PARASITISM AND FATTY LIVER DISEASE IN THE INVASIVE RED LIONFISH,

PTEROIS VOLITANS (LINNAEUS) ALONG THE GULF OF MEXICO

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by

Danielle M. Fails

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DEDICATION

This thesis is dedicated to my daughter, Abigail Esme Fails, who has been with me (nearly) every step of the way and who has pushed me to be the very best version of myself. You are a ray of sunshine in the thunderstorm that is life, and both your screams and your smiles have given greater purpose to this project. Although you arrived in the middle of this endeavor, you made finishing it so much more enjoyable. I hope that this serves as a way to inspire you in the future, and as an example of what you can achieve with a little bit of hard work and a lot of coffee.

In fact, I would also like to partially dedicate this work to all of the young minds out there—choose science. Specifically, choose parasites—they rule the world.

ABSTRACT

Fails, Danielle M., *Parasitism and fatty liver disease in the invasive red lionfish, Pterois volitans (Linnaeus) along the Gulf of Mexico*. Master of Science (Biology), December, 2017, Sam Houston State University, Huntsville, Texas.

Invasive species are detrimental to both the economy as well as to environmental stability. One of the most successful to date is the red lionfish, *Pterois volitans*, which first invaded the western hemisphere around 30 years ago. Lionfish have decimated native fish populations at roughly 7,500 lbs. per acre per year, have no natural predators, and seem fairly resistant to parasitism. Few species (<50) of parasites have been found in lionfish. Not only is parasitic prevalence low in lionfish, but they also seem to exhibit resistance to the effects of fatty liver disease. This research provides an updated parasite survey and reports six species of parasites, three of which are parasite species reported for the first time in lionfish; (1) one Cymothoid isopod: *Olencira praegustator*, (2) one Corallanidae isopod: *Excorallana truncata,* and (3) an acanthocephalan: *Serrasentis sagittifer*. Overall parasite prevalence and intensity was low for all hosts, and were significantly higher in males. A baseline study of fatty liver analysis in lionfish revealed that $>85\%$ of examined fish displayed evidence of fatty liver disease, and most exhibited moderate degrees of disease. Sex, location, and standard length of lionfish did not play a significant role in degree of disease, though slight disease differences were observed among locations. Outward condition (i.e. skin/scale integrity, coloration, observed mass) of specimens observed in relation to fatty liver disease and parasitism seemed relatively unaffected.

KEY WORDS: Lionfish, Parasites, Fatty liver disease, Predators, Resistance, Invasive species, Gulf of Mexico, Caribbean, Histology

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CHAPTER I

Introduction

Studies of invasive species have become increasingly important as they are often

detrimental to an ecosystem and its native species. It has been estimated that 50,000

invasive species have been introduced to North America over a period of 500 years, and

estimated costs associated with their removal average 140 billion dollars/year (Westbrook

and Brabson, 2008). Not only do invasive species bring alarming costs to the economy

and devastate native ecosystems, they also have the potential to expose native, naïve

species to exotic parasites (Albins et al., 2013).

The success of these invasive species is often a result of a general lack of

predation and reduced ecological or environmental constraints that shape the range,

density, and niche of native species (Torchin et al., 2005). Rather than intrinsic factors

(inherent ability to be a successful invader), success often relies on extrinsic factors:

release from ecological and genetic influences such as hybridization, natural enemies, or

other such interactions (Colautti et al., 2014). The combination of low predation, prey

naiveté, and optimal prey availability in ideal environmental conditions can serve to

heighten the fitness of the invader (Torchin et al., 2005; Sax et al., 2007). This often

results in their ability to outcompete trophically similar native species (Simmons 2014).

Lionfish (*Pterois volitans*) are exotic marine fish native to Indonesia, Australia,

French Polynesia, Japan, and the western Pacific Ocean (Figure 1). Although

morphologically and molecularly similar, the red lionfish (*Pterois volitans*) and the devil

firefish (*Pterois miles*) are both found in North America (Western Atlantic and Caribbean

Sea). However, *Pterois volitans* is far more prevalent in the invaded range, and roughly

93% of lionfish found are *P. volitans* (Hamner et al., 2007). This research targets *P. volitans* because of its rapid expansion, and the term "lionfish" will herein be used to describe this species (Lasso-Alcalá and Posada, 2010).
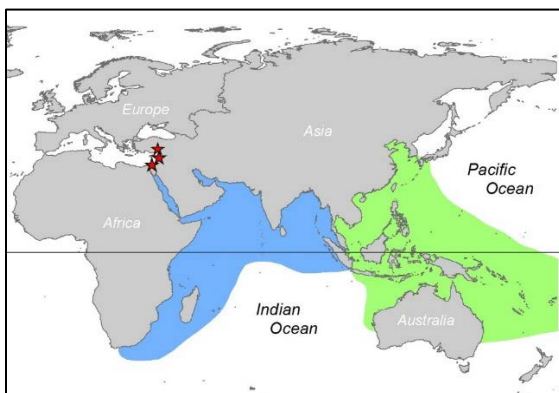
Figure 1. Map of the native range of lionfish. *Pterois volitans* is represented in green, *P. miles* is in blue, and the red stars indicate the migration of *P. miles* via the Suez Canal. Reprinted with permission from the U.S. Geological Survey (2014).

In their native habitat, lionfish occur most often near coral reef systems, are fairly tolerant to fluctuating water temperatures, and can withstand temperatures as low as 10˚C (Ruiz-Carus et al., 2006). Lionfish produce very large gelatin-like egg masses that facilitate dispersal and they reach sexual maturity in less than a year. Furthermore, lionfish possess the ability to reproduce every 4 days, year round. This results in an average annual fecundity of 2 million or more eggs (Butterfield et al., 2015).

The Invasion

The exact method of the introduction of lionfish was a matter of debate for some time, though scientists now attribute the invasion to accidental or incidental release of aquaria lionfish into the ecosystem (Betancur et al., 2011; Courtenay 1995; Morris et al., 2009; Ruiz-Carus et al., 2006). Molecular studies of lionfish populations suggest the present population to be a result of either a "single release event" or a "multiple release"

scenario of limited numbers of individuals; in either case, the small number of introduced individuals has resulted in relatively low genetic diversity within subsets of lionfish populations in the invaded range (Hamner et al., 2007; Betancur et al., 2011).

During the initial introduction, lionfish populations were largely restricted to areas of southern Florida and the eastern coast of the United States (Schofield 2010). Lionfish are now found in the following locations: the Atlantic coast of mainland United States, the Gulf of Mexico, Bermuda, the Bahamas, Cuba, Turks and Caicos, the Cayman Islands, Jamaica, Puerto Rico, the Dominican Republic, Costa Rica, Columbia, Venezuela, Brazil, and Central America (USGS-NAS 2017, Figure 2).

Figure 2. Map of the current distribution of lionfish from Pamela Schofield, PhD and Amy Benson, MS of the USGS (2017). Reprinted with permission from the U.S. Geological Survey.

Although a few sightings have been recorded off the coast of Mexico, it remains a largely unpopulated area where lionfish are concerned. The rapid expansion of lionfish is attributed to several factors, including their ability to reproduce year-round, broad

thermal tolerance, the ability to survive extensive periods of time with limited food intake, and the absence of natural predators (Johnston and Purkis, 2011).

Scientists hypothesize that currents are the primary dispersal mechanism of lionfish (Schofield 2010; Johnston and Purkis, 2015; Kimball et al., 2004). The first confirmed record of a lionfish sighting along the Gulf of Mexico was in 1985, in Dania, Florida. Conversely, the Florida Keys did not report any sightings of lionfish until 2009, though this doesn't necessarily suggest the absence of lionfish, as there may have been a lack of reports (Kimball et al., 2004). Research suggests, however, that due to intolerance to colder climates, proliferation of lionfish found north of North Carolina is dramatically reduced. Furthermore, as there are no singular-direction currents leading outward of the Gulf of Mexico, lionfish have no distribution barriers (Johnston and Purkis, 2015).

Lionfish removal efforts have included derbies at recreational diver and fisheries tournaments, citizen removals, and the development of an economic market for lionfish consumption. However, removal efforts appear to only reduce local populations (Barbour et al., 2011) and with the existence of lionfish populations at depths exceeding diver limitations, only a small percentage of total lionfish populations are being taken. Oftentimes, this is a subset of the population containing mature males and females, and continual removal efforts like this will only serve to continually increase lionfish numbers (Ramos-Ascherl et al., 2015). The effectiveness of removal efforts to date has been negligible, and sterilization approaches have not yet been considered.

Parasitism

Parasitism is a form of symbiosis in which one partner extracts some resource from another, thereby causing it some degree of harm. As a life-history strategy, parasitism is highly successful and has been adopted across all taxonomic groups. There are two main types of parasites: endoparasites (live/feed within host) and ectoparasites (live/feed on the outside surface of host), both of which can be either microparasites or macroparasites. The distinctive life cycles of parasites, often requiring diverse intermediate and determinate hosts, allow for interaction with hosts at multiple trophic levels (Simmons 2014). The ontogenetic shift in diet in lionfish (Villaseñor-Derbez and Herrera-Pérez, 2014) presents an opportunity for lionfish to acquire parasites from known arthropod intermediate hosts. Many processes can shape the abundance and distribution of parasite species, including dispersal, competition, and predation. Conversely, distribution and abundance can also be affected by factors due to interactions with their host. For instance, in many cases, parasite intensity varies significantly over time as a result of the effects that long-term or seasonal climatic changes place on their intermediate and definitive hosts (Poulin 2007).

The speed of lionfish establishment in the invaded range has been attributed to several explanations, including the enemy release hypothesis (Simmons 2014; Torchin et al., 2002). This hypothesis proposes that as invasive species arrive in a new location depauperate of natural enemies (i.e. sharks, cornetfish), an increase in population of the invasive species is expected due to prey naiveté (Colautti et al., 2004). However, parasites are often overlooked despite the detrimental effects they can cause to host populations.

Non-native species experience competitive advantages over native species due to a lack of parasitism, known as parasitic release: a phenomenon that many attribute the overall success of lionfish in the invaded range. Studies focusing on comparisons of parasite infections of invasive species in both native and invaded ranges have noted that invaders have fewer parasites. Individuals in the invaded range are also known to be less frequently infected (percent individuals infected subtracted from prevalence) compared to their native counterparts (Torchin and Mitchell, 2004; Loerch et al., 2015). These same studies suggest that "native" parasites are being left behind and parasites of the non-native range are slow to infect them. Host susceptibility, time and mode of introduction, and distance from the native range all play a role in the decrease of parasite diversity and abundance in invasive species (Blakeslee et al., 2009). Advantages of parasite release are short-lived, however, as native parasites eventually adapt to infect the invaders (Gendron et al., 2012).

Based on the molecular studies providing evidence that small numbers of individuals were initially released, the probability of these fish introducing native parasites along with their host is subsequently reduced (Simmons 2014). Furthermore, as these fish left the aquarium trade, despite temporal differences, they are frequently treated with antiparasitics (USDA-APHIS 2017). As a result, it is unlikely that native parasites are transferred with the additional required hosts necessary for the completion of parasitic life cycles (Torchin et al., 2003). Even in cases where invasive parasites are introduced into a new range, establishment does not occur unless there is a suitable host(s) to complete the life cycle (Torchin and Mitchell, 2004).

Parasites can influence the invasion process in three ways. First, introduced species can escape parasites that typically infect them in their native range, and infection by novel parasites do not make up the difference (Torchin et al., 2003). As a result, an increase in density, biomass, or overall body size in introduced populations is often seen. Furthermore, a reduction in parasites will serve to increase the invader's ecological impacts (Torchin et al., 2002). Second, parasites that are also transferred with non-native species may pose a threat to native species; if infected hosts invade a new ecosystem, the invasive parasites may then switch hosts and infect similar (ecologically, phylogenetically) native species with potentially devastating consequences (Hayward et al., 2001). Lastly, native parasites themselves can be locally extirpated as a result of an invasion, with the potential of cascading effects in subsequent trophic interactions in the native community (Torchin et al., 2005).

Lionfish are native to the waters of the Pacific and Indian Oceans, extending from the coast of Japan to Madagascar. The invaded range includes the entire eastern coast of the United States (Atlantic Ocean), the length of the Gulf of Mexico, and most of the northern coast of South America (inclusive of the Caribbean Sea). Parasite species richness of the invasive red lionfish in the invaded range differs greatly from its native range. To date, only one ectoparasitic species of *P. volitans* (*Trachelobdella lubrica*; Appendix A) overlaps between the two ranges, though several documented species are known to have worldwide distributions. Furthermore, seven of the eleven parasite species recorded from the native range are specialist parasites, having only been documented in *P. volitans* and other scorpaenids (Hassanine 2006; Dojiri and Ho, 1988; Diamant et al., 2004; Bullard et al., 2011). Many of the parasite species of the native range have mild to

severe pathological effects on the host, including blood loss, destruction of intestinal villi, mild fin rot, destruction of respiratory tissue, and stunted reproductive and growth processes (Dojiri and Ho, 1988; Paperna 1976). Previously documented species of parasites found in lionfish in their native and invaded ranges, respectively, are detailed in Appendix A.

Previous studies of lionfish parasitism in their native range is lacking, and parasite faunal description is largely limited to their invaded range (Diamant et al., 2004; Hassanine 2006; Ruiz-Carus et al., 2006). The earliest description of ectoparasites in native lionfish occurred in marine fish surveys in the Indo-Pacific, where copepods of *Acanthochondria* sp. (Leigh-Sharpe and Oakley, 1972), were found parasitizing gill structures (Dojiri and Ho, 1988). *Trachelobdella lubrica* (Grube 1840), a leech, was first described in lionfish in Japan (Paperna 1976); consequently, this was the first ectoparasite described in lionfish in the invaded range (Ruiz-Carus et al., 2006 renamed by Bullard et al., 2011). Several studies have compared parasitism of introduced species in their native and introduced ranges (Torchin and Mitchell, 2004; Blakeslee et al., 2009), though studies of this type are lacking in lionfish. The few available suggest that overall parasite diversity and abundance are low in invasive lionfish, and have subsequently resulted as a product of high prey availability and ideal environmental conditions (e.g., temperature, salinity) in the expanded range (Simmons 2014; Côté et al., 2013). Trophic ecology and feeding habits of lionfish in their native range are scarce, perhaps a result of stable, non-problematic native populations (Diamant et al, 2004).

Few conclusions have been made on the difference between parasite fauna of the invaded and native range, considering that most species documented in the red lionfish in

the invaded range have nearly worldwide distributions (i.e. *Acanthocondria sp., Anisakis sp., Tentacularia sp.*, etc.) while most documented in the native range are restricted to that geographic area (*Proneohelicometra aegyptensis, Taeniacanthus miles*). The overall prey diversity between the two ranges does differ, and many of the parasitic species previously documented have indirect life cycles, involving more than one intermediate host. However, overall prevalence of parasitism of lionfish in the invaded range has been consistently low (Simmons 2014; Fogg et al., 2016; Ramos-Ascherl et al., 2015). Although the red lionfish has a voracious appetite and consumes a variety of crustaceans, fish, and marine invertebrates, parasitic infection rates remain lower than expected compared to other areas where parasite species typically have higher infection rates (i.e.. cestodes, acanthocephalans) (Ramos-Ascherl et al., 2015). Most have hypothesized that the lower infection rates are due to the recent presence of lionfish in the invaded range, as lionfish have not had a very lengthy establishment, while others believe lionfish, as a species, possess a highly resistant immune system (Côté et al., 2013).

Adaptive immune responses have been known to aid in the control of parasitic infections, and could be the driving force behind the low intensity numbers previously documented. Despite immune response, environmental factors (overpopulation, stress, etc.) can also influence a host's susceptibility to parasitism and although small, there has been a slight increase in numbers of documented species of parasites in lionfish of the invaded range. Most baseline surveys documented one to two species, while surveys in recent years (2013-present) have recorded 13-27 species (Ramos-Ascherl et al., 2015; Fogg et al., 2016; Simmons 2014). It is also possible that overpopulation could be a driving mechanism to this small increase in parasite diversity, although intensity has

remained low (Morund and Poulin, 1998). A possible explanation of this situation could be the dilution effect hypothesis: in ecological situations where biodiversity is high, overall risk of infection by opportunistic, generalist parasites is diluted (Ostfeld and Keesing, 2000). Some believe that parasitism could act as a population control on the lionfish invasion, though no studies have been documented at length regarding the fitness of parasitized lionfish.

Fatty Liver Disease

Lionfish are very efficient hunters and decrease native fish recruitment by over 75% on coral reefs of the Bahamas (Arias-González et al., 2011). Lionfish are gluttonous consumers, and can expand their stomachs up to 30 times its original size to accommodate food influx. In times where food becomes scarce, the metabolic rate of a lionfish will slow. Lionfish can maintain this for up to three months, and only lose 10% of their overall body mass (Morris and Akins, 2009). Interestingly, lionfish in the invaded range have biomass estimates up to 13 times greater than those in their native range (Luiz et al., 2013). Recent studies have even shown lionfish in the invaded range to be cannibalizing each other, though the driving force behind this is undetermined (Dahl et al., 2017). Moreover, in order to obtain the obesity these fish are displaying, the intake of food must reach at least 7.5 times their normal dietary intake. This fact alone means that lionfish have the potential to consume over 7,000 pounds of prey per acre per year, and several of the prey items found in the stomachs of lionfish are endangered or threatened species (Wilcox 2013).

Such high consumption rates can cause liver damage in these fish in the form of non-alcoholic fatty liver disease (NAFLD), which is commonly associated with

aquarium-kept fishes (Meyer et al., 1983). In both aquarium and farmed fish, fatty liver disease (liver degeneration) commonly manifests as a result of a diet too high in fats (Leatherland and Woo, 2010). Other documented causes have been lysine deficiency leading to higher lipid accretion rates (Lie 2008) and genetic predisposition (Zhenyu 2014). Because fish do not possess the ability to assimilate fats well (Meyer et al., 1983), it accumulates in their tissues and organs in the form of adipocytes—most often in the liver, especially in sedentary species such as lionfish (Hemdal 2015). However, unlike many other species (mammals in particular), in times of starvation, fish cannot readily access this stored fat. Once stored in other tissues, the fat deposits are rarely if ever reconverted to usable energy sources (Hemdal 2015; Lall 2000). In fish with sedentary lifestyles, outward signs of obesity are difficult to detect, and thus fatty liver disease is usually only diagnosed post mortem. Upon necropsy, the liver in these cases contains oil droplets that are usually yellow in color, and the damaged tissue will likely float in water due to oil saturation. This disease is correlated with age, as younger fish have higher growth rates and avoid the deposition of unused fats, as excess caloric intake is often used to build muscle (Hemdal 2015). This has been understudied (Braunbeck et al., 1990; Kuwashiro et al., 2011; Asaoka et al., 2013) and is an important aspect of lionfish biology. A histological study of nonalcoholic fatty liver disease in lionfish of the invaded range could provide baseline knowledge of the overall presence/absence of disease, the extent of the damage, and whether sex and/or age influences the degree of the disease.

Lionfish diet also shifts with age. Juvenile lionfish feed almost exclusively on crustaceans, whereas adults forage on a diversity of reef-associated fish (Villaseñor-Derbez and Herrera-Pérez, 2014). As lionfish growth rates are density-dependent

(Benkwitt 2013), lionfish with delayed maturation (i.e. high density) will place considerable pressure on crustacean populations. Furthermore, lionfish densities in the invasive range (400 ind. ha^{-1}) are significantly higher than those in the native range (28.8 ind. ha^{-1}) (Kulbicki et al., 2012). This has extreme implications for the fisheries industry in that lionfish are decimating economically important species, and studies indicate that lionfish populations will continue to grow exponentially, until stabilized by the lack of resources (Benkwitt 2013).

Hypotheses

If the introduction of lionfish into North America was through the aquarium trade, it is unlikely that this invader brought with it its own parasites, as the aquaria trade is not capable of supporting most parasitic life cycles (no intermediate hosts) and fish are routinely treated with a suite of antiparasitics (USDA-APHIS 2017).

(1) Parasite richness and abundance of lionfish from the Gulf of Mexico will be comparatively less than specimens collected in the Caribbean.

Most studies conducted on lionfish parasitism have focused on the Caribbean, Atlantic Coast of the United States, and the Florida Keys. Studies in these localities document parasite richness and abundance values between 10-25% (Sellers et al., 2015). Though unclear, it is thought that these localities possess higher biodiversity in terms of ecological structure and therefore may be providing lionfish with more sources of infection. It is also thought that, as lionfish populations have been established at these localities for over two decades, time of establishment is allowing for greater parasite richness and abundance as compared to the Gulf of Mexico, where lionfish have been established for less than ten years (Schofield et al., 2010). Surveys in the Gulf of Mexico

(Simmons 2014), found very few parasites, and not enough data was present to determine why (temperature, lower biodiversity, parasite community differences, etc.). However, one study performed on thermal preferences of plerocercoid larvae of *Schistocepahalus solidus* in a three-spined stickleback fish host (*Gasterosteus aculeatus)* showed that warmer temperatures not only positively affected the parasite's growth, but that infected hosts preferred warmer waters (Macnab and Barber, 2011; Reimchen and Nosil, 2001). Based on the current literature, it is likely that samples obtained along the Gulf of Mexico will have lower parasite abundance and species richness than those from other localities.

(2) Small to mid-sized fish will be more parasitized than larger, more mature fish, as small fish consume more crustaceans which are the primary reservoir for the parasites known to infect lionfish.

Relatively few studies have been conducted concerning lionfish and their diet in the light of ontogeny. In those rare cases that have, research was concentrated in the Caribbean, and morphometric measurements were taken, but not directly correlated with rate of parasitism. Young lionfish primarily prey on crustaceans, and as they age, switch to a teleost diet (Villaseñor-Derbez and Herrera-Pérez, 2014). Maturation rates of lionfish are variable in terms of standard length, as habitat has been shown to have an effect on growth as well as dietary availability (Barbour et al., 2010). As such, the use of morphometry in this study will help determine the parameters for small and large fish for use in statistical analyses. Small to mid-sized fish have more parasites than larger fish, as these fish prey primarily on crustaceans—a known secondary host to most marine parasites previously documented in this species (Boxshall and Rohde, 1995).

(3) Fatty liver disease will increase with age (standard length).

Fatty liver disease in aquaria fish species is one of the most common causes of death. The disease often affects the host the most following three years of poor diet, wherein death is usually inevitable (Hemdal 2015). It has not been determined, however, if fatty liver disease in wild fish populations has the same lethal effects as it does in aquaria-raised fish species. Thus, it is important to determine if this disease occurs in lionfish in a natural setting, and if age (adult/juvenile), sex, length/weight, and habitat (location) play a role in the presence/absence and degree of disease.

Purpose and Objectives

This project's objectives are two-fold. The first is to conduct an updated parasite survey to include the Gulf of Mexico, Caribbean Sea, and Atlantic Ocean. It is important to better understand the parasites' updated geographic ranges, and provide data on whether these fish have acquired any parasite species previously undocumented in either their invaded or native range. Parasitism of lionfish could result in an eventual biological control of the overall population.

The second objective is to examine lionfish liver tissue for evidence and degree of fatty liver disease and determine whether sex, life cycle stage, or standard length play a role in the degree of disease. A histological analysis of non-alcoholic fatty liver disease (NAFLD) was conducted on salvaged lionfish, representing the first assessment of NAFLD in fish (specifically lionfish). Fatty liver disease is often fatal in aquaria lionfish, and this research could serve as a baseline study to determine what degree of disease lionfish are exhibiting in the invaded range, and whether it, too, might serve as a control to the invasion.

CHAPTER II

Materials and Methods

Study Area

Lionfish were collected from several sites along the Gulf of Mexico, Atlantic

coast of Florida, and Belize and each was designated a site number (Table 1). Each site

presents different environmental habitats, average water temperature, and diversity of

prey items (NOAA 2017). Lionfish were collected indirectly via collaborations with

researchers of non-profits and federal organizations.

Studies of parasitism in the Gulf of Mexico have been scarce and as such, this

location was of particular interest. The Flower Garden Banks National Marine Sanctuary

(FGBNMS), located offshore of Galveston, Texas, is comprised of three separate

artificial reef "banks". Of the three, fish were sampled from the two largest: east and

west. Alex Fogg of the Florida Fish and Wildlife Conservation Commission (FFWCC)

aided in the collection of fish from offshore locations in Carabelle, Florida (northern) and

Sarasota, Florida (southern). Fish collected from these sites were primarily deep-water,

continental shelf, or reef systems. These types of environments are considered natural

habitats for lionfish. An additional shipment of fish was received courtesy of the Emerald

Coast Reef Association (ECRA), located in Destin, Florida (northern panhandle). Fish

from this site were captured off artificial reef systems. However, as the sample size from

this location was small, and no livers were taken, this site was excluded from the fatty

liver disease portion of the study and as such, is marked as * in place of a site number.

Specimens from the Atlantic Ocean and Caribbean Sea were included in the Gulf

of Mexico study to provide both parasite and fatty liver disease information on differing

bodies of water. This was also done to further enhance the study, as these bodies of water had been previously surveyed, and this study wanted to provide an updated account. Fish were obtained from one location along the northern Atlantic coast near Flagler, Florida, courtesy of Alex Fogg. Specimens from this location were captured from natural habitats. Lastly, fish from one site in the Caribbean Sea, St. George's Caye, Belize, were collected courtesy of the Environmental Conservation Organization-Marine (ECOMAR). Lionfish collected from this location were captured in both mangrove systems and from the Belize Barrier Reef.

Table 1. Lionfish collection sites and abbreviations.

Collection Location	Water Source	Water Temperature Range, Low-High ºC	Source/Organization	Site
Galveston, Texas	Gulf of Mexico	12-31	Flower Garden Banks National Marine Sanctuary, NOAA	Site 1
Carabelle/Cape Coral, Florida	Gulf of Mexico	17-30	Florida Fish and Wildlife Conservation Commission	Site 2
Destin, Florida	Gulf of Mexico	13-30	Emerald Coast Reef Association	*
Flagler, Florida	Atlantic Ocean	13-29	Florida Fish and Wildlife Conservation Commission	Site 3
St. George's Caye, Belize	Caribbean Sea	26-30	ECOMAR	Site 4

*Note. Fish from Destin, Florida were excluded from liver histology data analyses, but included in parasite studies. Fish received from Carabelle, Florida and Cape Coral, Florida were combined for statistical simplicity.

Lionfish Collections

As a result of such proliferate lionfish numbers, researchers collaborated with fisherman, divers, and restaurants to establish an economic market. To help along the effort, the National Oceanic and Atmospheric Administration launched an "Eat 'Em to Beat 'Em" campaign in 2010—drastically increasing demand for the meat. In Belize, it was reported that these efforts were working so well that demand greatly exceeded supply (Chapman et al., 2016). Though lionfish capture is unrestricted, costs associated are immense (often thousands of dollars) just for one collection site. Lionfish removal efforts require the cooperation and funding of local fisherman, independent divers, and researchers not including equipment and travel costs. Due to the rising economic market, specimens collected from the various sites were salvaged, and as such, some arrived without heads, fillets, or tails. However, all internal organs remained intact and relatively undisturbed. This method was chosen in order to drastically reduce time and costs associated with removal efforts, but this sampling method does provide a degree of error. The method of capture, handling time (amount of time the fish were exposed to air prior to freezing), water temperature, depth, and salinity were several factors that varied across sites. As such, sources of error exist and must be considered when reviewing the results of this study. All samples were packaged, labeled, flash-frozen, and shipped overnight to prevent deterioration of microhabitats and parasites. The process of keeping the hosts frozen until necropsy allowed for the examination of helminths within their realized niche before the possibility of migration. The lionfish were then transported to the Texas Invasive Species Institute research laboratory at Sam Houston State University and remained frozen until necropsy.

Lionfish specimens from the Gulf of Mexico, Caribbean Sea, and Atlantic Ocean were received from the sites in Table 1. Total lionfish obtained from the invaded range was n = 372. Collection sites with respective *n* number of lionfish obtained is shown in Figure 3.
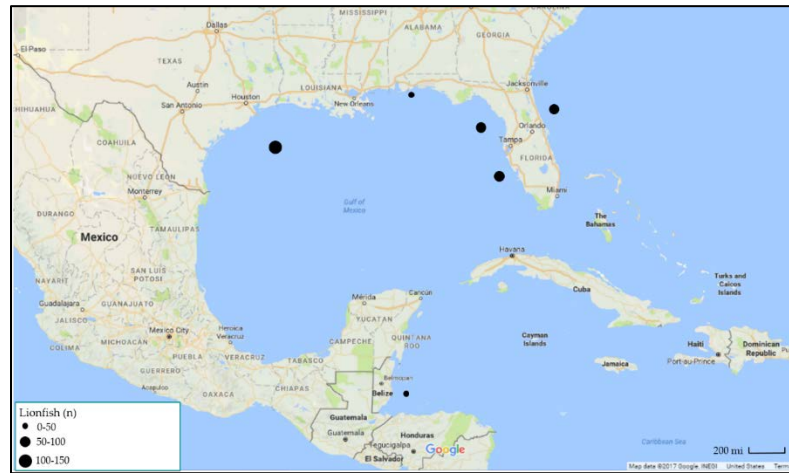
Figure 3. Map of collection sites. Graduated symbols indicate sample size collected (n). Note that the two symbols on the gulf coast of Florida were later combined to one sample site (Site 2) and that the smaller symbol on the northern coast of Florida was excluded from liver histology studies (Google Maps 2017).

The Flower Garden Banks National Marine Sanctuary provided 140 samples, though 100 initial samples were not used for liver histology and were thus excluded from that portion of the study. Thirty-three fish were obtained from Destin, Florida via ECRA and because only two livers were taken for observation, these too, were excluded from the liver study. A total of 105 fish were received from the gulf coast of Florida: fifty-eight specimens from the northern gulf coast of Carabelle, Fl, and 47 from the southwestern gulf coast near Cape Coral. Fifty fish were received from the northern Atlantic coast of Flagler, FL as well as 44 specimens from St. George's Cay, Belize. Comprehensive collection data can be observed in Table 4.

Helminth Collection, Processing, and Identification

Upon necropsy, fish were thawed in a water bath, whole fish weighed (±0.1g), and measured using standard length (±1.0 cm). External organs and tissues (eyes, tongue, and gill rakers) were examined for ectoparasites with the use of a dissecting microscope. Each fish was eviscerated and the sex documented. The viscera were divided into the following microhabitats: liver, gall bladder, stomach, spleen, swim bladder, and intestine. A gross examination of helminths was achieved with the use of a dissecting microscope, following the maceration of microhabitats to dislodge parasites contained within. This was achieved by removing each organ, cutting longitudinally if luminal, scraping the lumen with a spatula, and placing the contents in a 500 mL beaker with approximately 200 mL of deionized water. This mixture was then swirled vigorously for each microhabitat, and the remaining sediment from each cleared microhabitat was examined under a dissecting microscope (Smith and Fedynich, 2012). Parasites were quantified and preserved following the methods of Smith and Fedynich, (2012).

Parasites were fixed and preserved using standard techniques: acanthocephalans were fixed in AFA and stored in 70% ethanol, while nematodes were fixed in acetic acid and stored permanently in 7% glycerol. Any ectoparasites or annelids were examined whole and unstained and preserved in 70% ethanol. Parasites were identified using taxonomic keys and primary literature.

Liver Histological Processing

To maintain random sampling and limit biases, the liver of every fourth fish was extracted for histological processing (Tang et al., 2004). Following this method helped to ensure random sampling by taking a uniform and systematic count of *n* number of

samples, beginning with a randomly selected individual. The liver was fully extracted from the viscera, and each lateral lobe discarded. The center of each liver (caudate and quadrate lobes collectively) was trimmed to a 0.5 cm x 0.5 cm sample, ensuring consistency. Each sample was fixed in formalin and preserved in 70% ethanol prior to histological processing. Upon processing, liver samples were placed in glass vials and dehydrated according to standard histological methods (Carson, 2007).

After dehydration, the sample was embedded in paraffin using a metal mold and a histology cassette. This was done by pouring a small layer of paraffin on the metal mold, placing the dehydrated liver sample in the middle, and then placing the histology cassette on top. Each mold was then flash frozen on an ice pack until hardened and cool enough to pop out of the mold. Samples were trimmed along the edges with a blade, removing excess paraffin, and labeled. Fine sections (10μm) were made longitudinally with a Thermo Scientific Micro HM 340E microtome. Nine slices were taken of each liver sample and each slice was placed in a room-temperature water bath prior to mounting. Upon preparing the microscope slides, three sections were mounted on each. These slides were then stained using the standard histological reagents, dyes, and processing times shown in Table 2.

Table 2. Processing time and reagents for histological staining.

Step	Reagent	Stain Time (min)	Step	Reagent	Stain Time (min)
1	Xylene	3	14	Ammonia H_2O	7
2	Xylene	3	15	Tap H_2O	10
3	Xylene	3	16	Eosin	2
4	100% EtOH	3	17	50% EtOH	3
5	95% EtOH	3	18	70% EtOH	3
6	85% EtOH	3	19	95% EtOH	3
7	70% EtOH	3	20	100% EtOH	3
8	50% EtOH	3	21	100% EtOH	3
9	DI H_2O	5	22	100% EtOH	3
10	Harris Hematoxylin	15	23	EtOH/Xylene 50:50	3
11	Tap H_2O	3	24	Xylene	3
12	3% Acid Destain	7	25	Xylene	3
13	Tap H_2O	1	26	Xylene	Resting

Liver sections were examined for fatty liver content. This was achieved by selecting one section per slide (1/3) of all three slides prepared for each liver sample, and performing fat cell counts on a portion of each section (Figure 4). Of the three slides prepared for each liver sample, and the nine associated histological sections, only three sections were observed. The selection of the histological section was done based on the section with the least amount of artifacts (tears, rips, bubbling, etc.).
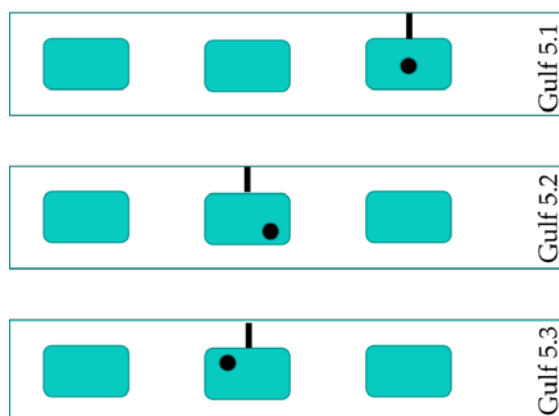
Figure 4. Method for randomized section selection of fat cell counting.

The small field of view of each section was then determined by selecting a field at 70X that exhibited the most representative tissue of the entire liver. This was determined by scanning the entirety of the histological section, until a field of view was present with any amount of fat cell content. Upon viewing fat cell content, if any, this field of view was then selected. This method ensured that the field of view chosen would show fat cell content, if any, while limiting bias by not searching for the "most fatty" section of the liver.

Pictures of the field of view of each histological section (3) were taken at 70X with the use of light microscopy and CellSens Software of each representative section. Photos were then processed in ImageJ/FIJI (NIH) to remove excess background noise. Noise is defined as any extraneous biological material, including hepatocytes, erythrocytes, leukocytes, smeared or partial adipocytes, and dye. This was achieved by converting the image to 8-bit grayscale then and by the use of the MRI Adipocyte Toolset. Three manual counts were taken on each photograph per slide for a total of three photographs per liver. Fat cell counts were then averaged on a per slide basis and then the

entire liver's average fat cell count was taken (Figure 5). This method of averaging

manual counts of adipocytes within samples follows that of Wang et al. (1977).

Fish ID	Stage	Sex	L (cm)	Wt (g)	Slide #	Count 1	Count 2	Count 3	TOTALS/3	AVG
ATL3	Adult	M	26.00	N/A	1	6	9	9	8	
					2	2	3	3	3	
					3	3	3	3	3	5
ATL7	Adult	M	23.20	N/A	1	24	24	24	24	
					2	19	19	21	20	
					3	20	20	20	20	21

Figure 5. Manual counting method of adipocyte cells in lionfish liver tissue histological sections. The average adipocyte count was determined by taking the mean of the three observed counts.

Upon completion of fat cell counts, each liver was given an overall grade of

disease based on the average number of fat cells recorded. This was done following

human medical (Kleiner et al., 2005) and clinical protocol (Vendhan et al., 2013), as

methods regarding fatty liver disease in fish have been scarcely documented (Braunbeck

et al., 1990; Kuwashiro et al., 2011; Asaoka et al., 2013). Per protocol, the degree of fatty

liver disease (grade) was arbitrarily determined by the average number of fat cells. An

arbitrary designation was made as this project was not concerned with the total extent of

the damage—but rather the absence/presence of disease of smaller randomized

subsections. Four grades of disease were utilized to describe the degree of fatty liver

disease (Table 3).

Table 3. Fatty liver disease grading system.

Grade	Average Number of Adipocytes	Description
1	<5	Little disease, relatively healthy
2	5-9	Mild disease
3	10-14	Moderate disease
4	15+	Heavy disease, degradation

The physical differences between degrees of disease can be viewed in Figure 6. Since this research is novel, methodologies mirror that of human and mammalian medical and clinical literature (Brunt et al., 1999).

Figure 6. Physical representation of non-alcoholic fatty liver degrees of disease in lionfish livers. Grades are in order of ascending disease.

Statistical Analyses

Summary & Descriptive Statistics

Prevalence was determined as the number of hosts infected with a particular parasite taxon/species divided by the number of hosts sampled, most often expressed as a percentage. Mean abundance was determined by taking the total number of a parasite species/taxon found in a sample of an individual host, divided by the total number of hosts sampled (Simmons 2014; Bush et al., 1997). For this study, mean abundance was used in place of mean intensity as this study includes both infected and non-infected hosts.

Comparative Statistics

Analyses of the effects of site, sex, life cycle stage (adult/juvenile) of the host, and standard length on both parasite prevalence and abundance were conducted. Sex, life cycle stage (adult/juvenile), and standard length were tested against fatty liver disease. These were done with the use of one-way ANOVAs as well as Kruskal-Wallis analyses. The use of Kruskal-Wallis analyses in conjunction with one-way analyses of variance were necessary, as fatty liver disease data was not normally distributed and the assumptions for parametric statistics could not be met. A two-way ANOVA was performed on the analysis of sex, site, and fatty liver grade in conjunction with the Friedman test for non-parametric data. Spearman correlations were used to test comparisons between life cycle stage and standard length on fatty liver grade, respectively. Significant correlation coefficients are +1 or -1, with data becoming less correlated the further away from either value.

One-way analysis of variance (ANOVA) was used to test standard length (cm) and sex distribution among hosts with diseased liver tissue. A two-way ANOVA and the Friedman test were used to test sex and degree of disease (grade) within sites. Seasonality differences of collections were ruled out by a one-way ANOVA and Kruskal-Wallis analyses.

All tests were considered significant at $P \leq 0.05$. Descriptive statistics are presented with ± 1 standard error (SE). All statistics were calculated with the use of the Excel descriptive statistics package, RealStats package, Solver Add-In, and Minitab.

Community Analyses

Percent similarity index (PS_i) was used for community analysis. The PS_i (Krebs, 1989) was used to compare degree of disease across location sites, as it pertained to fatty liver disease. The following equation was used to generate percent PS_i values: PS_i = minimum (P_{1i}, P_{2i}) where PS_i = percentage similarity between samples 1 and 2, P_{1i} = percentage of degree of disease i in location 1, and P_{2i} = percentage of degree of disease i in location 2.

CHAPTER III

Results

The overall range of length was 7.79-30.48 cm, and the mean standard length was

19.31 cm ± 4.1. The standard length of the fish varied significantly over the sites

(ANOVA, P = 3.28E^{-16}; Kruskal-Wallis, P = 1.52E^{-12}), inclusive of the specimens

received from ECRA. Over 1.5 x more males were sampled than females, and 6 x more

adults were received than juveniles. Sites 2 (Florida Gulf Coast) and 3 (Atlantic coast of

Florida) revealed no significance between standard length of adult females and males.

Total collection data is detailed in Table 4.

Table 4. Lionfish sample locations with total lionfish sampled, length (standard, cm), life cycle ratio, and sex ratio. Specimens with undetermined sex are excluded.

Site	n	Standard length range (cm)	Sex Ratio (M:F)	Life cycle stage ratio (Adult:Juv)
1	140	12-30.48 ± 3.7	76:64	140:0
2	105	8.38-28.14 ± 3.7	55:34	85:20
3	50	15.5-27.6 ± 3.1	39:9	48:2
4	44	7.79-28.32 ± 4.4	25:13	36:8
*	33	8.7-16.3 ± 2.4	17:11	9:25

*Note. Lionfish from ECRA, denoted by *, were excluded from liver studies.*

The Atlantic Coast specimens (Site 3) were relatively large while those from

waters of the Florida Panhandle (ECRA*) were smaller lionfish. Fish length can also

depend on sex and maturity, and although size (length) at maturity is estimated to be 10.0

cm for males and 17.5 cm for females, (Morris and Akins, 2009; Barbour et al., 2011),

this study did not possess enough data necessary to conduct a fully comprehensive adult

versus juvenile analysis. Furthermore, detectability of lionfish is dependent on habitat

complexity, size class, their cryptic behavior (Kulbicki et al., 2012; Green et al., 2013) influencing the efficiency of diver-based removal tactics (Barbour et al., 2011).

Parasitism

Seven parasites representing six species were found in the 372 fish examined: *Olencira praegustator, Aegichus tenuipes, Rocinela signata, Excorallana truncata, Serrasentis sagittifer* and *Trachelobdella lubrica*. These included parasites of the phyla Acanthocephala, Arthropoda, and Annelida. Arthropods represented the largest percentage of parasites found (74%), while acanthocephalans (14%) and annelids (14%) each represented the same proportion. The overall prevalence of parasites was 1.89% and the mean abundance was 1.17. Three of the six species are documented for the first time in the red lionfish (Table 5). All but one infected host had 1 parasite and 6 of 7 infected fish were male. The mean standard length of the parasitized fish was 19.43 cm $\pm$ 4.5. The buccal cavity was the primary location of parasitism with parasitic isopods dominating the overall parasite composition. Lionfish parasite distribution varied across sites geographically, though insignificantly.

Four of the six parasitized fish came from Site 2 (Gulf of Mexico), 1 from the Site 4 (Caribbean Sea), 1 from Site 3 (Atlantic Ocean), and Site 1 (Galveston, Texas) yielded no parasitized fish. Statistically, there was no significant relationship between latitude and parasite abundance (P = 0.169). A plot of parasite abundance and latitude, with linear regression (R^2 = 0.59) is detailed in Figure 7.
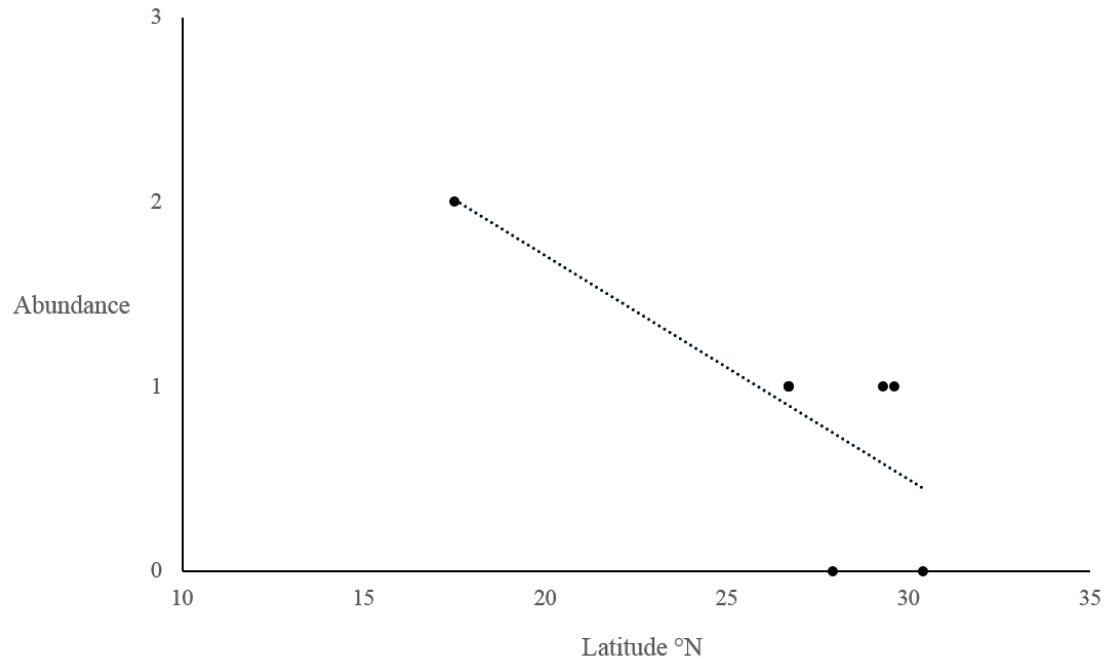
Figure 7. Effects of lionfish host latitude (°N) across all sites sampled (Caribbean Sea, Atlantic Ocean, Gulf of Mexico) on parasite abundance. N = 6 infected hosts of 372 sampled; linear regression ($R^2 = 0.59$).

Of the infected fish, adults outnumbered juveniles 5:1, and statistical analyses show a significant difference between standard lengths of the adults vs. juveniles (P = 0.022). This suggests that juveniles were smaller than adults that were infected, enforcing the use of the metric of size for general age of the fish. ANOVA and T-test analyses showed that prevalence and mean abundance of infected male lionfish was significantly higher (P = 0.022) than females. This mirrors the results of Al-Zubaidy and Mhasien (2012) and Poulin (1996), who also observed significantly higher infection rates in males of other teleost fish species.

*Table 5. Prevalence (%P), abundance (A), and infection site of parasites from red lionfish from the Gulf of Mexico, Caribbean, and Atlantic Ocean. * denotes new host record in the invaded range.*

Species	(%P : A)	Infection area	Collection Site (Body of Water)
Acanthocephala *Serrasentis sagittifer**	0.27 : 1	Intestinal fat	Site 3 (Atlantic Ocean)
Annelida *Trachelobdella lubrica*	0.27 : 1	Gill raker	Site 2 (Gulf of Mexico)
Arthropoda *Aegiochus tenuipes*	0.27 : 1	Gill raker	Site 2 (Gulf of Mexico)
Rocinela signata	0.27 : 1	Gill raker	Site 2 (Gulf of Mexico)
Excorallana truncata*	0.27 : 1	Gill raker	Site 2 (Gulf of Mexico)
Olencira praegustator*	0.27 : 2	Buccal cavity	Site 4 (Caribbean Sea)

A single acanthocephalan, *Serrasentis sagittifer* (Linton 1889), was obtained from the intestinal mesentery of a lionfish from the Atlantic Ocean. This species of parasite has been previously documented in fish of the Red Sea and Yemeni waters, the lionfish's native range (Al-Zubaidy and Mhaisen, 2012). However, this is the first account of this parasite in the red lionfish in the invaded range (new host record).

One member of the phylum Annelida, *Trachelobdella lubrica* (Meyer 1965), was found in the gill rakers of a single fish from the Gulf of Mexico. This ectoparasitic leech typically resides in the gills of many marine teleost fishes in various temperate and warm waters around the world.

Three species of ectoparasitic arthropods, *Excorallana truncata* (Richardson 1899), *Aegiochus tenuipes* (Schioedte and Meinert, 1879), and *Rocinela signata* (Schioedte and Meinert, 1879) were identified from lionfish from the Gulf of Mexico, and one species *Olencira praegustator* (Latrobe 1802) was identified from a lionfish from the Caribbean Sea. All parasitic isopods were removed from the host's gill rakers

except for the *O. praegustator* specimens*,* which were taken from the buccal cavity. Two

of four ectoparasite species, *E. truncata* and *O. praegustator*, represent new host records,

as well as a range expansion.

Fatty Liver Disease

Of the 372 fish obtained for parasitism studies, only 58 were used for fatty liver

analysis. Of these 58, the ratio of males to females was 42:12, and 4 specimens were too

young to determine gender. The adult to juvenile ratio was 51:7. Collection of fish

occurred over both spring and fall seasons, but the variability in disease was not

significant by Kruskal-Wallis analysis (P = 0.253), and one-way ANOVA analysis (P =

0.158). The Spearman correlation (coefficient = 0.781, P = 0.013) reinforced the claim of

no seasonality differences as highly correlated degrees of disease between the spring and

fall males were noted. The distribution of the data collected is non-normal, despite log-

normal and square-root transformation attempts. The frequency distributions of fatty liver

grade and average adipocyte counts are detailed in Appendix B.

Analyses of standard length between adult/juvenile and adult male/adult female

were conducted for each site. Overall, there appeared to be significant differences

between standard length of adult and juveniles and standard length of adult males and

adult females in all locations, with a few exceptions. Adult/juvenile analyses yielded

complete significance overall (ANOVA, P = $8.7E^{-23}$; Kruskal-Wallis, P = $8.73E^{-16}$).

However, Sites 2 (ANOVA, P = 0.059; Kruskal-Wallis, P = 0.079) and 3 (ANOVA, P =

0.059; Kruskal-Wallis, P = 0.064) revealed no significance between standard length of

adult males and females. Both one-way ANOVA (P = 0.456) and Kruskal-Wallis (P =

0.463) analyses revealed no significant differences between adult/juvenile stages and their respective degrees of disease over all locations.

There was a statistically significant difference between degree of disease and average adipocyte counts (ANOVA, P = $4.63E^{-19}$; Kruskal-Wallis, P = $5.38E^{-08}$). This ensured that degree of disease was an appropriate qualitative descriptor for the average adipocyte count. The breakdown of degree of disease for all individuals is detailed in Figure 8.
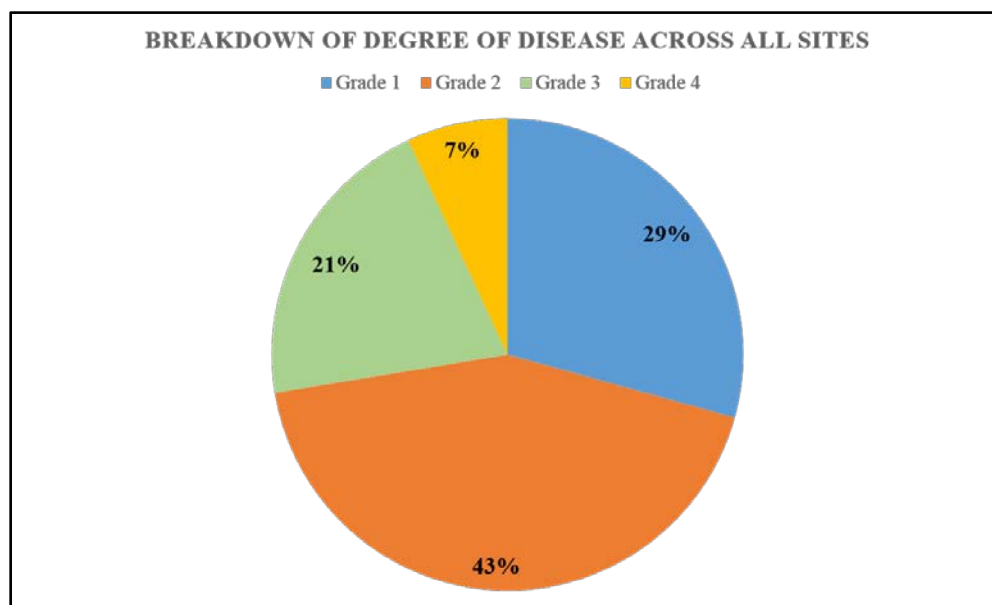
Figure 8. Percentage of each non-alcoholic fatty liver degree of disease (1-4) for all lionfish individuals (N = 58), across all sites sampled (Caribbean Sea, Gulf of Mexico, Atlantic Ocean).

Analyses made between standard length and degree of fatty liver disease also determined no significant differences. A Spearman's correlation test revealed a coefficient of 0.131, with one-way ANOVA (P = 0.798) and Kruskal-Wallis (P = 0.238) analyses confirming no significance. The plot of standard length for all individuals taken

for liver analysis and their respective average adipocyte counts was utilized to show the relationship between standard length and fatty liver disease (Figure 9).
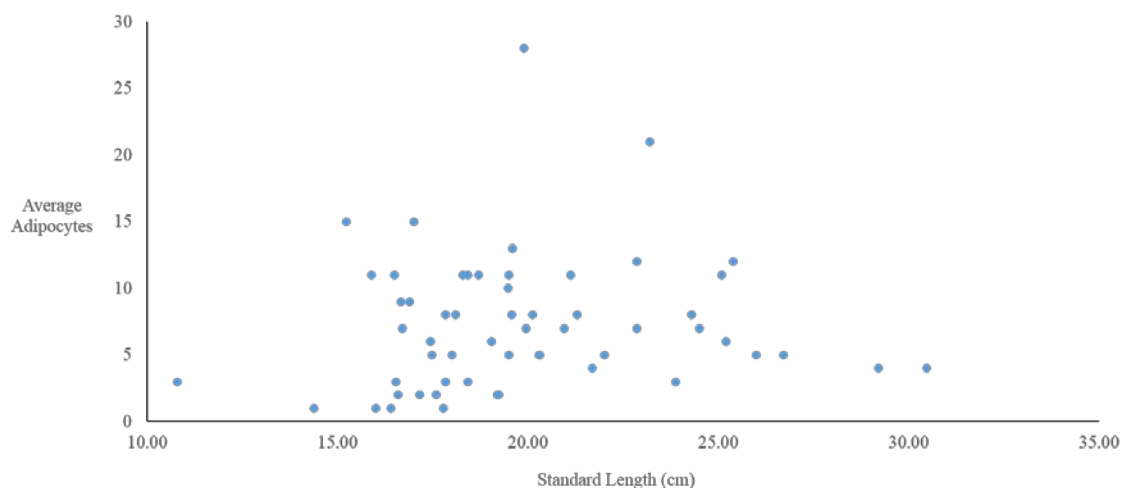
Figure 9. The effect of lionfish (N=58) standard length (cm) on the average adipocyte count in liver tissue histological sections, across all sample sites.

A T-test was used to evaluate significance between male and female fatty liver disease, and to determine if H_0 could be rejected (H_0 = The means of the two populations are equal). The T-test showed the means of males (7.42) and females (7.52) to be too close to reject the null hypothesis. The degree of disease was tested against males and females to determine significant differences across sites. A two-way ANOVA showed no statistical difference (P_{sex} = 0.695; P_{site} = 0.849; P_{inter} = 0.548), though some slight (statistically insignificant) differences were observed within locations. Males do show slightly higher rates of disease than females, although this could be due to sample size discrepancies—nearly four times as many males (42:12) were analyzed as females. Fatty liver degree of disease and its correlation with sex across all sites can be observed in Figure 10.
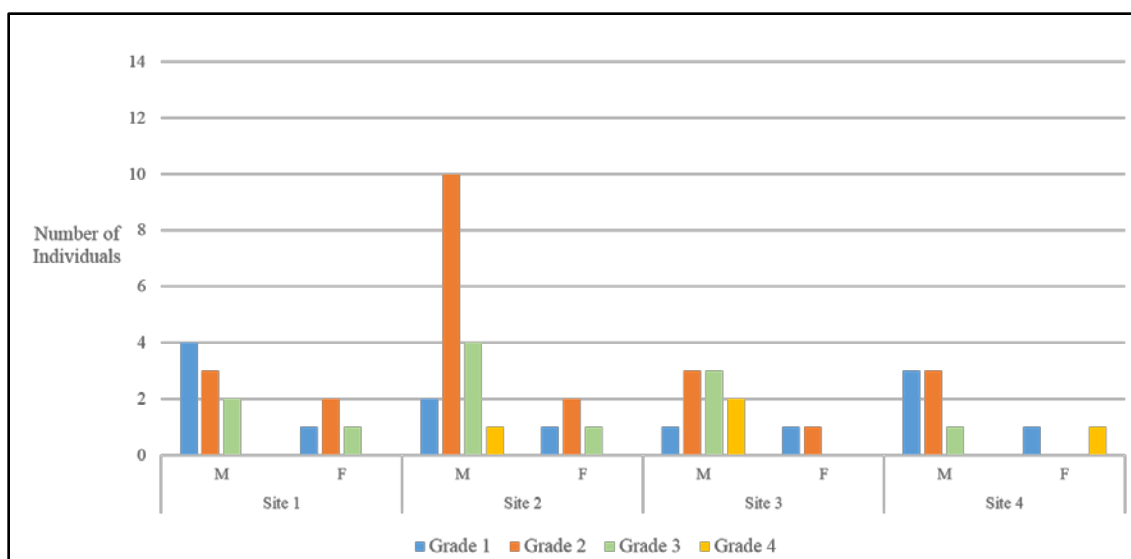
Figure 10. Distribution of the number of lionfish sampled (N=58), by sex and site, and the respective non-alcoholic fatty liver disease grades. Grades (1-4) are in order of increasing states of disease.

Percent similarity indices for fatty liver degree of disease and location were calculated between each site for a total of six indices. Overall, similarity was uniformly high (~75%), indicating no significant differences of disease between sites. The closest similarities observed were between Sites 1 and 2, and Sites 2 and 3. The least similar was the index between Site 2 (Gulf of Mexico, Floridian region) and Site 4 (Belize). This revealed that variability in fat grade differed within the same water site (Gulf of Mexico).

To cross-check individual analyses, a multiple analysis of variance (MANOVA) was conducted on fatty liver degree of disease, sex, adult/juvenile, and standard length in relation to site. The results show no significant differences: Pillai Trace, P = 0.668; Wilk's Lambda, P = 0.686; Hotelling Trace, P = 0.704.

CHAPTER IV

Discussion

Parasitism

Extirpation of lionfish populations is highly unlikely and the threat they pose to the native reef communities is great (Albins and Hixon, 2008). Predation by native fish is negligible, and removal efforts hindered by populations exceeding scuba depths, eradication of current lionfish numbers has been largely ineffective (Barbour et al., 2011). However, it is possible that, given enough time, parasitism could serve as a possible biotic control to the lionfish invasion.

In terms of parasitological significance, this study reports the first instance of *Serrasentis sagittifer* in lionfish in the invaded range, as well as the first instance of both *Excorallana truncata* and *Olenicra praegustator* in lionfish in the invaded range. A new distribution record is reported for *E. truncata*, as previous distributions restricted it to the Pacific coast of Mexico. Overall, only 7 parasites representing 6 parasite species were found among all microhabitats of the 372 fish studied. As this study surveyed many of the sites previously observed, these results suggest that lionfish are even less parasitized than they were two years ago (Simmons 2014). Furthermore, although prevalence and mean abundance of parasitized males was significantly greater than females, a sample size of 7 is not large enough to make a conclusive statement.

Most of the parasite species infecting lionfish were generalists and/or species that infect a wide range of carnivorous fishes. No species commonalities between sites were observed, and as such, this suggests that a suite of opportunistic parasites is infecting this invasive fish (Ramos-Ascherl et al., 2015). In previous comparative studies of native and

invasive fish parasites, it was noted that specialist parasites decreased as distance from the native range increased (Kennedy and Bush, 1994). Although this study applied to the introduced rainbow trout, this trend is applicable to the data reported in this study, as most of the parasites recorded in the lionfish of this study are generalist species.

With such low parasitism found across all bodies of water, no conclusive connections may be made between site and parasite prevalence or abundance. It was believed that annual water temperature extremes (NOAA 2017) and latitude (Sellers et al. 2015; Rohde and Heap, 1998) would positively correlate with parasite prevalence, richness, and abundance (Griffiths et al., 2014). This was not necessarily the case, as an acanthocephalan was found in a lionfish collected from the Atlantic Ocean, an annelid from the southern Gulf of Mexico, and parasitic copepods were found in fish from both the Caribbean Sea and Gulf of Mexico. The Atlantic Ocean (13-29°C) and the Gulf of Mexico (12-31°C) lay on the cooler end of water temperatures, the Floridian Gulf of Mexico (17-30°C) has slightly warmer water temperatures, and the Caribbean Sea (26-30°C) has the highest yearly water temperatures (NOAA 2017). The Texas end of the Gulf of Mexico (Site 1) has the largest range of extremes, while Belize maintains the lowest range of extremes. In terms of latitude, Belize (Site 4) is at the lowest end (17.5° N) while the ECRA site is at the highest end (30.4 °N). Parasite abundance in Belize was 2, 1 in the eastern Gulf of Mexico, and 0 in both the western Gulf of Mexico and the Florida panhandle. Statistically, latitude played a role in parasite abundance, though the sample size of this study nor the amount of parasites recovered were large enough to make any conclusive statements on this relationship. It is important that future parasite

faunal surveys continue to adequately assess the development of parasite species richness and abundance in *P. volitans* in the invaded range.

Acanthocephala

One acanthocephalan, *Serrasentis sagittifer* (Linton 1889)*,* taken from the intestinal mesentery of a lionfish, marks the first record of this parasite species in lionfish. *Serrasentis sagittifer* has been reported from various definitive hosts of various marine waters in the world including the tropical Atlantic and Gulf of Mexico inclusive of the range from Texas and Florida, extending upwards to coast of South Carolina.

All worms of the relatively small phylum Acanthocephala are parasitic, as adults, in the intestines of all vertebrate groups. Specifically, the class Palaeacanthocephala is the largest and most diverse class of acanthocephalans that parasitize fishes, amphibians, reptiles, birds, and mammals worldwide (Amin 1998). Eggs are passed with the definitive host's feces and remain free in the environment until ingested by an arthropod intermediate host. Aquatic intermediate hosts of *S. sagittifer* in both the native and invaded ranges include isopods, copepods, and some ostracods (Amin 1998). Definitive hosts in the invaded range are typically fishes including, but not limited to, silver perch (*Bairdiella chrysura*), hardhead catfish (A*rius felis*), summer flounder (*Paralichthys dentatus*), and cobia (Rachycentron canadum) (Felder and Camp, 2009). A small number of definitive hosts in the native range include the Japanese thread fin bream (*Nemipterus japonicus*), Coitor croaker (*Johnius coitor*), and the northern sand flathead (*Platycephalus endrachtensis)* (Maghami et al., 2008; Verweyen et al., 2011).

Acanthocephalans have complex life cycles in which the primary and intermediate arthropod (Maghami et al., 2008) hosts ingest eggs, which then mature into

infective cystacanths. These are then transmitted to an appropriate vertebrate definitive

host by predator ingestion. On occasion, vertebrates may serve as paratenic hosts and

harbor larval acanthocephalans until ingested by the appropriate definitive host (Al-

Zubaidy and Mhaisen, 2012; Santos et al., 2005). In paratenic hosts, immature worms

are usually encysted in or on body cavity organs (Espínola-Novelo et al., 2013). Known

reservoir (paratenic) hosts in the invaded range include lionfish prey species: *B. chrysura*

[American silver perch], *Centropristis striata* [Black sea bass], *Coryphaena hippurus*

[Mahi-mahi], *Orthopristis chrysoptera* [Pigfish/Grunt], and *Paralichthys albigutta* [Gulf

flounder] (Linton 1889; Amin 1998).

In the definitive host, the juveniles attach to the intestine, mature, and produce

eggs. Infection with this parasite often results in the destruction of the intestinal villi and

degeneration of the mucosal epithelium. In response to inflammation at the site of

infection, the host's immune system increases production of lymphocytes, epithelioids,

and mucus (Maghami et al., 2008). Human infection with acanthocephalans, in general, is

incidental but has occurred as a result of ingestion of undercooked fish or pig (CDC

2017), as these are either definitive or reservoir hosts, but most commonly in areas where

the ingestion of intermediate arthropod hosts is prevalent. Human infection, though rare,

is often severe and presents as nausea, vomiting, abdominal pain, and fever. As only four

species have been documented in humans, *S. sagittifer* not being one of them, it is

unlikely that this parasite would be of concern to those interested in consuming lionfish

(CDC 2017).

Annelida

The circumtropical distribution of *Trachelobdella lubrica* is inclusive of the Mediterranean Sea, Red Sea, the Caribbean, the coastal waters of Africa, South Carolina, Australia, Gulf of Mexico, and the Hawaiian Islands (Öktener and Utevsky, 2010; Sawyer et al., 1975). *Trachelobdella lubrica* has been previously documented in a suite of teleost fish species in the native range including, but not limited to, Serranidae [bass and grouper], Sciaenidae [drums], and Scorpaenidae [lionfish] (Sağlam et al., 2003; Paperna 1976). This generalist ectoparasitic leech is also only one of two parasites known to infect lionfish in both the native and invaded ranges. In the invaded range, *T. lubrica* has been documented in the invasive lionfish (Ramos-Ascherl et al., 2015; Ruiz-Carus et al., 2006; Fogg et al., 2016) in the gulf coast of Florida and Puerto Rico. Many species of native fish in the invaded range (snapper and grunt) that serve as major prey items for lionfish can harbor these leeches. In general, leeches have a simple, direct life cycle in which infection occurs by contact. Infection of a host with *T. lubrica* results in a significant decrease of mean urea, hematocrit, and leucocyte levels as well as the manifestation of fin rot and hemorrhaging of the gills in high intensity infections (Celik and Aydin, 2006). These effects are typically short-lived, however, as this leech detaches from the host to lay eggs and die after one or two blood meals.

The presence of *T. lubrica* in the lionfish specimen described is not atypical, as its distribution in the invaded range and suite of available teleost hosts is quite large. An abundance of 1 is also not surprising because lionfish are known to have low infection rates (Fogg et al., 2016; Ramos-Ascherl et al., 2015), and *T. lubrica* is a direct life-cycle parasite, in which the leech only parasitizes the host for a short time.

Arthropoda

Parasitic isopods are often restricted to warmer or temperate waters, most have direct life cycles, and most are opportunistic (aside from *O. praegustator*). Secondly, the presence of parasitic isopods in a fish species has been known to correlate with low or no ciguatera toxin (Bunkley-Williams et al., 2006). Ciguatera toxin has been a concern of not only lionfish consumption, but other fish species as well, as upon ingestion by humans, the toxin can induce nausea, abdominal pain, seizures, respiratory distress, and death in severe cases. The colorless, odorless ciguatoxin is produced by dinoflagellates and bioacummulated by fish (Wilcox and Hixon, 2015).

Excorallana truncata is an ectoparasitic isopod found in benthic habitats, often in sand deposits or on reef systems. This species of parasite has been previously documented in limited fish species of the Pacific Ocean and Caribbean, but this is a tentative new host record of this parasite in the red lionfish. This identification is tentative, as *Excorallana* has been suggested for revision (Delaney 1984; Bunkley-Williams et al., 1999) and many species are in need of re-description. Other related species have been known to associate with fishes, including *E. acuticauda* and *E. quadricornis* (Bunkley-Williams et al., 1999). Tentatively, this study also provides a range expansion of this parasite into the Gulf of Mexico, the lionfish's invaded range. Previous distribution records restricted this species to the northern Pacific Ocean, the coasts of Baja California and Mexico, and small parts of the Caribbean. Infection with this parasite results in minimal damage to the host, as they are typically commensals on marine fishes (Delaney 1984). A study in Bonaire reported a single *Excorallana* sp. (Stebbing 1904) isopod parasitizing the gills of a single lionfish (Poole 2011). The

infection of a red lionfish with *Excorallana truncata* is likely incidental, as these parasites are commensal and due to the tentatively new distribution record, probably new to the general ecosystem.

Olencira praegustator, of the family Cymothoidae, is an obligate direct life cycle fish parasite that resides in the mouth of the host. Distribution of this ectoparasite is restricted to the lionfish's invaded range, primarily the Atlantic coast of the United States, the southern Gulf of Mexico and northern Caribbean. *Olencira praegustator* is highly host-specific, with the only known hosts to be menhaden fish species, *Brevoortia patronus, Brevoortia tyrannus, Brevoortia gunteri, and Brevoortia smithi* (Hoffman 1999). Of these host species, only *B. patronus* is known to reside in Caribbean waters, so it is only likely that the lionfish infected with these parasites acquired them by predation on an infected *B. patronus* fish. Furthermore, no cymothoid parasites (i.e. *O. praegustator*) in aquaculture or aquaria conditions infect the same species in the wild (Smit et al., 2014). As such, it is unlikely that lionfish introduced this parasite, but rather that they acquired it from their prey items or environment. Fish infected with this parasite experience localized lesions at the site of attachment (tongue, buccal cavity), host behavioral problems, and reduced lifespan (Smit et al., 2014). Diversity and overall prevalence of cymothoid parasites decreases from the tropics to temperate/cold waters, explaining further why this species was only found in a host from the Caribbean Sea as opposed to the Gulf of Mexico or Atlantic Ocean.

Two members from the family Aegidae, *Rocinela signata* and *Aegiochus tenuipes,* were taken from the gill rakers of fish from the Gulf of Mexico. These parasites have direct life cycles, and fish are infected by coming in contact with the parasite. Both

species take blood meals from their hosts and leave, and as a result, are often considered micropredators. Consequences of infection with these parasites include damage to the gill filaments, physical irritants to the branchial tissues, and stunted growth of respiratory tissues in heavy infections (Rameshkumar and Ravichandran, 2014).

Rocinela signata is a generalist fish parasite previously described in lionfish in the invaded range. This ectoparasite has been documented in marine teleosts of the Gulf of Mexico, tropical Atlantic, and Caribbean Sea, as well as the lionfish's native range (Schotte et al., 1995; Schioedte and Meinert, 1879). This parasite is known to infect various fish species including *Pseudupeneus maculatus* [spotted goatfish], *Haemulan aurolineatum* [Tomtate grunts], *Otolithes ruber* [croaker], and many others (Cardoso et al., 2017) including commercially important species like snapper and grouper. This parasite is one of the only know isopods that frequently treats humans as a food source, its bite known to be very painful (Bunkley-Williams et al., 2006). Handlers of lionfish typically wear gloves, but the presence of this parasitic isopod should further enforce the necessity of them.

Aegiochus tenuipes is an ectoparasitic isopod of various fishes, though its distribution has been documented to only include the waters of Cuba, Cayman Islands, and the tropical Atlantic (Bruce 2009; Ramos-Ascherl et al., 2015). This marks only the second report for this parasite species in lionfish in the invaded range. *Aegiochus tenuipes* is not a well-documented species in the literature, and very little is known about its specific feeding strategies, life cycle, and overall life history (Poore and Bruce, 2012). The presence of this species in a lionfish from the Gulf of Mexico also marks a new

report for its distribution range, as this parasite species was not previously known from this locality (WoRMS 2017).

Most of the parasite species found have been described in marine teleost fish in lionfish's native and invaded range, but not in lionfish species. The overall low prevalence of ectoparasites could also be a result of the method of capture, handling process, and shipping timeframe. These factors could easily have resulted in the loss of ectoparasites and the disturbance (movement) of endoparasites, though immediate freezing of hosts was ensured to minimize disturbance of parasites in their realized microhabitats.

It can be tentatively said that location (i.e. high/low water temperature, latitude, time since establishment) does not significantly play a role in the rate of parasitism. It was believed that bodies of water with higher water temperatures year-round (Caribbean Sea), combined with other ecological and environmental factors, could have a positive correlation with parasite richness, prevalence, and abundance (Macnab and Barber, 2011). However, lionfish from the Gulf of Mexico had 4 species of parasites compared to those from the Caribbean Sea (1) and the Atlantic Ocean (1). Lionfish from Site 1 (Galveston, Texas) did not have parasites, despite being in the Gulf of Mexico, although this could be due to the greatest temperature range in that area (12-31°C). These numbers are not significant, statistically speaking, though other papers have reported very similar results (Simmons 2014; Fogg et al., 2016). The lack of parasites in Site 1 could be the result of lionfish caught at this location. They reside on a relatively new artificial reef system as opposed to coral reef tracts and older, more established artificial reefs (FGBNMS 2015). It could also be a result of different parasite diversity, or the temporal

lag in parasite establishment vs. the Florida coast (Site 2). Since the lionfish introduction began in coastal Florida in 1990, and the first reports of lionfish occurred off the coast of Texas 20 years later, it is not surprising that lionfish in the eastern gulf of Mexico would at least have greater parasite richness and/or abundance. Longer establishment of an invasive species into a new habitat provides greater opportunity for the parasite communities to adapt to and infect invaders (Alvarez-Pellitero 2008; Côté et al., 2013).

It was also hypothesized that smaller (younger) lionfish should have higher infection rates than larger (older) fish, as young fish prey almost exclusively on crustaceans while older fish transition to a teleost diet (Boxshall and Rhode, 1995). As most parasites of lionfish have indirect life cycles (Ramos-Ascherl et al., 2015; Fogg et al., 2016; Sağlam et al., 2003; Paperna 1976) and arthropod intermediate hosts, it was thought that smaller fish will be parasitized more heavily. This was not necessarily the case in this study. The mean standard length of parasitized fish was 19.43 cm and the length range was 11.33-25 cm. The juvenile fish (11.33 cm) from Belize did, in fact, have two parasites in contrast to the others with only one parasite per host. However, not enough data was obtained to support this claim. In future studies on parasite abundance and fish age, otolith analyses would be a better metric than standard length. With a more even sample set (i.e. total n per site, male: female, adult: juvenile), otolith age analyses, and control of both capture and handling methods, it could be more solidly determined whether age (standard length) and location affect the parasite prevalence, abundance, and richness.

The few infections observed in this study may be a result of the recent presence of lionfish in the range observed (Schofield et al., 2010; Torchin et al., 2013), or due to a

highly resistant immune system (Côté et al., 2013). Parasite infections of fish can be

controlled through innate and adaptive immune responses (Alvarez-Pellitero 2008), and

although not studied in length, it is possible that this is aiding the red lionfish in the

invasion. Compared to mammals, fish innate immunity is highly evolved with possible

enhanced functionality, whereas adaptive immunity may be less developed (Lieschke and

Trede, 2009). It is also important to note that this study focused on macroparasites, like

previous studies, and it is possible that the lionfish could have been carrying

microparasites. It is important that future studies focus on microparasites, as these studies

are lacking (Anderson et al., 2010).

To date, few parasite species are known to infect lionfish in the Pacific (Nagaty

and Abdel-Aal, 1962; Durio and Manter, 1968; Paperna 1976; Dojiri and Ho, 1988;

Ogawa et al., 1995; Hassanine 2006), probably due to successful immune responses in

these fishes in their native range. Conversely, in cases where overpopulation or other

environmental stressors are high, parasitic infection rates increase (Schultz et al., 2006).

In these cases, fish populations experience delayed growth, reproductive issues, and other

associated ailments (Ramos-Ascherl, et al., 2015). It is possible that, over time, lionfish

will succumb to parasitic infection at higher rates than currently observed—a process that

may ultimately serve to slow the invasion.

In order to relevantly study the comparison of parasite communities affecting

lionfish in their invaded range, future studies of parasitism in lionfish should also

investigate parasite communities of ecologically and trophically similar native fishes.

This will shed light on the availability of parasite species at different localities and will

also compare parasite intensity between lionfish and native reef carnivores (Ramos-

Ascherl et al., 2015). Furthermore, little is known about parasites infecting lionfish in their native range (Hassanine 2006; Paperna 1976; Ogawa et al., 1995; Dojiri and Ho, 1988). More studies of parasites of lionfish in the Indo-Pacific will potentially reveal the degree to which these fishes are naturally susceptible to parasitic infection, and definitively prove whether lionfish have introduced invasive parasite species (Ramos-Ascherl et al., 2015).

Fatty Liver Disease

The original hypothesis behind the study of fatty liver disease in lionfish was that older (larger) fish should have a higher degree of disease (Hemdal 2015). However, the results obtained from this study do not support this claim. It is possible, though, that age correlates with degree of disease as this study utilized standard length as the metric for age rather than otolith analysis, the more appropriate metric for measurement of age.

Life cycle stage of the host (adult, juvenile), did not play a role in the degree of disease exhibited by the fish. This was also part of the assumption from the original hypothesis, as standard length often correlates with age. However, there are a few reasons why this was not determined statistically different in this study. First, sampling size differed greatly, as adults outnumbered juveniles 51:7 and this could have contributed to the lack of significant difference. Second, it could be that adults, or older fish, are not susceptible to disease based on age alone. The studies supporting this claim were conducted on lionfish in aquaria systems (Hemdal 2015), so it is possible that lionfish in natural systems may not be susceptible to this same assumption. Although they eat voraciously, and stomach size does correlate with age, this study did not prove this

parameter, and no conclusions can be made based on this study. Further age and fatty liver disease studies could yield a significant difference between disease and overall age.

There was no difference between sex and degree of disease within and among sites. There are possible explanations for this, including the large ratio discrepancies where lionfish were concerned (males outnumbered females greatly). Males of Site 2 had extremely high numbers, but this is due to sample size differences among the sites (Figure 9). Sites 1, 3, and 4 included 13, 11, and 10 samples respectively, while Site 2 represented 24 samples. This was due to merging two very similar collection sites geographically, from the same donor. Gender can often play a role in other ecological aspects (i.e., parasitism), and as such, was thought to play a possible role in degree of disease. However, this study did not confirm this. It is possible, with future studies, that sex could still be determined to play a role in fatty liver disease.

These figures also show that the Gulf of Mexico site had no fish with Grade 4 liver disease, suggesting that fish in this body of water had either not yet reached that state of disease, were less prone to this degree of disease, or were not consuming as much. This could also be a result of the geographic time lapse that pertains to the lionfish invasion, as lionfish were initially documented in water sites near the Florida coast, and it has been determined that a spatial and temporal lapse in lionfish distribution occurred (Simmons 2014). It is also important to note that the data of Site 2 may be skewed due to higher sample size (24 versus 11). Overall, numbers suggest that Site 3 had more specimens with higher grades of disease as compared to the other sites. This suggests that fish from the Atlantic Ocean may have arrived with damaged livers as compared to those

of the Gulf of Mexico and Caribbean Sea. The fish with the least damaged livers at the time of dissection were taken from the Caribbean Sea.

Percent similarity indices were as expected. All sites have similar water temperatures (NOAA 2017) albeit different temperature ranges, and as previous analyses indicated no significant difference between sex or standard length in the overall degree of disease, then similarity should be relatively high. Sites 1 and 2 were most similar, and make up the Gulf of Mexico while Sites 2 and 3 were also highly similar and are both localities surrounding Florida.

The liver analysis portion of this research aimed to provide baseline data on the effects of sex, standard length, and life cycle stage (adult/juvenile) had on degree of fatty liver disease. Although this study shows that these factors do not play a statistically significant role in the degree of disease, this is the first study to monitor these factors in the invasive lionfish. As fatty liver disease in fish (farmed, aquaria, etc.) is often the result of a diet too rich in lipids (Leatherland and Woo, 2010), too low in lysine (Lie 2008), or due to a genetic predisposition (Zhenyu 2014), it is possible that lionfish are genetically predisposed to this condition. The results of this study are more in line with this hypothesis, though dietary nutrient composition was not observed and could also play a role in the observed fatty liver disease. As with all research, it is imperative that more studies be conducted to collect evidence to support these claims, especially in the native range. Studies of nutritional requirements and normal liver condition of lionfish in the native range are negligible (Fishelson 1997), and these studies would be crucial to determine the absolute cause of this disease. This study provides an important step in

determining the cause of fatty liver in lionfish, though any effects of this disease on the
fish have not been determined and it is unlikely that this disease will inhibit the invasion.

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APPENDIX A

Previously described parasites of lionfish from both the introduced and native ranges.

Parasite species with overlapping ranges are marked with *.

Invaded Range		Native Range	
Taxa	Species	Taxa	Species
Acanthocephala	*Dollfustentis sp.*	Ciliophora	Trichodinid ciliates
Acanthocephala	*Paracavisoma chromitidis*	Copepoda	*Acanthochondria sp.*
Acanthocephala	*Serrasentis sp.*	Copepoda	*Colobomatus pteroisi*
Cestoidea	*Nybelinia sp.*	Copepoda	*Taeniacanthus miles*
Cestoidea	*Otobothrium dipsacum*	Copepoda	*Taeniacanthus pteroisi*
Cestoidea	*Scolex pleuronectis*	Hirudinea	*Trachelobdella lubrica*
Cestoidea	*Tentacularia sp.*	Monogenoidea	*Benedenia epinepheli*
Copepoda	*Acanthochondria sp.*	Monogenoidea	*Haliotrema pteroisi*
Copepoda	*Caligus lobodes*	Myxozoa	*Sphaeromyxa zaharoni*
Copepoda	*Lernaeenicus cf. polyceraus*	Trematoda	*Cainocreadium pteroisi*
Digenea	*Bivesicula caribbensis*	Trematoda	*Proneohelicometra aegyptensis*
Digenea	*Helicometrina nimia*		
Digenea	*Lecithochirium floridense*		
Digenea	*Neotorticaecum sp.*		
Digenea	*Pachycreadium crassigulum*		
Digenea	*Stephanostomum sp.*		
Digenea	*Tergestia sp.*		
Hirudinea	*Trachelobdella lubrica*		
Isopoda	*Aegiochus tenuipes*		

(continued)

	Invaded Range	Native Range
Isopoda	*Alcirona krebsii*	
Isopoda	*Carpias serricaudus*	
Isopoda	*Eurydice convexa*	
Isopoda	*Excorallana sp.*	
Isopoda	*Gnathia sp.*	
Isopoda	*Nerocila acuminata*	
Isopoda	*Rocinela signata*	
Monogenoidea	*Psuedempleurosoma carangis*	
Monogenoidea	*Udonella caligorum*	
Nematoda	*Anisakis simplex*	
Nematoda	*Capillaria sp.*	
Nematoda	*Contracaecum sp.*	
Nematoda	*Goezia sp.*	
Nematoda	*Hysterothylaceum sp.*	
Nematoda	*Paracuria adunca*	
Nematoda	*Raphidascaris sp.*	
Nematoda	*Spirocamallanus sp.*	

Note. Paperna, 1976; Dojiri and Ho 1988; Ogawa et al. 1995; Hassanine 2006; Ruiz-Carus et al. 2006; Celik and Aydin, 2006; Bullard et al. 2011; Poole 2011; Simmons 2014; Fogg et al. 2016; Ramos-Ascherl 2015; Diamant et al. 2014; WoRMS 2009.

APPENDIX B

Frequency distributions of fatty liver degree of disease (grade) and average fat cell counts

for all specimens observed.
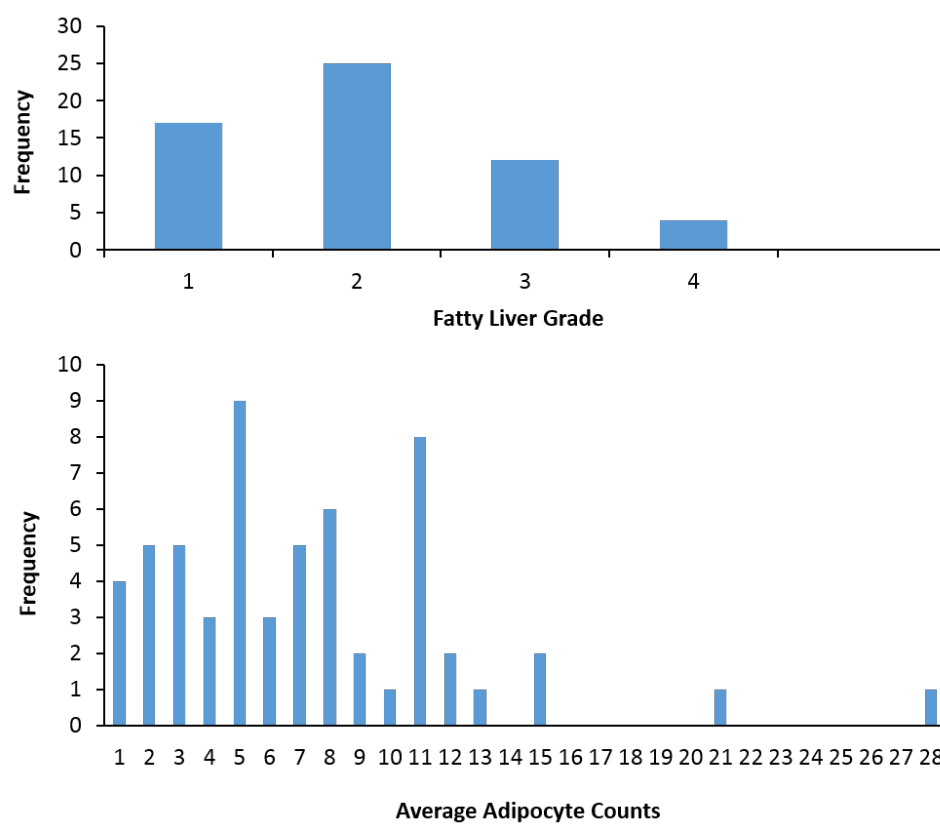
VITA

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<u>**Education**</u>
MS in Biology   **2015-2017**
    Sam Houston State University
BS in Biomedical Sciences   **2015**
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<u>**Research Experience**</u>
Research Assistant   **2015-2017**
Texas Invasive Species Institute, Sam Houston State University
- Graduate Assistant to Dr. Smith-Herron, conducting ecological and parasitological research on invasive lionfish

Research Assistant   **2013-2014**
TRIES Laboratory, Sam Houston State University
- Assistant to Dr. Hargrave, conducting ecological research on a variety of small freshwater fishes

<u>**Volunteer Experience**</u>
Undergraduate Research Symposium, SHSU   **2016**
- Served as a moderator for the poster and oral presentations

Shadowing Experience, Dr. Basil Long, DVM   **2013-2014**
- Assisted with surgeries, animal husbandry, and instrument care.

Brazos County Animal Shelter   **2011-2012**
- Responsible for assisting staff with cleaning, administering medicines, and animal restraint.

<u>**Work Experience**</u>
Veterinary Technician, Willis Animal Hospital   **2014-2015**
- Responsible for filling prescriptions, animal restraint, anesthesia monitoring, animal dentistry, X-ray processing, administration of vaccinations, and conduction of labs.

Laboratory Assistant, SHSU Biology Dept.   **2014**
- Assisted freshman biology courses by providing the necessary materials for each week.

<u>**Student Societies**</u>
Biological Sciences Graduate Student Organization, SHSU   **2015-Present**

Pre-Veterinary Society, SHSU   **2012-Present**

<u>Professional Memberships</u>

American Fisheries Society   **2017-Present**
Texas Invasive Plant and Pest Council   **2016-Present**
Southwestern Association of Parasitologists   **2016-2017**
Texas Academy of Science   **2015-Present**

<u>Scientific Conferences</u>

American Fisheries Society   **2017**
- Oral presentation

Texas Academy of Science   **2016-2017**
- Served as judge; submitted grant proposal

Texas Invasive Plant and Pest Convention   **2016**
- Poster presentation

<u>Honors and Awards</u>
- Best Poster – TIPCC Convention ($500.00)   **2016**
- Emma Normand Biological Sciences Scholarship ($2000.00)   **2014-2015**

<u>Skills</u>
- Proficient in Microsoft Word, PowerPoint, Excel, and Outlook
- Adobe InDesign, Dreamweaver and Photoshop experience
- Typing speed of 95 wpm
- Proficient in AMIRA programming for digital CT work