}\right) * \frac{s}{\bar{x}}$$

which is corrected for the small sample size. When the coefficient of variation between

the reads was $\leq$5%, one of the two reads was randomly selected as the increment count,

"age," for each lionfish juvenile. Age estimates with greater than 5% CV were included if

their CV was less than 15%. To compare reading axes, counts from the two reading axes

were compared to each other and assessed to determine which was most unambiguous

and consistent. The axis with the most precise estimates was used to create a growth

curve for the juvenile lionfish samples. The most precise axis was defined as having the

lowest CVs between the four otolith age estimates, and lacking a relationship between

higher CV and greater lionfish length.

Age estimates were used to back-calculate the birth dates of each fish, assumed to

be the first day that lionfish start accreting rings to their otolith. Birth dates were

computed for each individual by subtracting the age (increment number) in days from the

date of capture for each lionfish. *Dendrochirus bracypterus*, a close relative of *P. volitans* and *P. miles,* were shown to have visible otoliths when hatching at day 3 of development post-fertilization (Fishelson, 1975). For this reason, the day of hatching and birth date will be considered to occur on the same day for lionfish as well, as no published data describes the day otolith accretion and hatching occurs for the lionfish species examined in this study. The distribution of lionfish birthdates was created to find presence or absence of newly born lionfish during each month of the calendar year.

A linear model was fitted to the age estimates to estimate the growth of lionfish included in the sample. This fitted curve was compared to published curves to validate the accuracy of the ages estimated in this analysis. An ANCOVA was used to assess the differences in the age curve generated and previously published literature.

Results

The otolith collection used for this analysis contained 72 otolith samples, of these, only 52 of these otoliths could be accurately aged. Otoliths were also excluded from the analysis when no clear reading axis could be distinguished from their microstructures due to cracks, broken or chipped otoliths close to the otolith outer edges, and dark regions due to inconsistencies in otolith thickness. The juvenile lionfish aged had total lengths that ranged from 72 to 286 mm, with a mean of 147 mm (Figure 2.3). All but one of the fish included in this analysis had total lengths smaller than the mean total length estimated for an age 1 lionfish, 215 mm (Barbour et al., 2011).

Determination of Pelagic Larval Duration

Settlement marks were identified for all of the otoliths aged and the numbers of increments from the core to the settlement mark were counted to estimate pelagic larval

duration (PLD). The number of increments between the core and settlement ranged

between 21 and 39, with a mean of 30.2 increments. This corresponds to an estimated

average PLD of 30 days for lionfish, assuming they start accreting their otolith on the

same day they hatch (Figure 2.4).

Evaluation of Reading Axes

   Lionfish age increments were enumerated along the broad and narrow axes on

opposing sides of each otolith. A final age count, in increments, was randomly selected

from the two closest reads for the broad and narrow axes. The age counts ranged from 77

to 257 increments for the two axes. The mean increment count was smaller for the

broader axis as compared to the narrow axis, with 135 and 144 increments, respectively.

A t-test indicated that there was no significant difference between the means of the counts

between the two axes (p = 0.2068), and a linear regression of the axes counts showed a

highly significant correlation between the two reading axes (Figure 2.5). Distributions of

the final increment counts were constructed for the two axes (Figure 2.6).

   The dispersion between the two closest reads was estimated by calculating the

coefficient of variation (CV) for each sample. The CV between the two closest otolith

reads was lower than 10% for all samples, and less than 5% for all but 2 and 3 of samples

for the broad and narrow axes respectively (Figure 2.7). A linear model was fitted to the

coefficient of variation values to determine if there was a correlation between CV and the

size of the fish, but there was no significant trend in CV for size of lionfish when

comparing the two closest reads (p>0.05).

   When considering all four reads for each otolith, there was no significant trend in

CV for the broad reading axis, but a significant trend was evident for the narrow axis

(Figure 2.8). The significant trend was likely a product of the greater presence of sub-daily increments on the narrow axis in comparison to the broad axis which made analysis of the microstructure more difficult. The calculation of the final birth dates was based upon the reads from the broad axis, which maintained high precision and no trend in the deviations with increasing size in lionfish length.

Growth Rate Estimation

A linear regression fitted to the length at age data corresponded with a mean growth rate of 0.69 mm/day for lionfish juveniles. This growth rate was compared to two published growth rates for adult lionfish caught in North Carolina and Little Cayman (Figure 2.9). The estimated growth rate for lionfish juveniles in this study was higher than the two published growth rates completed using a combination of juveniles and adults.

The age estimates obtained from the broad reading axis were used to construct a distribution of birth months for the otoliths analyzed in this study (Figure 2.10). Lionfish juveniles were found to be born in every month, with the exception of September. The small number of samples utilized and uneven numbers of lionfish collected in each month prevents any definitive interpretation of seasonality of lionfish spawning, but these results do indicate that lionfish juveniles are born and settle to reef habitat in the Bahamas year round.

Discussion

The estimation of pelagic larval duration in this study closely matches the PLD designated by the analysis conducted by Ahrenholz and Morris (2010). The number of increments between the otolith core and settlement mark corresponded to the age of

lionfish at settlement, in days. For both studies, the minimum settlement age was 20 days; but our study found the maximum settlement age to be 39 days, four days more than previously reported (Ahrenholz and Morris, 2010). The mean settlement age was estimated to be 30.2 days. This value was higher than presented by Ahrenholz and Morris, but the average fell within the previous studies 20-35 day range of settlement ages (2010). The similarity in these ranges helped to validate the accuracy of the subsequent aging analyses in this study. The inflection points in the birth and settlement distributions appeared to be offset by one month, corresponding to the mean number of days to settlement (Figure 2.12).

This analysis identifies the most reliable reading axis for aging juvenile lionfish as the broader region from the core to the ventral edge. The reads from the narrower axis, from the core to dorsal edge, were significantly correlated with those on the broader axis; there were higher increment counts on average on the narrower axis. These higher values were a function of sub-daily increments and distortions along the reading axis on the narrower region of the otolith, which made discerning the increment count for each otolith more difficult. This was substantiated by the significant increasing trend in coefficient of variation with increased lionfish length when comparing reads made on the narrower reading axis of the lionfish. The counts from the broader reading axes maintained lower CV between the two closest reads and no trend in variance with increased lionfish length. The broad axis for aging juvenile otoliths should be designated as the preferred reading axis, unless only the narrow region of the otolith is visible. The only other study to identify the reading axis used a photo to identify annuli (Potts et al., 2010). While the directional reading axis is not stated in most lionfish aging studies, few

of them have focused on the aging of juvenile fish. The high correlation between the narrow and broad axis should allow the results from all lionfish aging analyses to be comparable.

Previous lionfish aging studies focused on aging adults of all age classes and estimating age with annual rings (Barbour et al., 2010; Potts et al., 2010; Edwards et al., 2014). In such analyses, fish that are less than a year old are lumped into the same age category. This is an acceptable method for estimating a growth curve over the entire life span of a lionfish, however, this technique does not describe the growth rate within the first year. This analysis examined the first year of growth by days, and fitted a growth model to the full range of daily ages available within the first year of life. This resulted in an estimated growth rate that is higher than those previously published using yearly age readings.

The analyses of juvenile lionfish presented here failed to collect and identify fish younger than 80 days. Settlement of lionfish occurs at 30 days, on average, which means most of the fish sampled had settled almost two months prior to collection. This study likely does not characterize growth over the first two months post settlement. Based on this analysis, assuming similar growth rates over that period, the estimated average length at settlement would be 74 mm total length. Similarly, this study only aged two fish older than 180 days, and neither of which was older than 240 days. This means that our growth estimates probably represent growth best between 2 and 7 months after settlement and are most reliable 2-5 months after settlement. This relatively narrow range of ages was partially influenced by the main objective of our study to collect young fish for which birth date would be easier to estimate.

Studies that aim at describing growth of the entire first year of life should collect larger and older fish than the sample used for this study. The age estimates obtained from the reading axis on the broad axis of each otolith yielded a distribution of birth dates that spanned the majority of the calendar year. The only month with no lionfish birth dates was September. This result is likely an artifact of the low sample size for the study and the uneven temporal sampling of lionfish. Even with the low sample size, the results strongly suggest lionfish are being born and recruiting to Bahamian reef habitat throughout the calendar year.

Prior work used age estimates to construct growth curves for lionfish in various regions within the invaded range (Barbour et al., 2011; Edwards et al., 2014; Potts et al., 2010), validated the daily accretion of otolith increments (Edwards et al. 2014), and estimated pelagic larval duration for lionfish (Ahrenholz and Morris, 2010). This study helped to verify the finding of previous histological studies by Morris et al. (2009) and confirm not only that lionfish spawn year round, but also that larvae successfully survive and recruit to Bahamian reef habitat throughout the year. Although the results suggest greater number of births in the summer this may be an artefact of low sample sizes. Future work should seek to see if this recruitment pattern holds over the latitudinal range where breeding lionfish are present, or if there are peaks in spawning success for more northerly areas of their distribution.

Table 2.1. Published growth rates from lionfish aging studies, compared to this study of juveniles collected from reef habitats in the central Bahamas.

Authors	Growth Equation	Growth Rate	Region
Current Study	$L_t=52.5+0.69t$	*0.67*	*Bahamas*
Potts et al. 2010	$L_t=455.1(1-e^{-0.32(t+1.22)})$	0.32	North Carolina
Barbour et al. 2011	$L_t=425.2(1-e^{-0.47(t+0.5)})$	0.47	North Carolina
Edwards et al. 2014	$L_t=349(1-e^{-0.42(t+1.01)})$	0.42	Cayman Islands
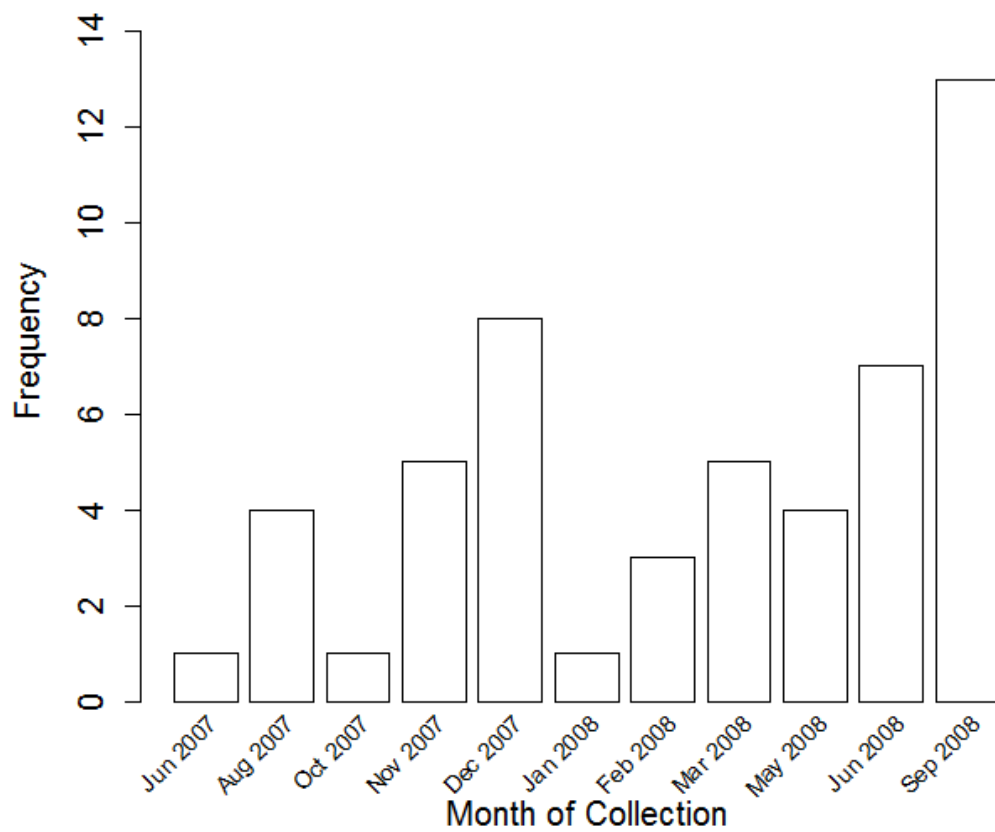
Figure 2.1. Number of lionfish juveniles collected from the Common wealth of the Bahamas, in each month of the calendar year between June 2007 and September 2008.
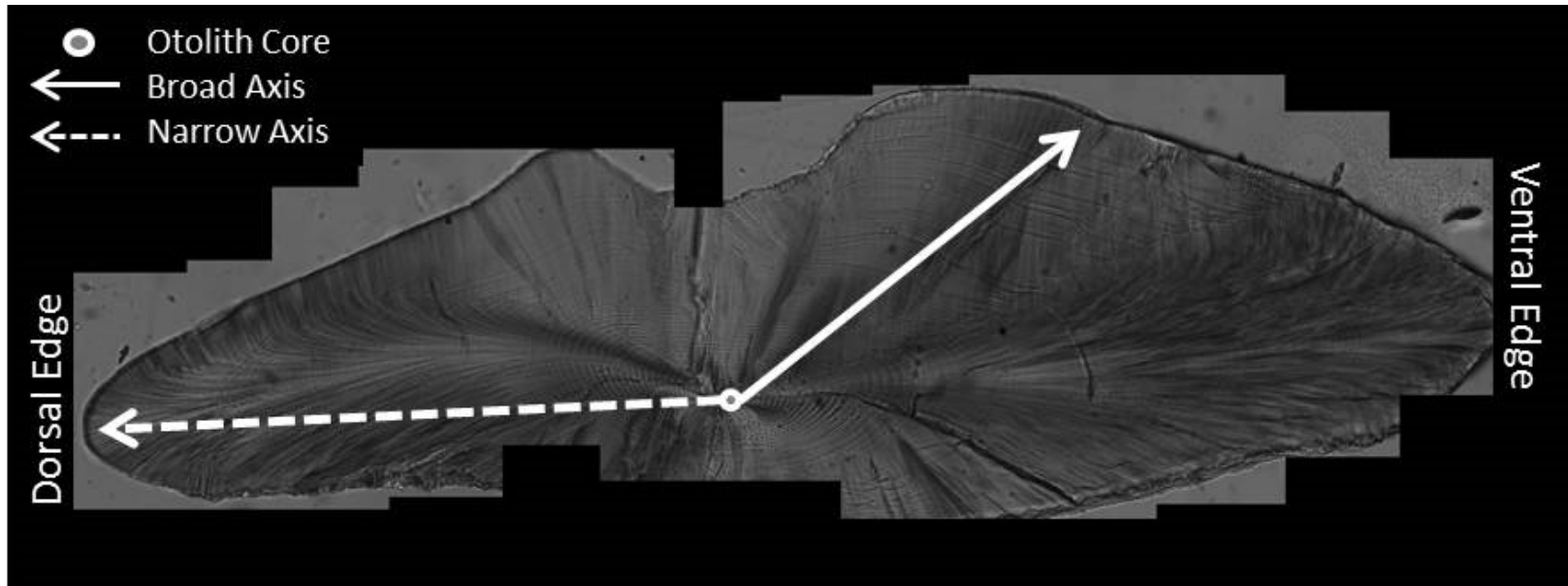
Figure 2.2 Sectioned juvenile lionfish otolith, of total length 107mm. Two reading axes used for analyses are denoted with the solid and dashed lines. The narrow axis gets slimmer toward the dorsal edge, while the broad axis widens along the top of the otolith toward the ventral edge.
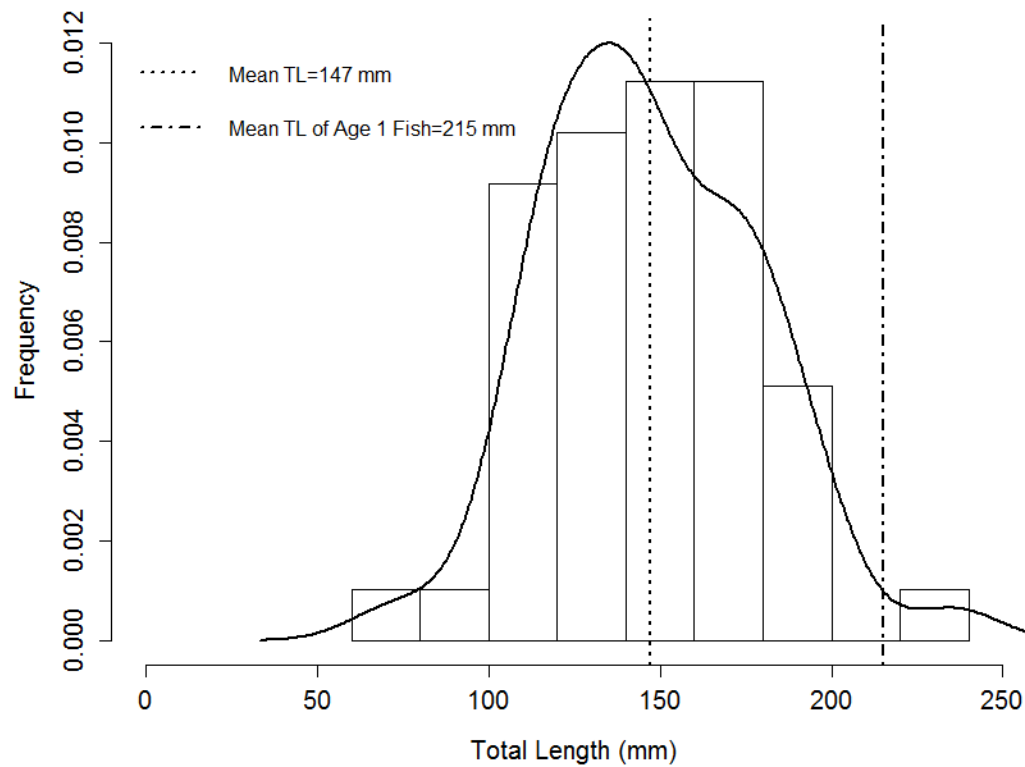
Figure 2.3. Frequency distribution of juvenile lionfish total lengths analyzed for aging (mm). The mean total length of lionfish in the sample is denoted by the dashed line, the mean total length of a one year old lionfish is denoted by the multi-dash line, and the solid line represents a smoother line for the frequency distribution.
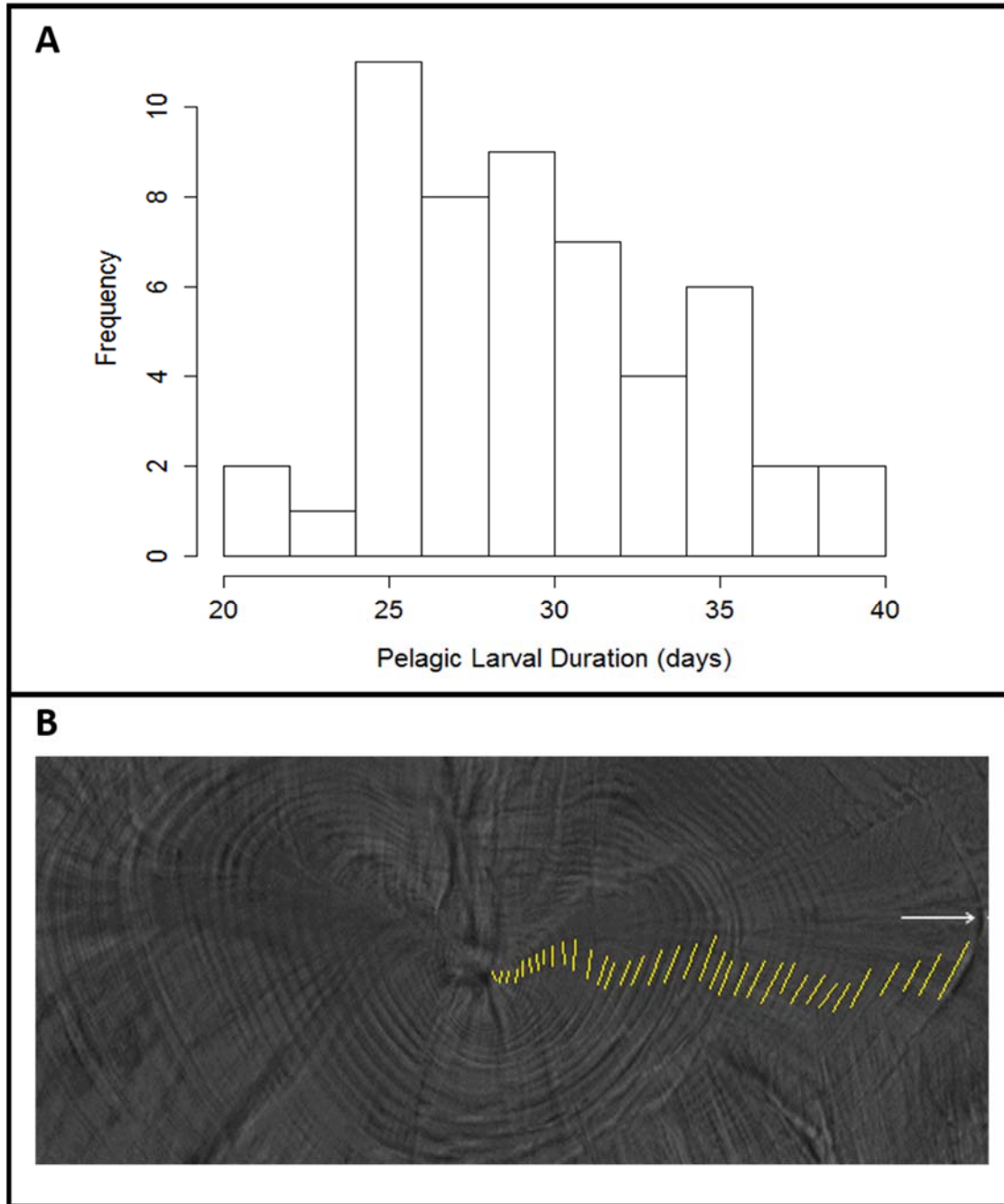
Figure 2.4. Histogram of estimated pelagic larval durations, in days, is shown in upper panel. Photograph of a juvenile lionfish otolith, with an estimated 35 day pelagic larval duration. Each increment is marked with a yellow line, and settlement mark is denoted by the write arrow.
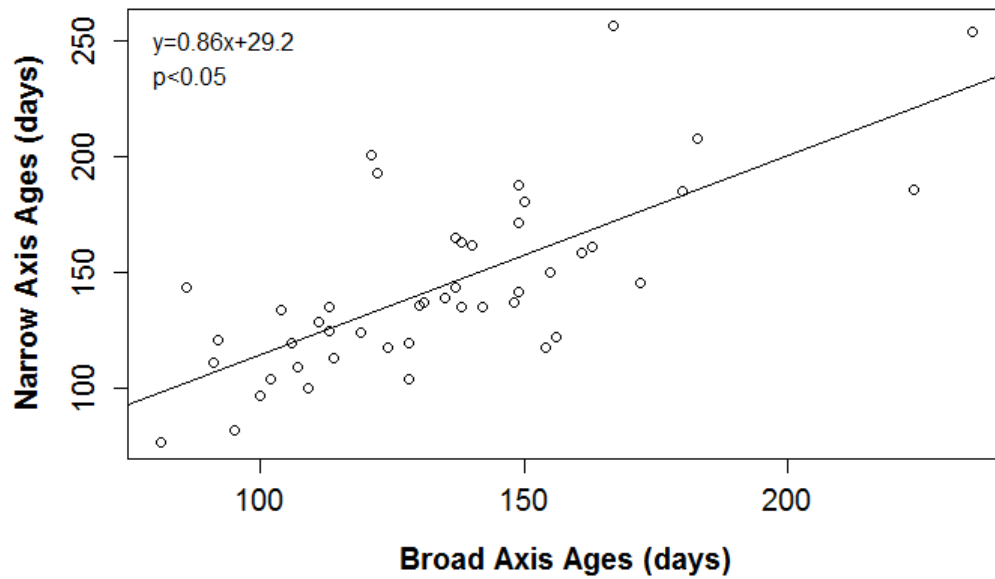
Figure 2.5. Linear regression representing the correlation between the ages estimated (in days) from the two reading axes. The narrow axis runs from the otolith core to the dorsal edge, while the broad axis runs form the otolith core to the ventral edge.
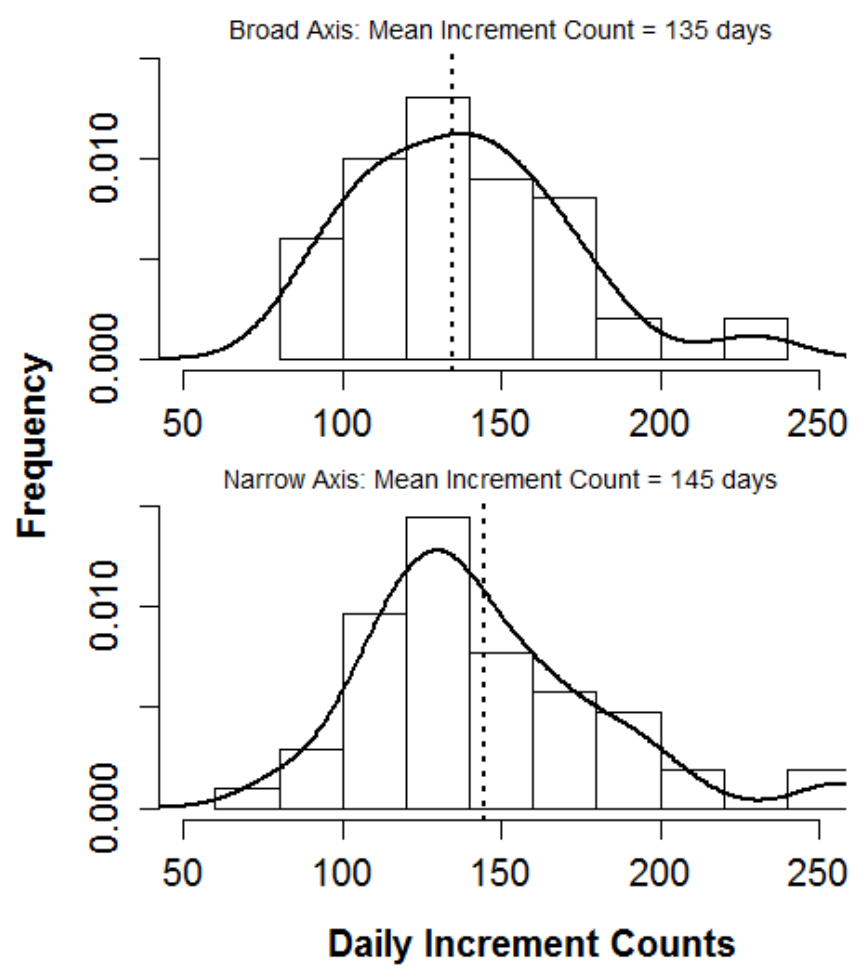
Figure 2.6. Frequency distribution of daily increment counts (ages) for lionfish sampled, described by both a histogram and smoothed density curve (solid line). Mean increment count for the sample is represented by the dashed vertical line. The top panels represents the age increment counts along the broad and narrow reading axes, respectively.
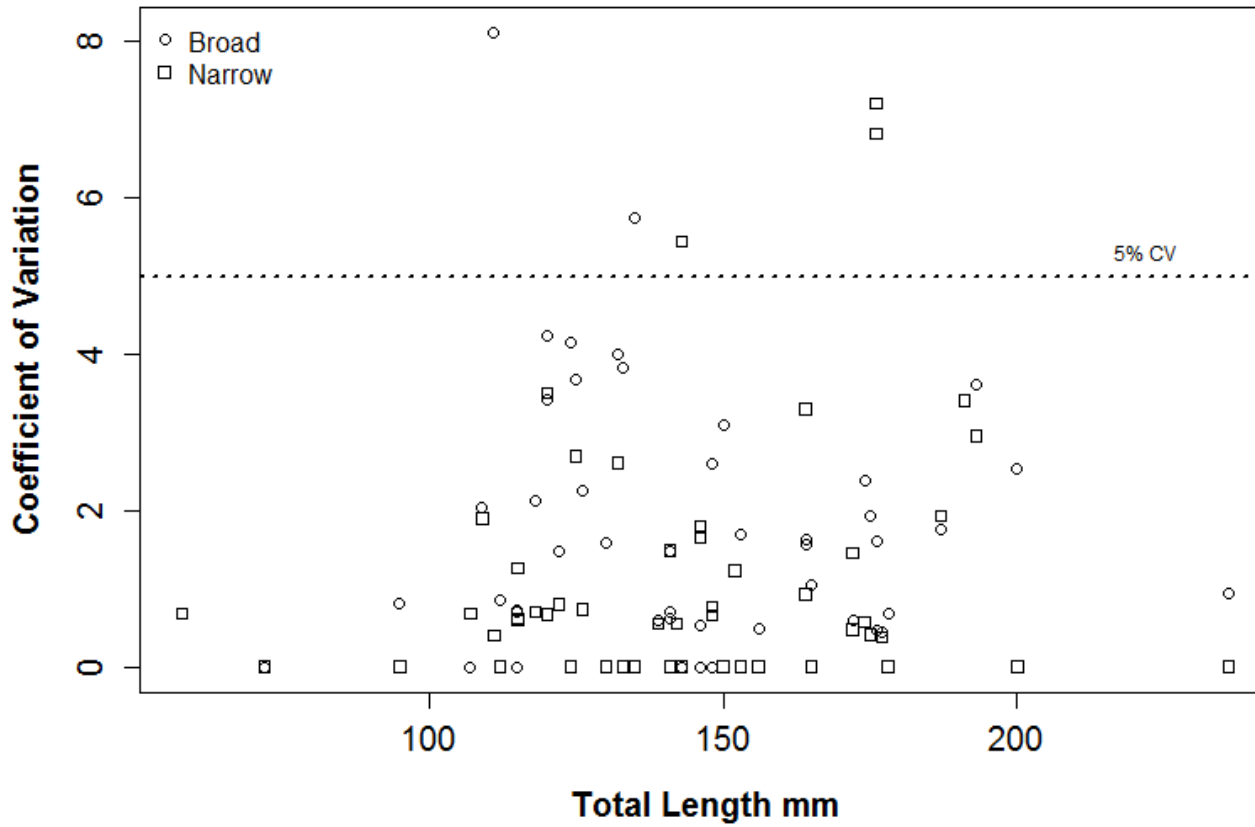
Figure 2.7. Coefficient of variation values, measuring the precision between the two closest reads, calculated for both the broad and narrow axes. A dotted line represents deviation of 5% between estimated values.
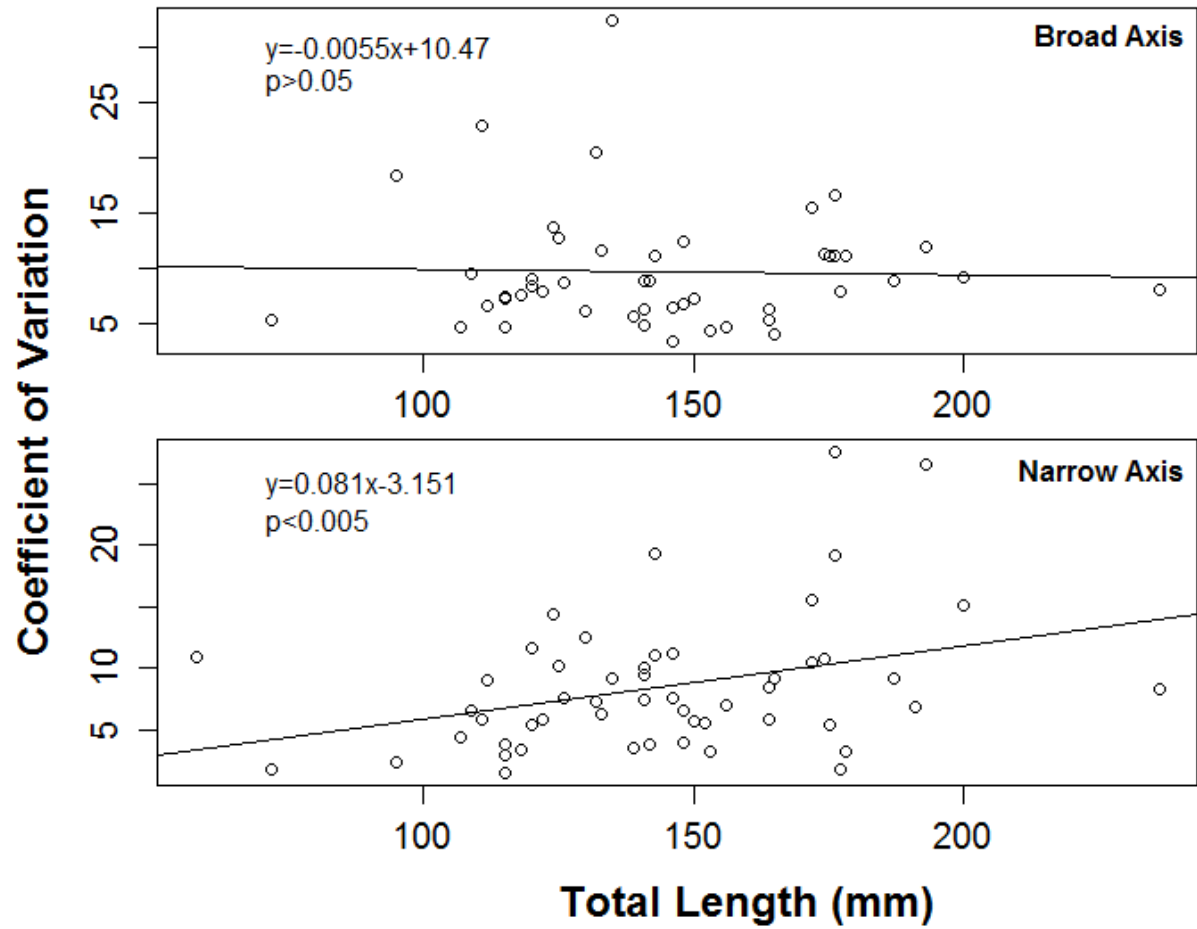
Figure 2.8. Coefficient of variation between the two closest reads for each otolith plotted against the total length corresponding with each otolith. The fitted lines represent the correlation between the total length and coefficient of variation for each of the axes read.
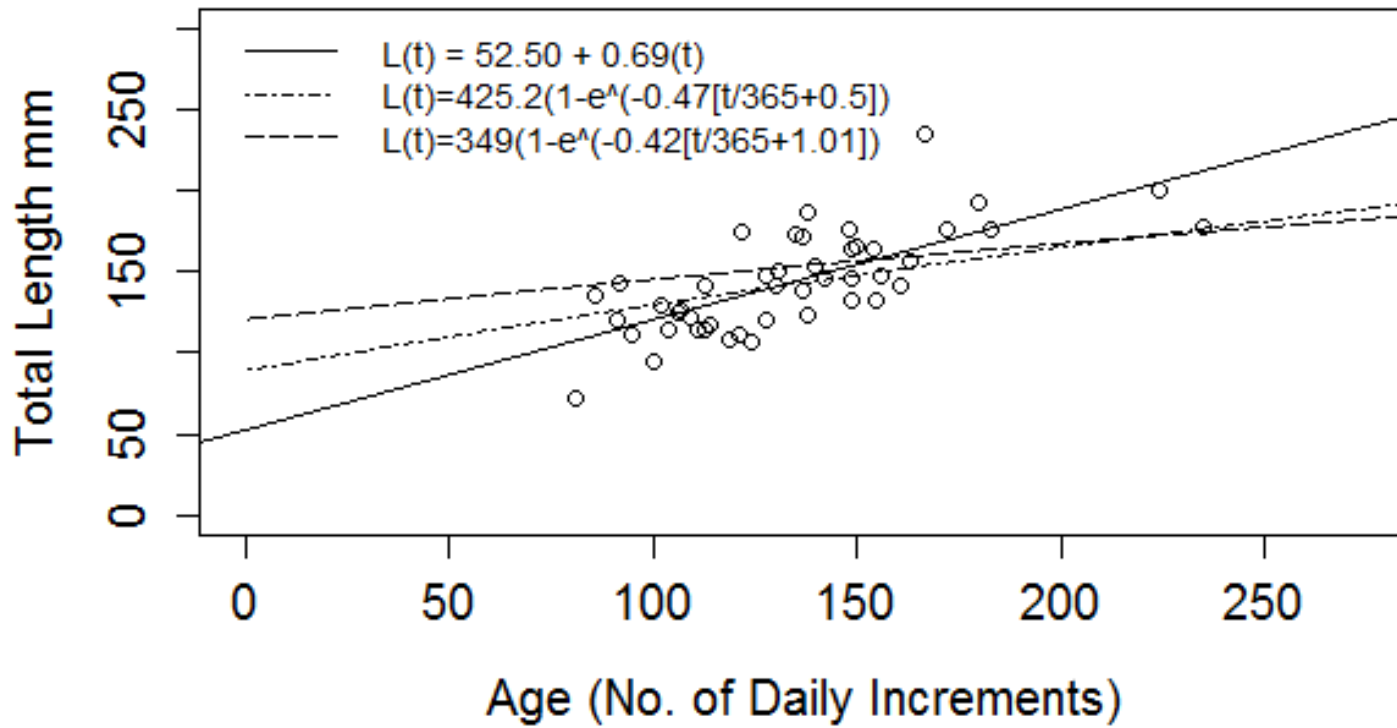
Figure 2.9. Correlations of lionfish total length as a function of age (in daily increments). The solid line is representative of a fit to the study data, the multi-dashed line is the age curve published by Barbour et al. (2011) and the dashed line is a published age curve of Edwards et al. (2014).
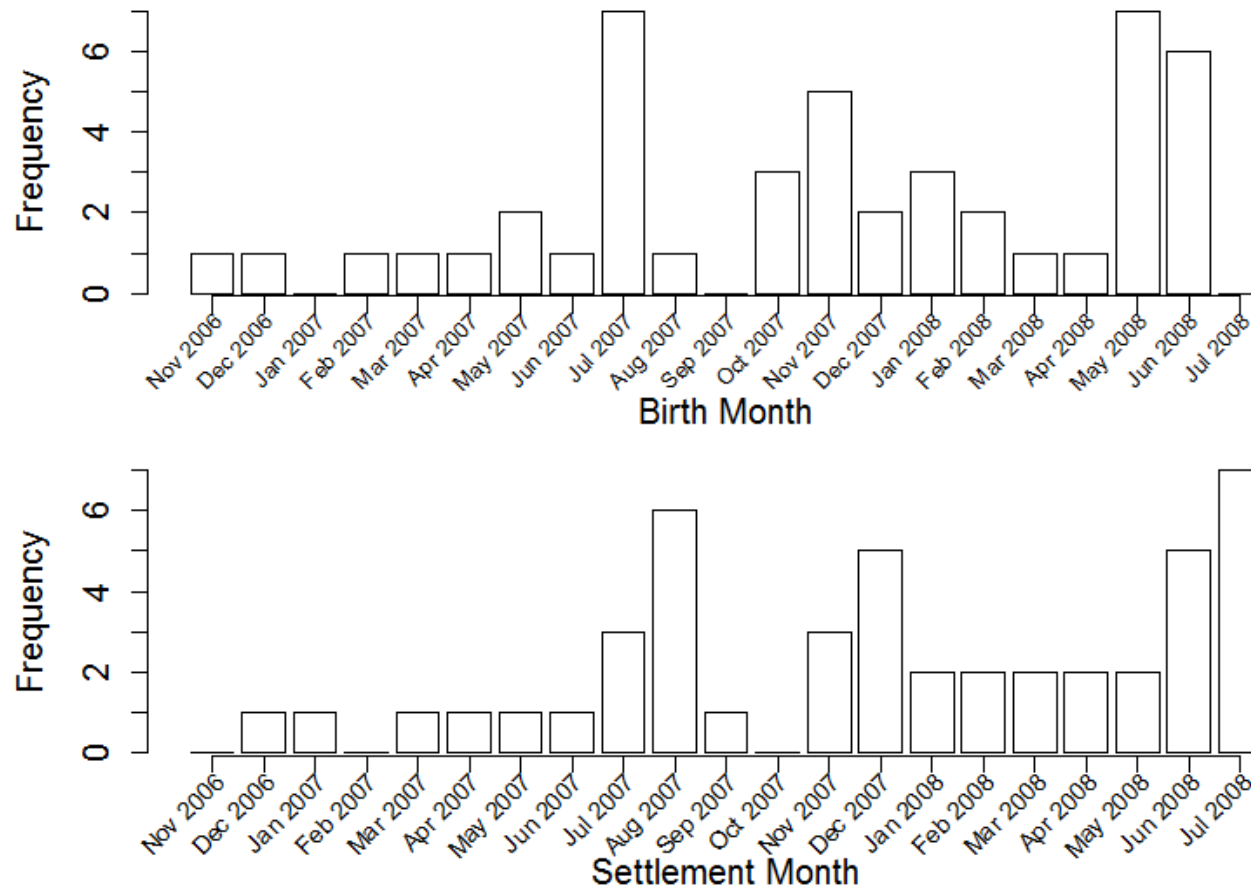
Figure 2.10. Frequency distribution representing the number of individuals born or settling in each month between November 2006 and July 2008.

Chapter 3 – Modeling the Larval Dispersal and Spread of Lionfish in the Tropical Western Atlantic

Background

Two introduced lionfish species, *Pterois volitans* and *Pterios miles*, have become established in the Tropical Western Atlantic Ocean (Whitfield et al., 2002). As early as the 1970s, recreational fishers provided anecdotal accounts of lionfish presence in the coastal waters off Broward and Miami-Dade counties in Florida and across the Gulf Stream in New Providence, Bahamas (personal communication Kathleen Sullivan-Sealey). Verifiable observations of lionfish were uncovered and reported by a fisherman off of Palm Beach, Florida in 1985 (USGS Database, 2014). By the year 2000, these species established a breeding population from coastal Florida to deep, hard bottom reef habitat along the North Carolina coast (Whitfield et al., 2002). Within a decade the population expanded its range to the Caribbean Sea and finally the Gulf of Mexico (Schofield, 2009).

Most invasive marine species are introduced by transport in ship ballast water or on the hulls of ships that reach new regions (Ruiz et al., 1997; Miller et al., 2011; Williams et al., 2013), but lionfish represent the first marine fish to become established after aquarium releases (Semmens et al., 2004). The average lionfish home range observed in mangrove-seagrass habits covers a linear distance of 28 meters (Jud and Layman, 2012), which makes it unlikely that migrating adult individuals are responsible for increasing the lionfish range. The expansion is better explained by multiple introductions of adults to new areas or as a result of larval dispersal from regions containing adult spawners to new recruitment areas. Lionfish larval dispersal occurs over larger distances, and represents the more plausible scenario for range expansion.

Many marine species in coastal Florida are relatively genetically distinct from the same species in the Caribbean Sea (Goetze, 2011; Hemond and Vollmer, 2009), with the Gulf Stream acting as a major physical barrier of larval flux between Florida and the Caribbean, specifically the Bahamas. However, the timeline of sightings records indicate that lionfish likely spread from Florida to other habitats in the Caribbean, somehow allowing larvae to overcome a major geographical barrier that has restricted the dispersal of many native species. Larval lionfish have been difficult to locate and identify in situ, as evidenced by the ten year gap between discovering an established population of lionfish in North Carolina and identification of the first larval invasive lionfish (Vasquez-Yeomans et al., 2011). These difficulties can be overcome by using dispersal models to describe the potential pathways and quantify flux of larvae between spawning and recruitment habitats.

Significant advances have been made in ocean circulation models, allowing researchers to accurately simulate the movement of particles, including larvae, in the water column. Early attempts to model larval dispersal used estimates of pelagic larval duration in conjunction with oceanographic models to track the movements of passive particles through the water column. These approaches neglected the wide range of swimming and navigating abilities that are now known for a variety of pelagic larval species (Paris et al., 2007; Staaterman et al., 2012), which can restrict long distance dispersal of larvae and promote greater natal retention (Cowen et al. 2006; Paris et al., 2007). Inclusion of biological and life history parameters in particle dispersal models helps to yield more accurate model results, and reduces overestimation of dispersal that results from using only physical parameters to control particle movements (Werner et al.,

2007). In particular, we must have an understanding of how the species in question behaves in three-dimensions to define a species dispersal potential (Delaney et al., 2012).

Genetic analyses have been the predominant method used to describe the relationships between lionfish communities in the invaded range, and have indicated the founding population was located on the United States east coast (Freshwater et al., 2009, Hines et al., 2009), specifically Florida (Betan-cur et al., 2011). These genetic analyses illustrate the genetic similarities of haplotypes within the invaded range, but do not clarify what factors contributed to the rapid spread or define the dispersal potential of lionfish between habitats in the native range. This study used the Connectivity Modeling System (CMS) to incorporate biological inputs representative of the invasive lionfish to simulate the dispersal of lionfish in the Caribbean Sea (Paris et al., 2013). The results of the model were compared to the recorded sightings records to identify the linkages that led to the current population of lionfish within the invaded range, and quantify the dispersal potential between regions.

Methods

Oceanographic Module and Habitat

The Connectivity Modeling System (CMS) is coupled offline with archived current data from Hybrid Coordinate Ocean Model (HYCOM) and Gulf of Mexico Hybrid Coordinate Ocean Model (GoM-HYCOM) to recreate the oceanographic conditions present from 2005-2008. The GoM-HYCOM and HYCOM models have resolutions of 1/24° (~4 km^2) and 1/12°latitude (7 km^2), respectively. These models were nested to allow particles to advect freely between model boundaries, drawing data from the highest resolution model available in each time step. A stochastic Lagrangian

framework introduced individual variability by choosing particles at random to represent

a wide variety of attributes. Particles were released from a series of 1,682 habitat

polygons, each with an area of 8 km^2, which serve the dual purpose of release and

recruitment zones (Figure 3.1). The grid of 8 km^2 boxes were superimposed over reef

habitat delineated by the Millennium Coral Reef Mapping Project, which identify suitable

benthic habitat for the the model (Andrefouet et al., 2006).

Biological Module Parameterization

A combination of life history traits from the literature and previously unpublished

work were used to provide the particles with lionfish like characteristics. "Lionfish"

particle were assigned to three distinct life stages: egg, preflexion and postflexion (Figure

3.2). During spawning, lionfish release buoyant, gelatinous egg sacs near the ocean's

surface; eggs are released into the water as the sacs deteriorate over ~ 2 days (Fishelson,

1975; Morris et al., 2011). In the model, particles in the "egg stage" were released at the

surface, and the majority of particles were restricted to the shallowest depth bin (1 meter)

for a two day period. Scorpaenidae larvae collected by ichthyoplankton tows in Barbados

were used as proxy organisms for describing the preflexion and postflexion larval life

stages. The vertical distributions of these stages were approximated by calculating their

probability of occurrence (P) for each life stage (i) within discrete depth bins (j): 1 m, 10

m, 30 m, 50 m, 70 m, and 90 m.

Equation 3.1:

$$P_{ij} = \frac{N_{ij}}{\Sigma N_{ij}}$$

As the particles were advected they were distributed in the vertical direction to reflect the

calculated proportions (Figure 3.3). Ahrenholz and Morris (2010) estimated lionfish

pelagic larval duration (PLD) at 20-35 days , and this study estimated 20-39 days before

juveniles settled to benthic habitat. (Chapter 2). The length of each life stage was

calculated as a function of PLD. The preflexion life stage was estimated to be 7 days, 1/3

of the minimum pelagic larval duration of 20 days. The postflexion larval stage was

calculated to be 28 days, the difference between the maximum PLD and estimated

preflexion stage length. Lionfish have been show to successfully spawn throughout the

calendar year (Morris et al., 2011; Chapter 2), which was reflected in the model by

releasing "lionfish" particles daily from each reef habitat polygon.

Particle Release and Data Analysis

The dispersal of lionfish throughout the Caribbean basin was simulated by

releasing "lionfish" particles over the time period of the invasion when lionfish range

expanded most rapidly, 2005-2008. One hundred particles were released daily, from all

1,682 habitat polygons. CMS tracked all particles in three dimensions, from release to

until they encountered a habitat polygon or were advected out of the domain of the

model. Any particle that did not reach a habitat polygon during the pre-determined

settlement period, 20-37 days, was omitted from further analysis. A connectivity matrix

was constructed to quantify the dispersal probability of "lionfish" particles (L) released

from each source polygon (i) to all recruitment polygons (j), for each year of the

simulation (t).

Equation 3.2:

$$M_t = \frac{L_{ijt}}{\sum L_{it}}$$

These matrices were post-processed to create connectivity matrices representative of the progressive spread of "lionfish" particles over time. These were compared to the list of confirmed lionfish sightings which was compiled by querying the most comprehensive sightings databases within the invaded region: the USGS Lionfish Sightings Database and REEF Visual Census Survey's lionfish sightings. The full connectivity matrix from each year (M_t) was multiplied by a vector of zeros and ones that represent the presence or absence of recruited lionfish in each habitat polygon in the previous year (R_{t-1}) to produce a model prediction for the year in question.

Equation 3.3:

$$Model\ Prediction_t = M_t * R_{t-1}$$

For the first year of the simulation, all sightings from 2004 were cross-referenced with the 1,682 8 km^2 reef- habitat polygons to identify source regions containing lionfish sightings prior to the start of 2005. Eight sightings fell within the defined habitat polygons in 2004, two polygons in Florida and six polygons in the Commonwealth of the Bahamas. For each successive year the presence/absence vector was generated from the habitat polygons "lionfish" particles successfully recruited to in the previous year.

The subset of connectivity matrices represented the locations and dispersal probabilities of lionfish between 2005 and 2008. The connections between source and sink habitats were described at the country / region level, with the dispersal probabilities relating the strength of the connections. These matrices were compared to the sightings database to determine the precision of the model predicting the lionfish invasion along the observed timeline. The sighting records represent presence of juvenile or adult aged lionfish, therefore the year corresponding to the first sighting in each Caribbean region

was not necessarily representative of the year each individual initially recruited. The age of the largest observed fish, from the five earliest sightings within each Caribbean country, was estimated using the age-at-length curve published by Barbour et al. (2011). Each fish's estimated birth date was calculated by subtracting their age in days from their observation date. The year corresponding to the earliest birthdate was used to represent the corrected year of recruitment for fish observed in the sightings database.

Results

In each year of the simulation, 36,500 particles were released from each habitat polygon, with 40-52% of particles successfully recruiting to some habitat polygon by the end of their competency period (Table 3.1). Connectivity matrices show the strength of the connections between source and sink habitat polygons for each year of the simulation and indicate strong self-recruitment of particles (Figure 3.4 – 3.7). The four-year simulation resulted in dispersal of "lionfish" particles from habitat polygons in two countries to eleven additional countries throughout the Caribbean basin, encompassing ~60% of the habitat polygons available (Table 3.2). The proportion of newly invaded habitat polygons increased through the third year of the simulation, but appeared to become saturated in the final year (Figure 3.8).

Progression of Lionfish Spread

In Year 1 (2005) particles released from the Bahamas showed self-recruitment and dispersal to new habitat polygons in Cuba, while particles released from Florida showed only self-recruitment (Figure 3.9). In Year 2 (2006) "lionfish" particles from Florida continued to show self-recruitment and revealed a new connection to the

Bahamas (Figure 3.10). Source polygons in the Bahamas and Cuba during this time period resulted in the spread of particles to new regions, including settlement of particles in Hispaniola and Jamaica. The third year of the simulation exhibited the greatest expansion of "lionfish" particles in terms of number of new habitat polygons and countries reached (Figure 3.11). Connections developed both within the Greater Antilles and between the Greater Antilles and the Bahamas. Turks and Caicos was newly invaded by particles originating from the Bahamas, Cuba, Hispaniola and Jamaica. The Cayman Islands was reached by particles originating from Jamaica and Cuba. The third year also started the extension of "lionfish" particles to the Western and Southwestern Caribbean, with Jamaica as the sole source of particles to Mexico, Colombia, Honduras and Nicaragua. The final year of the simulation resulted in the spread to Panama and Belize for the first time (Figure 3.12). The newly invaded countries in the Southern and Southwestern Caribbean Sea dispersed particles widely, spreading particles within the southern region of the basin and further to Florida, the Bahamas and Cuba. The Bahamas and Cuba were major sinks of "lionfish" particles receiving from nine and ten countries respectively.

Model Predictions vs Observed Sightings

The dates of the earliest lionfish sightings were adjusted to reflect the date each individual would have recruited to the reef habitat. The average size of the earliest observed fish was 21.5 cm in total length, with age estimates from 0.35-2.1 years in age. A comparison between the years the model predicted first settlement to each country overlapped with the corrected year of first sightings for most countries. The strongest agreement between model predictions and sightings records occurred in countries more

centrally located within the Caribbean Basin. The model predicted recruitment to Jamaica, Mexico, Honduras and Panama one calendar year earlier than corrected lionfish sightings indicated their arrival in those countries. The recruitment of lionfish to Nicaragua occurred 2 years after the model predicted first recruitment of "lionfish" particles to the country. While the model predicted recruitment of lionfish to some countries earlier than the sightings records, the timeline of the lionfish invasion was largely replicated by the simulation model. Maps overlaying the model predicted habitat of lionfish to the USGS and REEF sightings records showed that the model produced a more widespread distribution within each country than noted by the sightings alone (Figure 3.13).

Connectedness of Founding Population

The model revealed limited dispersal from Florida to other regions within the Caribbean; particles spread to the Bahamas starting in 2006 and to Cuba in 2008. Florida served as a major sink of "lionfish" particles from the rest of region, by the end of the simulation. Particles were exported to Florida from seven other countries, most of which originated from the Western and South Western Caribbean, starting in 2008. The Bahamas, Cayman Islands and Cuba were the only countries in the northern region of the Caribbean that had particles recruit to Florida. The Bahamas and Cuba were major sinks of "lionfish" particles receiving from nine and ten countries respectively.

Discussion

The release of "lionfish" particles from just 8 habitat polygons led to the colonization of lionfish into new Tropical Western Atlantic ecoregions, largely along the

established timeline of the lionfish invasion. Each discrepancy between the model predictions and sighting records suggested lionfish arrived earlier than the observed sightings. The variable nature of sightings data could mask the initial arrival of lionfish from observers. The USGS Database provides a reliable source of lionfish locations and presence data over time, but these data of opportunity were not generated from a systematic monitoring of the region. The amount of effort used to gather the sightings information cannot be ascertained and there was no accounting for instances when lionfish were not observed. The REEF database provides both presence and absence data within each region sampled, but these visual censuses do not necessarily include all possible habitats that lionfish could recruit to. The lack of presence and absence data from all regions in the invaded range make it difficult to infer detection probability of lionfish early in the invasion.

Early colonizers recruiting to regions with lower monitoring frequency or low awareness of the lionfish issue could easily be missed by the opportunistic efforts that identified new lionfish sightings. The high standard set for sighting records to be included in the USGS sighting database may have delayed the reporting of credible lionfish sightings in some regions. Lionfish are found in a variety of habitats and depths, some of which are less accessible to divers and snorkels that monitor lionfish presence. Additionally, lionfish juveniles have cryptic coloring and hovering behaviors that can make them more difficult to detect during this earlier life stage.

During the four year simulation, the model showed the spread of "lionfish" particles to 11 new countries. The model identified Jamaica as a major hub of dispersal, serving as the first country to link the Greater Antilles with the Western and

Southwestern Caribbean. Particles originating from Jamaica can be influenced by the North Equatorial and Caribbean Current travel that passes the Jamaican coastline or anti-cyclonic eddies that add variability to the region before passing through the Yucatan Channel (Alvera-Azcarte et al., 2009). The southern region of the Caribbean then supplied additional flux of "lionfish" particles to Florida, the Bahamas and Cuba, the major sinks within the invaded range, likely due to advection from the Yucatan and Florida Current. Removals of lionfish have been shown to reduce lionfish populations at local scales (Green et al., 2014), but the high connectivity within the region will continuously supply larvae from a variety of sources.

The connections observed between Florida and the Bahamas may indicate that lionfish have an advantage of dispersal that many native species lack. The buoyant egg masses exuded by lionfish deteriorate over a two-day period (Fishelson, 1975; Moyer and Zaiser, 1981), while native snappers and groupers utilize spawning aggregations to find mates and release gametes directly into the water column (Domeier and Colin, 1997; Claro and Lindeman, 2003). Some of these spawning aggregations occur at great depths (Coleman et al., 1996), but lionfish larvae have greater potential for wind-driven transport. The model shows that while dispersal between Florida and the Bahamas exists, these events are rare. It is unlikely lionfish invasion spread directly from Florida to the Bahamas, at least from the portions of Florida that are represented by the model domain.

The Connectivity Modeling System was able to re-create the spread of lionfish and identify a life history trait that may have enhanced the spread of lionfish throughout the Tropical Western Atlantic Ocean. This tool can be used to help identify the probabilistic spread of future marine invaders, using relatively small amounts of

biological information about the potentially invasive species. An increase in basic research on the life history traits of potential invaders would arm scientists with important information that can be used to model the potential spread of new introductions.

Table 3.1. Particle release summary, detailing the number of habitat polygons particles were released from, number of particles released, and the proportion of particles that recruit to a habitat polygon during the simulation for each year.

Year	Release Polygons	No. of Particles Released	Prop. of Particles Recruited
2005	8	292000	0.40
2006	400	14600000	0.52
2007	750	27959000	0.49
2008	1007	36755500	0.47

Table 3.2. Summary of the dispersal spread to new regions.

Sources	Sink Regions			
	2005	*2006*	*2007*	*2008*
Florida		Bahamas	Bahamas	Bahamas, Cuba
Bahamas	Cuba	Cuba, Hispaniola, Jamaica	Cuba, Florida, Hispaniola, Jamaica, Turks & Caicos	Cuba, Florida, Hispaniola, Jamaica, Turks & Caicos
Cuba		Bahamas, Hispaniola, Jamaica	Bahamas, Cayman Islands, Florida, Hispaniola, Jamaica, Turks and Caicos	Bahamas, Cayman Islands, Florida, Hispaniola, Jamaica, Turks and Caicos
Hispaniola			Bahamas, Cuba, Jamaica, Turks & Caicos	Bahamas, Colombia, Cuba, Jamaica, Turks & Caicos
Jamaica			Bahamas, Cayman Islands, Colombia, Cuba, Hispaniola, Honduras, Mexico, Nicaragua, Turks and Caicos	Bahamas, Cayman Islands, Colombia, Cuba, Hispaniola, Honduras, Mexico, Nicaragua, Panama, Turks and Caicos
Turks & Caicos				Bahamas, Cuba, Hispaniola
Nicaragua				Bahamas, Belize, Cuba, Florida, Honduras, Mexico, Panama
Colombia				Bahamas, Belize, Cayman Islands, Cuba, Florida, Honduras, Mexico, Nicaragua, Panama
Cayman Islands				Cuba, Florida
Honduras				Bahamas, Belize, Cuba, Florida, Mexico, Nicaragua, Panama
Mexico				Florida, Bahamas, Cuba

Table 3.3. Comparison of the model predictions of lionfish recruitment to each region with first confirmed sightings of lionfish and the earliest sighting with the oldest estimated individual. Lionfish confirmed sightings were corrected to reflect the year sighted fish would have been spawned.

Region	Earliest Sighting Date	Corrected Sighting Year	Model Predicted Year
Florida	**10/16/1985**	-	-
Bahamas	**10/26/2004**	-	-
Turks and Caicos	**7/0/2007**	**2007**	**2007**
Cuba	**6/27/2007**	**2005**	**2005**
Hispaniola	**2/24/2007**	**2006**	**2006**
Cayman Islands	**2/5/2008**	**2007**	**2007**
Jamaica	3/25/2008	*2007*	*2006*
Mexico	1/26/2009	*2008*	*2007*
Belize	**12/11/2008**	**2008**	**2008**
Colombia	**12/26/2008**	**2007**	**2007**
Honduras	5/22/2009	*2008*	*2007*
Panama	3/30/2009	*2009*	*2008*
Nicaragua	11/11/2009	2009	2007
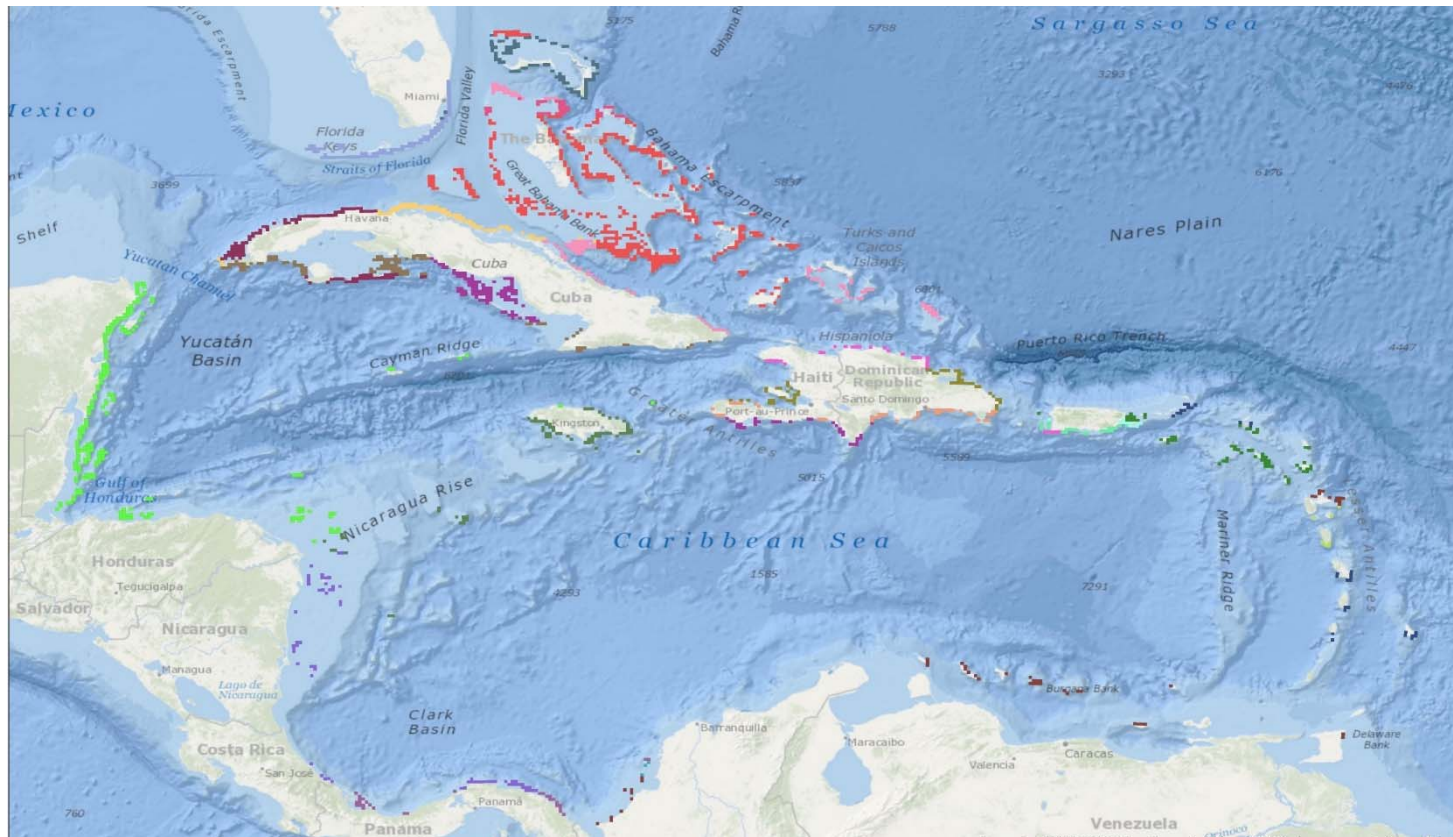
Figure 3.1. Map of each 8 x 8 km^2 habitat polygon available in the model, 1,682 in total. Each grid box overlays coral reef habitat delineated by the Millenium Coral Reef Mapping Project.
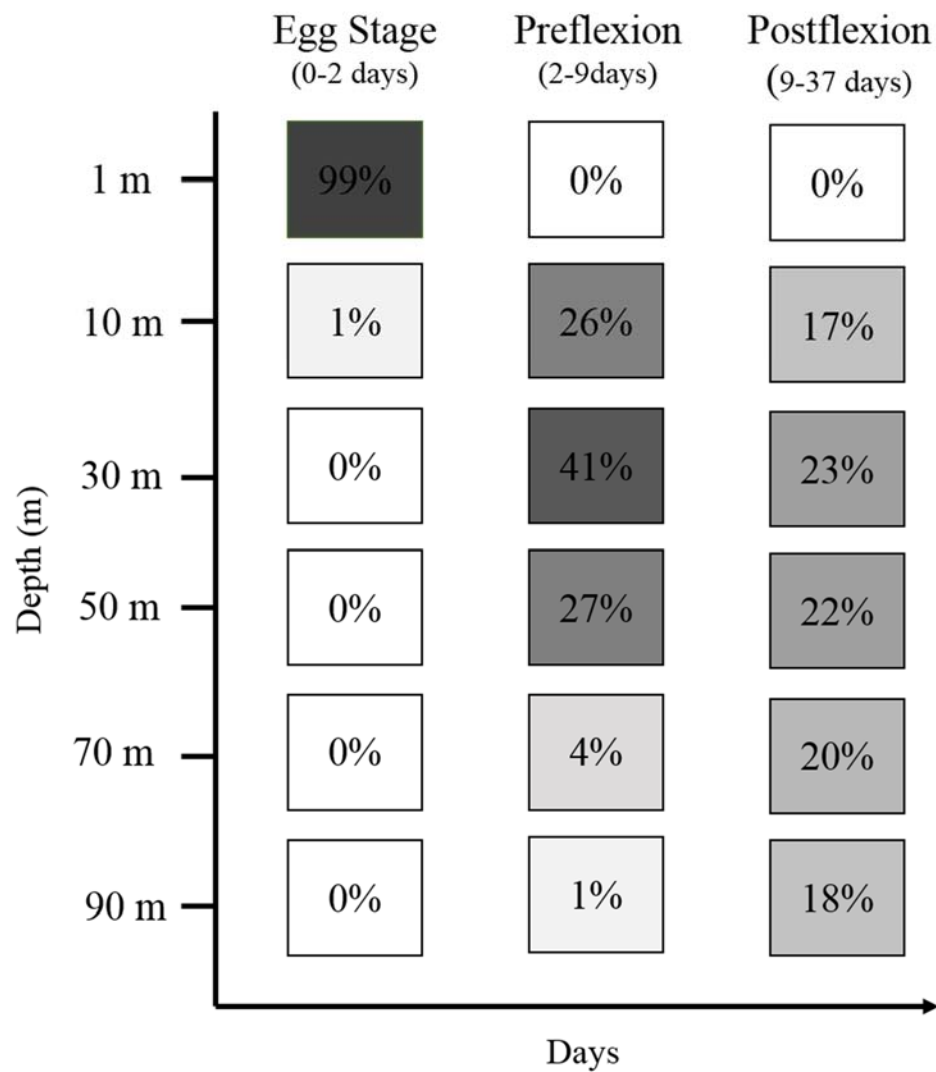
Figure 3.2. Representation of the vertical matrix used to designate the position of "lionfish" particles in the water column over time, in each simulation of CMS. Each box represents a depth bin, with the centroid labeled on the y-axis. Columns represent the three life stages: egg stage, preflexion and postflexion, with the shading signifying the proportion of particles found in each depth bin during each life stage.

Scorpaenid Distribution
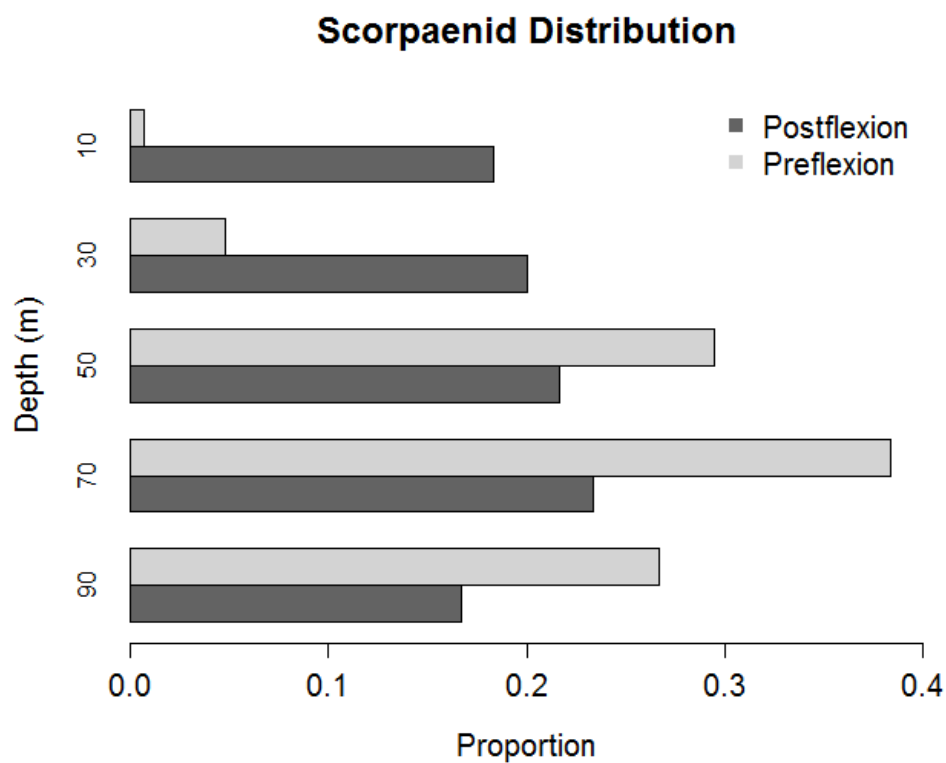
Figure 3.3. Vertical distribution of preflexion and post flexion Scorpanidae larvae collected in icthyoplankton tows at discrete 20 meter depth bins. Preflexion "lionfish" particles range from 2 – 9 days of age and postflexion "lionfish" particles are 9 – 30 days old (*Paris unpublished data*).
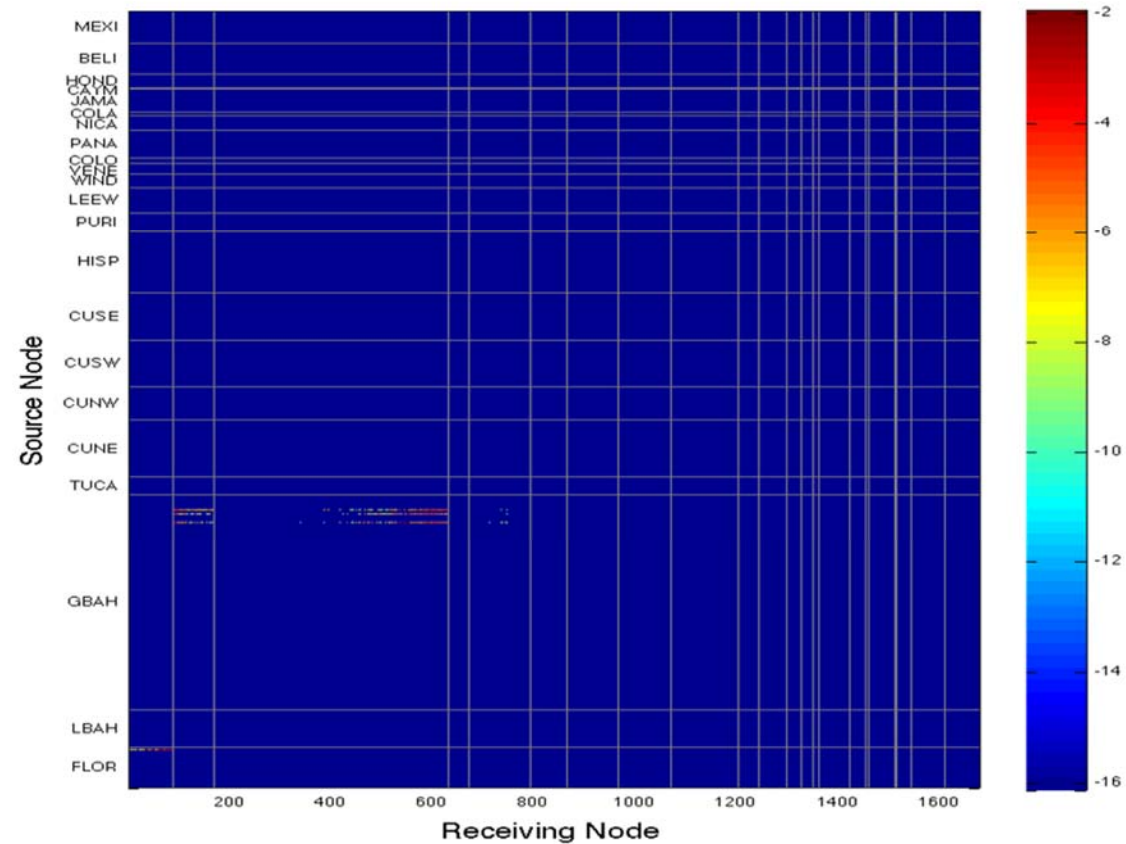
Figure 3.4. Connectivity matrix corresponding to the strength of source to sink connections in 2005. Warmer colors represent higher dispersal probabilities. The abbreviations for the source nodes are: Florida (FLOR), Bahamas (LBAH, GBAH), Turks and Caicos (TUCA), Cuba (CUNE, CUNW, CUSW, CUSE), Hispaniola (HISP), Puerto Rico (PURI), Leeward Islands (LEEW), Windward Islands (WIND), Venezuela (VENE), Colombia (COLO, COLA), Panama (PANA), Nicaragua (NICA), Jamaica (JAMA), Cayman Islands (CAYM), Honduras (HOND), Belize (BELI), and Mexico (MEXI).
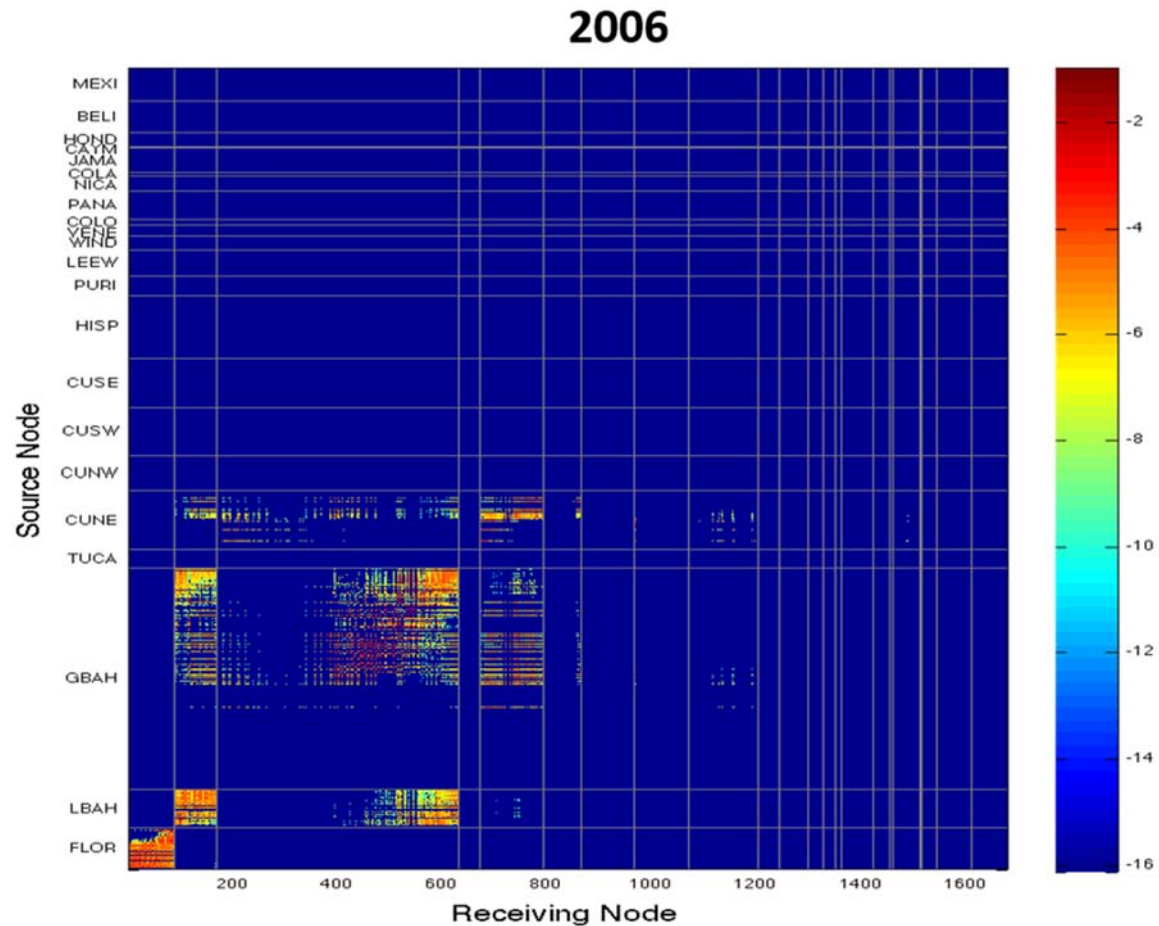
Figure 3.5. Connectivity matrix corresponding to the strength of source to sink connections in 2006. Warmer colors represent higher dispersal probabilities. The abbreviations for the source nodes are: Florida (FLOR), Bahamas (LBAH, GBAH), Turks and Caicos (TUCA), Cuba (CUNE, CUNW, CUSW, CUSE), Hispaniola (HISP), Puerto Rico (PURI), Leeward Islands (LEEW), Windward Islands (WIND), Venezuela (VENE), Colombia (COLO, COLA), Panama (PANA), Nicaragua (NICA), Jamaica (JAMA), Cayman Islands (CAYM), Honduras (HOND), Belize (BELI), and Mexico (MEXI).
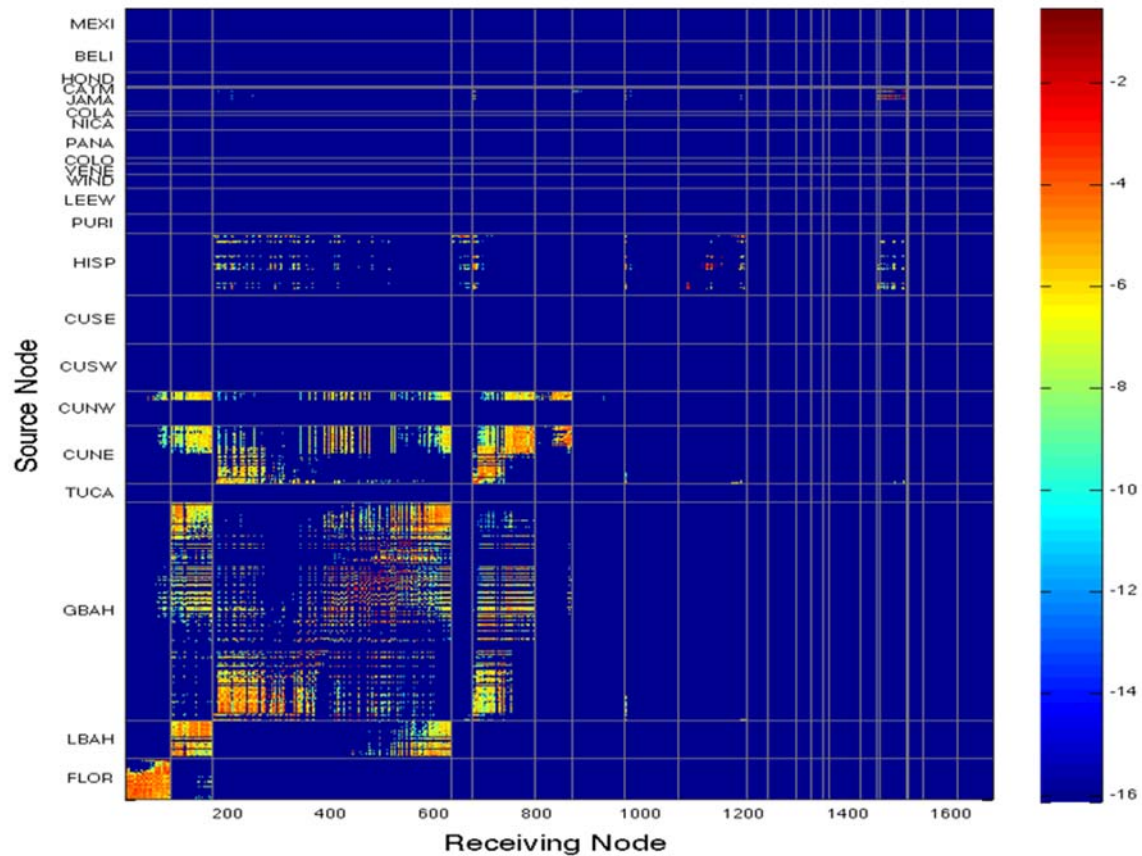
Figure 3.6. Connectivity matrix corresponding to the strength of source to sink connections in 2007. Warmer colors represent higher dispersal probabilities. The abbreviations for the source nodes are: Florida (FLOR), Bahamas (LBAH, GBAH), Turks and Caicos (TUCA), Cuba (CUNE, CUNW, CUSW, CUSE), Hispaniola (HISP), Puerto Rico (PURI), Leeward Islands (LEEW), Windward Islands (WIND), Venezuela (VENE), Colombia (COLO, COLA), Panama (PANA), Nicaragua (NICA), Jamaica (JAMA), Cayman Islands (CAYM), Honduras (HOND), Belize (BELI), and Mexico (MEXI).
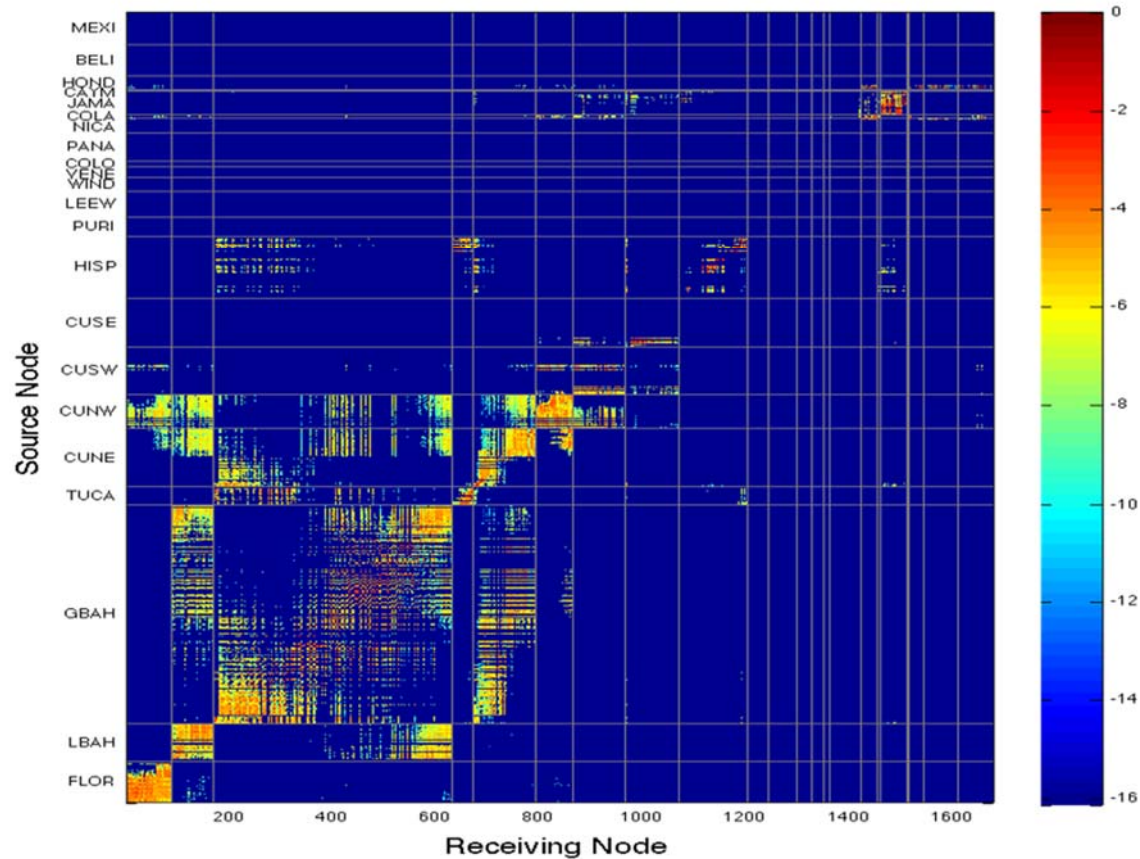
Figure 3.7. Connectivity matrix corresponding to the strength of source to sink connections in 2008. Warmer colors represent higher dispersal probabilities. The abbreviations for the source nodes are: Florida (FLOR), Bahamas (LBAH, GBAH), Turks and Caicos (TUCA), Cuba (CUNE, CUNW, CUSW, CUSE), Hispaniola (HISP), Puerto Rico (PURI), Leeward Islands (LEEW), Windward Islands (WIND), Venezuela (VENE), Colombia (COLO, COLA), Panama (PANA), Nicaragua (NICA), Jamaica (JAMA), Cayman Islands (CAYM), Honduras (HOND), Belize (BELI), and Mexico (MEXI).
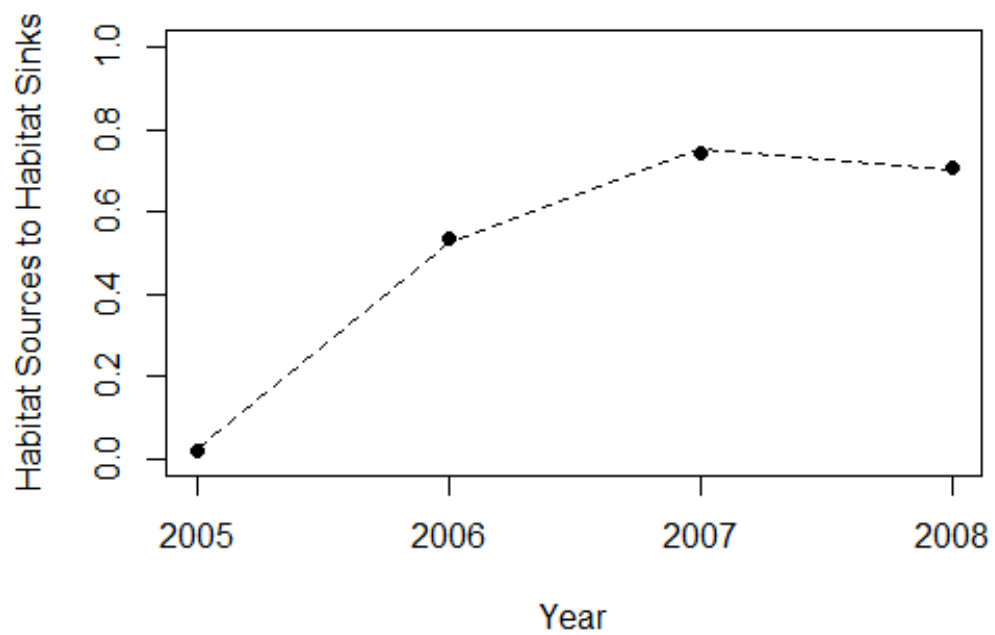
Figure 3.8. The ratio of the number of habitat polygons that particles were released from to the number of habitat polygons that particles recruited to.
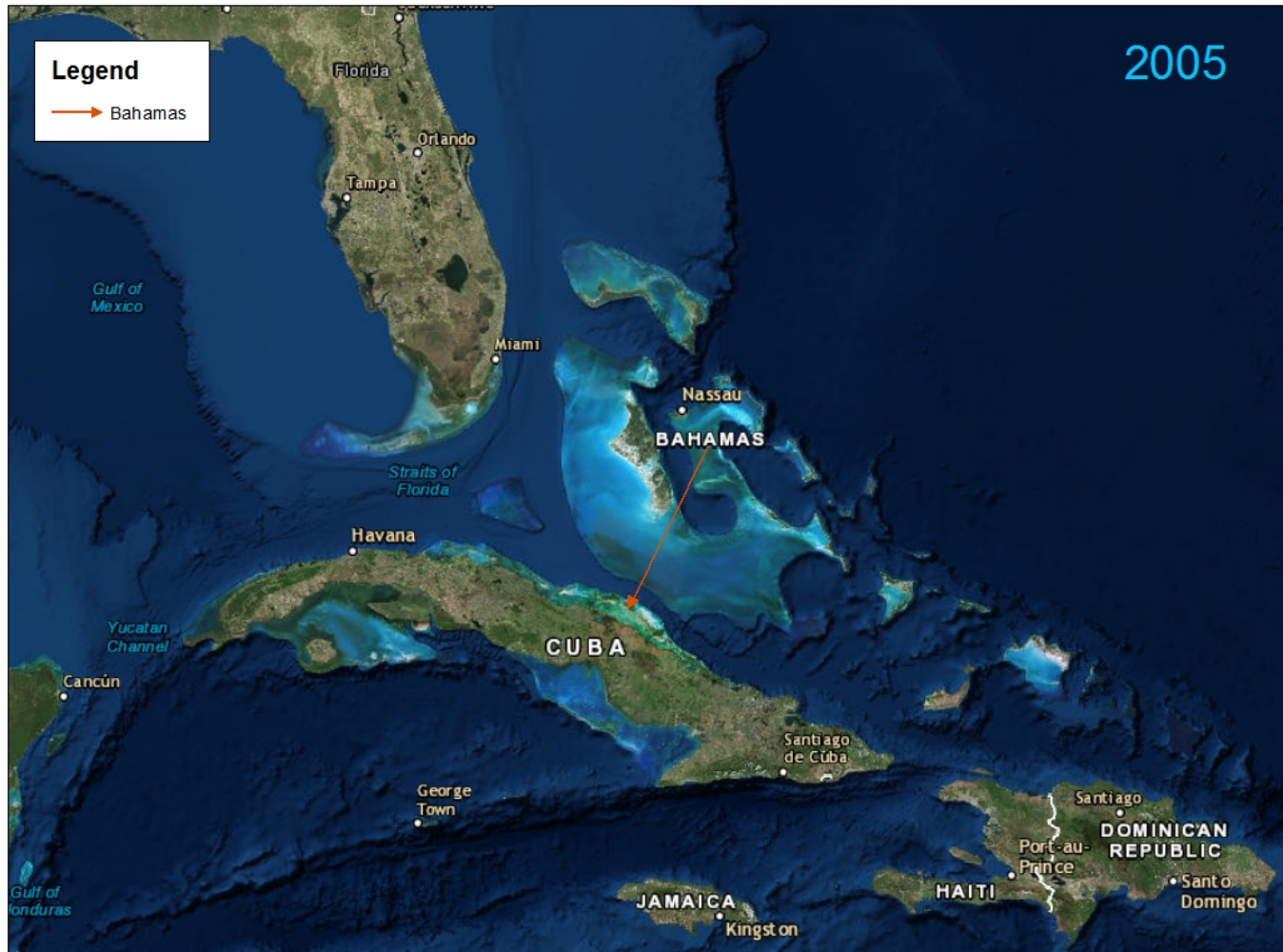
Figure 3.9. Map depicting the direction of "lionfish" larval transport between countries in 2005. Self-recruitment occurred in each year of the simulation, but is not represented with any arrows.
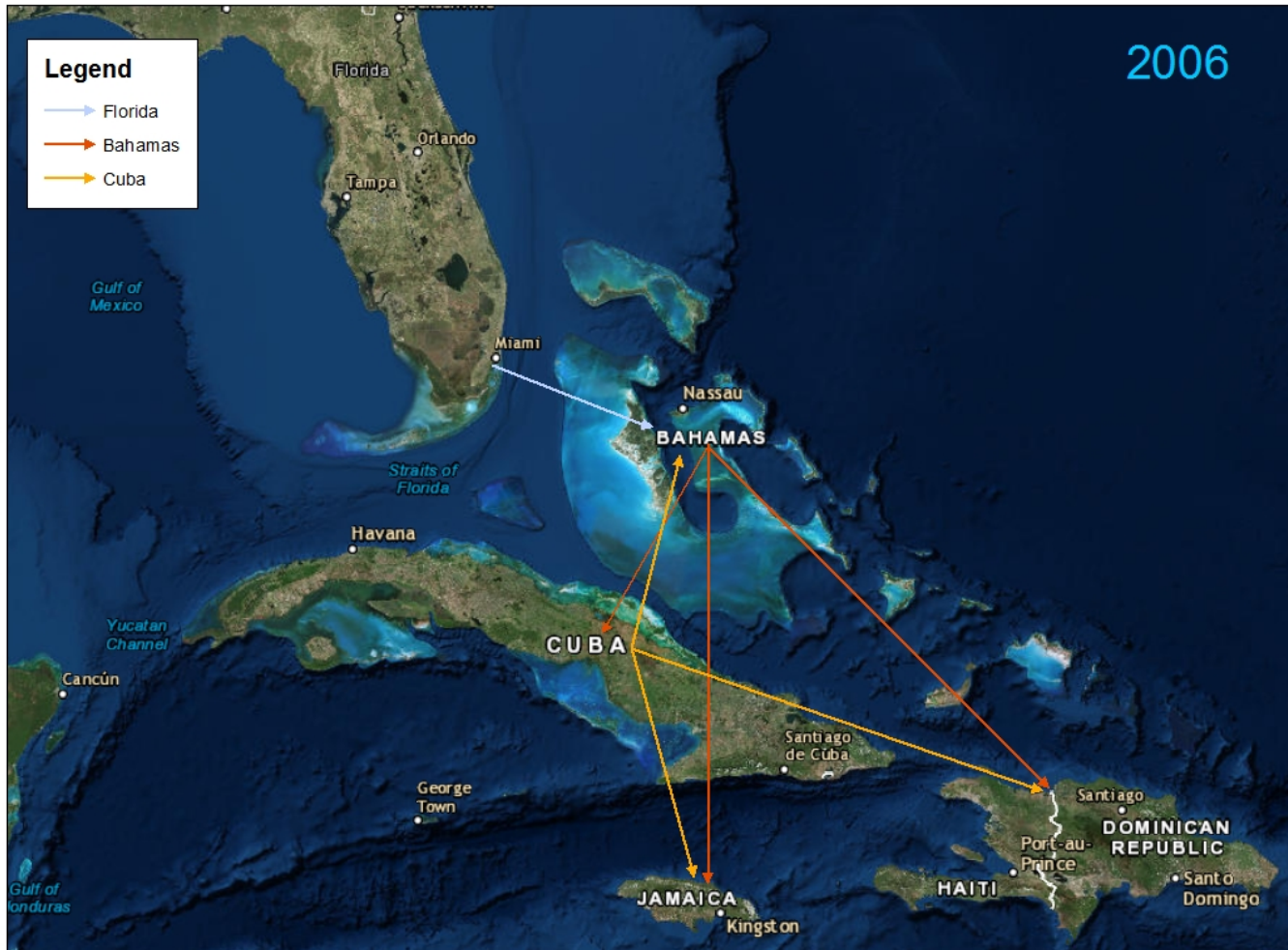
Figure 3.10. Map depicting the direction of "lionfish" larval transport between countries in 2006. Self-recruitment occurred in each year of the simulation, but is not represented with any arrows.
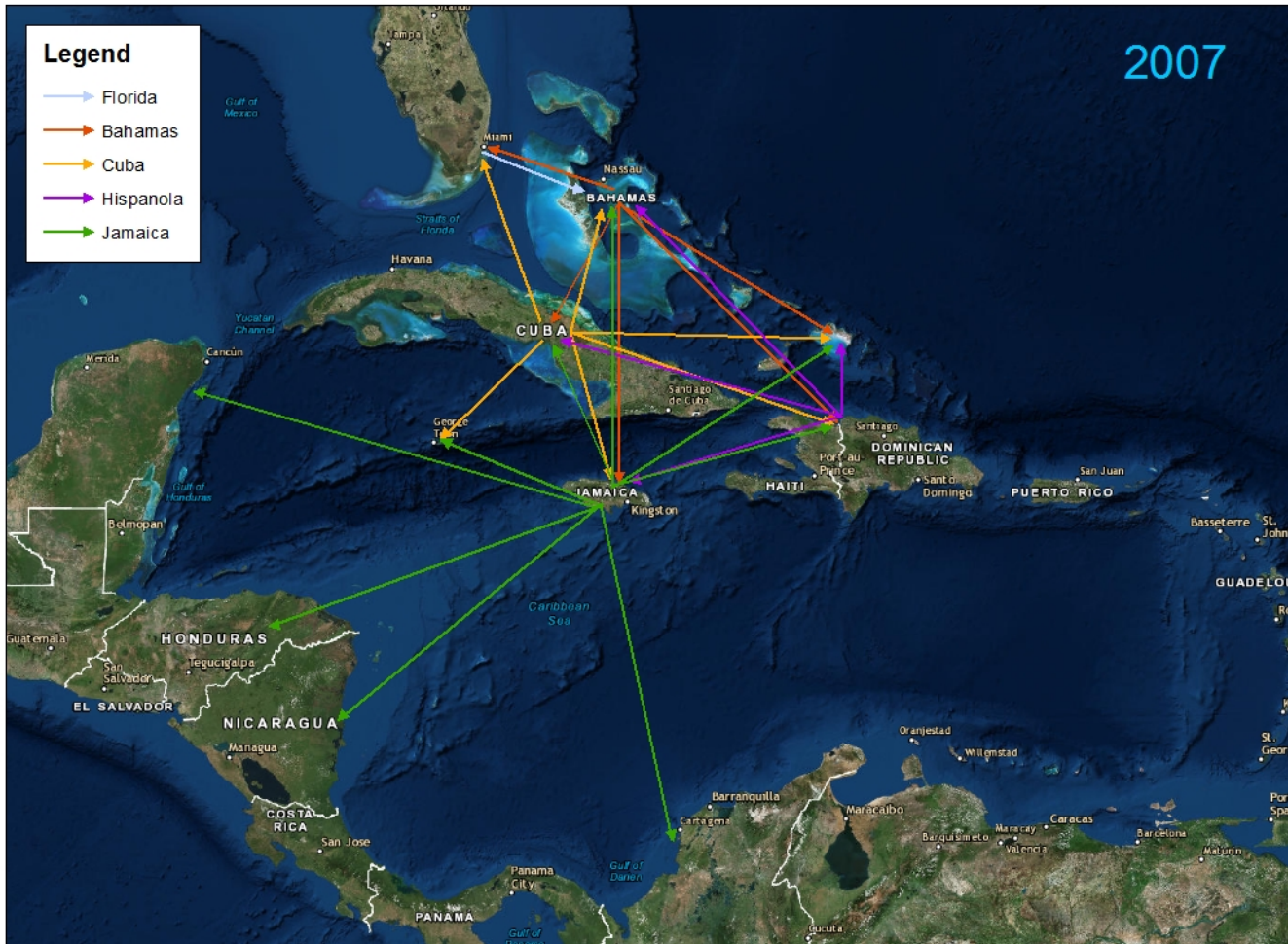
Figure 3.11. Map depicting the direction of "lionfish" larval transport between countries in 2007. Self-recruitment occurred in each year of the simulation, but is not represented with any arrows.
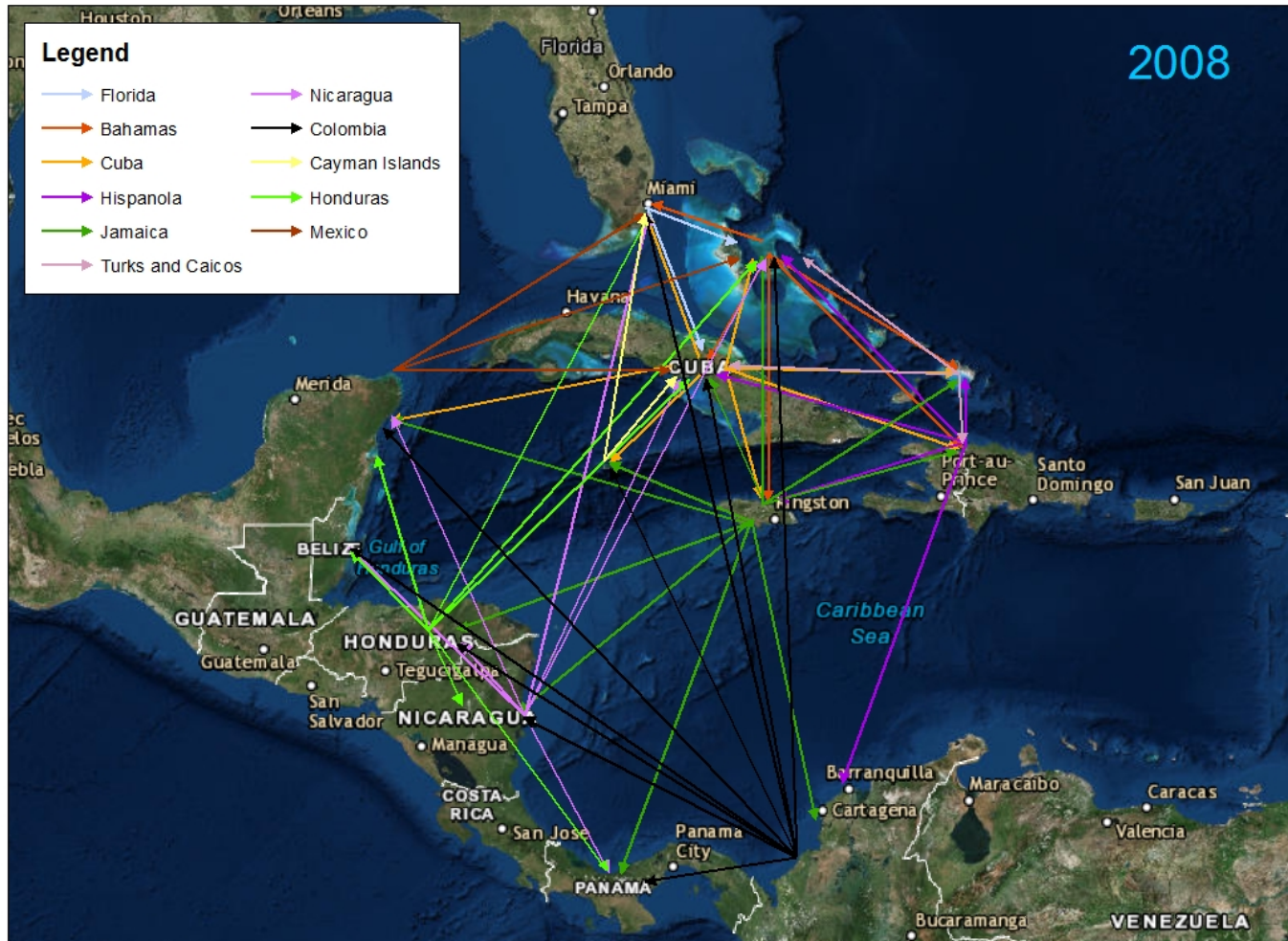
Figure 3.12. Map depicting the direction of "lionfish" larval transport between countries in 2008. Self-recruitment occurred in each year of the simulation, but is not represented with any arrows.

Chapter 4 – Deepwater Commercial Spiny Lobster Fishery Provides Lionfish Refuge and Optimum for Improving Removals

Background

Two species of lionfish, *Pterois volitans* and *Pterois miles*, were introduced to the Tropical Western Atlantic and are now amongst the most successful invasive marine fish species on record. The earliest reported sightings of invasive lionfish occurred along the east coast of Florida in 1985, with a handful of additional sightings recorded in the 1990s (Whitfield et al., 2002; Schofield, 2009). By 2000, a breeding population was discovered off the coast of North Carolina (Whitfield et al., 2002). High fecundity and slowly deteriorating egg masses have aided the dispersal of lionfish larvae through a well-connected network that links adult populations (Chapter 3; Morris et al., 2011). Lionfish sightings and spatial coverage of the species increased rapidly, with lionfish now found throughout the Caribbean Sea and Gulf of Mexico (Ruttenberg et al., 2012).

While most introduced species fail to become established, lionfish have been able to overcome predation and competition pressures from native species to develop successful breeding populations (Lockwood et al., 2008; Morris et al., 2009). As lionfish populations have grown, their negative impacts on native reef fish abundance has been documented extensively. Lionfish can be linked to the reduced recruitment of juvenile and small-bodied fish species in habitats that span from mangrove forests to coral reefs (Albins and Hixon, 2008, 2011; Morris and Akins, 2009; Green et al., 2011). Many culling and research initiatives have been developed to understand and counteract the impacts of lionfish on native reef fish abundance (Morris, 2012). However, these efforts are generally restricted to regular SCUBA diving depth limits (30-40 meters), while

lionfish have been observed in depths greater than 300 meters (Nuttall et al., 2014). This leaves a large proportion of potential lionfish habitat under-studied.

Lionfish were first observed on coral reef habitats of the Florida Keys in January 2009, and by the beginning of 2010, were observed in the offshore areas of the spiny lobster fishery (Gary Nichols, personal communication). Fishers deploying traps in deeper fishery use areas (> 20 m) reported increasing numbers of lionfish as bycatch, and fishers believed that lionfish are reducing their catch of legal-sized spiny lobster. Presence of incidental lionfish catch in deeper fishing areas (20-60 meters) provides an opportunity to study lionfish at depths beyond the convenient diving range where the majority of lionfish research occurs. This study describes the length frequency and spatial distribution of lionfish in offshore lobster fishing areas and estimates catch rates of lionfish throughout the shallow and deep water fishing areas for the first time. Additionally, this study determined the relative contribution of lionfish to the bycatch community in the fishery. Lastly, the study evaluated the effectiveness of using lobster traps as a lionfish control method.

Methods

Lobster Fishery Background

Lobster fishers in the Florida Keys rely on trapping and SCUBA diving to collect spiny lobster, *Panulirus argus*. Trap fishers use two main trap designs; wooden traps and wire traps, allocated to the shallow water and deep water fishing areas, respectively (Figure 4.1). Wood traps are predominantly used in shallow fishing areas, less than 23.5 meters in depth, and are arranged individually in single-file "lines" of approximately 50 traps per line. Wire traps, which are less prone to deterioration and breakage, are used in

deep water fishing areas between 20 and 57.5 meters. Wire traps are tethered together in 20 trap strings or "trawls", the additional weight of which prevents the traps from dragging along the bottom and makes them easier to recover after inclement weather events. For these analyses, the deeper fishing areas were divided into three3 depth bins: Trawl 1 – 20 to 32.5 meters, Trawl 2 – 32.5 to 45 meters, and Trawl 3 – 45 to 58 meters.

Observer Study Protocol

Observational surveys were conducted by two observers aboard a commercial lobster vessel operating in the Middle Florida Keys during the 2011-2012 lobsters (Season 1) and again for the 2012-2013 season. Trap pulls in each season began on August 6th and continued through March 31st. The fishing areas included coastal Atlantic Ocean waters, bounded by Upper Matecumbe Key to the north and Bahia Honda Key to the south (Figure 4.2). Traps were deployed at the discretion of the fishing captain in the two depth regimes described above, at an approximate ratio of 8:1 (deep fishing areas to shallow fishing areas). Observers were allowed aboard the fishing vessel to conduct surveys at least twice each month during the fishing season, unless prohibited by inclement weather or mechanical issues on the vessel.

The observers entered data directly into a Panasonic Toughbook and used the internal GPS to record the location of each trap sampled. The depth of each trap was estimated from the sounder on the vessel, at the beginning of each line in shallow waters or each trawl in deeper waters. Trap catch composition data were collected following a similar sampling scheme as Matthews et al. (1994). Crew members removed all organisms from every third trap and passed them off to observers who identified and measured every organism to the lowest taxonomic level possible. Amorphous, non-

lobster invertebrates, such as crabs, sponges, shrimp, octopi, etc., were not measured due to their ambigous body shape or small size. All of the lionfish caught from both sampled and non-sampled traps were separated from the catch to obtain counts and total length measurements for each trawl or line sampled on a fishing day. At the end of each survey trip the total pounds of landed lobster and all lionfish were also recorded.

Catch Analysis

Lionfish catch data were used to describe the length distribution and catch rates of lionfish within the shallow and deep water fishing areas in each season. Trap data were used to quantify the proportion of the catch comprised of the target species versus each of the bycatch species captured. The weight per trap of the three most common bycatch fish was estimated using parameters from the literature (Table 4.1). The catch was aggregated into categories describing the most abundant species groups: lobster, bycatch fish (excluding lionfish), and lionfish. The catch rates of each of these groups were estimated for the depth bins within the fishery, between the two fishing seasons. Additionally, the catch rates and sizes of lobsters were compared for traps as a function of the species composition within the traps. The possible species combinations included: (1) Lobster Only; (2) Lobster and Bycatch Fish (excluding lionfish); (3) Lobster and Lionfish; (4) Lobster, Bycatch Fish, and Lionfish. These catch rates were compared statistically with an analysis of variance. The average catch rate of lionfish per day was used to compare the removals from a fishing vessel to the removals from culling events over a year. Finally, the catch rates of legal lobster catch were standardized using a generalized linear model applied to the log catch rates of positive catches of legal lobsters. As expected, the catch data was zero-inflated; a small number (0.0001) was added to all zero values in the

data to enable the log transformation. A set of models was constructed to determine

which factors were the most significant in explaining the variance in the lobster catch

data (Table 4.2).

Results

During the two lobster fishing seasons (2011-2012 and 2012-2013), observers

sampled 3,659 traps over 30 fishing days. The majority of these traps were deployed in

the deep water fishing areas (N=3213), with only 12% of the total traps set in the shallow

water fishing areas (N=446). The fishing effort was not equal between the two seasons;

20% more traps were deployed in the second fishing season. Observers identified 20,438

total organisms from 130 species; 7,731 organisms in Season 1 and 12,707 during Season

2.

Spiny lobsters were present in 88.1% of the traps sampled, with 178 kg (SD=29

kg) and 210 kg (SD=53 kg) landed per day in each season respectively. Bycatch species

were found in 65.6% of the traps deployed, but few species comprised a significant

proportion of the catch (Figure 4.3). No species of bycatch fish was found in higher

proportion of traps than lionfish, 21.4% of traps sampled. White grunts (*Haemulon

plumieri*i) were the most abundant bycatch species caught in traps located in shallow

grounds, found in 7.3% of traps. In the deeper fishing areas, lionfish were second only to

littlehead porgies (*Calamus proridens*) in number, found in 17.3% of traps. The average

weight per trap of the three most commonly observed bycatch was estimated to be: white

grunts – 0.295 kg/trap, littlehead porgies – 0.316 kg/trap, and lionfish – 0.484 kg/trap.

The average weight of lionfish landed per trip was 34 kg (SD =5 kg) in Season 1 and 38

kg (SD=8 kg) in Season 2. Spotted scorpionfish (*Scorpaena plumieri*), the most closely related reef fish species to lionfish, was only captured in 1.5% of traps. A mixture of invertebrates and fish dominated the bycatch of the shallow traps, whereas bycatch in deeper traps predominantly fish species (Table 4.3).

Lionfish were predominantly captured in the deeper fishing areas (N=3929), with only 3 lionfish caught in the shallow fishing areas where lines of wooden traps are deployed (Figure 4.4). Lionfish total length ranged from 28 mm to 412 mm (Figure 4.5). The median length of lionfish in the catch increased from 249 mm (SD = 38.1 mm) to 260 mm (SD = 50.6 mm) between Seasons 1 and 2; the largest lionfish caught in each season was 370 mm and 412 mm, respectively. An overall broadening and shift of the distribution to the right is also seen in the second season, reflected by an increase in the number of larger fish caught. Mean lionfish total length varied significantly by depth strata; the largest fish weremcaught in the deepest depth strata (ANOVA, $p<0.05$). This relationship varied by season, as indicated by the smallest fish found in the intermediate depth strata during the first season. During Season 2, the mean size of fish showed an increasing trend with depth (Fig 4.6).

Catch rates for a subset of the species composition categories containing spiny lobsters were calculated for each season and depth strata: (1) Lobster Only; (2) Lobster and Bycatch Fish (excluding lionfish); (3) Lobster and Lionfish; (4) Lobster, Bycatch Fish, and Lionfish (Figure 4.7). Lobster catch rates varied significantly by depth and season (ANOVA, $p<0.05$), with no clear pattern where catch rates were the highest. Season 1 had high catch rates in the shallow water fishery, with an average 6.1 lobsters per trap. Over both seasons, the mean lobster catch rate varied between 2.6 and 3.5

lobsters per trap in the deep fishing areas. Mean legal lobster catch rates ranged from 0.58 to 2.8 lobsters per trap. Bycatch fish had the lowest catch rates in the shallow water traps, but bycatch catch rates decreased as depth increased in the trawl fishing areas. Lionfish catch rates were extremely low in the shallow water trap fishery. No lionfish were caught in the shallow line traps during Season 1 (N=86 traps), and catch rates only increased to 0.01 lionfish per trap in Season 2 (N=360 traps). In the deeper fishing areas, the mean catch rate of lionfish per trap ranged between 0.25 and 0.61 lionfish. On average, 131 lionfish were caught in lobster traps per day. This which would represent 26,724 to 44,540 lionfish removed each season, by a lobster fisherman hauling traps 3 to 5 days per week, respectively.

Legal lobster catch rates varied based on the combinations of species groups in the traps (Figure 4.8). The major trap classifications were: (1) Lobster Only - SO; (2) Lobster and Bycatch Fish (excluding lionfish) - BS; (3) Lobster and Lionfish - LS; (4) Lobster, Bycatch Fish, and Lionfish - ALL. These trap categories include both legal and sub-legal sized lobsters. When comparing the mean catch rates in the trap categories that involved lobster, the catch rates were highest when lobsters were alone. In both seasons, traps containing only lionfish and lobsters (LS) had mean catch rates that were lower than the mean catch rates for the traps containing only bycatch fish and lobsters (BS). The lowest legal lobster catch rates were found in traps containing legal lobsters, lionfish, and bycatch fish. Legal lobsters were larger on average in the second season, regardless of the presence of other bycatch in the trap. In addition the average size of legal lobsters was larger when caught in traps without bycatch species. The average size of legal lobsters was greater in traps with bycatch fish and lobsters than in traps with lionfish and lobsters

for both seasons. The size of lobster in traps within the previously defined species group combinations (SO, BS, LS, ALL) varied greatly between the two seasons. In Season 1, mean carapace lengths were shortest in the trap categories containing lobster, but during Season 2, the mean carapace length fell between that of the bycatch/lobster and lionfish/lobster traps (Figure 4.9).

Of the generalized linear models assessed, the best model for fitting the catch of legal lobsters included the explanatory factors: season, month, depth strata, number of lionfish, and number of sub-legal sized lobster (Model AIC 50.37, Table 4.2). Without the factors of lionfish and sub-legal lobster, the AIC value for the models increased by a factor of 2-3. There is some evidence that inclusion of soak time and bycatch fish also decreased the AIC of the models tested, but the most parsimonious model and model with lowest AIC only included factors related to lionfish and sub-legal sized lobster abundance (Figure 4.10). Lobster catch rates are quite variable in the first season, with no trend season through the season. Season 2 showed high catches in the early months of the fishing season, in August, and then a declining trend as the season progressed. The catch rates of legal lobster appeared lower on average in Season 2 as compared to the first season.

Discussion

The majority of lobster traps examined caught spiny lobsters, but bycatch species were present in almost two-thirds of traps. Grunts, porgies, parrotfish and hermit crabs have become a more dominant component of the catch in lobster traps retrieved from the shallow grounds of the fishery, a shift from bycatch dominated more by stone crabs, grunts, spider crabs and urchins in the 1990s (Matthews et al., 1994). In deeper water,

porgies, grunts and lionfish were the most abundant species groups in the catch, similar to catch composition in the 1990s, with the exception of the addition of lionfish. The differences in catch rates between the two studies could be due to a variety of factors, including changes in reef fish species abundance over time resulting from an environment experiencing heavy fishing pressure, pollution, increased ocean acidification, and disease (Ault et al., 1998). The emergence of lionfish does not appear to have displaced any of the previously abundant species groups from the traps, but they are now one of the largest components of the bycatch species captured in the deeper fishing areas.

The large median size of lionfish, 254 mm, indicated that the majority of lionfish caught were reproducing individuals; males and females mature at 100 mm and 175 mm total length respectively (Barbour et al., 2011). Removals of lionfish from deep fishing areas by lobster traps may help reduce spawning stock of lionfish from the environment. The size distributions of all lionfish captured showed an increase in the retention of larger individuals, either due to older lionfish moving into deeper water in the second season or the growth of individuals found in deeper waters. When the size structure of lionfish was parsed between the three depth categories of the deeper fishing areas, the sizes of lionfish vary by depth. In both seasons, the average size of lionfish was largest increased depths.

The presence of lionfish was not uniform among depth strata. Lionfish catch rates were highest at greater depths; this trend was especially clear in Season 1. Virtually no lionfish were caught in waters shallower than 23.5 meters, an unexpected observation based on the large numbers of lionfish found by divers and snorkelers in similarly shallow depths. Lionfish may be using the structure provided by traps in greater depths as a refuge. The ocean bottom in deeper fishing areas is characterized by silty mud and sand

(Walker et al., 2008), with few rocks and rubble dispersed along the bottom. Conversely, the shallower grounds are closer to coral reef habitat, patch reefs, and man-made refuse that provides habitat for marine fauna and may provide more attractive habitat to lionfish than traps.

The large quantity and biomass of lionfish caught in the lobster fishery has created an additional source of revenue for fishers that land and sell them. The novelty of an invasive species and high price per pound ($6-$7 per pound – ungutted) make lionfish highly sought after and one of the most valuable bycatch species found in the catch from deeper fishing areas. Lobster traps have the potential to remove large numbers of lionfish from deeper fishing areas in the Florida Key, 10 to 20 times more fish than are normally collected in the REEF Lionfish Derby Series in each year (Table 4.2). While derbies encourage the removal of lionfish from all size ranges and raise awareness about the damage that lionfish can cause, the number of derbies conducted each year would need to increase to match the removal yields from commercial lobster traps. These derbies are also limiting in the number of individuals required to capture large numbers of fish, and hours in the water necessary for removals and collections are limited by depth restrictions of SCUBA and snorkeling.

Lobster catch rates vary greatly when considering depth strata alone, but a clearer pattern is evident when considering the additional species within the traps. The number of legal lobsters per trap was lower when other species were found in traps alongside lobsters. Legal lobster catch rates were lowest when both bycatch fish and lionfish were found together with lobsters. When comparing traps that contain lobsters and these

species combinations separately, the combination of lionfish with lobsters yield slightly lower catch rates than with any other bycatch fish and lobsters combined.

The best fit model for removing the variation in the legal lobster catch rates showed that the most important factors for explaining legal lobster catch rate were the season, month, depth, number of lionfish, and number of sub-legal sized lobsters. The removal of lionfish and sub-legal sized lobsters greatly increased the AIC value for competing model configurations. This indicates that lionfish presence was an important factor in explaining the number of legal lobsters caught in traps. While this does not prove that lionfish reduce the catch of legal lobsters, as fishers contend, it confirmed the negative correlation between the catch rates of both species in the trap. Five hypotheses could explain this phenomenon: A) lionfish and lobsters occupy different habitats; B) lobsters exclude lionfish from traps (lobster dominance); C) lionfish exclude lobsters from the traps (lionfish dominance); D) presence of both is largely incompatible (antagonism without dominance); or E) size of the trap limits the number of lobster and lionfish that can fit in the trap at one time. Only behavioral experiments combined with habitat characterization can reveal which of these best describes lobster and lionfish interactions. If the drivers behind the negative correlation between lobster and lionfish catch are not C or D, the high price received for lionfish should mean that lionfish catches are beneficial to the fishers. Even if the reason for the negative correlation is C or D, any losses incurred by fishers would be dependent on the strength of the loss of revenue from a reduction in the lobster catch rates and the compensation produced by the landing of lionfish.

Lionfish control efforts have been focused in shallow waters that can be easily reached by divers and snorkelers. While continual removals have been shown to keep numbers of lionfish low on small patch reefs (Green et al., 2014), these removals do nothing to address the problem of lionfish in deeper waters. This study provided more perspective on the size structure, spatial structure, and yields of lionfish that can be removed opportunistically through the spiny lobster fishery. Further studies are necessary to monitor lionfish in deep water regions, and continue to improve culling measures in these regions.

Table 4.1. Growth Coefficients used to estimate the weight of the three most common bycatch species captured.

PARAMETERS FOR LENGTH - WEIGHT RELATIONSHIP ESTIMATION				
Common Name	Species	α	β	Reference
White Grunt	*Haemulon plumieri*	6.3×10^{-5}	2.73	Silva and Murphy 2001
Littlehead Porgy	*Calamus proridens*	1.413×10^{-2}	2.8	Froese and Pauly 2015
Lionfish	*Pterois volitans*	2.89×10^{-5}	2.89	Barbour et al. 2011

Table 4.2. Model configurations tested to explain the variation in legal lobster catch rates from the lobster trap catch. The bolded model indicates the most parsimonious model, with the lowest AIC.

LEGAL LOBSTER CATCH RATE MODELS

MODEL	AIC
Catch Rate of Legal Lobster~Year+Month+Depth+Month*Depth+No. Lionfish+No. Sub-legal Lobsters+Soak Time+No. of Bycatch	57.95
Catch Rate of Legal Lobster~Year+Month+Depth+No. Lionfish+No. Sub-legal Lobsters+Soak Time+No. of Bycatch	53.83
Catch Rate of Legal Lobster~Year+Month+Depth+No. Lionfish+No. Sub-legal Lobsters+Soak Time	53.07
Catch Rate of Legal Lobster~Year+Month+Depth+No. Lionfish+No. Sub-legal Lobsters	**50.37**
Catch Rate of Legal Lobster~Year+Month+Depth+No. Sub-legal Lobsters	63.20
Catch Rate of Legal Lobster~Year+Month+Depth+No. Lionfish	132.28
Catch Rate of Legal Lobster~Year+Month+Depth	152.83

Table 4.3. Comparison of the number of individuals of each species or species group per 1000 traps sampled for the Matthews et al study and the current study. N references the number of traps sampled for each trap type (wood – shallow water or wire – deep water). Only the top 10 species groups for each column are shown below. The dashes below represent the lack of lionfish present in the fishery during the time of the Matthews et al study.

SPECIES	Matthews et al. - Wood	Current - Wood	Matthew's et al. - Wire	Current - Wire
	N=18773	N=446	N=496	N=3153
SPINY LOBSTER	3253	3305	875	3189
STONE CRAB (all sizes)	235	137	4	19
LIONFISH	**-**	**9**	**-**	**428**
GRUNT (Blue-striped, Caesar, Cottonwick, French, Pinfish, Porkfish, Sailor's Choice, Tomtate, White, White Margate)	94	300	875	272
SPIDER CRAB	72	0	4	1
URCHINS	67	0	4	11
COWFISH (Honeycomb and Scrawled)	17	43	673	192
HERMIT CRAB	14	132	438	238
TRIGGERFISH (Gray, Ocean, Queen)	10	34	109	157
ANGELFISH (French, Gray, Queen)	10	7	36	9
TRUNKFISH (Common, Scrawled, Smooth, Spotted)	9	20	16	23
SNAPPER (Glasseye, Gray, Lane, Mangrove, Mutton, Red, Silk, Yellowtail)	7	40	20	144
PORGY (Grassy, Jolthead, Littlehead, Red, Saucereye, Sheepshead)	4	229	486	575
PUFFERFISH	4	0	379	0
PARROTFISH (Blue, Green, Rainbow, Redtail, Stoplight)	4	184	379	23
GROUPER (Black, Gag, Graysby, Red, Snowy)	4	9	26	49
FILEFISH (Orange-spotted, Planehead, Pygmy, Scrawled, Slender,Unicorn)	3	20	87	63
ARROW CRAB	2	2	214	6
HOGFISH	2	65	0	22

Table 4.4. The number of lionfish removed from the REEF Lionfish Derby Series
conducted in South Florida in each year.

Year	Number of Derbies	Number of Teams	Lionfish Removed	Catch Rate per Team
2010	3	-	664	-
2011	4	67	2224	33
2012	3	47	1923	41
2013	4	46	1745	38
2014	4	50	1912	38
2015	4	44	1141	26
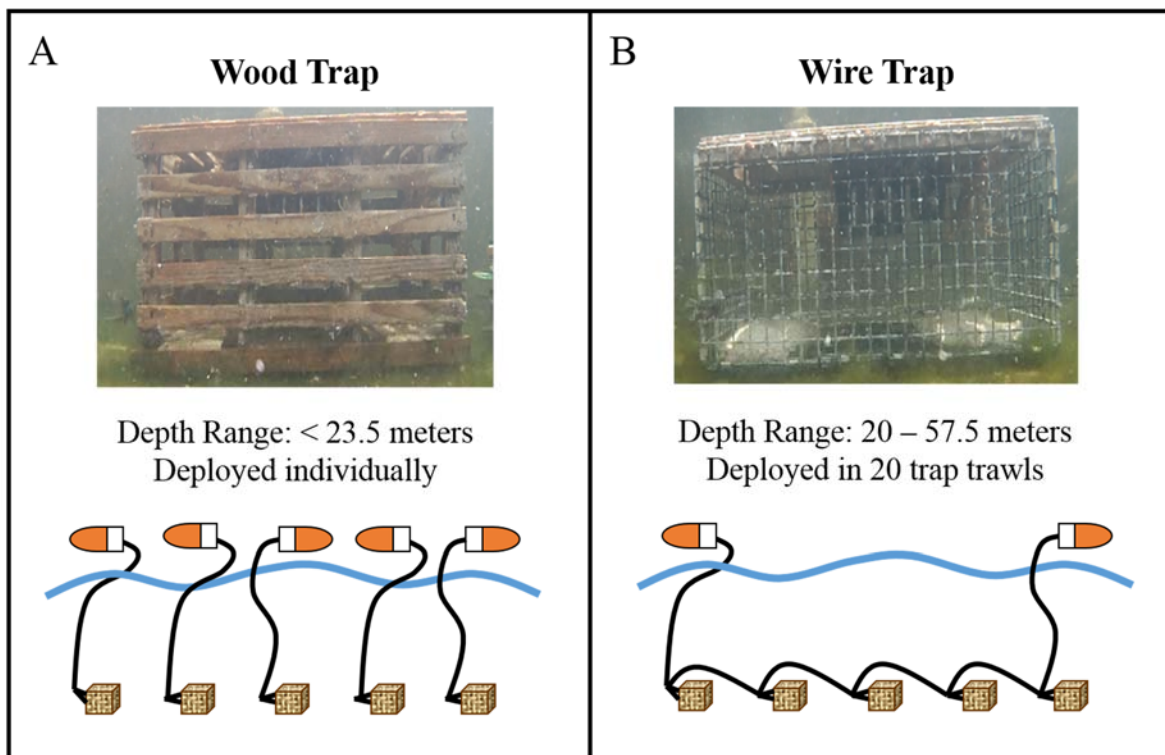
Figure 4.1. Two main trap types used in the commercial spiny lobster fishery, depth range for each trap, and deployment method.
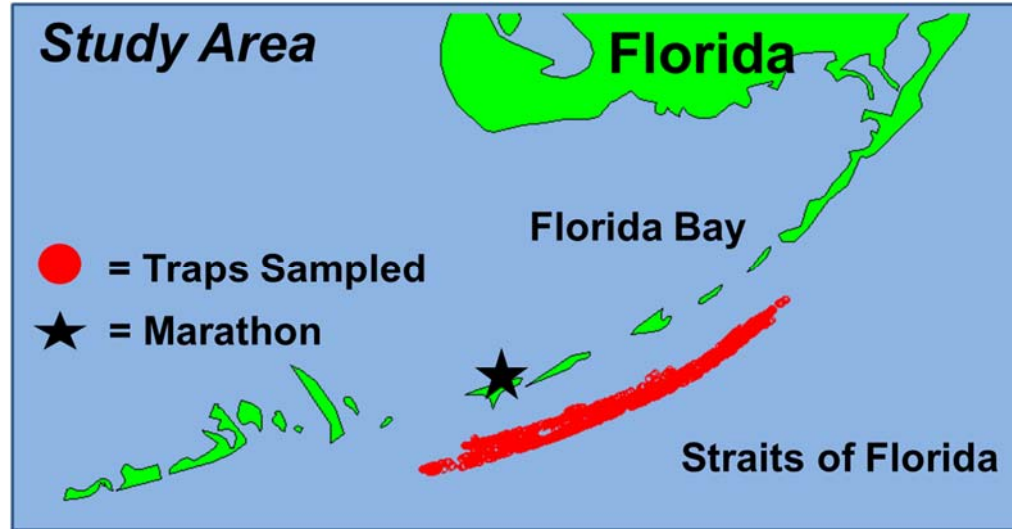
Figure 4.2. Lobster traps were deployed in coastal waters from Upper Matecumbe Key to Bahia Honda Key, the northern and southern boundaries respectively. During the two year study, traps were deployed between August 6th and March 31th for the 2012-2012 and 2012-2013 fishing season.
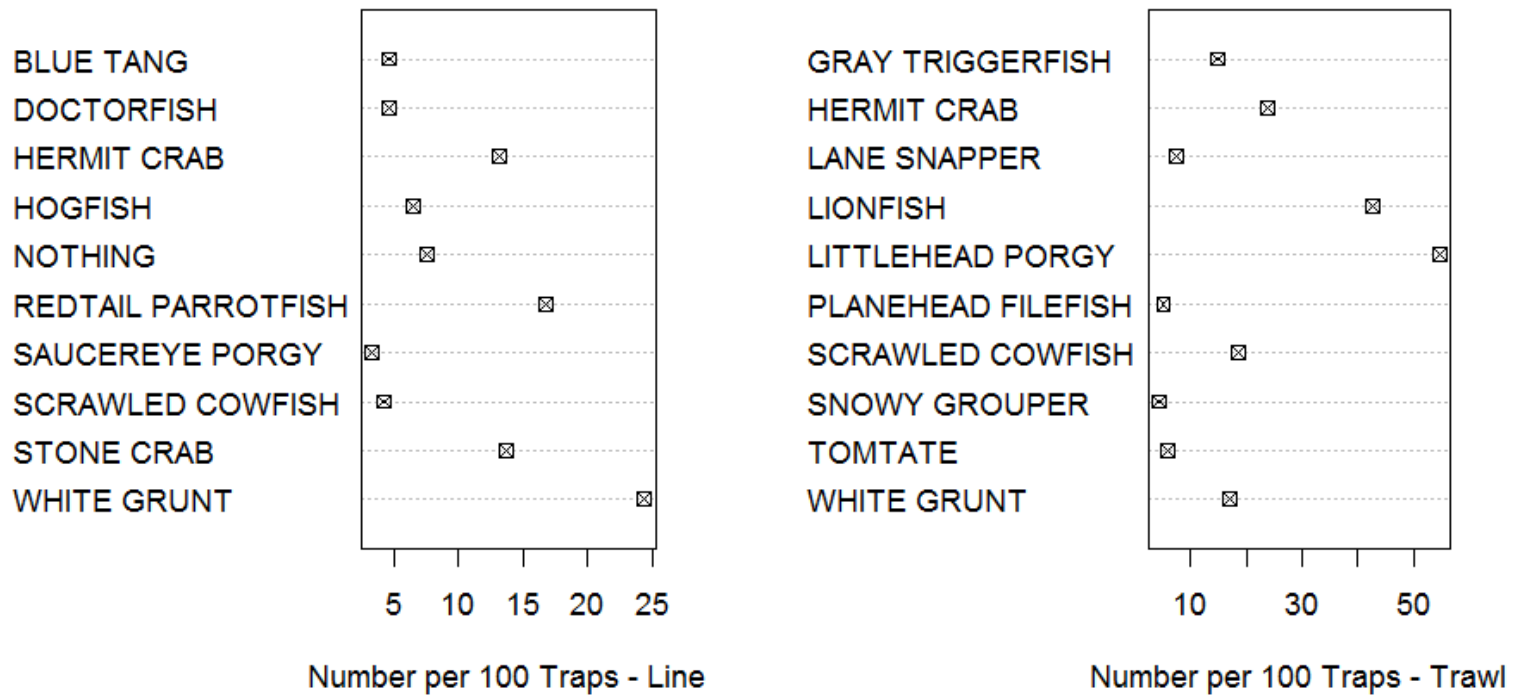
Figure 4.3. Ten most common bycatch species found in the catch, in numbers per 100 traps sampled, for the shallow (Line) and deep (Trawl) water fishing areas.
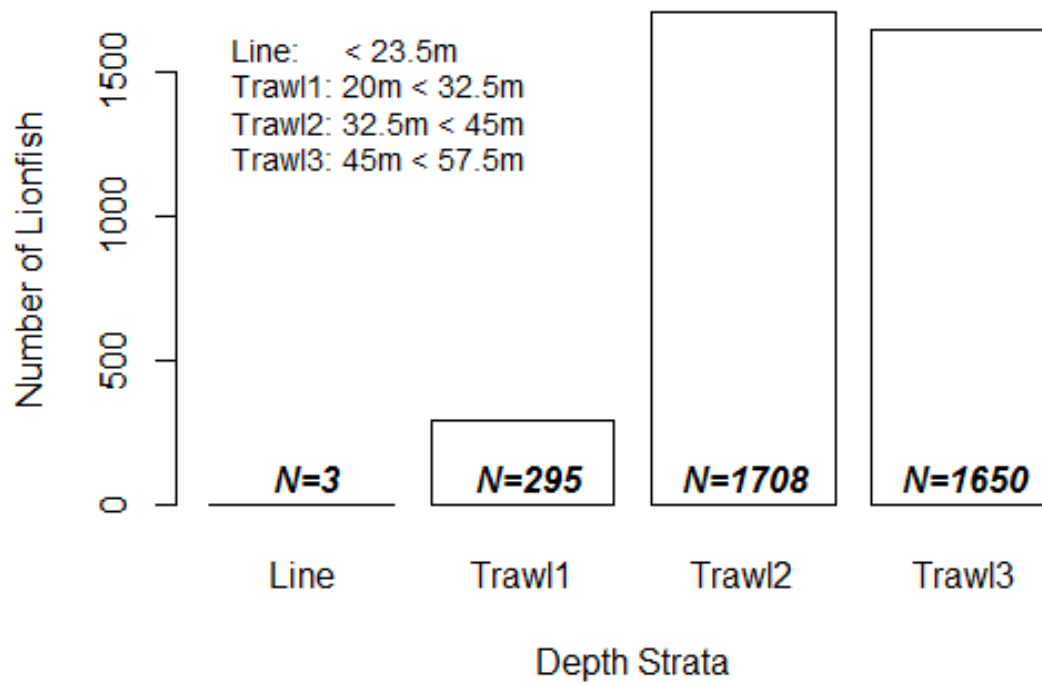
Figure 4.4. Number of lionfish caught by fishing ground and depth strata.
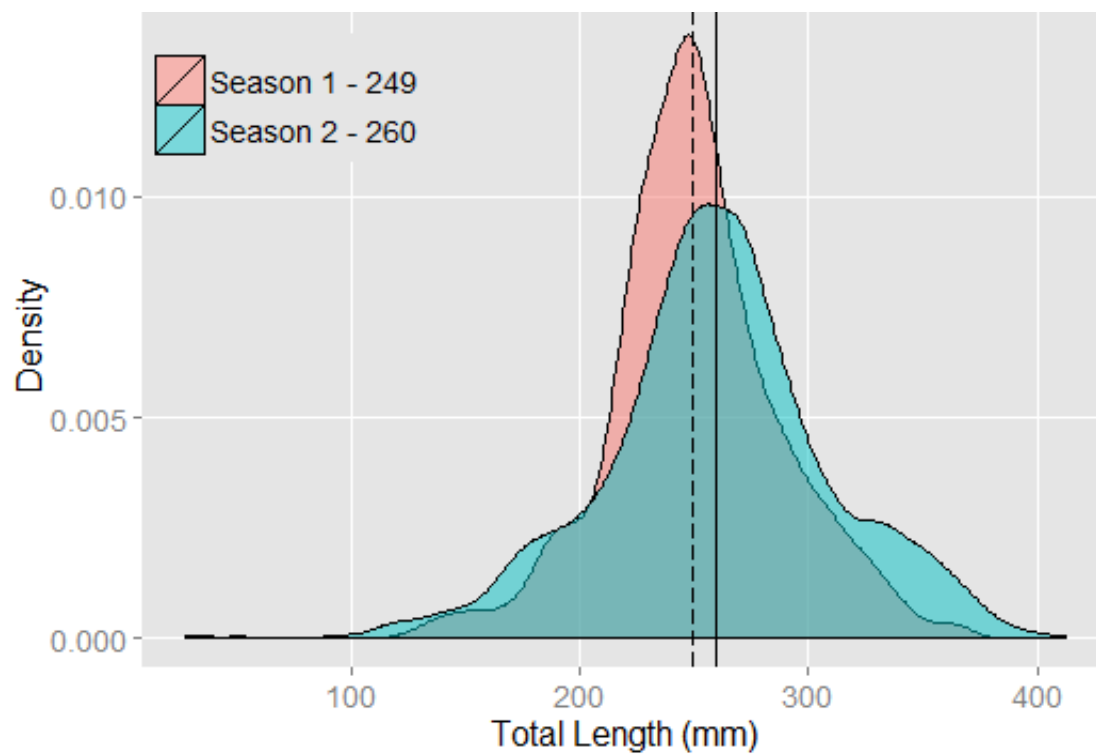
Figure 4.5. Frequency Distributions of lionfish total length by season. Median lengths are indicated by the vertical lines for Season 1 (dashed) and Season 2 (solid).
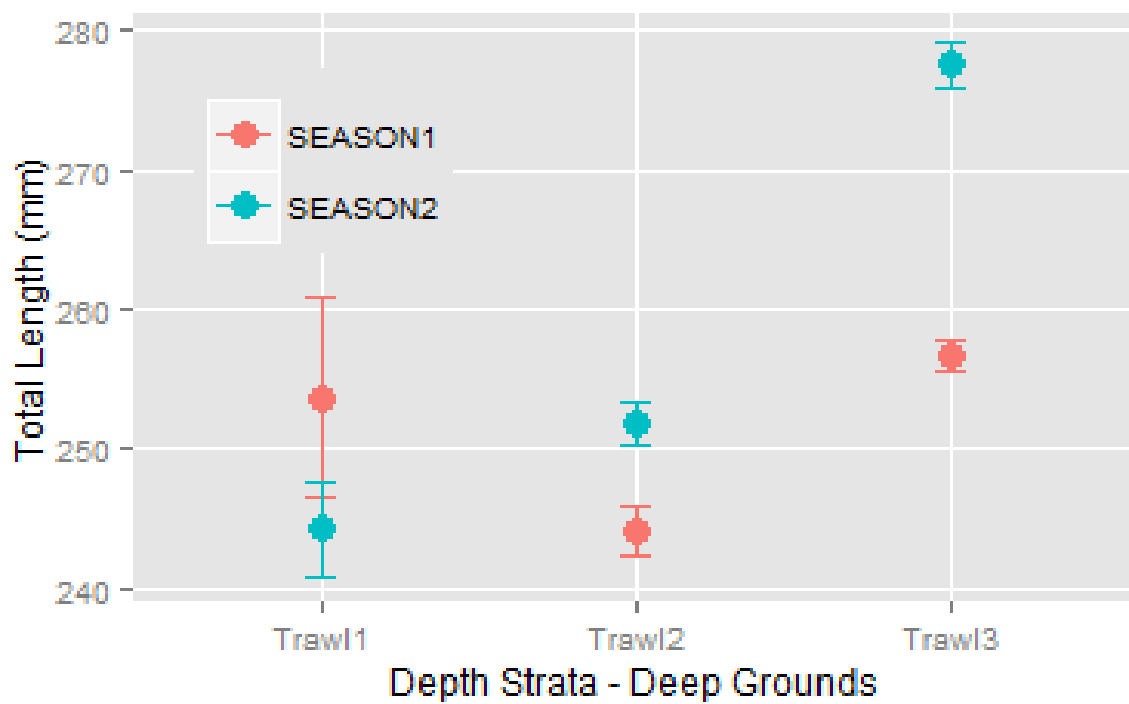
Figure 4.6. Represents total length of lionfish in the three depth categories within the deep fishing areas. Error bars represent one standard error from the mean.
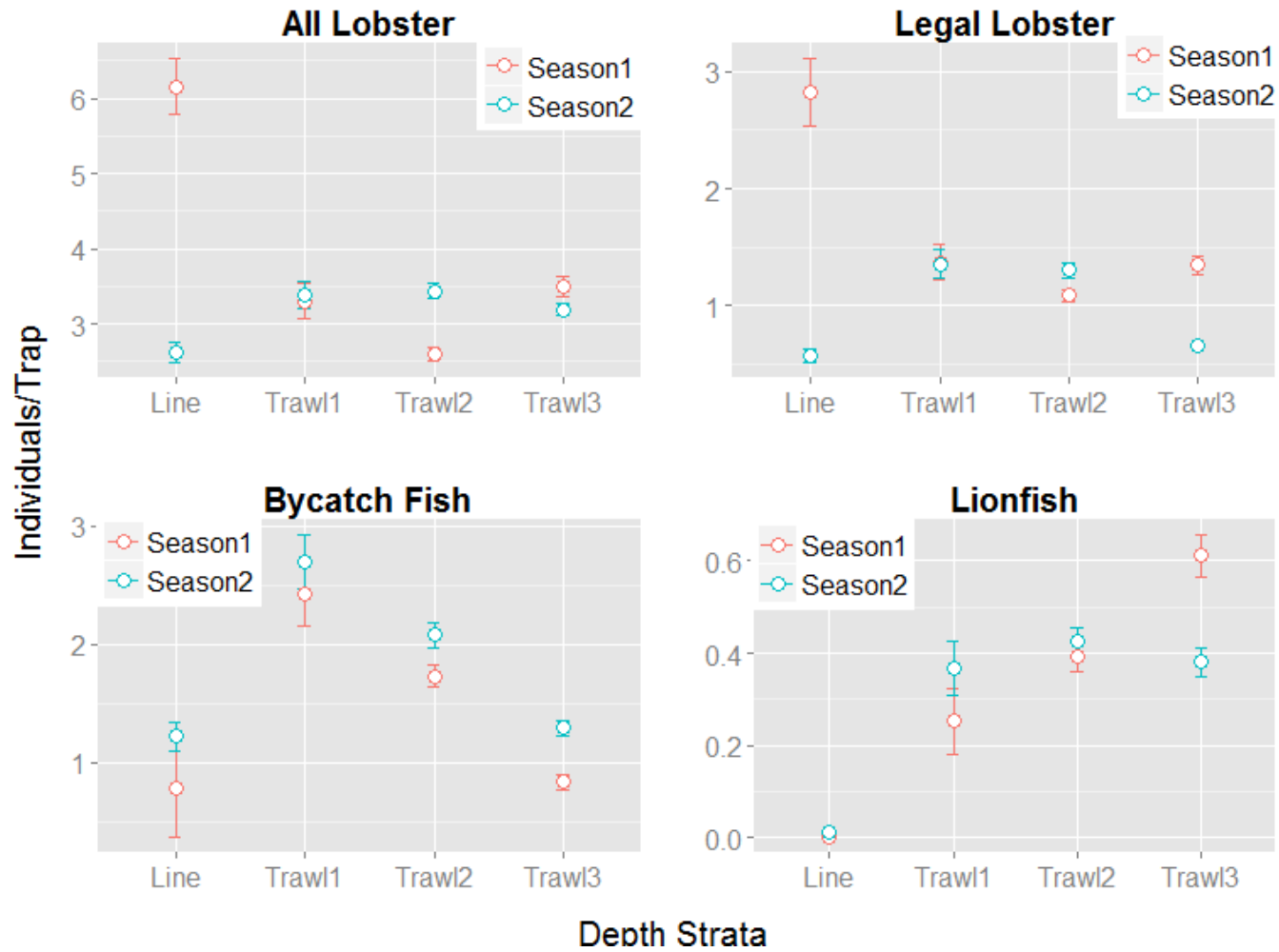
Figure 4.7. Mean catch rates for most abundant species groups in the catch by season and strata. Standard error bars represent one standard error from the mean.
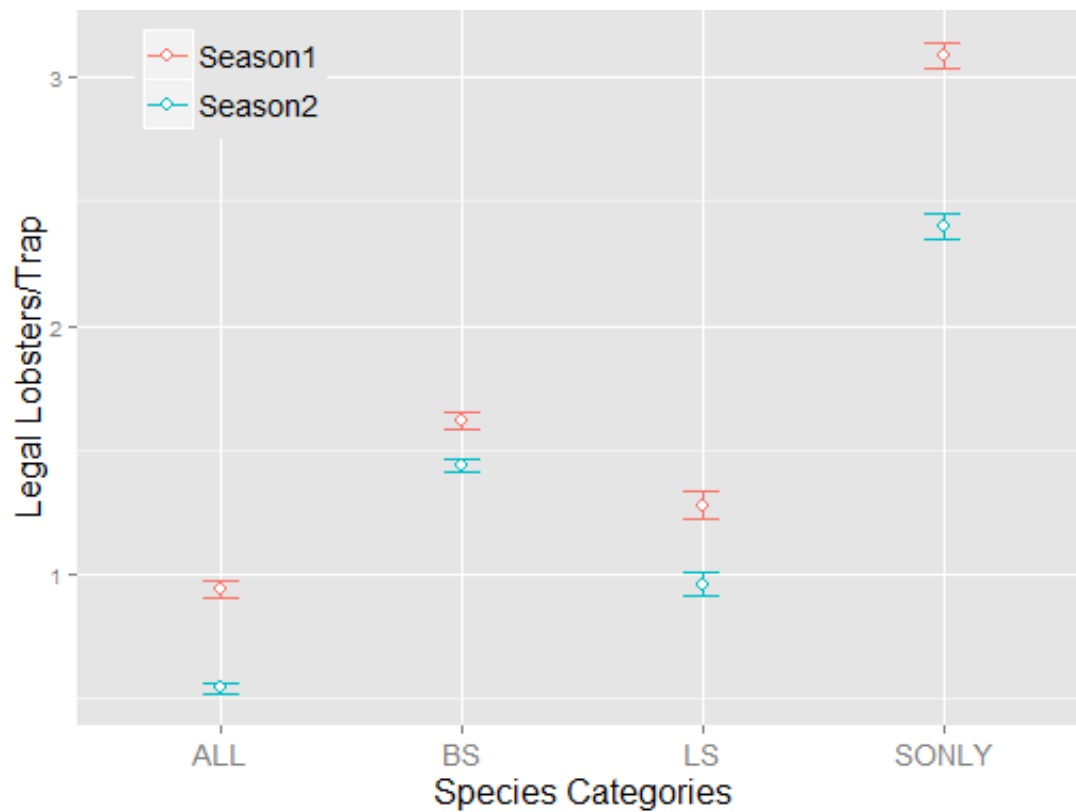
Figure 4.8. Mean legal lobster catch rates for traps containing Lobsters, Lionfish and Bycatch Fish (ALL), Bycatch Fish and Lobsters (BS), Lionfish and Lobsters (LS), Only Lobster (SONLY) for Seasons 1 and 2. Error bars represent one standard error from the mean.
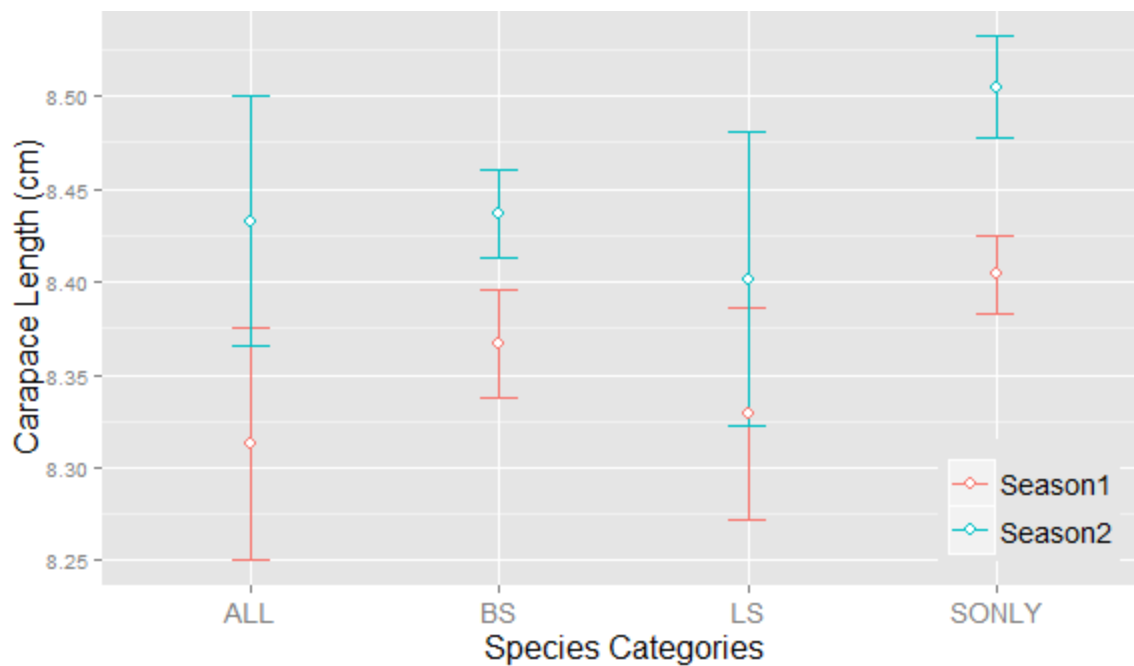
Figure 4.9. Mean carapace length of legal lobsters for traps containing Lobsters, Lionfish and Bycatch Fish (ALL), Bycatch Fish and Lobsters (BS), Lionfish and Lobsters (LS), Only Lobster (SONLY) for Seasons 1 and 2. The error bars represent one standard error from the mean.

Figure 4.10. Standardized legal lobster catch for both seasons; each season starts in the month of August. Red observation points represent an average for each fishing day, and the blue line represents the fitted model.

Chapter 5 – Behavioral Response of Lionfish to Benthic Structures Found in the Spiny Lobster Fishery in a Controlled System

Background

The introduction of the lionfish species *Pterois volitans* and *Pterois miles* has led to the reduction of small-bodied and juvenile fishes in the Tropical Western Atlantic Ocean (Albins and Hixon, 2008; Albins and Hixon, 2011). As lionfish populations have grown and spread, their negative impacts have been observed and quantified throughout the region. The risk of lionfish disrupting community reef structure has prompted the development of lionfish awareness programs, monitoring, and control efforts to reduce the abundance of these invasive predators (Akins, 2012[a]). Most removals require SCUBA divers or snorkelers to use spears and nets to collect lionfish from shallow nearshore waters (Akins, 2012[a]). This methodology is limited by short bottom times for divers, restrictions on maximum diving depths, and the difficulty of handling and transporting venomous fish underwater (Akins, 2012[a]). Catch of lionfish in deeper waters (20 to 180 meters) has been characterized by capture with traps (Chapter 4), and sporadic reports of lionfish being caught on hook and line (Akins, 2012[a]).

Since 2010, lionfish have become a major component of the bycatch in the spiny lobster trap fishery. Lobster fishers deploy traps from shallow inshore waters to deeper water fishing areas offshore to take advantage of lobster migrations on and offshore between benthic reef habitats. Results from our two year observer study determined that catch rates of lionfish increase at greater depths (Chapter 4). Additionally, lobster and lionfish catch rates were negatively correlated. The low catch rates of lionfish in shallow water raises questions about how lionfish distribute themselves along the depth gradient that exists when traveling away from shore.

Lionfish Habitat Usage

Lionfish have been observed at various depths (Morris, 2012), salinities (Jud et al., 2010; Jud et al., 2014) and inhabit many habitat types (Barbour et al., 2010; Biggs and Olden, 2011; Claydon et al., 2012). The capacity for long range migration in adults is limited, but they can travel between local habitat patches. Tracking studies have shown lionfish generally exhibit high site fidelity, but can travel up to distances of 1.35 kilometers (Tamburello and Cote, 2015). These movements may demonstrate attempts by some individuals to search out preferred habitat, as it is commonly believed that species choose habitat to improve their chances for survival (Fretwell and Lucas, 1970; Morris, 2003)

Habitat Preferences of Reef Fish

Environmental factors can alter the attractiveness and quality of benthic habitat that support the daily survival and growth of fish species. Past studies have shown reef fish species prefer live coral to dead coral, indicating that while recruitment to reef habitats as can be seen as a partially random process (Sale and Dybdahl, 1975), habitat choice has also been demonstrated at older ages (Talbot et al., 1978). Some species are capable of travelling longer distances to search out desired habitat, whereas less mobile species settle for the resources available where they have recruited, which may be sub-optimal (Ault and Johnson, 1998). As a result, both the quality and complexity of habitat can determine the number of fish an ecosystem contains (Hixon and Beets, 2000; Syms and Jones, 2000; Talbot et al., 1978).

The drivers responsible for the differences in lionfish distribution across depth strata cannot be determined from observations made on-board fishing vessels alone. This

study investigates how benthic structures may impact the distribution of lionfish across the depth strata within the lobster fishery. Benthic habitat along South Florida coast lines is arranged linearly, parallel to shore, with sandy patches between various reef types (Walker et al., 2008). The natural benthic structure is comprised of linear, spur and groove, patch reefs, colonized pavement and ridge habitats. Additionally man-made structures and artificial reefs are dispersed along the coast line as well.

Assessment of Lionfish Preference

Benthic mapping in South Florida is most comprehensive in depths up to 35 meters, but shows a trend towards declining amounts of benthic structure with increasing distance from shore and greater depth (Walker et al., 2008). It is possible that traps act as structured habitat in deep water fishing areas where less relief is available. This study aims to investigate a theory that lionfish catch rates observed in the lobster fishery do not differ as a function of depth per se, but as a function of the benthic structure available at each depth. This study aims to determine if lionfish have a preference for a series of proxy structures that represent the major benthic habitats available in the trap fishery, both alone and in the presence of the target species from the trap fishery.

Methods

Specimen Collection

Lionfish were collected with nets from reef habitat in South Florida, specifically the Florida Keys and Key Biscayne. Lionfish that were hauled from depths greater than 4.5 meters were placed in a small wire cage and brought to the surface slowly to reduce barotrauma. Once on the surface, lionfish were monitored for barotrauma and decompressed with a venting tool if signs of barotrauma were seen (e.g. distended

abdomen from over-expanded air bladder). All fish were transported in an aerated cooler

to a circular tank (~4.5 m diameter and 1 m height) in Miami, Florida. Lionfish total

length ranged in size from 196 to 313 mm (Figure 5.1).

Legal-sized lobsters (>76.2 mm carapace length) were purchased from a Florida

Keys lobster fisher and transported to Miami, Florida in the same manner as lionfish.

Lionfish and lobsters were acclimated to tanks for a minimum of five days. After this

period all animals demonstrated normal feeding behaviors and activity levels, including

buoyancy control for lionfish.

Tanks were shaded to prevent over-exposure to the sun. Plastic fencing was used

to partition the tank into two halves, with different species treatments occurring on either

side of the tank. Both sides of the tank were aerated and filtered with air-powered filters.

The seawater was filtered by 10 micron filter socks as it entered the flow-through tank. A

sump pump was used to circulate water from the tank through both a gravity and fabric

filter (100 microns) for additional filtration of water. This experimental design was

approved by the University of Miami Institutional Animal Care and Use Committee,

Protocol 12-203.

Experimental Treatments

The experiment consisted of two species treatment: lionfish alone on one side of

the tank partition (N=6 lionfish) and lionfish and lobsters on the other side (N= 6 lionfish,

N=9 lobster). After the acclimation period, both species treatments: Lionfish Only and

Lionfish and Lobster were presented with 4 bottom structure combinations over a one-

week period. There are four bottom structures used to represent the main benthic habitat

available in the spiny lobster fishery. The coralline rock simulates the irregular shapes

and crevices available in live coral reef habitat. The concrete blocks represent the angularly shaped surfaces with regularly shaped openings that correspond with many man-made objects. The wooden and wire lobster traps were obtained directly from a lobster fisherman; wooden traps are deployed in shallow depths and wire traps are deployed in deeper fishing areas.

The bottom structures were combined to assess lionfish preference for these habitats that occur different depth strata: 1) Concrete and Coral (CC) represent fixed benthic structures found mainly in shallow fishing areas; 2) Concrete, Coral, and Wooden Trap (CCWO) represent two fixed a and one temporary benthic structures found in shallow water; 3) Coral, Concrete, and Wire Trap (CCWI) represents two fixed benthic structures from shallow fishing areas and one temporary benthic structure from deep fishing areas; 4) Wire (WIRE) represents a temporary structure found in the deep fishing areas. Experimental animals were exposed to each species treatment for 2 days, with the exception of the Coral, Concrete and Wooden Trap treatment, which was only exposed for one day. The concrete and coral structures were placed at opposing ends of the half tank enclosures, with an empty transition area between them (Figure 5.2). When a lobster trap was introduced, it was placed in the central transition zone between the two structures. The CCWO structure combination was only set for one day based on the low affinity for lionfish catch in wooden traps in the lobster fishery, and lower visibility into the wooden traps (Chapter 4).

Video Analysis and Coding

Animal activity around the structures was monitored with high-resolution video cameras set facing each of the structures. Each camera recorded for two minutes every 15

minutes, 24 hours/day for the duration of the experiment. Cameras were modified to record at night in conjunction with UV red lights that illuminated the tank. Each recording was analyzed to count the number of animals in front of each structure, activity level in each video, and any intra- or inter-specific antagonistic behaviors were noted. How each individual spent their time was defined as one of three behaviors: Structure Time, Frame Time and Out of Frame Time. Structure Time referred to the amount of time an individual spent actively using a structure; touching or hovering within 0.3 meters of the structure. The Frame Time referred to the amount of time the individual was within the frame of the camera, but not actively using the structure. Lastly, Out of Frame Time referred to any time an individual spent outside the frame of the camera.

Some of the structures, coral and concrete, provide crevices that might lead to an underestimation of the number of individuals on or using the structures. A separate set of observations were collected to determine how effective each camera was at detecting the number of individuals of each species present. A human observer monitored the tank, while video cameras were recording, which gave the observer the ability to track all fish and lobsters, and count the numbers around each structure. The number of individuals seen during 2-minute videos was compared to the number observed in person to determine any deficiency in the cameras at tracking lionfish and lobsters accurately (N=85 for Concrete, N=89 for Coral). In person observations were considered the unbiased standard; the observer could track the position of all lionfish at one time.

Data Analysis

The activity level of lionfish was defined as the number of times fish entered or exited the frame of each video. An ANOVA was used to compare these quantities during

the day and at night, to determine if day and night observations could be grouped together for future analysis. It was assumed that if there was no significant difference in the movements of lionfish, the behaviors seen during the day and night could be analyzed together. The preference of lionfish for a structure was quantified by the proportion of lionfish available in the tank associated with each structure within a structure combination experiment. An estimate of autocorrelation was calculated and plotted comparing the serial collection of lionfish proportions. Autocorrelation was considered removed when the estimate of autocorrelation did not statistically deviate from zero (Appendix 2). This occurred when analyzing data points collected every 30 minutes instead of every 15 minutes. Difference in these proportions was determined using Dirichlet regression, a model construct with a distribution that is suited for use with proportions. Additionally, the proportion of each two-minute interval each individual fish spent actively using each structure was a compared using the Dirichlet regression. The probability of detection by video cameras was estimated for coral and concrete structures with a linear model with intercept equal to zero. A slope of one in such case would correspond with 100% chance of detection.

Results

Activity Level

The total number of entries and exits by lionfish were used as a proxy for the activity level of the lionfish in each two-minute video (Figure 5.3). According to the ANOVA there was no significant difference based on the factor of time of day (e.g. day versus night), p=0.338 (Appendix 2). The lack of difference between the means of day

and night allowed us to pool the day and night observations in further analyses of lionfish structure preferences.

Probability of Detection

The more complex habitats, coralline rock and concrete, did not drastically reduce the probability of detection, and the estimated probability of detections for these structures were 96.7% and 97.0%, respectively (Fig 5.4). This showed that the cameras only slightly under-estimated the numbers of lionfish present within the frame of the camera, therefore the counts of lionfish observed on the videos were used without correction in all further analysis.

Habitat Preference Analysis

A series of Dirichlet regression models were used to compare the proportion of lionfish in a tank associated with different benthic structures. The results below represent the differences between the Lionfish Only and the Lionfish and Lobster species treatments. The best fit models for each bottom structure combination included the added factor of structure type to explain the variance in lionfish proportions around the benthic habitat presented to the fish.

Coral and Concrete

In this bottom structure experiment, lionfish were given the choices of either coralline rock or concrete structure as refugia. The regression of the Lionfish Only species treatment showed a significant difference in the mean proportion of lionfish in front of different regions of the tank (Appendix 2). The highest proportions of lionfish were seen around the coralline structure, 63.1% of the lionfish in the tank (Figure 5.5). The other two regions of the tank, where the concrete structure was housed or the central

transition area between the two structure treatments had similar proportions of lionfish associated with them, 37.9 and 39.8% of the lionfish during 2-minute video observations.

When lobsters were present in the tank; lionfish shifted away from the coralline structures and the highest proportions were seen in the center section of the tank in between the coralline rock and concrete structures (Appendix 2, Figure 5.5). In terms of time spent actively using structures, lionfish associated themselves with coral for the longest time periods, in both the lionfish only and lionfish and lobster species treatments, 39.4 and 23.7 % of a two-minute video interval (Appendix 2, Figure 5.9). The amount of time closely associated with the structures was almost 2 times higher in the Lionfish Only treatment as compared to the Lionfish and Lobster treatment.

Coral, Concrete, and Wood Trap

The second structure treatment exposed the individuals to a coralline rock, concrete block structure and wood lobster trap. The Lionfish Only species treatment showed the highest proportions of lionfish in front of the coralline rock structure, at 52.3% of the lionfish present (Appendix 2, Figure 5.6). The model predicted less than a quarter of the lionfish in the tank were observed around the concrete structure and wooden trap when lionfish were alone (Appendix 2).

Lionfish were distributed differently around these structures when lobsters and lionfish were present concurrently. The proportion of lionfish remained highest in front of coralline structures, while increasing around the concrete structure and wood trap, in the Lionfish and Lobster species treatment. Lionfish proportions were highest in front of the wooden lobster traps, at 46.2% of available lionfish (Figure 5.6). Lionfish usage of the structures showed an affinity for coralline rock as compared to the other structure

types in this experiment, unless lobsters were present. When lionfish were alone, the time spent using the structures was almost doubled (Appendix 2).

Coral, Concrete, and Wire Trap

Experimental subjects were presented with coralline rock, concrete block and a wire trap as benthic habitat in the third bottom structure combination. The pattern of lionfish distribution was the same in the Lionfish Only and Lionfish and Lobster Treatment, but the magnitudes of fish varied by species treatment (Appendix 2). The highest proportions of lionfish were seen around coral rock habitat, followed by the wire trap, and lowest in front of the concrete structures (Figure 5.7). While the rank order of the proportions were the same for each structure type, there was a greater than 2-fold difference between the wire trap and coral rock proportions in the Lionfish Only treatment as compared to the Lionfish and Lobster species treatment. Lionfish also associated closely with coralline rock for the longest time periods, in both species treatments (Appendix 2, Figure 5.10).

Wire Trap Only

All structures, with the exception of wire lobster traps, were removed from the tank, but the tank continued to be monitored with the same cameras. Regressions showed predicted proportions of lionfish to be highest around the wire trap in the Lionfish Only treatment, but the predicted proportion was a third less when lobsters were present around that structure type. The lionfish that did not associate closely with the trap, hovered close to the cameras monitoring the barren regions of the tank that previously held the concrete and coral. The highest proportions of lionfish were observed in close proximity to the cameras being used to monitor the area where the coralline structures had been, 57.6 and

58.5% for the Lionfish Only and Lionfish and Lobster species treatments respectively. Lionfish spent large portions of each two-minute video hovering in regions of the tank that had previously housed the coralline rock and concrete structures especially in the treatment where lobsters were present.

Discussion

The highest catch rates of lionfish in the spiny lobster fishery were found in the deeper regions of the fishery, likely a function of the behavior exhibited by lionfish to associate with whatever type of structure is available to them. Future studies that employ traps to catch lionfish should consider the distance from the traps to established coralline or man-made habitats and the densities of lionfish within these habitats. Additionally, long term monitoring efforts aiming at understanding abundance of lionfish should consider habitat type as an indicator for lionfish abundance and stratify survey effort to increase efficiency of census efforts (Ault et al., 1999).

Proxy structures were created to represent the main benthic habitat types found in the spiny lobster fishery and were deployed in the tanks in a variety of combinations. The results of the Lionfish Only species treatment provided an assessment of lionfish preferences for these structures, with coralline rock attracting the largest numbers of individuals. The time spent actively hovering around or within a structure was also observed, with lionfish spending more time closely associated with coralline rock structures when they were available in the Lionfish Only Treatment. The coralline rocks used to create the structures allowed lionfish to hide in crevices and under rock ledges, providing the fish with a more amorphous shape, congruous with reef habitat found in

situ. These results may indicate a preference for coral reef habitat over other benthic habitat when present.

In situ, live coral has been shown to host greater biodiversity (Coker et al., 2012; Luckhurst and Luckhurst, 1978, McCormick, 1994), and the increased complexity of the reef habitat creates more feeding opportunities for resident predators (Almany, 2004, Gratwicke and Speight, 2005). Additionally, density of lionfish has been reported to be between 0 and 53 fish per 100 m^2 on reefs in Caribbean (Hackerott et al., 2014), and from 0 to 38.5 fisher per 100 m^2 on natural and artificial reefs in the Gulf of Mexico (Dahl and Patterson., 2014). Lionfish are readily available in reef habitat, but catch rates of lionfish in lobster traps posted near those habitats yields few lionfish (Chapter 4). In shallow waters, more available benthic habitat, particularly coral, is available (Walker et al., 2008), and could provide more desirable habitat than wooden traps normally deployed in these depths.

The bottom structure combination with only wire traps is representative of the habitat that would be available in the deeper fishing areas, fairly barren flats until lobster traps are deployed and create structure. In these regions with low natural benthic structure, lionfish are likely attracted to the wire traps, just as the highest proportions of lionfish were seen around wire traps when they were the only available structure in the tank experiment. When deployed by commercial fishers, these traps are baited, enticing fish and lobster species to the traps. Lionfish are not attracted to the bait in the trap, however, they might take advantage of these traps as aggregating devices to find prey and/or for protection from strong currents found at the greater depths offshore.

The species treatment that included both lionfish and lobsters provides insight into the relationship between these two species in the wild. The presence of lobsters appeared to change the distribution of lionfish around the structures available in the tanks. Lionfish proportions were lower around the preferred coralline rock structures, generally occupied by lobsters, increasing around the other available structures. The dominance and aggression of lobster towards lionfish was observed on numerous occasions during the experiment; lobsters were seen actively chasing lionfish away from structures or attempting to catch lionfish. Prior studies have shown lobster aggression or avoidance to be a function of the perceived threat from other species (Eggleston and Lipcius, 1992; Weiss et al., 2008); the behaviors exhibited by lobster used in this study suggest that the lobster did not see lionfish as a threat. There were no incidences of lionfish displaying any aggressive movements towards lobsters in any of the videos analyzed in the study.

The majority of spiny lobster fishers deploy their traps in shallower regions. The high catch rates of lobsters in wooden traps in these depths in addition to the frequent availability of coral and artificial structures in shallower depths may provide alternative shelter to lionfish and contribute to the low catch rates of lionfish seen in the shallow fishing areas of the spiny lobster fishery. On the other hand, the limited availability of shelter in deeper waters suggest that if lobsters are absent from the area where the trap is located, lionfish are more likely to enter it. This would explain the high catch rates of lionfish in deep waters and the negative relationship between catch rates of lobsters and lionfish reported in Chapter 4. Careful management of the lobster fishery could arm

shallow water habitats with an organism that could influence the distribution of lionfish on reef habitat.

While tank experiments like these can help to identify some patterns and preferences of the species being assessed, the limitations of confined mesocosms can prevent the scaling of the results shown here to the reality of the field (Spivak et al., 2010). This experiment provided species with constant food and competition from a single species, to assess the factor of affinity to a structure type alone. Conditions in situ provide a much more complex food web that could impact the decision making of the species (Taylor et al., 2001). Moving forward, additional experiments should be conducted to explore additional factors that make traps attractive to lionfish in deeper water habitats. The use of a combination of mesocosm and field experiments can be used to systematically add abiotic and biotic factors that influence choices made by individuals. Continuing to investigate the behaviors that govern trap selection by lionfish can be helpful in the efforts to design control methods making culling efforts more efficient.
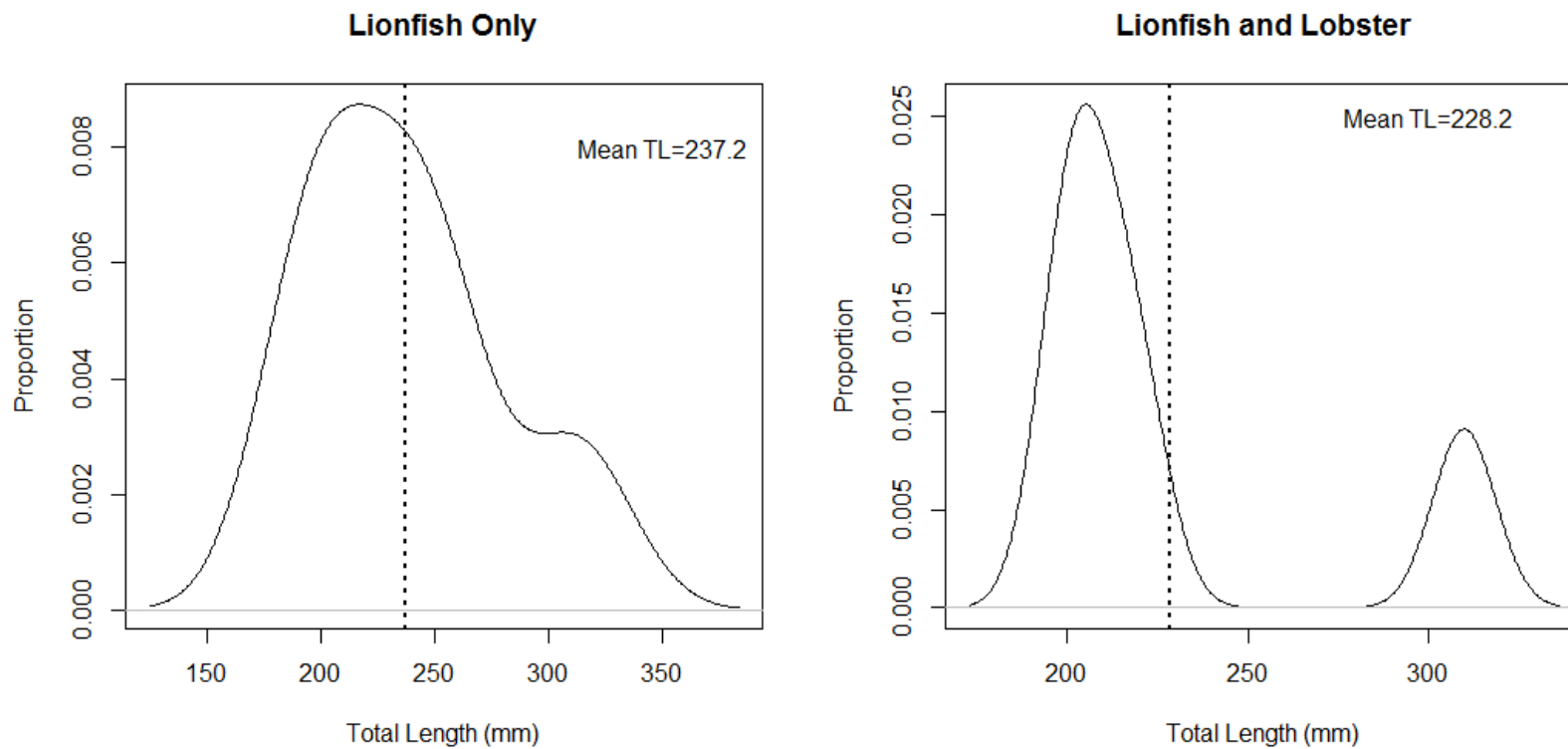
Figure 5.1. Total length distributions for lionfish in the Lionfish Only and Lionfish and Lobster species treatments. T-test shows lionfish total lengths are not significantly different from each other (p=0.7484), with mean total length of 237 mm and 228 mm for the Lionfish Only and Lionfish and Lobster species treatment, respectively.
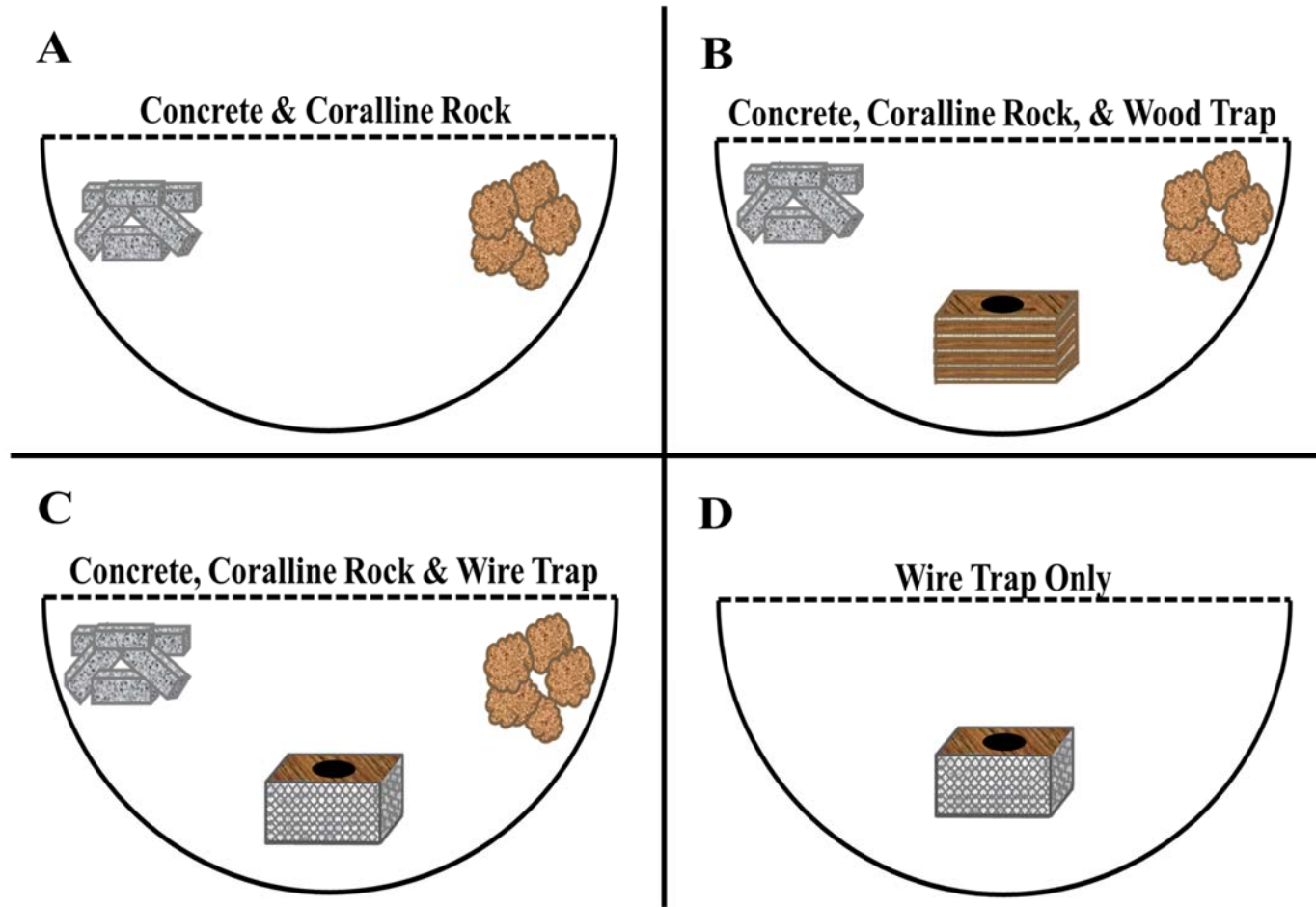
Figure 5.2. The four bottom structure combinations: Coral and Concrete; Coral, Concrete, and Wire Trap; Coral, Concrete, and Wood Trap; and Wire Trap Only. Each was presented to the two species treatments, Lionfish Only and Lionfish and Lobster, on either half of the 4.6 meter diameter circular tank housing animals.
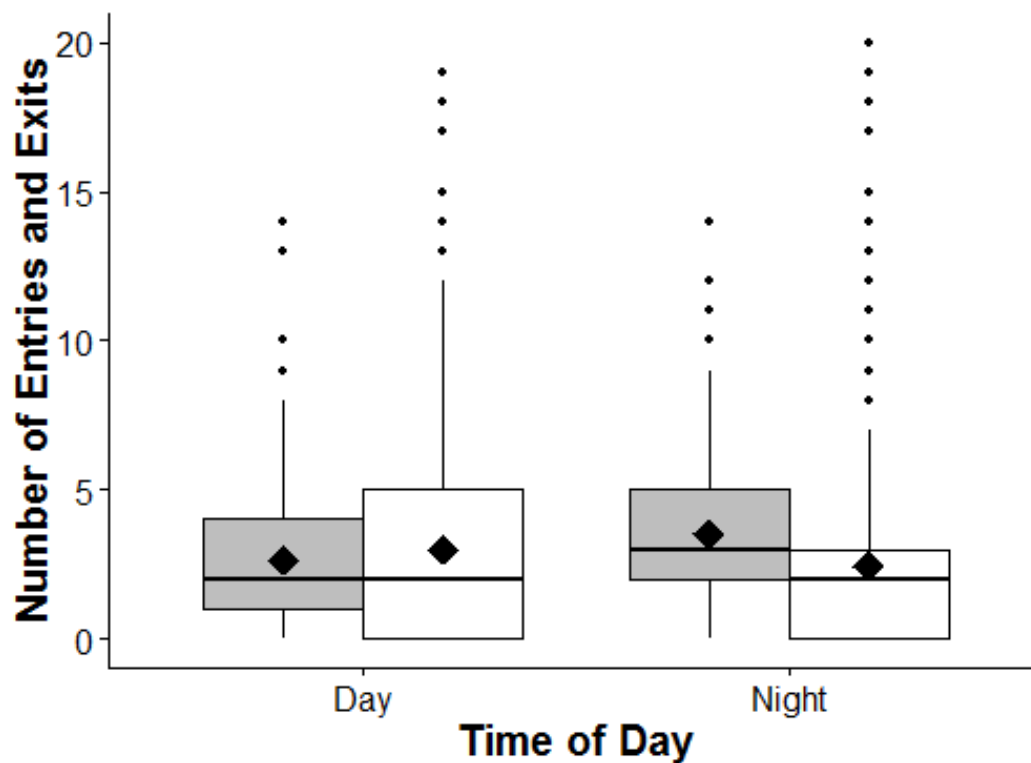
Figure 5.3. A comparison of the activity level of lionfish for the two species treatments: Lionfish and Lobster has gray fill and Lionfish Only has white fill, occurring at different times of day. Day corresponds to the hours between sunrise and sunset, and Night corresponds to the hours between sunset and sunrise. The mean number of exits and entries into the portion of the tank housing each structure treatment is represented with a black diamond. The horizontal line in the box plot represents the median.
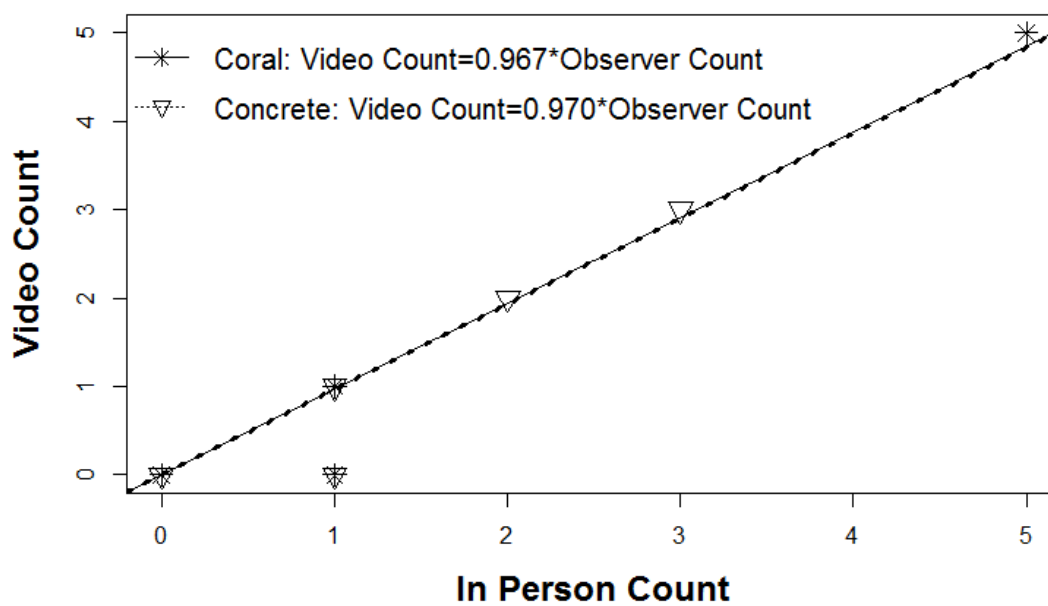
Figure 5.4. A comparison of in person and video lionfish counts to determine the probability of a camera capturing lionfish presence accurately in front of structures with partially obstructed regions (Coral and Concrete). The blue dotted line represents a 1 to 1 relationship between counts or 100% probability of detection.
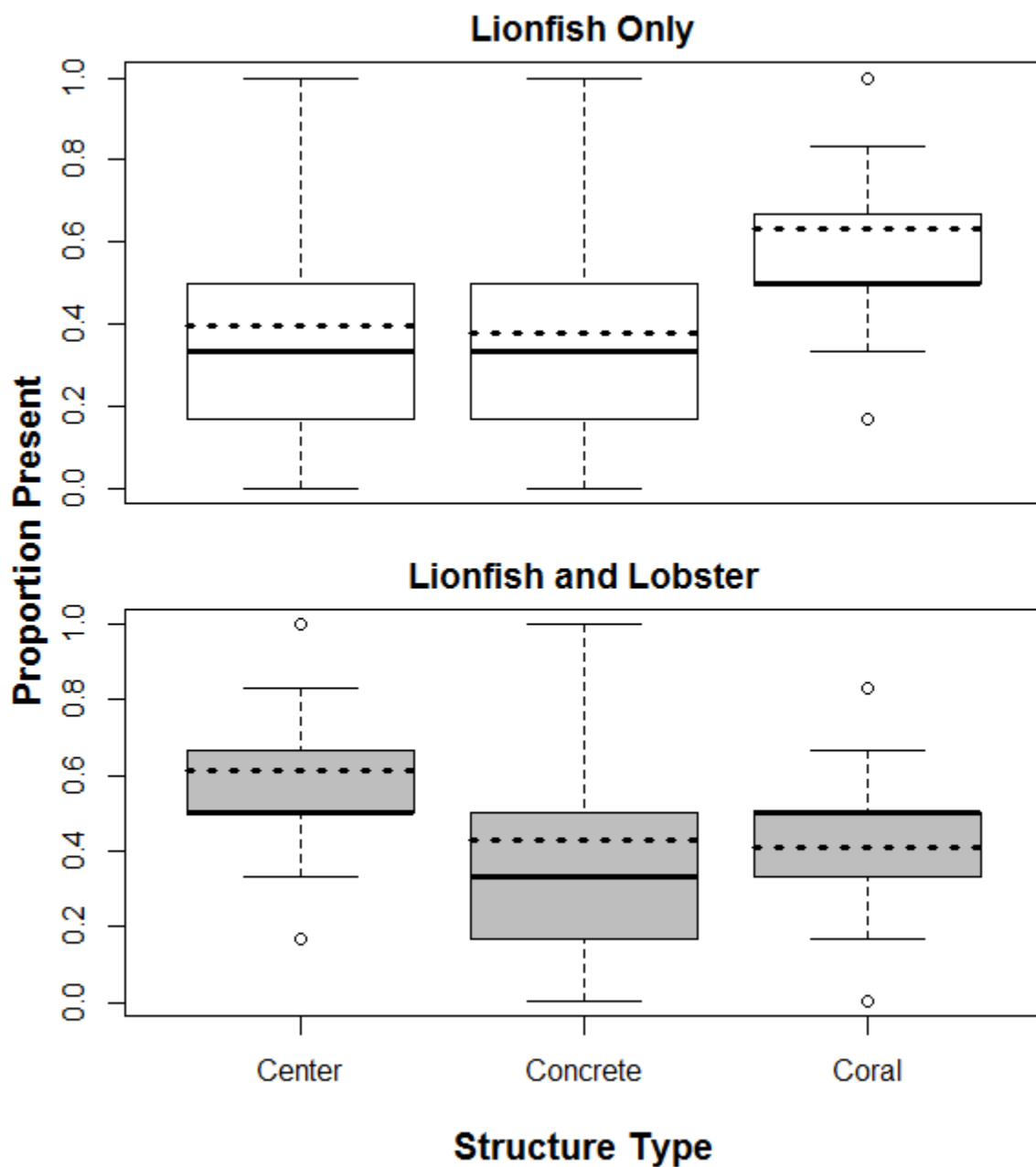
Figure 5.5. Proportion of lionfish present in front of available structures for the Coral and Concrete bottom structure combination for the two species treatments. The predicted mean values from Dirichlet regression are represented with dashed lines around each box.
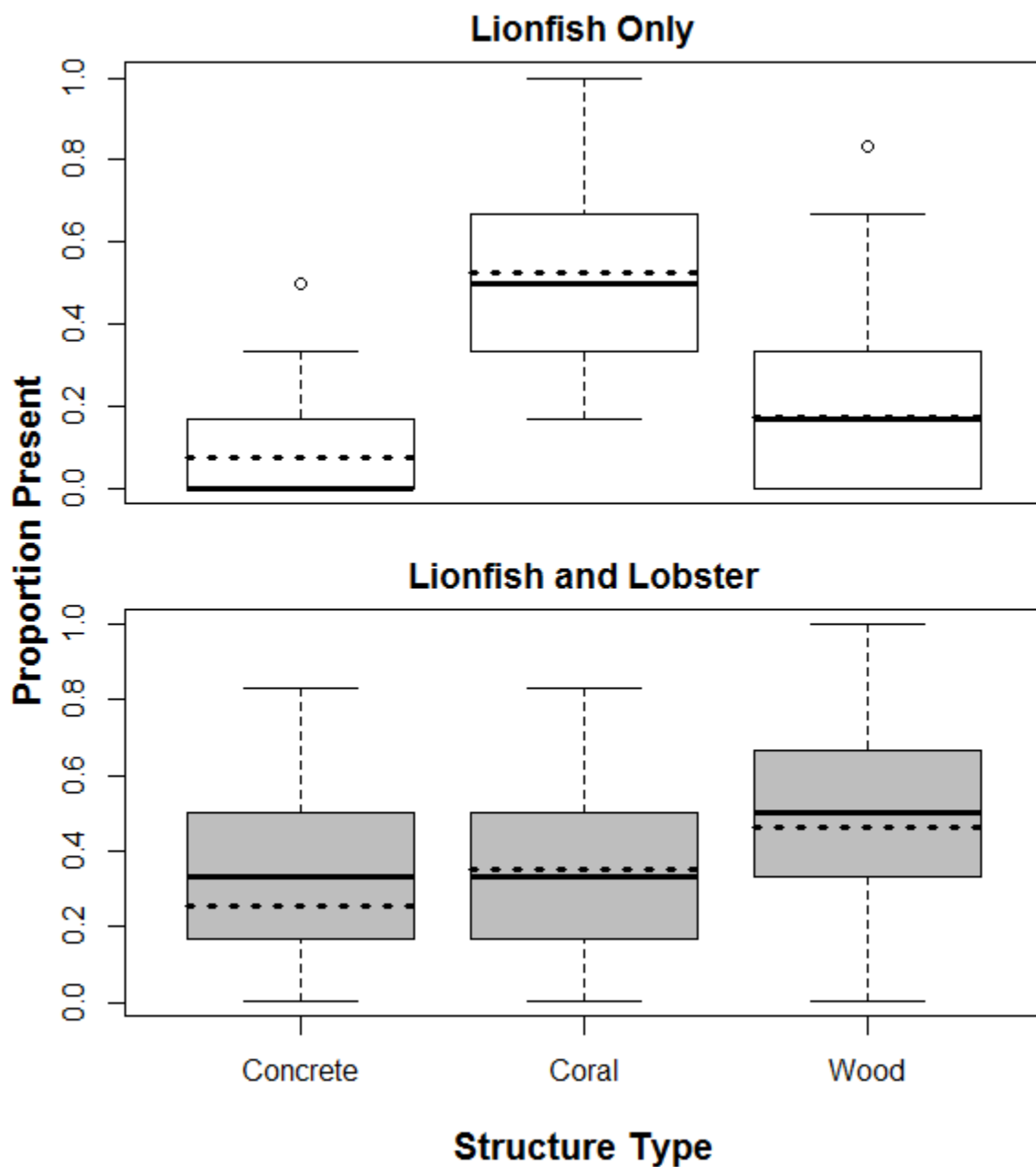
Figure 5.6. Proportion of lionfish present in front of available structures for the Coral, Concrete, and Wood Trap bottom structure combination, for the two species treatments. The predicted mean values from Dirichlet regression are represented with dashed lines around each box.
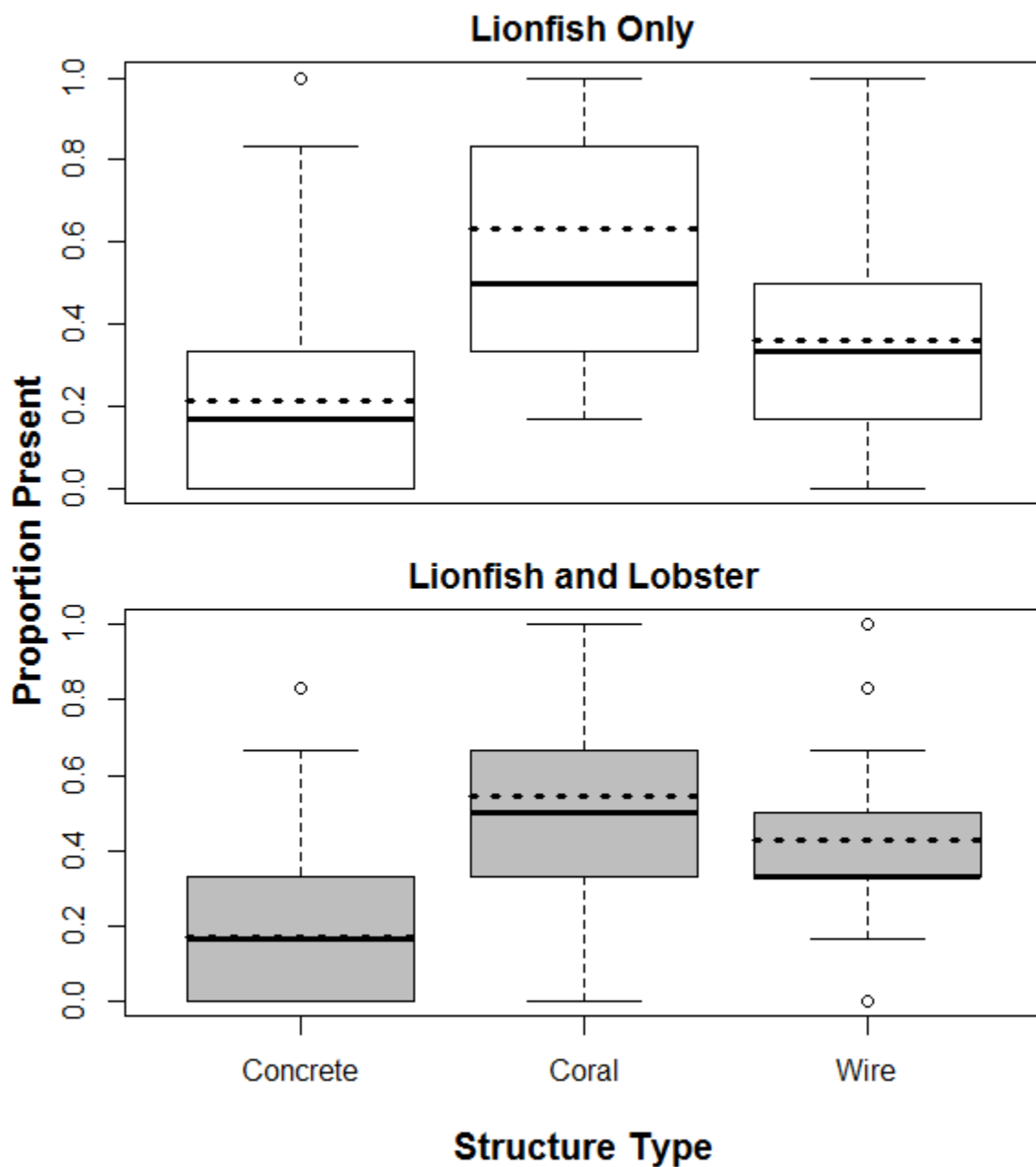
Figure 5.7. Proportion of lionfish present in front of available structures for the Coral, Concrete, and Wire Trap structure combination, for each of the two species treatments. The predicted mean values from Dirichlet regression are represented with dashed lines around each box.
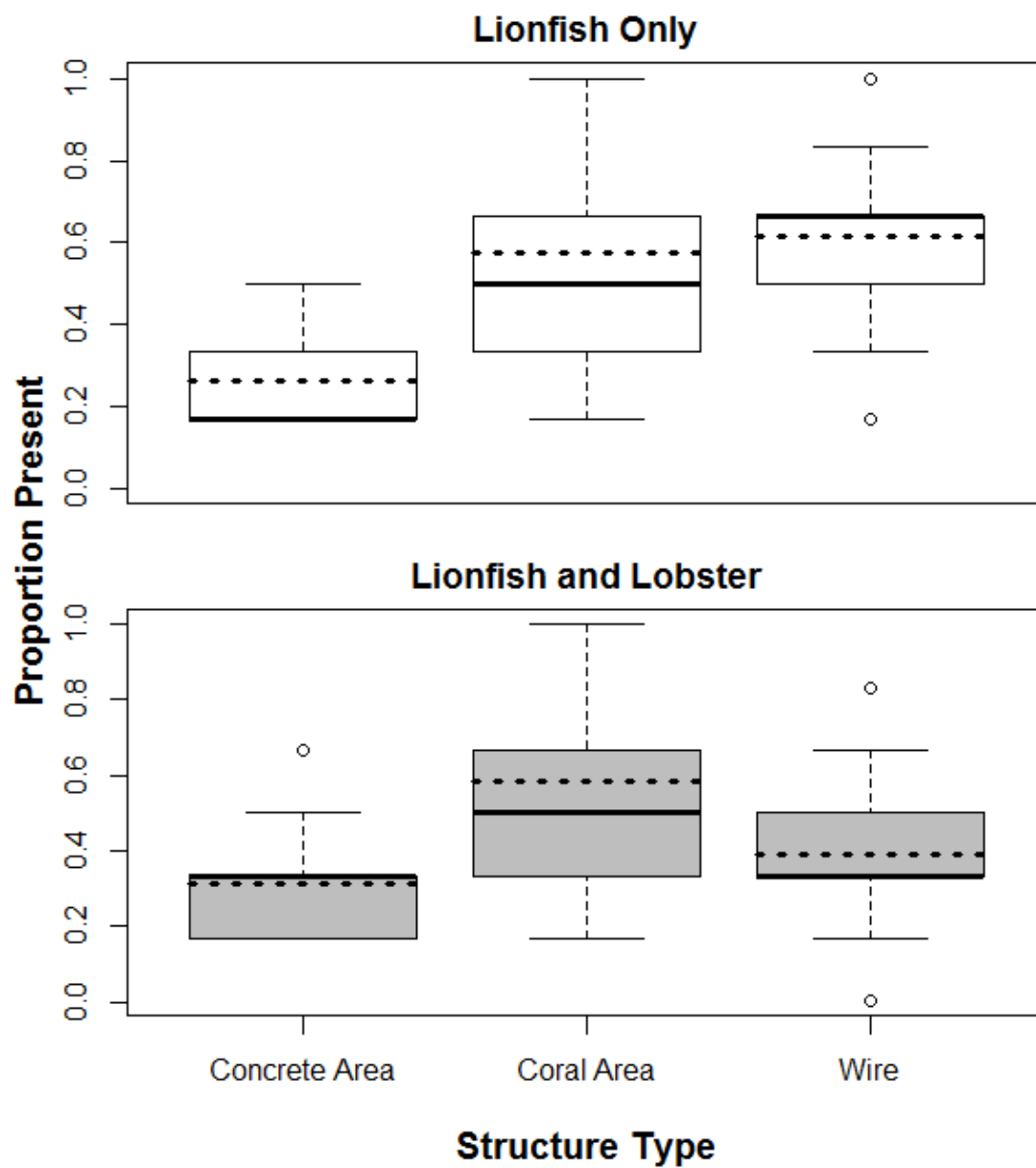
Figure 5.8. Proportion of lionfish present in front of available structures for the Wire Trap Only bottom structure combination, for each of the two species treatments. The predicted mean values from Dirichlet regression are represented with dashed lines around each box.
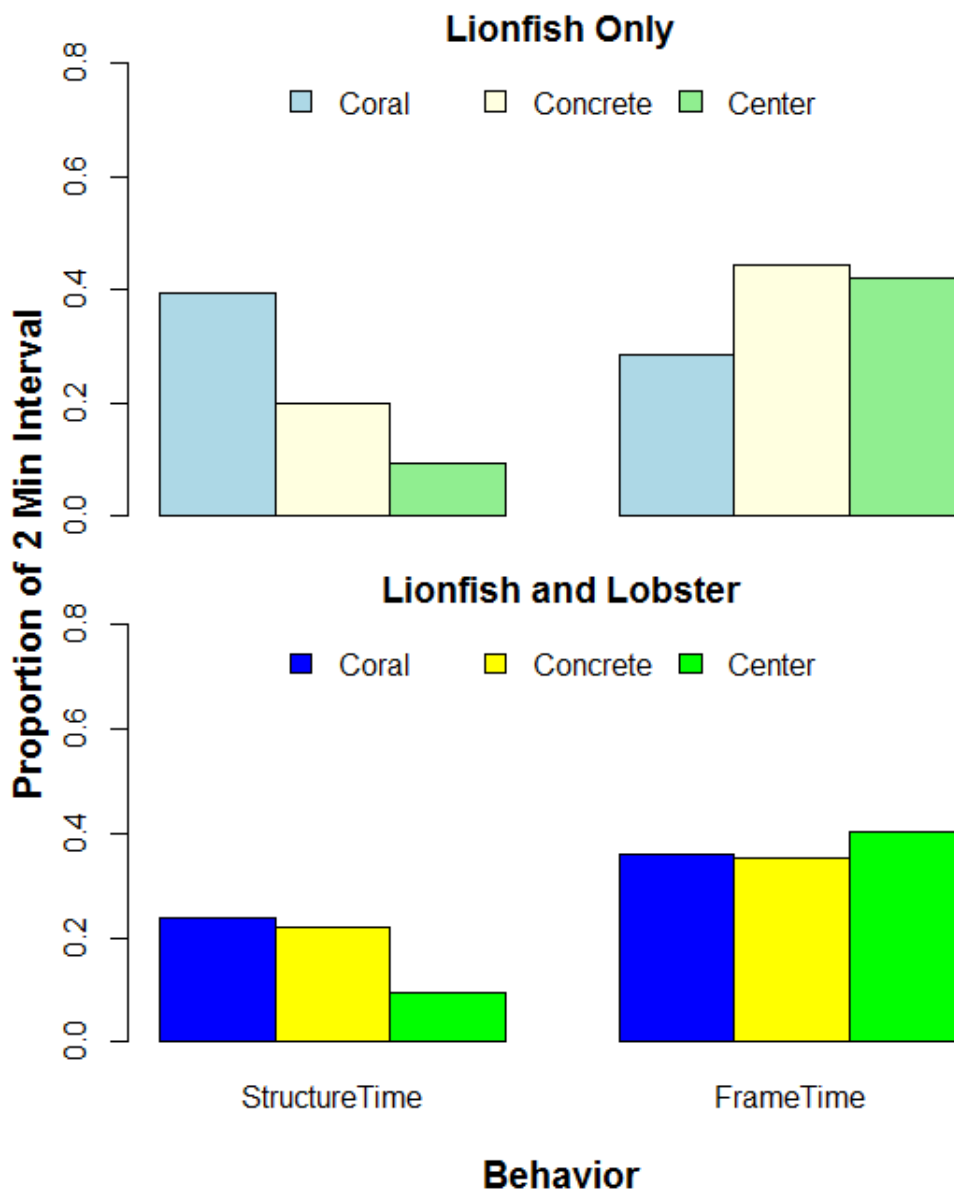
Figure 5.9. Comparison of predicted means for the best fit Dirichlet regression of the time spent by lionfish actively using each structure (Structure Time) and the time spent within the frame of the video camera but not using each structure (Frame Time), for the bottom structure combination including: coralline rock, concrete blocks and a center transition zone. The lighter colored bars correspond with the Lionfish Only species treatment and the darker colored bars correspond with the Lionfish and Lobster species treatment.
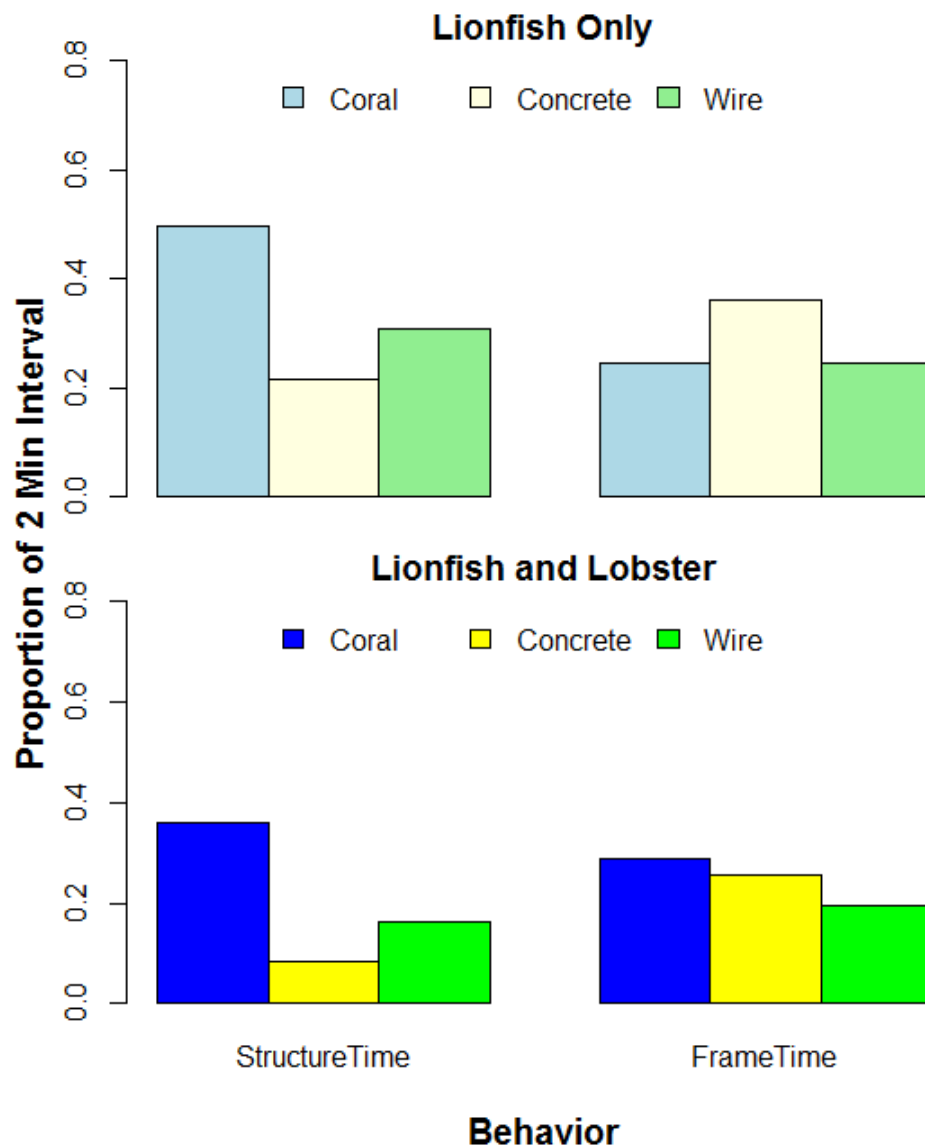
Figure 5.10. Comparison of predicted means for the best fit Dirichlet regression of the time spent by lionfish actively using each structure (Structure Time) and the time spent within the frame of the video camera but not using each structure (Frame Time), for the bottom structure combination including: coralline rock, concrete blocks and wire trap. The lighter colored bars correspond with the Lionfish Only species treatment and the darker colored bars correspond with the Lionfish and Lobster species treatment.
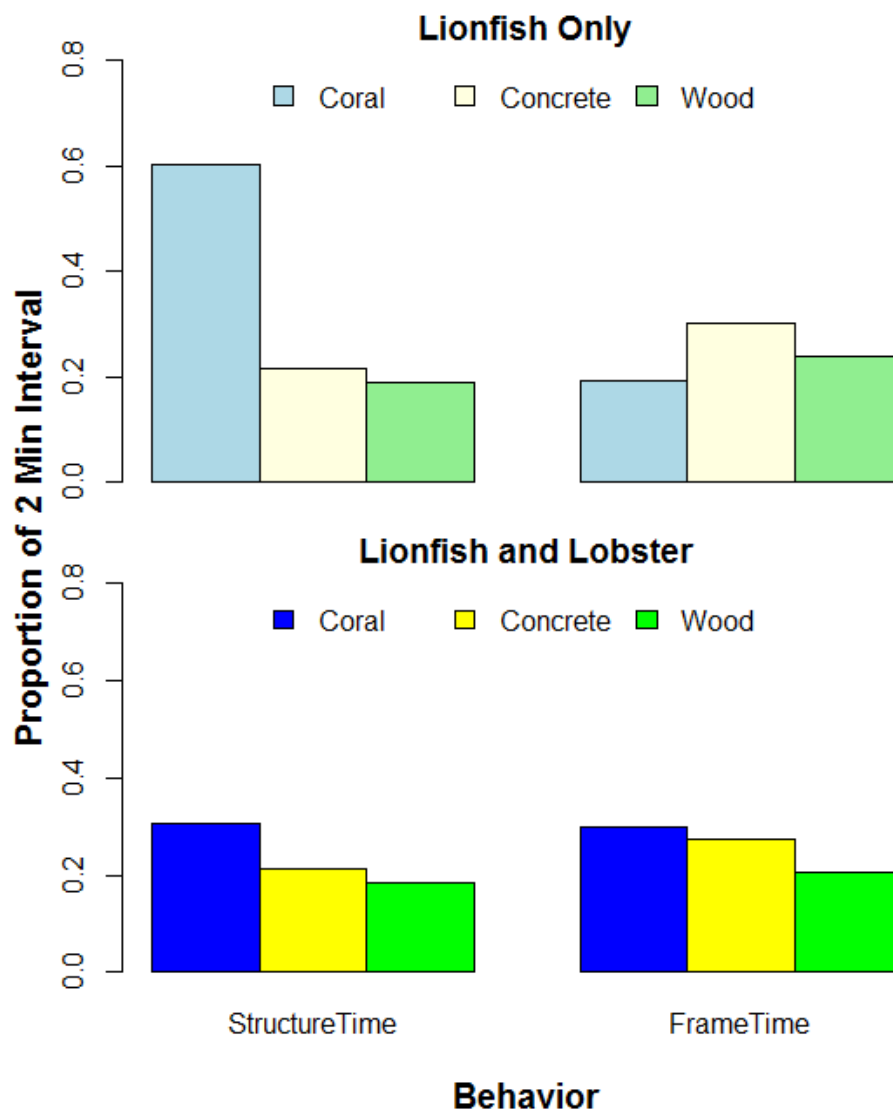
Figure 5.11. Comparison of predicted means for the best fit Dirichlet regression of the time spent by lionfish actively using each structure (Structure Time) and the time spent within the frame of the video camera but not using each structure (Frame Time), for the bottom structure combination including: coralline rock, concrete blocks and wood trap. The lighter colored bars correspond with the Lionfish Only species treatment and the darker colored bars correspond with the Lionfish and Lobster species treatment.
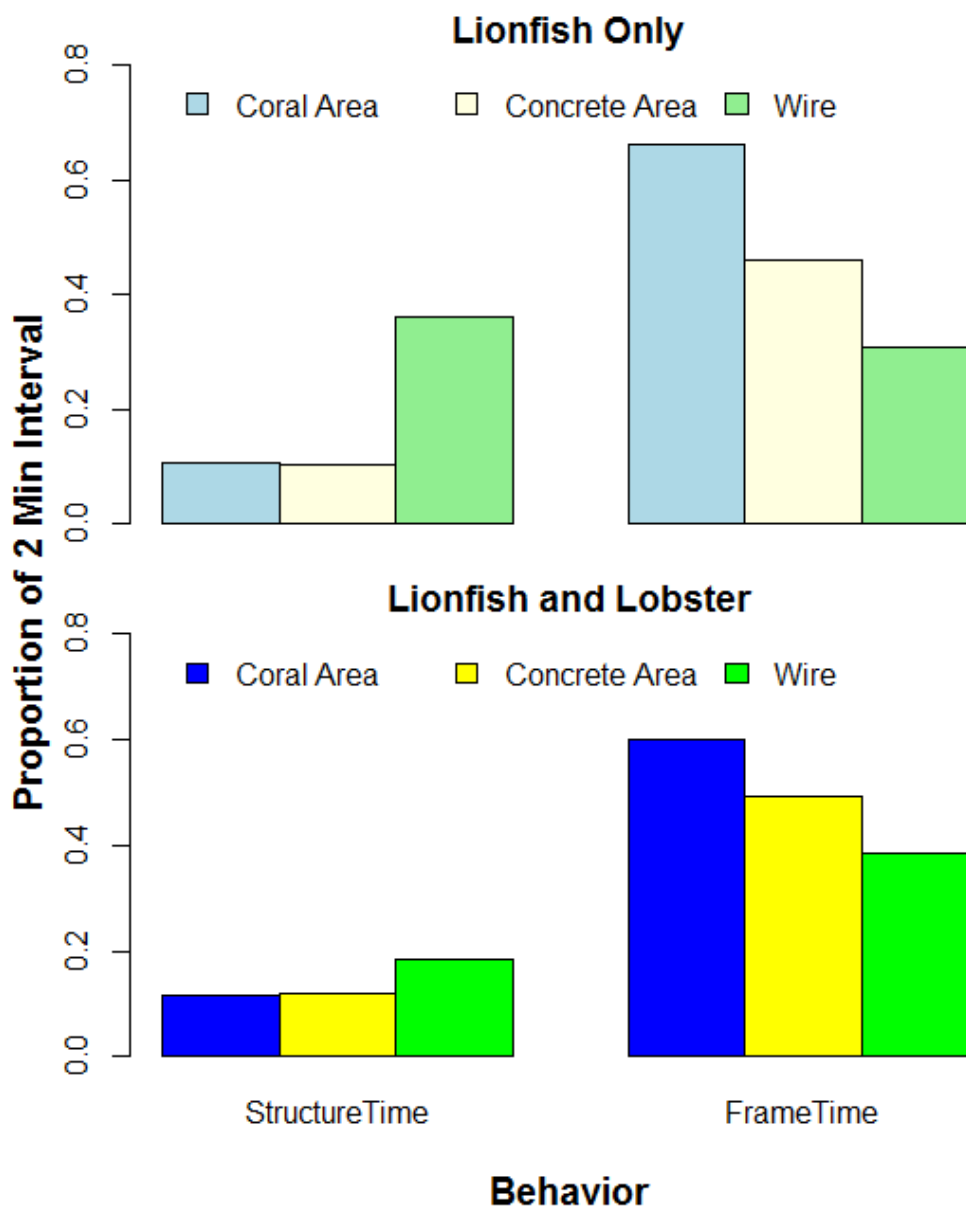
Figure 5.12. Comparison of predicted means for the best fit Dirichlet regression of the time spent by lionfish actively using each structure (Structure Time) and the time spent within the frame of the video camera but not using each structure (Frame Time), for the bottom structure combination including: coralline rock area, concrete block area and wire trap. The lighter colored bars correspond with the Lionfish Only species treatment and the darker colored bars correspond with the Lionfish and Lobster species treatment.

Chapter 6 – Management Implications and Future Work

Summary

The introduction of invasive species has become more prevalent as the world becomes more globally connected and climate change expands the potential habitat range for some species (Bax et al., 2001). Lionfish were introduced to the Western Atlantic, and have rapidly extended their range to include the wider Caribbean Sea and Gulf of Mexico. These species have reduced the abundance of small-bodied and juvenile fishes native to the invaded range (Albins and Hixon, 2008; Morris and Akins, 2009; Green et al., 2011; Albins, 2013).

Reef fish assemblages are in non-equilibrium states that vary as a function of recruitment to the benthic habitat and loss of individuals from predation, competition or fishing pressure (Sale, 2004). Natural controls in the form of disease or predation do not appear to be curbing the growth of the populations, instead lionfish act as an example of the enemy release hypothesis in action. These fishes host a generalist parasite community with relatively low parasitic abundance as compared to other species (Simmons, 2014). Predation by native species has been unable to keep lionfish populations in balance up to this juncture (Hackerott et al., 2014), but increased reports of native predators eating lionfish enhances the likelihood that these species will adapt to routinely prey upon lionfish in the future. Culling methods used by humans have been shown to reduce local populations of lionfish, but these measures alone are not enough. While the ecosystem acclimates to the presence of a new species, there is a need to improve lionfish management plans that will be effective in reducing the abundance and spread of lionfish.

This research aimed to enhance our understanding of the ecology of invading lionfish so that control and monitoring strategies can be made more effective. This was accomplished by 1) studying unknown aspects of the larval life and seasonal settlement patterns; 2) collating this new knowledge with other available data about larval life to parameterize a biophysical model of the invasion; 3) investigating the life history traits that aid in the spread of lionfish; 4) studying lionfish population in deep areas of the reef by collecting data on board a commercial spiny lobster fishing vessel; and 5) testing assumptions about lionfish habitat preferences with laboratory experiments.

Spawning frequency of lionfish

Lionfish females successfully spawn year round in the Bahamas. Previous studies indicated that lionfish had the potential to spawn every 4 days, and females exhibited to gonads representing all stages of egg development after histological analysis. However, this study takes one step further and shows that lionfish not only have the potential to spawn, but also that juvenile lionfish are successfully born and reach settlement habitat throughout the year. This exemplifies the r-selected reproductive strategy common to invasive species across a wide range of taxa (Sakai et al., 2001). The availability of constant larval flow to regions in the Western Atlantic in conjunction with the lack of predation removes barriers of population growth.

This study determined the most accurate reading axis for estimating ages of juvenile lionfish. The narrower axis of lionfish appears to have higher numbers of sub-daily marks that inflated the ages as compared to the broader reading axis. Additionally the study confirmed the length of the larval phase, from 20 to 39 days, and a competency period for larval settlement of almost three weeks. It is important to acknowledge that

the data collected on recruitment is preliminary; future work is needed to calculate

reliable recruitment rates and spawning frequencies. My research indicated future studies

would benefit from larger and more broadly distributed samples of juvenile lionfish to

estimate and quantify seasonal spawning rates throughout the invaded range; attempts to

reduce lionfish population may be more successful if culling efforts can be coordinated as

a function of peak spawning/ recruitment periods

Connectivity of lionfish subpopulations within the invaded range

Connectivity modeing can be used to produce predictions of range expansion

between settlements regions for an invasive species. Life history traits of lionfish,

specifically their vertical distribution and pelagic larval duration are important in

distinguishing the spread potential of these invasive species. This analysis indicated that

dispersal between the Florida and the Bahamas was rare but possible. This modeling

effort focused on recruitment to reef habitat, but lionfish are capable of recruiting to a

wide variety of habitats from nearshore mangroves to deep mesophotic reefs. The next

phase of this modeling effort could expand the habitat polygons used in the model to

investigate the connections between lionfish in various biomes and the greater basin.

Lastly, this type of modelling can be used to test theories of potential spread for new

invasive species, as well as be used to evaluate the theories presented by genetic analysis

of the connectedness of marine populations.

Bycatch of lionfish in the commercial spiny lobster trap fishery

Researchers acknowledge that lionfish are found at great depths, however, the

majority of monitoring and culling practices are conducted in shallow dive-able waters.

The presence of lionfish in the deeper fishing areas of the commercial spiny lobster

fishery of the Florida Keys provides a mechanism for monitoring and studying lionfish populations through fisheries-dependent surveys. Continued cooperation with fishers operating at greater depths can also provide a means for reducing the lionfish population in deeper waters. This serves both as a means of reducing the standing stock of adults and by reducing the flux of lionfish larvae coming from deep locations. The lobster fishery has the potential to remove a greater number of individuals than standard culling methods in shallow waters. More emphasis needs to be put into monitoring and removing lionfish from deeper waters to affect the overall success of reducing lionfish populations in the Western Atlantic Ocean.

Habitat preferences of lionfish

This study showed that lionfish are more commonly caught in deep water lobster traps than in those set in shallow waters. Moreover, lionfish and spiny lobsters were often caught together in traps but there was a negative correlation between the catch rates of lionfish and that of legal size lobsters, which has led fishers to argue that lionfish negatively affect the catch of lobsters.

The lack of lionfish captured in shallow water lobster traps called into question the habitat choices lionfish make when associating with bottom structure. Furthermore, the negative correlation between lobsters and lionfish suggests a possible behavioral interaction between the crustacean and fish. The tank experiment provided lionfish with multiple habitat structure options: coralline habitat, man-made structures (concrete blocks), wooden lobster traps and wire lobster traps. Lionfish will choose to associate with any structure when no other option is available, but have a preference for the coralline habitat when the coralline habitat is an available choice for refuge. This

preference changed when lionfish were in the presence of spiny lobster, a potential

antagonist competitor and possibly a predator for these invasive fish. The negative

relationship between trap fishery catch rates of lionfish and lobsters is more a result of

antagonism of lobsters towards lionfish rather than the opposite. This may be an

important factor as the ecosystem adapts to the lionfish invasion, identifying a species

that may play a role in altering excluding lionfish from coveted reef habitat.

Conclusions and Implications

This study has, for the first, time provided the following evidence:

- Lionfish can successfully recruit to the reef throughout the year, which ensures the Tropical Western Atlantic circulate a constant flux of larvae

- A biophysical model can accurately recreate the geographical range expansion of the invading lionfish in the Caribbean and Gulf of Mexico, demonstrating the ability of lionfish to break the geographical barrier that has created a genetic break between reef fish in Florida and the wider Caribbean basin

- Lionfish occupy deep waters of the Florida reef track, and are a more common bycatch of lobster traps set in deep water than those set in shallow water

- Spiny lobster vessels can be used for the dual purpose of collecting data on relative abundance of lionfish in waters that are beyond the reach of divers and reducing the lionfish population in these understudied regions

- Lionfish catch rates on lobster traps are negatively correlated with the catch rates of legal-size spiny lobsters

- Negative correlation between catch rates of legal-size spiny lobsters and lionfish on spiny lobster traps are a likely result of the antagonistic relationship between these two species. Lobsters, however, are likely to exclude lionfish from traps rather than the other way around, Therefore; the negative correlation does not reflect a negative impact of the presence of lionfish on the catch rate of lobsters

Lionfish have become enmeshed in the coastal ecosystems spanning the southeastern United States, Caribbean Sea, and Gulf of Mexico; the negative impacts from their presence are well documented. Research and monitoring efforts should be focused on improving the efficiency of monitoring and culling measures to try to counteract their effects on the ecosystem. Examining and understanding how lionfish life history traits improve their survivability can aid in designing and strengthening monitoring and management plans. More specifically, attention needs to be paid to populations of lionfish in deeper waters that are likely contributing to the larval flux that sustains lionfish populations. The spiny lobster fishery should be used as a monitoring tool as these traps contribute to the removal of lionfish from deep fishing areas. Additionally, this study shows that maintaining a healthy population of lionfish competitors, like spiny lobsters, may contribute to reduction of abundance of this invasive species. The latter highlights the importance of considering management of fisheries in an ecosystem context, relying on understanding the trophic and habitat relationships between species. Finally, while the lionfish invasion is not likely to be reversed, this study has provided lessons that can help prevent or mitigate new invasions.

Appendix 1

These figures show the autocorrelation estimate between video data points taken 20 minutes apart.

Graphs of autocorrelation for each structure within the Lionfish Only species treatment, when observations are culled from every 15 minutes to every 30 minutes. Each column refers to a different structure combination experiment: Coral and Concrete (CC); Coral, Concrete, and Wire Trap (CCWI); Coral, Concrete, and Wood Trap (CCWO); Wire Trap Only (WIRE).

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Graphs of autocorrelation for each structure within the Lionfish Only species treatment, when observations are culled from every 15 minutes to every 30 minutes. Each column refers to a different structure combination experiment: Coral and Concrete (CC); Coral, Concrete, and Wire Trap (CCWI); Coral, Concrete, and Wood Trap (CCWO); Wire Trap Only (WIRE).

The probability densities of the proportion of lionfish for each structure within the Lionfish Only species treatment. Each row corresponds to a different structure combination: Coral and Concrete (CC); Coral, Concrete, and Wire Trap (CCWI); Coral, Concrete, and Wood Trap (CCWO); Wire Trap Only (WIRE).

The probability densities of the proportion of lionfish for each structure within the Lionfish and Lobster species treatment. Each row corresponds to a different structure combination: Coral and Concrete (CC); Coral, Concrete, and Wire Trap (CCWI); Coral, Concrete, and Wood Trap (CCWO); Wire Trap Only (WIRE).

Appendix 2

This appendix summarizes the model results from the ANOVA and Dirichlet regressions conducted in Chapter 5. Each table corresponds to a different statistical comparision.

Table 5.1. ANOVA table for model comparing the mean activity level of lionfish (Total of Entries and Exits) by species treatment (Lionfish Only vs Lionfish and Lobster) and time of day (Day vs Night).

ANOVA Table: Effects of Species Treatment and Time of Day on Activity Level

Model Factors	df	Sum Sq	Mean Sq	F value	Pr(>F)
Species Treatment	1	236	236.1	30.382	3.84E-08
Time of Day	1	7	7.1	0.916	**0.338**
Interaction Term	1	387	386.6	49.749	2.15E-12
Residuals	3074	23889	7.8		

Table 5.2. A deviance table comparing Dirichlet regression models that compare the proportion of lionfish present for each structure available within each bottom structure combination. The two models compared are the grand mean proportion with the mean proportion of lionfish present for each structure. These models reflect only the data in the Lionfish Only species treatment. *** represents the significance of the calculated statistic, corresponding to a value < 0.001.

Structure Combination	Deviance	No. of Parameters	df	Pr(>Chi)
Coral and Concrete				
Model 1: Prop. Lionfish ~ 1	-7.50	2		
Model 2: Prop. Lionfish ~ Structure	-92.63	6	4	< 2.2e-16 ***
Concrete, Coral and Wire Trap				
Model 1: Prop. Lionfish ~ 1	-394.74	2		
Model 2: Prop. Lionfish ~ Structure	-568.52	6	4	< 2.2e-16 ***
Concrete, Coral and Wood Trap				
Model 1: Prop. Lionfish ~ 1	-629.32	2		
Model 2: Prop. Lionfish ~ Structure	-994.48	6	4	< 2.2e-16 ***
Wire Trap Only				
Model 1: Prop. Lionfish ~ 1	-18.09	2		
Model 2: Prop. Lionfish ~ Structure	-139.20	6	4	< 2.2e-16 ***

Table 5.3. A comparison of Dirichlet regression models that compare the proportion of lionfish present for each structure available within each bottom structure combination. The two models compared are the grand mean proportion with the mean proportion of lionfish present for each structure. These models reflect only the data in the Lionfish and Lobster species treatment. *** represents the significance of the calculated statistic, corresponding to a value < 0.001.

Structure Combination	Deviance	No. of Parameters	df	Pr(>Chi)
Coral and Concrete				
Model 1: Prop. Lionfish ~ 1	-1.42	2		
Model 2: Prop. Lionfish ~ Structure	-84.60	6	4	< 2.2e-16 ***
Concrete, Coral and Wire Trap				
Model 1: Prop. Lionfish ~ 1	-181.43	2		
Model 2: Prop. Lionfish ~ Structure	-397.66	6	4	< 2.2e-16 ***
Concrete, Coral and Wood Trap				
Model 1: Prop. Lionfish ~ 1	-73.59	2		
Model 2: Prop. Lionfish ~ Structure	-112.17	6	4	8.5e-08 ***
Wire Trap Only				
Model 1: Prop. Lionfish ~ 1	-24.40	2		
Model 2: Prop. Lionfish ~ Structure	-113.37	6	4	< 2.2e-16 ***

Table 5.4. A deviance table comparing Dirichlet regression models that compare the proportion of time spent actively using each structure available within each bottom structure combination. The two models compared are the grand mean proportion with the mean proportion of lionfish present for each structure. These models reflect only the data in the Lionfish Only species treatment. *** represents the significance of the calculated statistic, corresponding to a value < 0.001.

Structure Combination	Deviance	No. of Parameters	df	Pr(>Chi)
Coral and Concrete				
Model 1: Prop. of Time ~ 1	-3106.7	3		
Model 2: Prop. of Time ~ Structure	-3292.5	9	6	< 2.2e-16 ***
Concrete, Coral and Wire Trap				
Model 1: Prop. of Time ~ 1	-2500.5	3		
Model 2: Prop. of Time ~ Structure	-2575.6	9	6	3.7e-14 ***
Concrete, Coral and Wood Trap				
Model 1: Prop. of Time ~ 1	-2630.6	3		
Model 2: Prop. of Time ~ Structure	-2774.2	9	6	< 2.2e-16 ***
Wire Trap Only				
Model 1: Prop. of Time ~ 1	-1799.6	3		
Model 2: Prop. of Time ~ Structure	-1922.4	9	6	< 2.2e-16 ***

Table 5.5. A comparison of Dirichlet regression models that compare the proportion of time spent actively using each structure available within each bottom structure combination. The two models compared are the grand mean proportion with the mean proportion of lionfish present for each structure. These models reflect only the data in the Lionfish and Lobster species treatment. *** represents the significance of the calculated statistic, corresponding to a value < 0.001 and ** corresponds to a value <0.01.

Structure Combination	Deviance	No. of Parameters	df	Pr(>Chi)
Coral and Concrete				
Model 1: Prop. of Time ~ 1	-2798.1	3		
Model 2: Prop. of Time ~ Structure	-2873.3	9	6	3.4e-14 ***
Concrete, Coral and Wire Trap				
Model 1: Prop. of Time ~ 1	-3409.9	3		
Model 2: Prop. of Time ~ Structure	-3586.0	9	6	< 2.2e-16 ***
Concrete, Coral and Wood Trap				
Model 1: Prop. of Time ~ 1	-899.35	3		
Model 2: Prop. of Time ~ Structure	-918.39	9	6	0.004094 **
Wire Trap Only				
Model 1: Prop. of Time ~ 1	-1692.0	3		
Model 2: Prop. of Time ~ Structure	-1724.5	9	6	1.27e-05 ***

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