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LIFE HISTORY OF THE NON-NATIVE INVASIVE RED LIONFISH (*PTEROIS VOLITANS*) IN THE NORTHERN GULF OF MEXICO

by

Alexander Q. Fogg

A Thesis
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ABSTRACT

LIFE HISTORY OF THE NON-NATIVE INVASIVE RED LIONFISH (*PTEROIS VOLITANS*) IN THE NORTHERN GULF OF MEXICO

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May 2017

Invasive Red Lionfish (Pterois volitans) were first detected in the northern Gulf of Mexico (nGOM) in 2010 and since then their numbers have increased dramatically. From 2010 to 2015, more than 15,000 Red Lionfish were collected opportunistically from the nGOM for this study. Length and weight relationships differed significantly among ecoregions by sex and there was clear sexual dimorphism in size with males being larger and heavier. Red Lionfish age ranged from 0-4.5 years old and males achieved greater growth rate (K) and asymptotic maximum lengths (Linf) compared to females and these parameters were also different by ecoregion. Total length at 50% maturity was greater for females compared to males. Histological examination of female gonads resulted in the confirmation of asynchronous oocyte development. Additionally, the accuracy of reproductive phases identified macroscopically were significantly different than the corresponding phase identified microscopically. Gonadosomatic index values were elevated for both males and females from May-October, coinciding with elevated water temperatures. On average, a female Red Lionfish in the nGOM is capable of spawning every 2.49 days, 11 months out of the year. Mean batch fecundity was 26,904 eggs and mean relative batch fecundity was 92.2 eggs/g of gonad free body weight and peaked during the warmer months. From this information, an average size mature female of 188.6g is capable of producing 2,332,490 eggs/year. This study provides the most comprehensive description of invasive Red Lionfish reproduction, age and growth, to date, in the nGOM and will be used in creating management plans.

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Charters and Canyon Coolers for their logistical support collecting lionfish specimens. Specific individuals I would like to thank as this work would not have been possible without them; Bryan Clark, Anna Clark, Grayson Shepard, Allie ElHage, John McCain, Chris Barton, Meaghan Faletti, Andy Ross, Lawren McCaghren, Gary Emerson, Mark Christy, Candy Hansard, Dennis O'Hern, Paul Grammer, Trevor Moncreif, Trey Driggers, Bill D'Antuono, William Stein, Dylan Hubbard, Bryon Holland, Wil Demuth, Terry Miguad, Steve Hartley, Craig Newton, Greg Onorato, Tommy Phelps, Bob Brown, Paul Cozic, Lenny Maiolatesi, Barry Shively, Carrie Jones, Alex Page, Brad Riles, Brian Belzer, Andre Debose, Chris Stafford, Kristen Dahl, Rick Warren, Phil Horen, Ritchie Zacker, Lynn Wilson, Cory Trier, Brittany Barbara, Heyward Mathews, Stephen Sanders, Caitlin Wessel, Walter Ingram, Patrick Green, Butch Ayala, Scott Bartell, Clint Retherford, Mark Konz, Lew Bullock, Julia Reeves, Lynn Wagenseil, Jeff Phillips, Mark Miller, Chad Toner, Troy Boudreaux, Forrest Phillips, Joe Haire, Charlie Meyling, Kevin Trusler, Paul Miller, JD Ellington, Scott Forbes, David Murphy and all other volunteer divers and fishermen for their support. We also thank all of the undergraduate interns Brandi Willis, CJ Duffie, Cody Jones, Alicia Monroe, Judith Gonnello, Kristen Alley, Meagan McKenzie, Jennifer Gross, Eileen Gibson, and Aimee Rust for laboratory assistance in processing lionfish. Histological processing was completed by L. Bustamante, Texas A&M Veterinary Pathology Laboratory.

DEDICATION

To my parents; Glenn, Linda, John and Ellen who not only encouraged me to pursue my graduate degree but were also there to provide support, especially during the hard times. I also want to dedicate my thesis to my aunt Alice and uncle Paris who were very supportive of my work, especially while I was traveling in southwest Florida. Lastly, I would like to dedicate this thesis to my girlfriend Meaghan who always encouraged me to complete my graduate degree after moving to Florida. I love you all!

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BF	Batch fecundity
CA	Cortical alveolar
CI	Confidence interval
DFRF	Degrees of freedom from the full model
DFRR	Degrees of freedom from the reduced model
EMM	Estimated marginal means
ETOH	Ethanol
g	Gram
GFBW	Gonad free body weight
GOM	Gulf of Mexico
GSI	Gonadosomatic index
GW	Gonad weight
H	Hydrated
K	Instantaneous growth rate
K-S	Kolmogorov-Smirnov
L_{inf}	Asymptotic maximum length
L-W	Length and weight
mm	millimeters
MSRF	Mean sum of squares from the full model

NBF	Neutral buffered formalin
NE	Northeast
nGOM	Northern Gulf of Mexico
OM	Oocyte maturation
PG	Primary growth
POF	Postovulatory follicle complex
RBF	Relative batch fecundity
rmANOVA	Repeated measures analysis of variance
SE	Southeast
SL	Standard length
SSRF	Residual sum of squares from the full model
SSRR	Residual sum of squares from the reduced model
SST	Sea surface temperature
SW	Spineless weight
t_0	Time at which length is zero
TL	Total length
TL_{50}	Total length at 50% maturity
TW	Total weight
U.S.	United States
VTG1	Primary vitellogenic
VTG2	Secondary vitellogenic

VTG3	Tertiary vitellogenic
W	West

CHAPTER I – INTRODUCTION AND SUMMARY OF INVASIVE LIONFISH (*PTEROIS VOLITANS*) IN THE NORTHERN GULF OF MEXICO

Terrestrial and aquatic non-native (as defined in Occhipinti-Ambragi and Galil 2004) invasive species are a growing problem around the world and can have both immediate and long-lasting effects on native ecosystems, as well as economic impacts (Pimentel et al. 2005). In the United States (U.S.) alone, there are more than 50,000 non-native invasive species costing more than $120 billion a year (2001 estimate) from their effects and implemented control measures (Pimentel et al. 2005). Within aquatic ecosystems, introductions of non-native species can result from both intentional and unintentional releases (Jenkins 1996). The most common intentional releases include introductions to replenish a stock in rivers, for biological control of another non-native species, stock enhancement for recreational fisherman, or habitat restoration activities. An example of an intentional release occurred when non-native Chinook Salmon (*Oncorhynchus tshawytscha*) were introduced into the Great Lakes as a biological control of Alewife (*Alosa pseudoharengus*) that invaded in 1954 (Madenjian et al. 2002). Unintentional releases often result from movements of species through canals, transport in ballast water, and escape of aquarium and aquaculture species. For example, the Brown mussel (*Perna perna*) has found its way to the Gulf of Mexico (GOM) through international shipping ballast water (Hicks et al. 2001), whereas in the 1990's, Nile Tilapia (*Oreochromis niloticus*) were accidently released into Mississippi waters from an aquaculture facility (Peterson et al. 2005, Grammer et al. 2012) and they quickly adapted to the surrounding environment. Although little to no direct feeding competition has been seen between Nile Tilapia and native species (Peterson et al. 2006), there is the possibility that Nile Tilapia may be preying on native fish eggs (Martin et al. 2010), thus negatively impacting the native ecosystem.

The occurrence and proliferation of non-native aquatic organisms is of concern, as the ecological impacts on native aquatic organisms are usually negative. For example, Northern Snakehead (*Channa argus*) is clearly a detrimental species of fish that invades and overtakes inland waters of the U.S. They can survive a wide range of environmental conditions, are piscivorous with no natural predators, and have the potential to alter entire ecosystems relatively rapidly (Gascho Landis et al. 2011). Alterations to these food webs and ecosystems are from top-down mechanisms where native top predators are removed and replaced by the non-native Northern Snakehead (Madenjian et al. 2002, Gascho Landis et al. 2011).

There have been instances of marine fishes establishing themselves in non-native habitats. In the Hawaiian Islands, there was a program instituted in the mid-1950's to introduce snapper and grouper species to the local waters as a way to enhance nearshore fisheries (Randall 1987).   Peacock Hind (*Cephalopholis argus*) were among the introduced species and it is now an apex predator in heavily fished near shore fishing areas. The main reason for their high ecological position is they were no longer valued as a food fish when found to be a carrier of ciguatoxin (Dierking 2009). While not established, non-native species have been detected in marine waters offshore the southeast U.S.

Panther Grouper (*Chromileptes altivelis*), for example, have been detected and removed on a number of different occasions offshore in Florida (USGS-NAS 2015), although it is not likely that breeding populations have been successfully established (Johnston and Purkis 2013). Panther Grouper are thought to have been introduced through the aquarium trade (Semmens et al. 2004).

From May 2004 to May 2005, more than 11 million marine fish (1,802 species) were imported to the continental U.S. for the aquarium trade, and 33 of those species have been successfully introduced into continental U.S. waters (Rhyne et al. 2012). Of those 33 introduced species, only lionfish (*Pterois volitans* and *P. miles*) have become established (Morris and Akins 2009). Lionfish are native to the Indo-Pacific and have been collected and sold worldwide as aquarium fishes (Albins 2011). Lionfishes are now widely reported to occur in U.S. waters of the western North Atlantic Ocean and have become established along the Atlantic coast of the U.S. and Caribbean (Morris and Akins 2009). The two lionfish species can only be separated genetically (Hamner et al. 2007) although, recent genetic work by Johnson et al. (2016) only detected *P. volitans* in the nGOM.

The first documented capture of a lionfish (*Pterois* spp.) in the western North Atlantic was in 1985 off Dania Beach, Florida (USGS-NAS 2015). Genetic analysis revealed that the most likely vector of introduction was a result of multiple aquarium releases off the southeast coast of Florida (Betancur-R et al. 2011). The next reports occurred in southeast Florida in 1992 although reports were not common until the early 2000's (USGS-NAS 2015). Once introduced, lionfish initially spread from Miami into Bahamian waters. Today, lionfish can be found from Massachusetts to the Florida Keys, the Bahamas and Caribbean Sea (Morris 2009, Schofield 2009, 2010), although thermal tolerance prevents lionfish from overwintering in the northern reaches of their range (Kimball et al. 2004). Due to the relatively rapid spread of this species through the waters of the east coast of the U.S. and Bahamas, there was major concern that the lionfish would spread into the GOM (Whitfield et al. 2007). This concern proved valid as the first lionfish sighting and collection was reported in 2006 off St. Petersburg, FL, although this collection is questioned as the specimen was found dead (Schofield 2009) and likely not a migrant from the Caribbean Sea or east coast of the U.S. The next documented sightings in the GOM were in 2009 off the west coast of Florida and the northern Yucatan peninsula (Aguilar-Perera 2010; USGS-NAS 2015). Lionfish have been slow to invade areas south of Venezuela likely due to the Amazon-Orinoco discharge plume (discharge from the Amazon River), which may act as a natural barrier and can potentially slow the spread of lionfish into the region (Luiz et al. 2013). However, the first record of lionfish south of the Amazon-Orinoco discharge plume was recently reported off the coast of Brazil and it is thought that this was as a result of a long-distance larval dispersal event (Ferreira et al. 2015).

Lionfish are now found in the nGOM in higher densities than any other invaded region, particularly on artificial reefs (Dahl and Patterson 2014). Within the nGOM, hard bottom reef habitats are much less common than in more southern regions (Parker et al. 1983), but thousands of artificial reefs and oil production platforms of varying size and depths have been deployed throughout the nGOM (Szedlmayer and Shipp 1994, Kaiser and Pulsipher 2005) to provide additional habitat for native species, thus creating structural habitat. It is uncertain if the presence of artificial reefs throughout the region has facilitated the spread of invasive lionfish into the nGOM where suitable reef habitat may not have been otherwise present.

To help better understand lionfish impacts and manage their effects, lionfish derbies are held to remove lionfish from local waters; derby activities are shown to decrease lionfish densities on a localized scale (Barbour et al. 2011, Green et al. 2014). This idea that ‘culling' works has spread throughout their invaded range and recently into the nGOM. Fogg et al. (2017) showed that the number of lionfish derbies and other spearfishing tournaments that have included a lionfish category have increased over the last five years in the nGOM and there are differences in lionfish total length by year and location. Although lionfish are captured in hook and line, trap, and trawl fisheries, SCUBA divers armed with spears is the most efficient means of capture (Fogg et al. 2015).

Fortunately, lionfish are considered a delectable food fish and in recent years, the presence of lionfish on the menu of restaurants around their invaded range has increased partially due to their superior nutritional value to many other native fish (Morris et al. 2011). Pasko and Goldberg (2014) addressed the numerous potential benefits and roadblocks associated with of the commercialization of invasive species and according to their criteria, invasive lionfish are a prime species for commercialization on localized scales.

In the present study, lionfish were collected throughout the nGOM from the Florida Keys to South Padre Island, Texas. Due to the large geographic area of these collections, three sampling ecoregions were specified: southeast (Florida Keys to north 28.25°), northeast (north 28.25° to west 88°), and west (west of 88° to the Mexico border) (Figure 1).

These ecoregions were identified based on known biogeographic criteria. The northern tip of Anclote Keys, FL was defined by Beck and Odaya (2001) as a north-south break, and Mobile Bay, AL is an east-west break (Balsam and Beeson 2003), and these definitions are used in the present work. The western ecoregion can extend as far south as north 22.25° (Beck and Odaya 2001), but for the purpose of this study, only U.S. waters were considered. These ecoregions were used to make comparisons across the nGOM in later chapters. Because of the large geographic area compared in this study, seasons were categorized for each ecoregion based on similar thermal conditions. To do this, sea surface temperatures (SST, °C) were compiled from the NOAA's National Data Buoy Center database using four years of data from four buoys in each ecoregion with the exception of the southeast ecoregion where only three buoys were available with four years of data (Figure 1, Table 1). Mean SST was calculated for each ecoregion, month and day to determine reasonable seasons and ensure similar seasonal comparison of life history characteristics among ecoregions (Table 2). Based on these data, seasons were defined for all ecoregions as spring (March–April; mean SST 19.0–25.0 °C), summer (May–

October; mean SST > 25.0 °C), fall (November– December; mean SST 19.0–25.0 °C), and winter (January–February; mean SST < 19.0 °C).

Red Lionfish were collected opportunistically throughout the nGOM from the three ecoregions by fishers, spearfishers (divers using pole spear or speargun), commercial trawl operations, and during fishery-independent bottom trawl surveys such as those conducted in Switzer et al. (2015). Since 2012, spearfishing tournaments that have added Red Lionfish as a category and Red Lionfish specific derbies in the nGOM have been on the rise (Figure 2).

Interest in Red Lionfish derbies have increased likely due to dive industry engagement resulting from outreach, education and the noticeable increase of lionfish sightings on recent dives. From 2012 to 2014, 11,783 Red Lionfish were collected during 14 Red Lionfish specific derbies throughout the nGOM, although the majority of the derbies were held east of the Mississippi River in the northeast and southeast ecoregions (Table 3). At a minimum, collection date, location (<5 km), and depth (m) associated with capture were provided with each specimen. After collection, many specimens were frozen and subsequently thawed prior to processing in the laboratory; however, about 52% of specimens were processed in the field shortly after capture. Total length (TL, mm), standard length (SL, mm) and total weight (TW, g) were measured to the nearest 0.1 g. In instances where Red Lionfish were provided without spines, the equation TW = [spineless weight (SW) + 0.6100] / 0.9581 (Fogg et al. 2013) was used to calculate total weight**.**

Chapter II discusses Red Lionfish age and growth across the nGOM. More than 4,000 otoliths were removed from Red Lionfish over the course of this study; a subsample of 1,607 otoliths (744 males, 716 females, 147 unknown or unsexed) or 40% of all otoliths collected, were processed for age and growth information (Table 3). The range in sizes and mean size of Red Lionfish across ecoregions was comparable (Table 3) and will be compared in more detail in Chapter II.

In Chapter III, information on Red Lionfish reproductive life history characteristics is presented as well as comparisons of these metrics across ecoregions in the nGOM. This comprehensive chapter used a number of different subsamples from the overall 16,000 Red Lionfish collected (Table 3). For this study, 4,527 gonads (2,280 male, 2,247 female) were processed to calculate gonadosomatic index values for each month and region. A total of 71 fecundity samples (10 southeast, 43 northeast, 18 west) were taken and represent the largest number of fecundity samples of any previous study (Table 3). For verification of macroscopic reproductive phase classification accuracy, 547 histological samples were processed. These histology samples were also used to calculate length at 50% maturity (L_{50}) for both males and females and identify the sex of smaller immature fish.

In Chapter IV, I synthesize the invasive life history characteristics including the most complete age, growth, and reproductive parameters. This information will help guide future research projects and provide invaluable data to help mitigate the potential affects this species is having on native ecosystems.

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Table 1

Buoys used to estimate sea surface temperature

Latitude	**Longitude**	**Buoy ID**	**Ecoregion**	**Years of SST Data Used**
27.340	-84.275	42099	Southeast	4
27.498	-83.721	42022	Southeast	4
27.169	-82.920	42013	Southeast	4
30.065	-87.555	42012	Northeast	4
28.794	-86.006	42039	Northeast	4
29.408	-84.858	SG0F1	Northeast	4
28.500	-84.517	42036	Northeast	4
28.982	-94.899	42043	West	4
27.896	-93.597	42047	West	4
28.867	-90.483	SPLL1	West	4
29.212	-88.207	42040	West	4

List of buoys used to estimate mean sea surface temperature for seasonal differentiation.

Table 2

Mean sea surface temperature by month

Month	Southeast	Northeast	West	Pooled
January	19.76	18.08	17.46	18.43
February	19.25	17.73	17.76	18.25
March	20.33	18.99	19.17	19.50
April	22.91	21.83	21.80	22.18
May	25.84	25.07	24.93	25.28
June	28.45	28.56	28.62	28.54
July	29.47	29.39	29.46	29.44
August	30.18	29.95	30.05	30.06
September	29.21	28.86	28.90	28.99
October	27.31	26.20	26.14	26.55
November	24.08	23.06	22.79	23.31
December	21.76	20.47	20.18	20.81

Mean sea surface temperature (°C) by month using four years of data from four buoys in each ecoregion with the exception of the southeast ecoregion where only three buoys were available with four years of data

Table 3

Summary of Samples

	SE	NE	W	All
Red Lionfish collected (Male)	944	1,184	368	2,496
Red Lionfish collected (Female)	721	1,245	354	2,320
Red Lionfish collected (Unsexed or Unknown)	4,487	10,657	72	15,216
Range in TL (mm) and mean (Male)	148-434 (276)	116-426 (270)	155-419 (276)	116-434 (273)
Range in TL (mm) and mean (Female)	86-353 (223)	96-368 (232)	92-361 (241)	86-368 (231)
Range in TL (mm) and mean (Unsexed or Unknown)	62-426 (255)	47-419 (236)	45-389 (236)	45-426 (239)
Range in TL (mm) and mean (All)	62-434 (255)	47-426 (239)	45-419 (257)	45-434 (243)
Histology samples (Female)	48	266	33	347
Batch fecundity	10	43	18	71
Gonads weighed for GSI (Male)	835	1,099	346	2,280
Gonads weighed for GSI (Female)	669	1,230	348	2,247
Otoliths removed (Male)	627	723	317	1,667

Otoliths removed (Female)	537	761	286	1,584
Otoliths removed (Unsexed or Unknown)	524	236	29	789
Otoliths processed (Male)	278	259	207	744
Otoliths processed (Female)	204	284	228	716
Otoliths processed (Unsexed or Unknown)	55	72	20	147
Number of lionfish-specific derbies from 2012-2014	4	9	1	14
Number of spearfishing tournaments with lionfish category from 2012-2014	14	10	8	32

Summary of samples collected for use in this study in the northern Gulf of Mexico. SE = southeast ecoregion; NE = northeast ecoregion; W = west ecoregion.

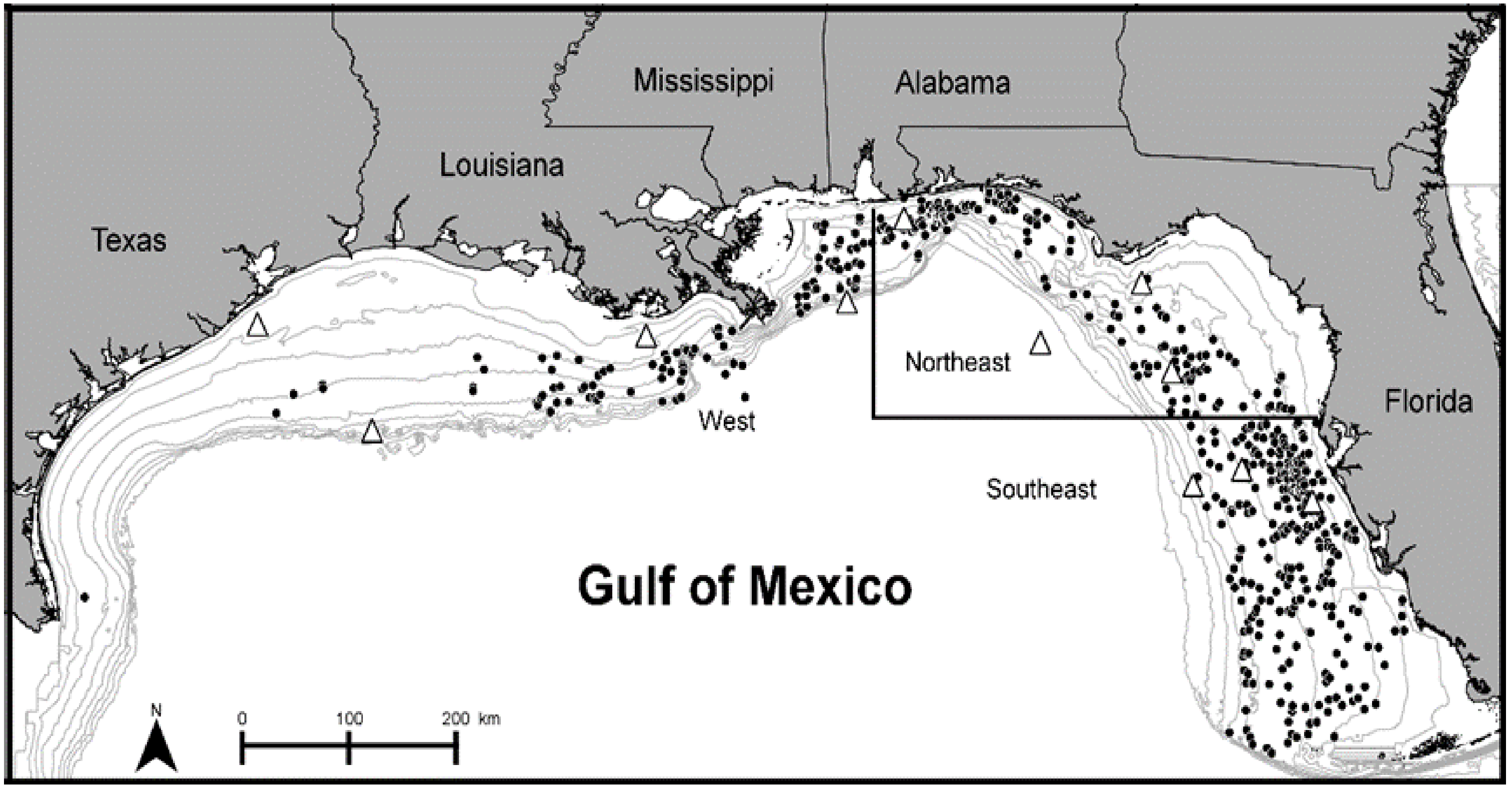

Figure 1. Map of established sampling ecoregions

Map of established sampling ecoregions. Southeast (Florida Keys to north 28.25°), northeast (north 28.25° to west 88°), ad west (west of 88°). Black dots represent sample locations and triangles indicate buoy locations used for ecoregional sea surface temperature.

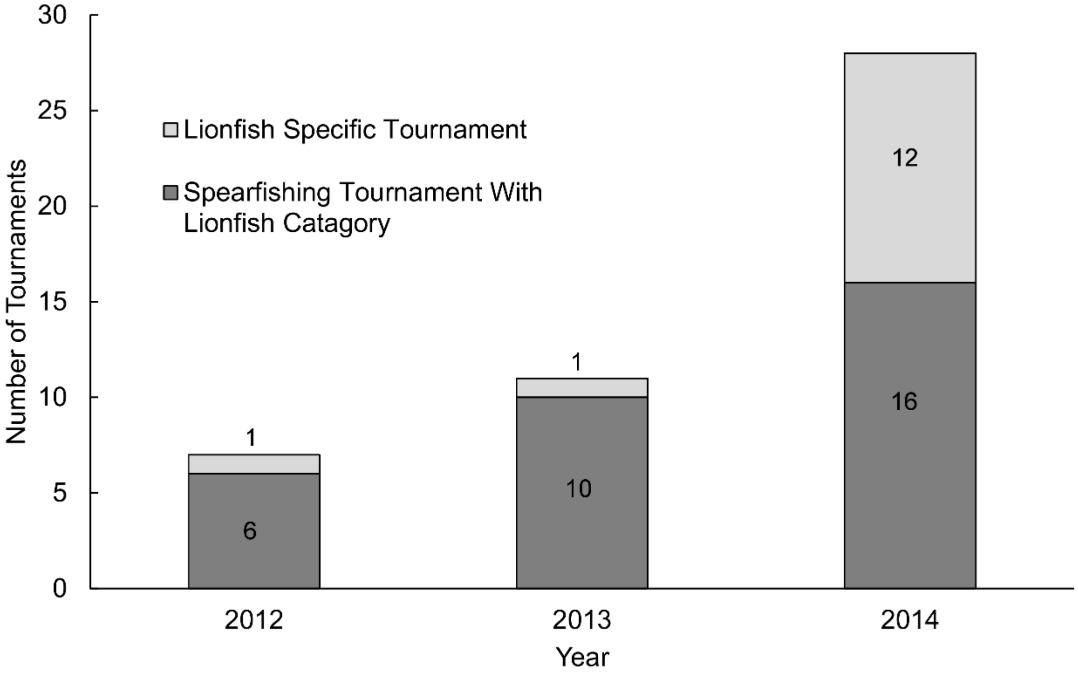

Figure 2. Lionfish spearfishing tournaments by year

Number of spearfishing tournaments with a Red Lionfish category and Red Lionfish specific tournaments in the northern Gulf of Mexico since the first event in 2012.

CHAPTER II - AGE AND GROWTH OF INVASIVE LIONFISH (*PTEROIS VOLITANS*) IN THE NORTHERN GULF OF MEXICO

Introduction

Information on length-weight (L-W) relationships and age and growth patterns are important for the successful management of species and can be used to assess the effects of invasive species on native species and the ecosystem they inhabit. Changes in size structure over time may be a useful indicator for management success (i.e., decrease in body size of invader) although metrics such as L-W are generally examined to quantify changes in size structure relating to potential over-exploitation of a species (Dulvy et al. 2004). Changes or truncation of age structure is another common indicator of overfishing (Berkeley et al. 2004), and in the case of invasive species could be used as an indicator of management success (Pasko and Goldberg 2014).

While there have been a number of studies that report length and weight data for invasive lionfish (Barbour et al. 2011, Fogg et al. 2013, Dahl and Patterson 2014, Edwards et al. 2014, Sabido-Itzá et al. 2015), few make comparisons between regions or sexes. Published comparisons range from reporting pooled L-W data across a range of locations (Barbour et al. 2011; Edwards et al. 2014; Sabido-Itzá et al. 2015) to comparing pooled L-W data by year showing a significant difference between the first and last year (2011-2013) (Dahl and Patterson 2014). In contrast, Fogg et al. (2013) compared L-W relationships between male and female lionfish in the northern Gulf of Mexico (GOM) and found no differences for fishes collected in 2012. However, the invasion into the nGOM was relatively recent (2010), and maximum sizes for each sex were likely not reached. Finally, Pusack et al. (2016) showed that lionfish in their native range grow at a slower rate and achieve smaller maximum sizes compared to those in the invaded range. These authors suggested that lionfish in their invaded range may be less susceptible to predation due to their larger size and would also be able to consume larger prey items.

In addition to regional comparisons of L-W relationships within their invaded range, age and growth relationships are important metrics to describe the life history of a species as well as to make sound management decisions. Two lionfish collected off the South Carolina coast (352 mm and 389 mm TL) were determined to be 5 and 6 years old, respectively (Meister et al. 2005), whereas lionfish captured in Onslow Bay, North Carolina had a maximum age of 8 years, with more than 90% of the fish (n = 814) being < 3 years old (Potts 2010, Barbour et al. 2011). However, these are not relatively old ages as lionfish in captivity can live up to 30-33 years (Potts 2010). Rodríguez-Cortés et al. (2015) and Johnson and Swenarton (2016) produced age and growth data for lionfish from the southern GOM and offshore northeast Florida / Florida Keys, respectively, using length-based modeling. Rodríguez-Cortés et al. (2015) provided the first age and growth parameters for the southern GOM region although the modeled parameters were not verified using otoliths. Johnson and Swenarton (2016) verified their model outputs with ages determined from a subsample of sectioned otoliths. Edwards et al. (2014) provides one of the most comprehensive invasive lionfish age and growth studies to date where a total of

499 lionfish (110 males and 128 females) from offshore Little Cayman were collected and aged using otoliths. The maximum age reported was five years old and once-yearly annuli formation was confirmed in the Caribbean region which had not been reported in earlier studies.

Because lionfish invaded different regions of the GOM and Caribbean Sea at different times (Schofield 2010) and can be found in vastly different ecosystems (Barbour et al. 2010, Jud et al. 2011, Claydon et al. 2012, Ruttenberg et al. 2012) and at various densities (Dahl and Patterson 2014), it is expected that age and growth parameters may vary by location. Additionally, rapid growth rates generally lead to the successful invasion of invasive species and thus are important to estimate (Copp and Fox 2007). Although age and growth has been reported for portions of the invaded range, it has been suggested that the growth of lionfish in more southern regions of their non-native range could differ (Barbour et al. 2011). The age structure of lionfish in the nGOM is expected to be much younger than other invaded regions where they have been established for a longer time period. The goal of this study was to determine if differences in L-W and age and growth relationships exist among ecoregions and by sex.

Methods

Red Lionfish were collected from the nGOM following methods described in Chapter I. Total length-total weight (TL-TW; TL in mm, TW in g) relationships were calculated by sex and ecoregion and these data were used to estimate their power function. The power function used was $TW = aTL^b$, for which a is the intercept and b is the slope (Anderson and Neumann 1996). The TL-TW data were $\log_{10}$ transformed prior to analysis with an Analysis of Covariance (ANCOVA), with TL used as a covariate. An ANCOVA was first completed for each sex separately comparing the TL-TW relationships across ecoregions ($n = 3$). If no significant differences were found, sex was pooled by ecoregion and a second ANCOVA was completed comparing males and females pooled by ecoregion. If the TL-TW relationships for any ANCOVA analysis violated the homogeneity of slopes assumption (parallelism) of ANCOVA, then separate models were used. Estimated marginal means (EMM) were also used to make observations of TW adjusted for mean TL between sexes. All data were tested for normality (Shapiro-Wilk's) and homogeneity of variance (Levene's test), and if violated a log transformation was applied and the data were reanalyzed. Relationships were considered significant when $\alpha < 0.05$.

Following Secor (1991), otoliths were removed by making a dorso-ventral incision from the top of the head to the preopercle. Gripping the head and body, the incision was widened to expose the brain. With a pair of forceps, the brain was removed exposing the two sagittal otoliths. The otoliths were then extracted, rinsed with water and allowed to dry on a paper towel. Once dry, the otoliths were stored in labeled vials to prevent damage prior to sectioning.

The left sagittal otolith was used to determine the age of Red Lionfish across ecoregions. Small 22 x 22 x 20 mm embedding molds (Poly Sciences) were used to mount the otoliths in a resin block. A small layer of resin mixture (West Systems Resin and Hardner) was placed in the embedding mold and allowed to dry/cure for a minimum of 24 h. The left sagittal otolith was centrally oriented in the labeled embedding mold and the resin mixture was poured into the tray until it completely covered the otolith.

After drying, the resin block was coarsely sanded to smooth the edges so that it could be steadied for the sectioning process, and a straight line was drawn across the block to indicate the ideal location for a representative section for aging. This ideal section resides near the junction of the ostium and sulcus (VanderKooy and Guindon-Tisdel 2003) revealing a “V” shaped grove in the otolith with distinct annuli radiating outward from this point. A Diamond Wheel saw was used to cut sections from the block containing the otolith. The block was mounted in the holding vice on the saw and the saw blades lined up on the line that was created earlier. Due to the small size of the otoliths, a three-blade system was used. Each blade was spaced with 300 µm spacers and one single cut was made resulting in two sections. Sections were mounted on glass slides using cytoseal and allowed to dry for 24 hrs. After drying, sectioned otoliths were viewed under a microscope to see which section captured the best record of the fish’s age.

The prepared otoliths were examined by two independent readers to determine age. When the otolith section was viewed through the microscope there were two distinct bands that can be seen. One band is opaque and is formed during slow growth periods while the other band is translucent and is formed during periods of faster growth (Vanderkooy and Guindon-Tisdel 2003). After both readers aged all of the samples independently, the ages were compared. Any discrepancies between the two readers were reexamined to determine the age. If a consistent age could not be determined, the otolith was removed from analysis. Following agreement, marginal increments were measured (0.001 mm) and compared to the width of the previous complete annuli using the following equation (Tanaka et al. 1981): $C = W_n/W_{n-1}$, where C is the index of completion, W_n is the width of the marginal increment, and W_{n-1} is the width of the previous complete annulus. This method was used to confirm the periodicity of annuli formation and to determine higher resolution age data at an accuracy of 0.25yr. Since daily rings were not read, it is likely there was an underestimation of growth.

Because our sample collections lacked smaller fishes (< 100 mm), a truncated normal distribution was used to fit a three-parameter von Bertalanffy growth curve to the age data, and separate model parameters were determined for each sex and ecoregion for comparison (Diaz et al. 2004). Due to the von Bertalanffy growth curve being non-linear, a sum of squares reduction test (Schabenberger and Pierce 2002) was used instead of a traditional ANOVA to determine if there were differences in growth between ecoregions and sex by comparing non-linear trends between groups ($\alpha=0.05$). A sum of squares reduction test is done by fitting a full and reduced model to the data. The test statistic (F) is calculated following the equation: $F = [(SSRR-SSRF) / (DFRR-DFRF)] / MSRF$, where SSRR and SSRF are the residual sum of squares from the reduced and full model, respectively, and DFRR and DFRF are the residual degrees of freedom for the reduced and full model, respectively. Lastly, MSRF is the mean sum of squares from the full model. This test evaluates the SSRF when the SSRR is removed from the model.

Results

While TL was recorded for more than 15,000 Red Lionfish, only 4,670 measurements had a corresponding TW. Male Red Lionfish (ANCOVA: $F_{2,2406}$ = 4.174, p = 0.015) and female Red Lionfish (ANCOVA: $F_{2,2264}$ = 15.882, p < 0.001) showed significantly different slopes by ecoregion, and therefore have different TL-TW relationships (Figure 3, Table 4; see Appendix 1 for plot of $\log_{10}$ transformed data sets). Although TL-TW relationships were significantly different by ecoregion, the differences were minimal (females differences = 18.21g; males differences = 22.75g; Table 5) and male Red Lionfish achieve a greater mean TW (333.62 ± 3.58) compared to females (195.13 ± 3.69) (Table 5). Pairwise ecoregional comparisons of TL-TW relationships (Table 5) revealed a significant difference for male Red Lionfish collected in the southeast and northeast ecoregions (ANCOVA: $F_{1,2038}$ = 8.159, p = 0.004; Table 4B), while female Red Lionfish showed significant differences in all ecoregional comparisons except between the southeast and west ecoregions (ANCOVA: $F_{1,1025}$ = 0.606, p = 0.436; Table 4B). Pooled ecoregional Red Lionfish TL-TW data revealed significantly different slopes by sex (ANCOVA: $F_{1,4670}$ = 21.96, p < 0.001; Table 4B) suggesting females have a steeper TL-TW relationship than males (Figure 4, Table 4A; see Appendices 2 for plot of $\log_{10}$ transformed data sets) and thus have a greater TW at a given TL. However, males attain larger TW overall than females (Table 5).

A total of 4,250 pairs of otoliths were extracted and a subset of 1,609 otoliths were randomly selected and processed from Red Lionfish ranging from 81-434 mm TL. Of those, age agreement was reached on 1,412 pairs of otoliths (87.8%). Table 3 in Chapter I highlights the breakdown of samples collected for age and growth analyses. Annual increment formation was confirmed using marginal increment analysis with marginal increments being most complete in May and gradually decreasing until reaching a minimum index of completion in October and November, indicating the beginning of annuli formation (Figure 5). Red Lionfish ages ranged from 0.50 to 4.50 years old (Figure 6) with 93% of aged lionfish being < 2 years old (see examples in Figure 7).

There were significant differences in age and growth parameters by sex and by ecoregion (Tables 6 and 7, all $p < 0.01$; see Figure 8). Female Red Lionfish from the southeast ecoregion had the highest growth rate (K) and asymptotic maximum length (L_{inf}) followed by the northeast and west ecoregions. Female Red Lionfish from the southeast ecoregion achieved a greater length-at-age than in the other two ecoregions (Figure 8A, Table 7B). Similarly, male Red Lionfish from the southeast ecoregion also had the highest K and L_{inf} followed by the northeast and west ecoregions. As with females, male Red Lionfish from the southeast ecoregion achieved a greater length-at-age than in the other two ecoregions (Figure 8B, Table 7B).

Data were pooled by sex and ecoregion to allow for comparisons with previous studies (Table 6). Data pooled by sex revealed the same pattern as separate male and female analyses, with the southeast ecoregion exhibiting the highest K and L_{inf} values and the west ecoregion showing the lowest (Figure 8C, Table 6 and 7). Separate comparisons were made by sex for each ecoregion (Figure 9, Table 7A). Male Red Lionfish achieved higher K and L_{inf} values in all three ecoregions compared to females (all $p < 0.01$; see Figure 9A-C, Table 7A). Model parameters determined from data pooled by ecoregion suggested males exhibited higher K and L_{inf} than females (Figure 9D, Table 6 and 7).

Discussion

The current study revealed significant regional and gender patterns in lionfish age and growth and TL-TW relationships. These metrics are important to document for Red Lionfish in order to measure potential changes to the population due to implemented management plans. For example, Chagaris et al. (2015) recently modeled potential lionfish management strategies and how lionfish will impact several native recreationally and commercially important species on the West Florida Shelf. Lionfish life history data (e.g., age and growth relationships) used in the model were largely from other regions and thus may not have accurately reflected lionfish population dynamics for the West Florida Shelf. There have also been a number of other management plans drafted from around the invaded region (Morris 2012, ANSTF 2014, Johnston et al. 2015) that cite age and growth research and parameters. While Morris (2012) and Johnston et al. (2015) specifically mention the need for age and growth, ongoing research is only mentioned in Johnston et al. (2015), and no mention of current age and growth parameters are reported from Red Lionfish invaded range. Information reported in ANSTF (2014) highlights a number of invasive lionfish age and growth studies and their reported growth parameters. While the information presented in these plans is helpful, updates will need to be made so that current research and findings are being used in future work.

Length-weight, age and growth metrics may be used for developing region specific age-structured population models that can be used to evaluate potential effects of targeted removals on the lionfish population such as what was conducted in Barbour et al. (2011). Further, the data presented here can also be coupled with other life history data to inform management decisions that will help mitigate the effects Red Lionfish are having on the native fishes and their ecosystems in the nGOM. The data presented here may also be used to predict future impacts of invasive Red Lionfish to the native ecosystem as well as provide insights to managing other potential marine invasive fishes that pose a similar threat to the region. Identifying spatial and temporal patterns to determine harvest vulnerability of older and larger lionfish has proven to be an effective management strategy to protect native species, as targeted regions and seasons can be more heavily protected or regulated as needed (Zhou et al. 2010, Tobin et al. 2013). The opposite management technique can be employed for lionfish if these locations or times of year can be identified. Age, growth and TL-TW relationship metrics are important to document for invasive species like Red Lionfish in order to measure potential changes to the population due to implemented management plans.

Marginal increment analysis for Red Lionfish in this study showed that annuli were most complete in the spring (March-May) and least complete in the fall (Sept-Oct). This observation is similar to that seen for another scorpaenid, the native Blackbelly Rosefish, off the coasts of North Carolina and South Carolina (White et al. 1998). Unlike what was observed for Blackbelly Rosefish and Red Lionfish, Black Scorpionfish from the Adriatic Sea showed the most complete annuli in the late summer (July-Sept) (La Mesa et al. 2010). Comparable thermal regimes (nGOM vs. Carolinas) are likely the reason for the similar annuli formation trends between invasive Red Lionfish and native Blackbelly Rosefish. Black Scorpionfish from the Adriatic Sea likely see delayed annuli completion compared to Red Lionfish and Blackbelly Rosefish from the nGOM and Atlantic Ocean due to relatively cooler spring SST (~11°C) that warm to above 20°C in July (La Mesa et al. 2010). The SST in July in the Adriatic Sea is similar to SST observed in March in the nGOM. Thus, water temperature seems to be driving annuli completion in these species.

Age determination was difficult to assess for invasive Red Lionfish in this study, as demonstrated with the 87.8% agreement in age determination between readers. This was similar to what was observed offshore northeast Florida where 93% reader agreement was reported (Johnson and Swenarton et al. 2016). However, our age determination agreement is relatively high compared to Edwards et al. (2014) who reported only 42% agreement between readers for lionfish collected in the Caribbean. Regional differences may be expected in accuracy, as annuli in lionfish collected in tropical waters (where minimal change in water temperature throughout the year results in relatively consistent growth;

Pitcher and Hart 1982) will likely be harder to distinguish vs. lionfish living in more seasonal regions (with slow and fast growth periods).

Ages of nGOM Red Lionfish ranged from 0 to 4.5 years ($\bar{x}$ = 1.35), which is significantly lower than the maximum reported age of 30 years for an aquarium specimen (Potts et al. 2010). It's not apparent whether lionfish could achieve this age in the Gulf of Mexico, however. Interestingly, the oldest back-calculated age confirms lionfish presence in the nGOM as early as 2008, two years prior to first detection in 2010. Edwards et al. (2014) also back-calculated a single lionfish (*Pterois* spp.) to before their first detection in Little Cayman in 2010. The documented delayed detection of invasive Red Lionfish from this study and Little Cayman is expected as invasive species are often not detected immediately after introduction due to lag times associated with invasive species expansion (Crooks et al. 1999).

Age distribution of marine fish species is an import metric for assessing the health of a population (Berkeley et al. 2004). Typically, an established, healthy population will exhibit a 'well balanced' age structure (Brunel and Piet 2013) with numerous larger, older individuals. In the nGOM, all three ecoregions showed an age distribution of 93% of fish $\leq$2 years. Similar results were reported in Little Cayman (Edwards et al. 2014), northeast Florida (Johnson and Swenarton 2016) and North Carolina (Barbour et al. 2011), where the majority of fish were $\leq$3 years old (>90%). The higher proportion of relatively older fish found in Little Cayman, northeast Florida and North Carolina is likely the result of lionfish having invaded those locations earlier. While Red Lionfish live much older than the majority of the population that has been sampled in their invaded range, the observed age class distribution is further indication that the population may still be stabilizing in the region, as the older individuals are not present or at least observed. Red Lionfish that were aged from the southern GOM (Rodríguez-Cortés et al. 2015) appeared to be much younger, as age and growth parameters were much lower than those reported in this and all previous studies. This is likely a result of Red Lionfish being collected within the first two years of the invasion in the southern GOM. It may also be due to the difference in aging techniques as this study determined age based on otoliths while Rodríguez-Cortés et al. (2015) estimated ages based on size-frequency analysis and was not verified using otoliths. It will be important to examine age and growth parameters in the future when the invasion has theoretically stabilized in the region.

Lionfish collected from the southeast ecoregion exhibited higher growth parameters compared to the northeast and west ecoregion. While it is expected that K would be greater in the southeast ecoregion, L_{inf} in southern regions is usually lower than more northern regions (Boehlert and Kappenman 1980). This anomaly may be a result of density-dependent growth as Red Lionfish collected from the northeast ecoregion especially, came from much smaller and isolated artificial and natural reefs and were thus found in much higher densities compared to the southeast region (Fogg, unpublished data) where density-dependent growth may not have been as much of a factor.

While the Black Scorpionfish (S*corpaena porcus*), native to the eastern Atlantic, Mediterranean and Black Sea, achieve a smaller maximum size (TL = 320 mm; Bilgin and Celik 2009), there are clear differences in maximum size by sex as females achieve large sizes compared to males. This is the opposite for invasive Red Lionfish found in the nGOM, as males achieve greater TL and TW than females. A potential reason that male Red Lionfish grow larger than females is a result of male rivalry and thus physical combat to increase opportunities for mating (Shine 1989). Additionally, evidence of combat between large Red Lionfish (presumably male) has been observed in the nGOM in the form of abrasions across the body (Fogg, per. observation). Another similar species, the Blackbelly Rosefish (*Helicolenus dactylopterus dactylopterus*), is a scorpionfish native to the western Atlantic Ocean with a similar body size compared to Red Lionfish (White et al. 1998). Like invasive Red Lionfish, male Blackbelly Rosefish achieve greater sizes compared to females and can achieve ages of up to 30 years old (White et al. 1998).

Sexual dimorphic growth was documented for invasive lionfish by Edwards et al. (2014), although this was done by otolith analysis only and TL-TW relationships were not evaluated. Early work in the nGOM showed no statistical difference in TL-TW relationships between male and female Red Lionfish (Fogg et al. 2013), although since data collected for that study was from early in the invasion, lionfish size distribution had likely not reached an asymptotic value.

Though von Bertalanffy growth parameters were not reported by sex in the other age and growth studies on invasive lionfish, age and growth and TL-TW data quantified in this study confirms that sexual dimorphism exists as males grow larger and faster than females. Male Red Lionfish in this study achieved a greater length-at-age than females, and similar to Edwards et al. (2014), had a significantly larger K and L_{inf} compared to females. The differences observed between male and female Red Lionfish age and growth and TL-TW relationships is likely a result of increased resource allocation going toward reproductive output in females (Gadgil and Bossert 1970). In the case of Red Lionfish, females mature at an early age and are capable of reproducing every few days eleven months out of the year (see Chapter III). This elevated and constant reproductive rate, likely results in more energy shunted to reproduction rather than growth, and therefore growth in female Red Lionfish is reduced. This trade-off of reduced growth in females as the energy is redirected to reproduction has been extensively described in numerous fish species (Reznick 1983, Roff 1983, Parker 1992). This information, coupled with reproductive life history information from the nGOM presented in Chapter III, verifies that Red Lionfish are capable of reproducing within the first year of life.

Growth rate and other life history traits have been shown to vary by region in other marine fishes (Choat and Axe 1996, Ruttenberg et al. 2005). The growth rate of Red Lionfish in the nGOM is greater than those reported from Little Cayman (Edwards et al. 2014), North Carolina (Barbour et al. 2011), and NE Florida (Johnson and Swenarton 2016), although K reported from the Florida Keys (Swenarton et al. 2015) and Yucatan, Mexico (Rodríguez-Cortés et al. 2015) was much higher than all studies (Table 6). The higher growth rate observed in the nGOM could explain why Red Lionfish densities are higher than anywhere else in their invaded range (Dahl and Patterson 2014). It also appears that nGOM Red Lionfish age and growth is most similar to what has been reported from NE Florida and North Carolina. Thus, similarities and differences among studies could be related to environmental thermal regimes rather than other biological and ecological factors. However, age and growth will need to be re-evaluated in the future as the current study consists of samples from early in the invasion for this region. Age and growth and TL-TW relationship metrics of Red Lionfish in the nGOM have not been comprehensively reported. This study not only provides vital life history metrics useful for sound management decisions, it also provides the first statistical comparison of Red Lionfish age and growth.

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Table 4

Total length-total weight regression equations and pairwise comparison results

A)		Male		Female	Stats
Ecoregion	N	Regression Equation	N	Regression Equation	
Southeast	857	$TW = 2.00x10^{-6}(TL)^{3.34}$	671	$TW = 1.00x10^{-6}(TL)^{3.44}$	$F_{1,1528} = 12.677, p < 0.001$
Northeast	1181	$TW = 3.00x10^{-6}(TL)^{3.26}$	1239	$TW = 3.00x10^{-6}(TL)^{3.30}$	$F_{1,2420} = 2.319, p = 0.128$
West	368	$TW = 3.00x10^{-6}(TL)^{3.30}$	354	$TW = 2.00x10^{-6}(TL)^{3.41}$	$F_{1,722} = 6.838, p = 0.09$
Pooled	2406	$TW = 3.00x10^{-6}(TL)^{3.29}$	2264	$TW = 2.00x10^{-6}(TL)^{3.37}$	$F_{1,4670} = 21.957, p < 0.001$

B)

Ecoregional Comparison	Males	Females
Southeast vs Northeast	$F_{1,2038} = 8.159, p = 0.004$	$F_{1,1910} = 27.721, p< 0.001$
Northeast vs West	$F_{1,1549} = 1.425, p = 0.223$	$F_{1,1593} = 10.186, p = 0.001$
Southeast vs West	$F_{1,1225} = 0.860, p = 0.354$	$F_{1,1025} = 0.606, p = 0.436$
Pooled Ecoregions	$F_{2,2406} = 4.174, p = 0.015$	$F_{2,2264} = 15.882, p < 0.001$

A) Total length-total weight regression line equations and pairwise comparison results for each ecoregion between sex and B) pairwise comparisons between ecoregions by sex for Red Lionfish from the northern Gulf of Mexico

Table 5

Estimated marginal mean values

Sex	Region	n	Mean ± SE
Female	Southeast	671	187.98 ± 1.69
Female	Northeast	1,239	193.88 ± 1.22
Female	West	354	206.19 ± 2.35
Female	Pooled	2,264	195.13 ± 3.69
Male	Southeast	857	325.20 ± 2.36
Male	Northeast	1,181	334.50 ± 2.01
Male	West	368	347.95 ± 3.61
Male	Pooled	2,406	333.62 ± 3.58
Pooled	Southeast	1,512	258.75 ± 4.79
Pooled	Northeast	2,328	253.52 ± 3.86
Pooled	West	694	294.96 ± 7.07

Estimated marginal mean total weight (g) adjusted for total length (mm) for males and females in each ecoregion and pooled. SE = standard error of the mean.

Table 6

Von Bertalanffy growth curve parameter estimates by region

	Northern Gulf of Mexico									
	Southeast	Northeast	West	Males	Females	Pooled	North Carolina	Little Cayman	Florida Keys / NE Florida	Yucatan, Mexico
L_{inf} (mm TL)	423.0	393.0	389.0	405.2	368.4	400.2	425.2	349.0	411.0 / 448.0	420.0
K	0.569	0.544	0.539	0.550	0.508	0.560	0.470	0.420	0.700 / 0.470	0.880
t_0	-0.155	-0.079	-0.341	-0.414	-0.482	-0.210	-0.500	-1.010	0.000 / 0.000	-0.107

Von Bertalanffy growth curve equation parameter estimates by ecoregion (southeast (Florida Keys to north 28.25°), northeast (north 28.25° to west 88°), and west (west 88° to Mexican border)) and sex. Due to truncated datasets, a sum of squares reduction test (Schabenberger and Pierce 2002) was used to compare model parameters between all ecoregions and sex. All comparisons were significantly different ($p < 0.01$). Parameters included from North Carolina (Barbour et al. 2011), Little Cayman (Edwards et al. 2014), Florida (Swenarton et al. 2016, Johnson and Swenarton et al. 2016), and Yucatan, Mexico (Rodríguez-Cortés et al. 2015) for comparison although parameters were calculated from pooled sex in these studies

Table 7

Von Bertalanffy-growth curve parameter by ecoregion

A)

Parameter	Pooled Sex			Male			Female		
	SE	NE	W	SE	NE	W	SE	NE	W
K	0.569	0.544	0.539	0.576	0.547	0.543	0.574	0.549	0.542
L_{inf}	423.0	393.0	389.0	426.0	394.4	390.7	382.0	366.8	360.9
t_0	-0.155	-0.079	-0.341	-0.170	-0.086	-0.354	-0.165	-0.089	-0.350
	$F_{12,1412} = 27.143$, $p < 0.001$			$F_{12,695} = 12.606$, $p < 0.001$			$F_{12,626} = 7.303$, $p < 0.001$		

B)

Parameter	Southeast		Northeast		West		Pooled Ecoregion	
	Male	Female	Male	Female	Male	Female	Male	Female
K	0.576	0.574	0.547	0.549	0.543	0.542	0.550	0.508
L_{inf}	426.0	382.0	394.4	366.8	390.7	360.9	405.2	368.4
t_0	-0.170	-0.165	-0.086	-0.089	-0.354	-0.350	0.414	-0.482
	$F_{8,453} = 2.412$, $p = 0.008$		$F_{8,489} = 2.012$, $p = 0.030$		$F_{8,379} = 2.362$, $p = 0.010$		$F_{8,1321} = 16.226$, $p < 0.001$	

Von Bertalanffy growth curve equation parameter estimates by ecoregion (southeast (SE; Florida Keys to north 28.25°), northeast (NE; north 28.25° to west 88°), and west (W; west 88° to Mexican border)) and sex. Comparisons made A) among ecoregion by sex and B) between sex by ecoregion

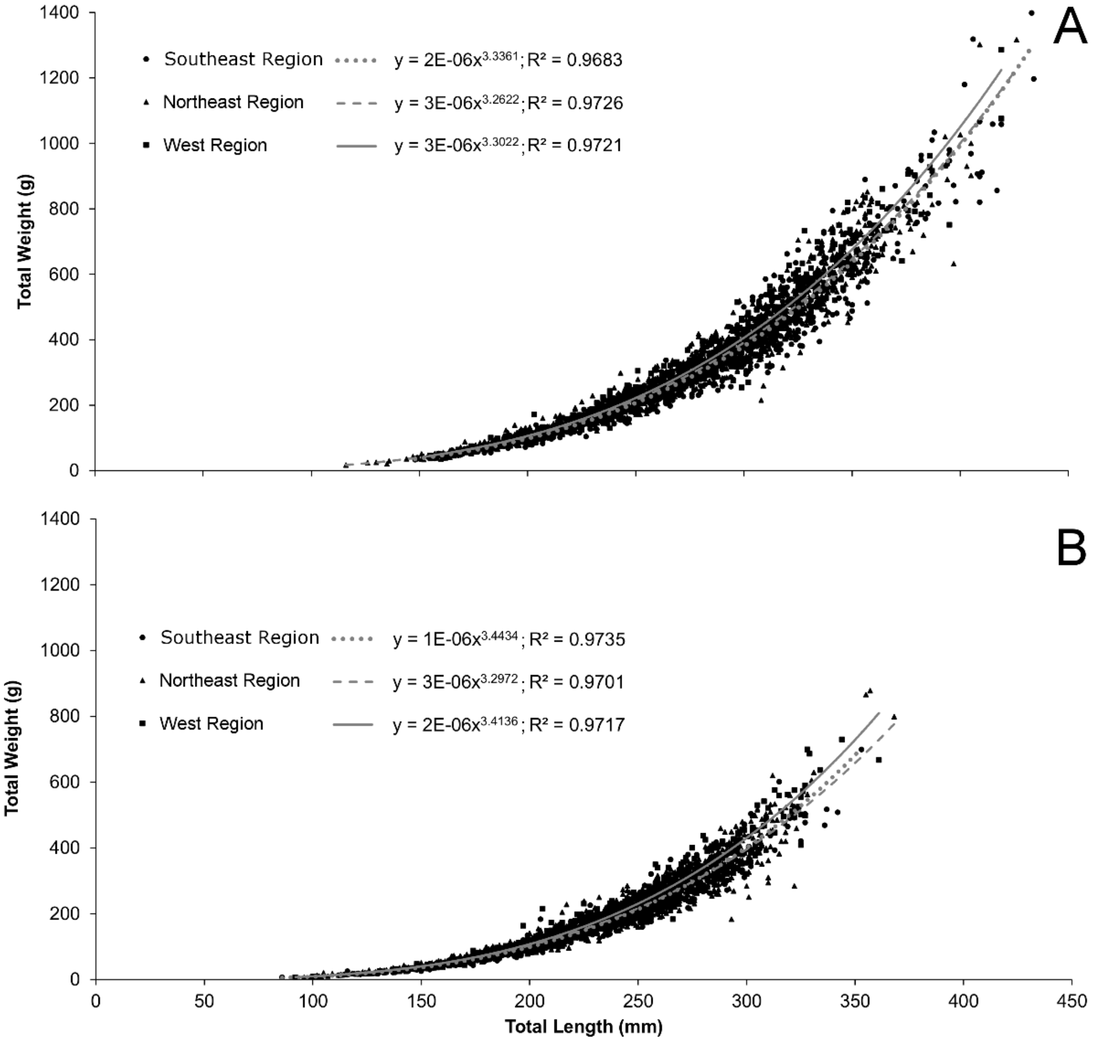

Figure 3. Total length-total weight relationship by ecoregion

Total length-total weight relationship by ecoregion: ● = southeast (Florida Keys to north 28.25°), ■ = northeast (north 28.25° to west 88°), and ▲ = west (west 88° to Mexican border) for A) male and B) female Red Lionfish.

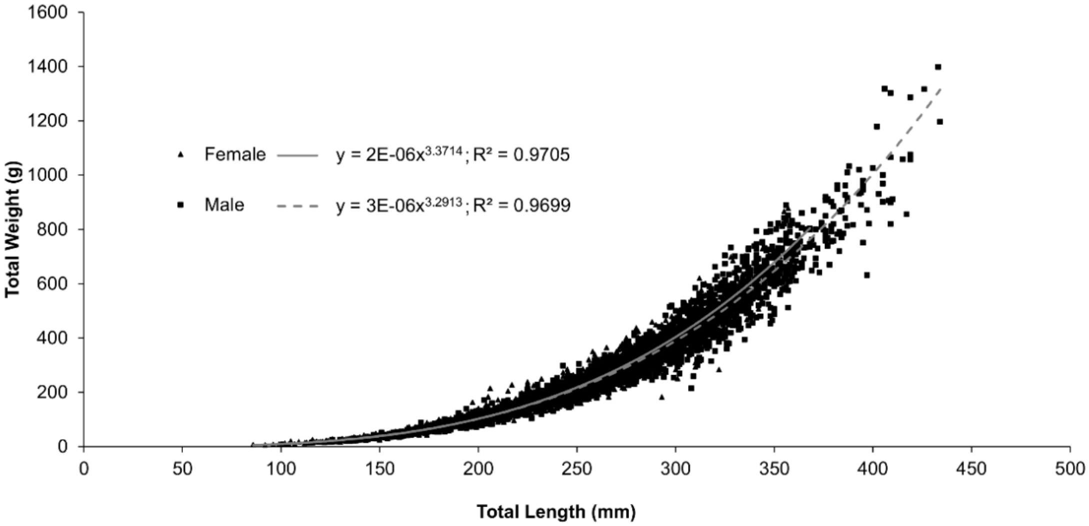

Figure 4. Total length-total weight relationship by sex

Total length-total weight relationship by sex (▲ = female; ■ = male) for all ecoregions pooled.

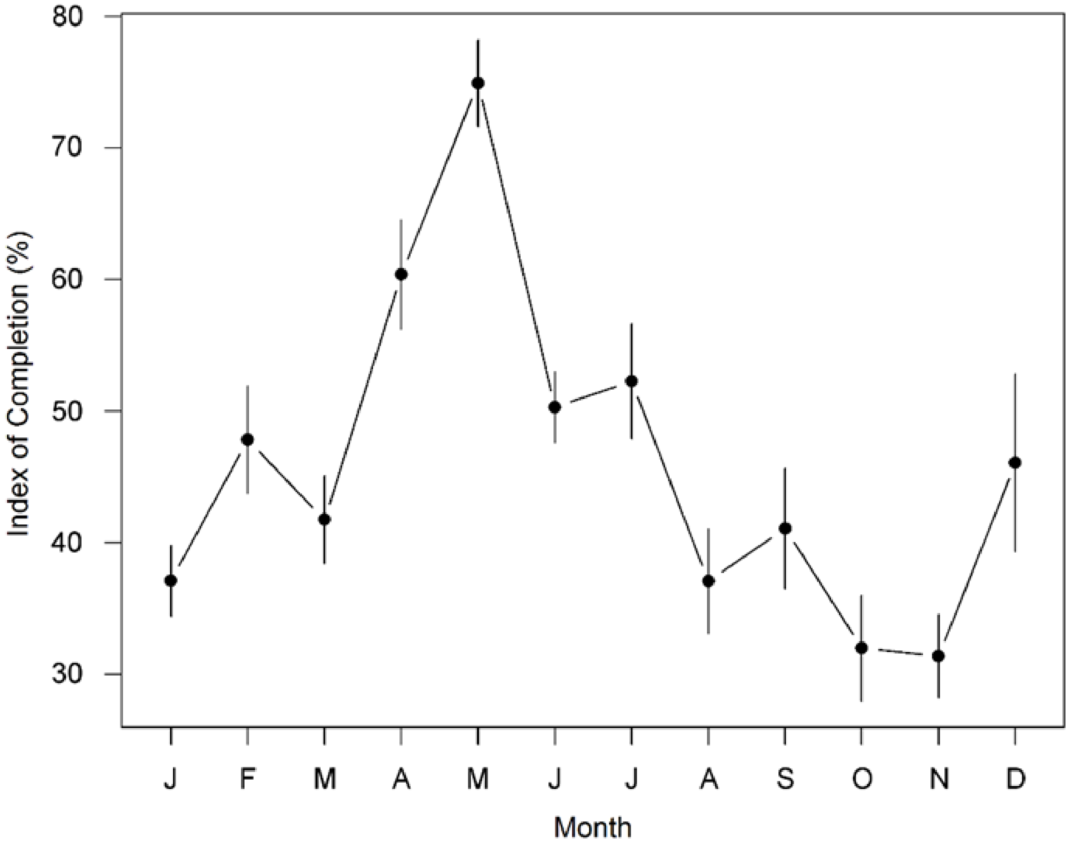

Figure 5. Mean index of marginal increment completion by month

Plot of the mean index of marginal increment completion by month (with 1 standard error of the mean). Number of samples ranged from 30 in December to 192 in June.

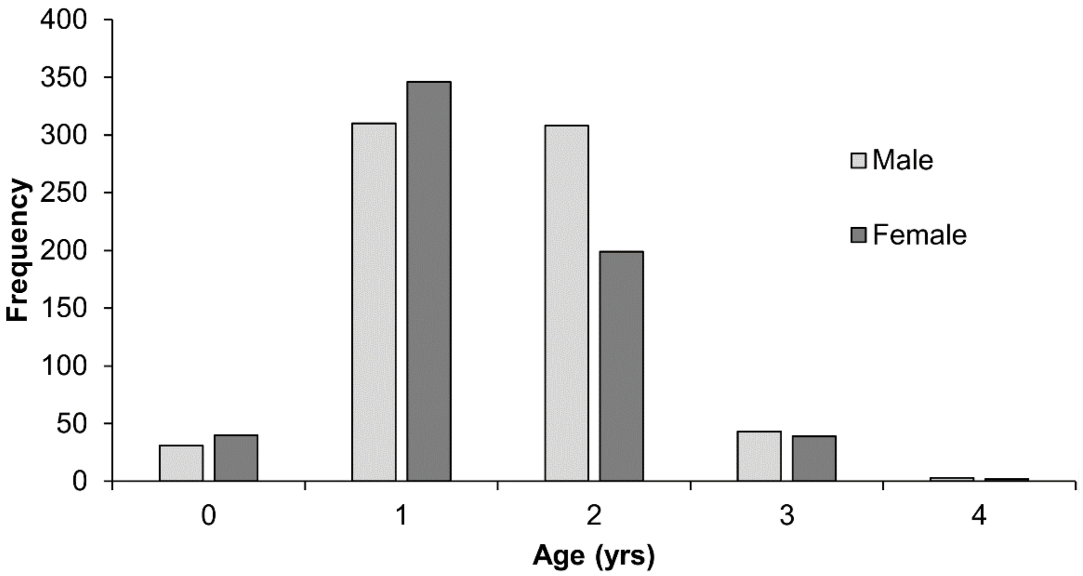

Figure 6. Age frequency distribution

Age frequency distribution (counts) of male and female lionfish collected from the northern Gulf of Mexico

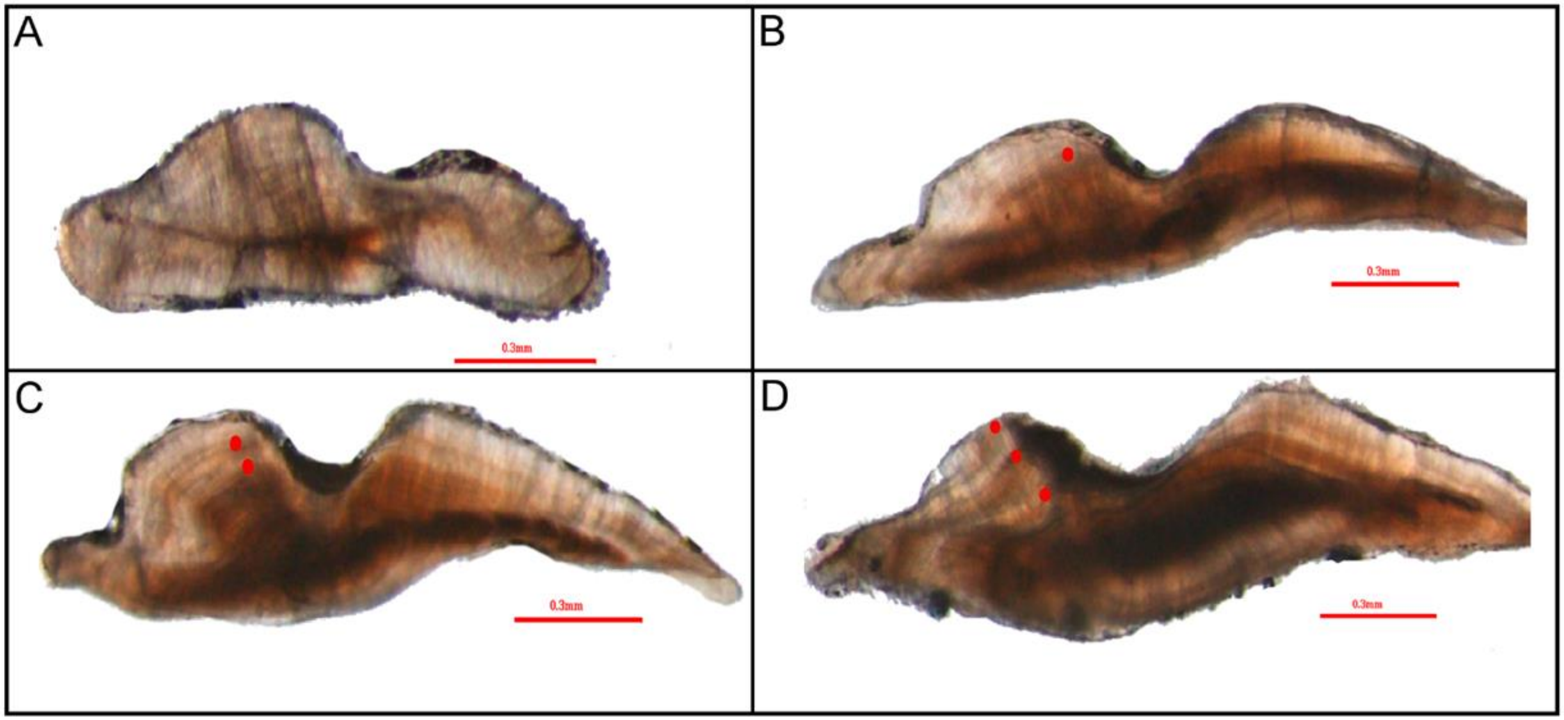

Figure 7. Annotated images of sectioned otoliths.

Annotated images of sectioned otoliths for four different ages. Red dot represents annuli. Percentages represent age distribution for regions and sexes combined. A. Age zero (8.6%). B. Age one (48.9%). C. Age two (36.2%). D. Age three (5.8%).

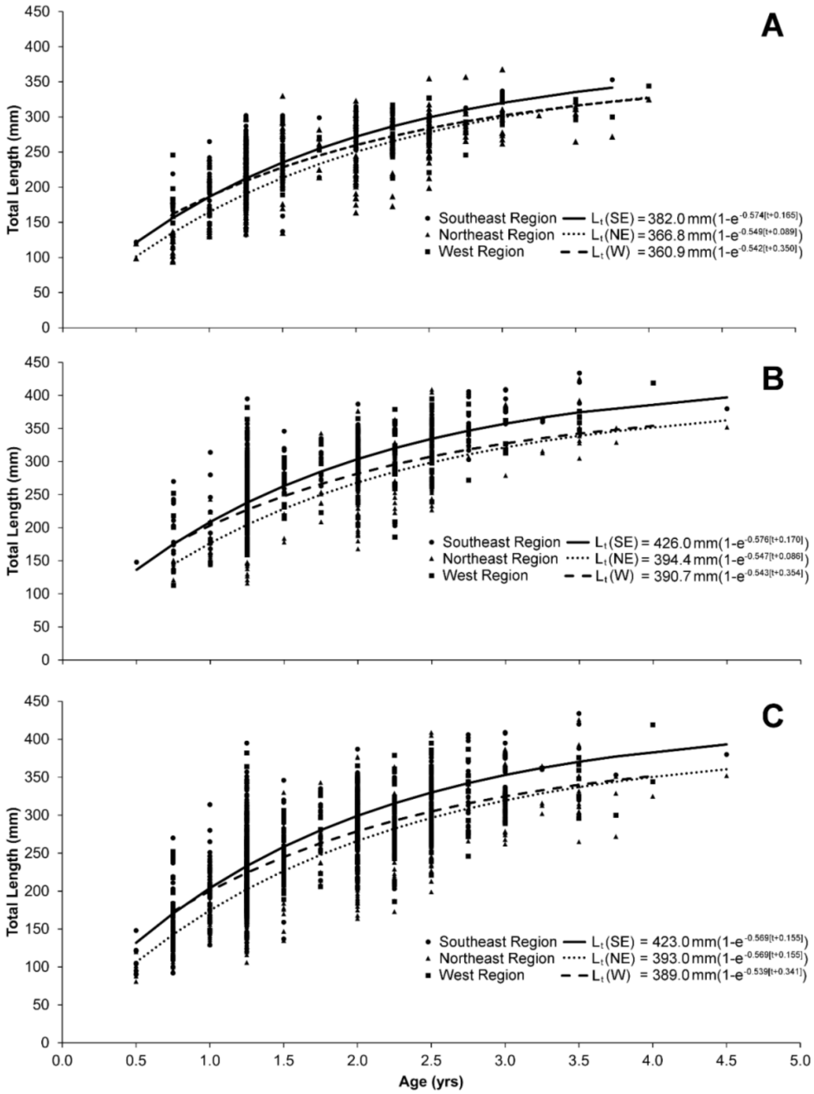

Figure 8. Von Bertalanffy growth curve by ecoregion

Von Bertalanffy growth curve and associated equations plotted with observed length-at-age by ecoregion: ● = southeast (Florida Keys to north 28.25°), ▲ = northeast (north 28.25° to west 88°), and ■ = west (west 88° to Mexican border) for A) Females, B) Males, and C) All sexes pooled.

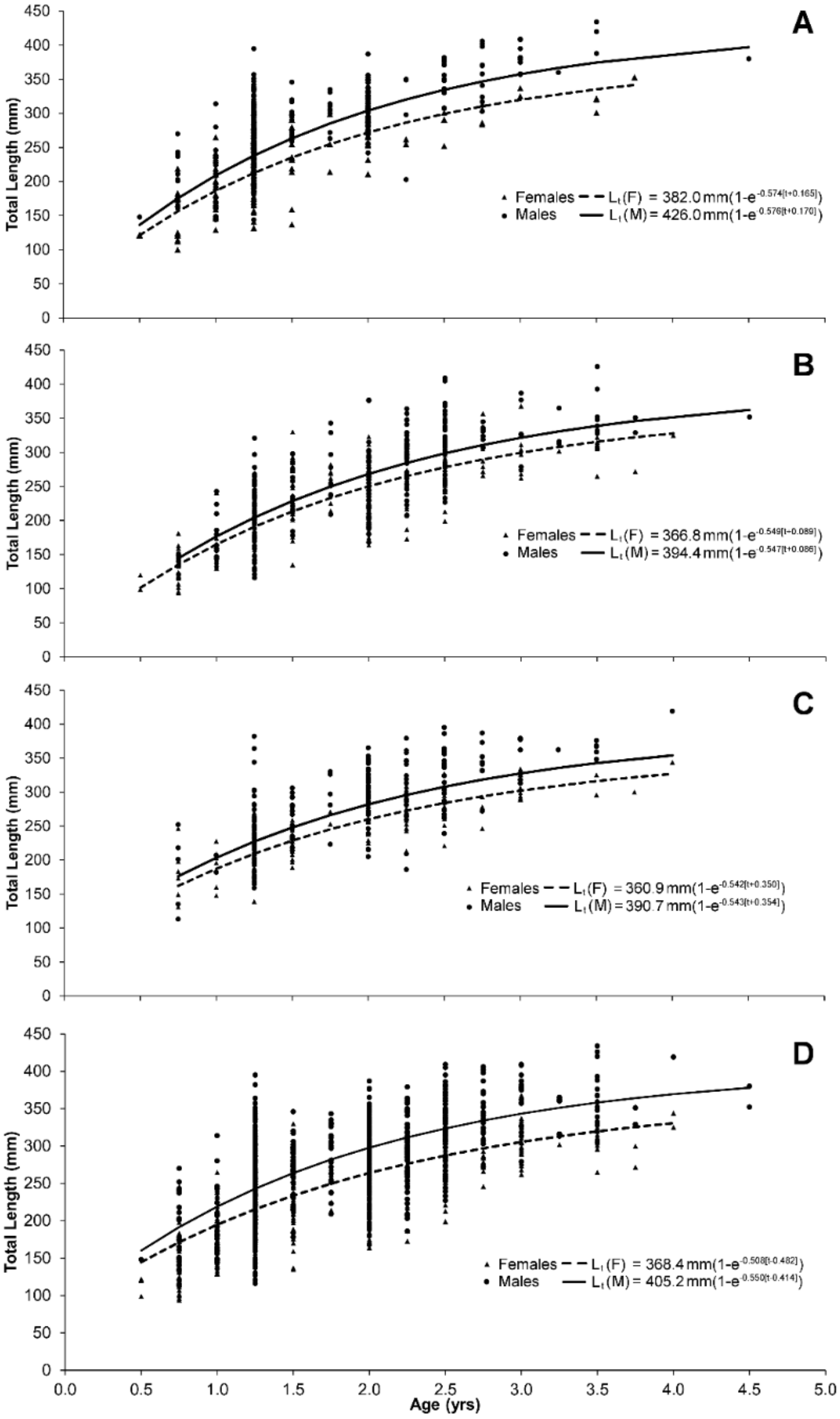

Figure 9. Von Bertalanffy growth curve by sex

Von Bertalanffy growth curve and associated equations plotted with observed length at age by sex (▲ = female; ■ = male) for the A) southeast ecoregion (Florida Keys to north 28.25°), B) northeast ecoregion (north 28.25° to west 88°), C) west ecoregion (west 88° to Mexican border), and D) all ecoregions pooled.

CHAPTER III – REPRODUCTIVE LIFE HISTORY CHARACTERISTICS OF INVASIVE RED LIONFISH (PTEROIS VOLITANS.) IN THE NORTHERN GULF OF MEXICO

Introduction

Invasive lionfish *Pterois volitans* and *P. miles* are established in U.S. waters of the western North Atlantic Ocean, northern Gulf of Mexico (nGOM) and Caribbean Sea (Morris and Akins 2009, Schofield 2010, Fogg et al. 2013). However, *P. miles* has not yet been detected in Gulf of Mexico waters (Johnson et al. 2016). Their reproductive capacity is thought to be an important factor in their invasion success (Morris et al. 2009; Gardner et al. 2015) and thus a detailed understanding of their reproductive biology is critical for managing the continuing invasion. Unfortunately, little is known of the reproductive biology of lionfish in their native range (Donaldson et al. 2011). Fishelson (1975) reported that Pacific Dwarf Lionfish *Dendrochirus brachypterus* (Cuvier, 1829) spawn every 6-8 days, 8 months out of the year in captivity, and the ovarian histology of Red Lionfish from the southeast coast of India has been described (Priyadharsini et al. 2013). The mean fecundity of Red Lionfish from India has been reported to be 75,547 eggs/spawn (Priyadharsini et al. 2013). However, no information on the reproductive seasonality of lionfish in their native range is available. Additionally, the unique reproductive strategy of specialized peduncular structures or stalks that support oocytes in the ovary and likely help to provide additional nutrients and oxygen, as well as prevent crowding of oocytes (Morris et al. 2011). Like many other scorpaenids, lionfish spawn their eggs in a buoyant gelatinous mass, which not only maximizes dispersal throughout their invaded range via ocean currents but also facilitates increased fertilization by reducing sperm dispersal (Morris et al. 2011).

Differences in the reproductive biology of lionfish have been apparent throughout their invaded range. Female Red Lionfish from the Atlantic Ocean (North Carolina and Bahamian waters) are reported to spawn about every four days, year around (Morris 2009). In contrast, female lionfish in Caribbean waters off Little Cayman were found to spawn every two to three days and have elevated (2.0+) gonadosomatic index (GSI) values throughout the calendar year (Gardner et al. 2015). Preliminary results in the nGOM suggest a similar but shorter period of peak spawning activity of May to October, though spawning capable females have been collected throughout all twelve months (Fogg et al. 2014, 2015, 2017). Interestingly, GSI values of Red Lionfish from the nGOM were lower than those values reported in by Gardener et al. (2015) from Little Cayman. While some histology has been completed on lionfish collected from Little Cayman (Gardner 2013) as it relates to spawning seasonality; reproductive histology documenting spawning seasonality in the nGOM has recently been completed confirming spawning capable Red Lionfish are found throughout the year and there is a greater proportion of spawning capable females in the warmest season (summer) compared to cooler seasons (fall, winter, and spring)(Fogg et al. 2017).

Perhaps one of the most important parameters for fisheries management and an accurate understanding of the reproductive potential of any species is batch fecundity. Dwarf Lionfish has a batch fecundity of only 3,000-6,000 eggs in captivity (Fishelson 1975), although these fish were captured in their native range and are generally much smaller than Red Lionfish. Red Lionfish batch fecundity has been estimated to be 1,800-41,945 eggs (female total length (TL) = 204-332 mm) for invaded areas of Little Cayman (Gardner et al. 2015) and 10,790-41,392 eggs (female TL = 250-350 mm) for the Bahamas and Carolinas (Morris 2009), although Morris (2009) determined batch fecundity from egg masses collected from spawning events in captivity. In contrast, batch fecundity of Red Lionfish found in the Indian Ocean, their native range, averages 75,547 eggs (Priyadharsini et al. 2013) but was determined by counting all eggs rather than only hydrated eggs as done in Morris (2009) and Gardner et al. (2015). Thus, batch fecundity values in the Indian Ocean may be grossly overestimated, so care must be taken when comparing those values to invaded regions.

The total length at 50% maturity (TL_{50}) of invasive lionfish has also been shown to be variable among different invaded areas. Gardner et al. (2015) found that TL_{50} of female lionfish in Little Cayman was 190 mm TL, which was 15 mm larger than lionfish that were pooled from North Carolina, South Carolina, the Bahamas, and the Philippines (Morris 2009). Gardner et al. (2015) stated that although the length at 50% maturity in Little Cayman differed from those values reported by Morris (2009), it may not be biologically significant since lionfish mature relatively early in life. Finally, size at 50% maturity was recently reported in the nGOM to be 166.6 mm TL for females and for145.2 mm TL, males (Fogg et al. 2017); this is smaller than reported in other parts of their invaded range.

The objective of this chapter is to provide detailed information on the reproductive biology of Red Lionfish in their invaded range. Specifically, 1) size and age at maturity; 2) sex ratios; 3) the effects of freezing and thawing on gonad weight; 4) comparison of macroscopic and histological ovarian assessments; 5) spawning seasonality as assessed macroscopically and histologically; 6) spawning frequency and 7) batch fecundity are reported for Red Lionfish in the nGOM. These results will be compared among the three distinct ecoregions. Additionally, these results will be compared to previous reports from both their native and invaded ranges.

Methods

Field Sampling

Red Lionfish were collected from locations throughout the nGOM within three distinct ecoregions as described in Chapter I. The TL, standard length (SL, mm) and total weight (TW, 0.1 g) of all Red Lionfish were recorded, macroscopic sex was determined, and both lobes of the gonads were removed and weighed (GW, 0.01 g). Depending on the size of the gonad, the entire gonad was removed or a small portion of gonadal material was removed from the center of the gonad and preserved in 10% neutral buffered formalin (NBF) for histological analysis. Reproductive phases were determined macroscopically when possible using terminology following Brown-Peterson et al. (2011; see Table 8).

Macroscopic discrimination among the early developing, regressing and regenerating phases for females was not possible, so fish in these reproductive phases were combined as a single “reproductively inactive” phase, but are considered sexually mature. Females in the developing, spawning capable and actively spawning phases are considered to be reproductively active fish. Males were macroscopically classified as only immature or mature, based on appearance of testicular material. Ovaries of fresh (not frozen) females macroscopically assessed as actively spawning were collected for batch fecundity analysis. A subsample of about 20% of the total gonad weight was removed, weighed (0.01 g), cut into smaller portions, and preserved in individual jars of Gilson’s Fluid (Bagenal and Braum 1978) for a minimum of three months.

Length and Age at Maturity

A binomial classification system was developed for immature and mature Red Lionfish that were classified microscopically. Immature Red Lionfish were labeled as 0 and mature Red Lionfish were labeled as 1 and separated by each ecoregion. TL50 was determined by fitting a two-parameter logistic regression model (2015 RStudio team; http://www.rstudio.com) following McBride et al. (2002) to the binomial maturity data:

$\text{Maturity}=1/(1+\exp(-A(X-B))$; where A = slope or instantaneous rate of increase; X = TL, B = TL at which 50% of the population is mature.

Total length at 50% maturity was considered significantly different if the 95% confidence intervals (CI: two times the standard errors) did not overlapped between sexes and among ecoregions (Zar 1999). In addition, the size of the smallest mature Red Lionfish of each sex was determined for each ecoregion. Age was not determined for all Red Lionfish that were classified microscopically. Therefore, the age of 50% maturity was determined by identifying what age Red Lionfish were estimated to be at TL50 using the Von Bertalanffy growth equations from Chapter II.

Sex Ratio

Sex ratio was calculated for each ecoregion and all ecoregions pooled and a Chi-square test was used to determine if the ratio was different from 1:1. Additionally, sex ratios were compared among ecoregions using a Chi-square test and considered significant when $P < 0.05$, unless otherwise noted.

Effects of freezing

Since Red Lionfish were collected across the nGOM in large numbers, the examination of fresh specimens was not always feasible. Thus, some Red Lionfish were frozen prior to data collection even though freezing may negatively impact reproductive biology analyses (Ramon and Bartoo 1997). To address what effect short and long term freezing may have on gonad weight, the fresh weights of the right lobe (0.01 g) were taken from 41 males and 33 females (varying reproductive phases), and gonads were placed back into the body cavity to be frozen for 24 hours. After 24 hours, the gonads were removed, weighed, thawed and reweighed prior to being refrozen within the body. A paired-t-test was used to determine differences between fresh, frozen and then fresh and thawed gonad weights by sex. If there is no significant difference between frozen and thawed gonads by sex, then gonads do not need to be thawed prior to being reweighed. Gonads were weighed every month for six months and monthly GW by sex was compared to fresh GW using a repeated measures ANOVA (rmANOVA) to determine if there was a significant change in GW over time. Normality and homogeneity of variance were first examined using the Anderson-Darling and Cochran's tests, respectively, and data were arcsine square root transformed if needed to meet these assumptions (Field 2013). If there was a significant difference estimated, means were separated with a Sidak posthoc test (Field 2013). All analyses were done with IBM SPSS (Vers. 20) and considered significant when $P < 0.05$ unless otherwise noted.

Comparison of Macroscopic and Microscopic Reproductive Phases

A subset of 548 gonads (341 female, 110 male, 97 unknown) were analyzed histologically to determine accuracy of macroscopic classifications and to assign sex when unknown. Due to funding constraints, the majority of samples were selected from the Northeast ecoregion, which had the most fish collected over the course of this study. Samples collected from the Southeast and West ecoregions were selected from months around peak reproductive activity based on GSI values. These gonad samples were rinsed overnight in running water, dehydrated (60% ETOH for two hours and two changes of 70%

ETOH for two hours each), embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin following standard histological protocols. All histological processing was performed at Texas A&M University following standard techniques. Reproductive phases were determined microscopically only for female and unknown samples following terminology from Brown-Peterson et al. (2011; see Table 9). Females were considered sexually mature if cortical alveolar (CA) oocytes were present in the ovary. Thus, all females in the early developing sub-phase were considered sexually mature for purposes of calculating 50% maturity.

I compared the proportion of ovarian samples that were classified correctly both macroscopically and microscopically using a Chi-square test. All comparisons were considered significant if $P < 0.05$ unless otherwise specified. Due to female gonads in the early developing, regressing and regenerating phases looking very similar macroscopically, they were all classified as the same reproductively inactive phase and for the purpose of this analysis, were considered correct if histological analysis resulted in any of those three phases.

Spawning Seasonality

The gonadosomatic index (GSI) was calculated for both males and females to determine the reproductive preparedness, and thus reproductive season in the nGOM. GSI was calculated as $GSI = [GW/[TW-GW]] \times 100$. Immature fish were not included in GSI calculations. If adjustments were needed for changes in GW due to freezing, they were applied prior to calculating GSI.

Monthly GSI values were calculated and plotted by sex and ecoregion. The percentage of females determined as reproductively active and inactive was determined for each season (for seasons, see Chapter One) to obtain additional insights into spawning seasonality by ecoregion.

To determine if GSI can be accurately used to determine spawning preparedness, transformed (arcsine square root) GSI and gonad-free body weight (GFBW, g) by sex was examined using linear regression as described in Jons and Miranda (1997). If no relationship existed, GSI can be used to determine spawning preparedness (Jons and Miranda 1997). If a significant overall relationship existed, the data were reanalyzed by two pooled macroscopic phases (reproductively active and inactive) to determine if a specific group of phases was driving the relationship. Subsequently, monthly GSI values over the 12 month period were compared by ecoregion with a two-sample Kolmogorov-Smirnov (K-S) test in a pair-wise manner to determine if differences in mean GSI 'patterns' (shape of mean GSI curve) were apparent (e.g., compare ecoregions southeast (SE)-northeast (NE), SE-west (W), and NE-W). Subsequently, calculated P-values were compared to a Bonferroni adjusted alpha level (0.05 / 3 = 0.0167) for multiple comparisons to control for a Type-1 error rate (Field 2013). If there were no differences among ecoregions, GSI values were pooled across ecoregions and compared by month using a one-way ANOVA and a Tukey’s posthoc test to determine homogeneous subsets, after assumptions of normality and homogeneity of variance were checked. If assumptions were not met, data were arcsine square root transformed. If GSI patterns differed significantly by ecoregion, each ecoregion was analyzed individually.

The proportion of “pooled” female macroscopic reproductive phases (reproductively active and inactive) was analyzed using a Chi-square test by season (n = 4) to determine if there were differences across ecoregions. Additionally, female macroscopic reproductive phases were analyzed by ecoregion (n = 3) to determine if there were differences across seasons. These same comparisons were made between the proportion of females in the reproductively active phase to females in the actively spawning sub-phase. All statistical analyses were done using SPSS software (vers. 20) or R (2015 RStudio team; http://www.rstudio.com), and results were considered significant if P < 0.05 unless specifically noted.

Spawning Frequency

Spawning frequency, defined as the number of days between spawning events, was calculated by season and across ecoregion using macroscopic observations and is calculated from the proportion of actively spawning females to spawning capable females as described in Hunter and Macewicz (1985). Spawning frequency was determined using macroscopic observations of females with hydrated oocytes.

Spawning frequency estimates were compared across season (n = 4) within ecoregion and by ecoregion (n = 3) within season using a Chi-square test. All statistical analyses were done using SPSS software (vers. 20) or R (2015

RStudio team; http://www.rstudio.com), and results were considered significant if $P < 0.05$ unless specifically noted.

Fecundity

Batch fecundity was determined volumetrically following Bagenal and Braum (1978). Ovarian tissue preserved in Gilson's fluid was rinsed under running tap water for 12 hours to ensure all Gilson's fluid was removed. The rinsed eggs were placed in an appropriate volume of water (50 - 200 mL) and stirred until eggs were evenly distributed throughout the solution. Six 1 mL sub-samples (with replacement) were taken from the solution and all hydrated oocytes in the sample were counted under a microscope. Total batch fecundity was estimated using $BF = (nV/v) \times (G_w/S_w)$ (Holden and Raitt 1974), where BF = Batch fecundity, n = mean number of eggs in the subsample, V = volume in which the total number of eggs was diluted, v = volume of the sub-sample, G_w = total ovary weight, and S_w = weight of the sub-sample. Relative batch fecundity (RBF) was calculated as RBF = BF / GFBW (Hunter and Macewicz 1980).

To determine the size of hydrated oocytes to count for fecundity analysis, an oocyte size frequency distribution was constructed by counting all oocytes > 100 µm in three 2 mL subsamples taken from a spawning capable and an actively spawning female. Ooctyes were measured and counted according to 50 µm size bins (see Morris et al. 2011). The distinct batch of largest oocytes in the actively spawning size distribution are hydrated oocytes, and only oocytes this size were counted for fecundity estimations.

A one-way ANOVA was used to compare BF and RBF estimates among months for ecoregions combined after assumptions of normality and homogeneity of variance were checked. If assumptions were not met, data were arcsine square root transformed. If a significant F-value was obtained, a Tukey b posthoc test was used to separate mean values. The various relationships between BF, RBF, TL and GFBW were determined using simple linear regression and all variables were $\log_{10}$ transformed prior to regression analysis. All statistical analyses were done using SPSS software (vers. 20).

Yearly egg production for the nGOM was calculated by dividing the total number of days in each month actively spawning Red Lionfish were detected by spawning frequency and multiplying this number by mean RBG. This number is the mean number of eggs Red Lionfish are capable of producing in a given year.

Results

Length and Age at Maturity

The 95% TL_{50} CI overlapped adjacent mean values among all ecoregions, and therefore TL_{50} was not significantly different among ecoregions for either sex (Table 10), and therefore data were pooled for ecoregions for each sex. The pooled TL_{50} for male Red Lionfish (N = 209) was 145.18 ± 6.18 mm while TL_{50} for female Red Lionfish (N = 344) was 166.61 ± 4.95 mm, and the slope or instantaneous rate of increase was -0.045 and -0.044 for males and females, respectively (Figure 10). The smallest mature male and female Red Lionfish was 94.0 mm TL and 150.0 mm TL, respectively. Pooled ecoregional male and female Red Lionfish TL_{50} correspond to age 0.5 to 1.0, based on information presented in Chapter II. Thus, Red Lionfish reach sexual maturity within their first year of life.

Sex Ratio

Sex ratios were calculated for each ecoregion individually and the northeast and west ecoregion sex ratios were not significantly different from 1:1 (both $P > 0.05$). However, there was a significant difference from 1:1 in the southeast ecoregion, with more males than females ($X_{1,3008} = 9.189$,$P = 0.002$; Table 11). Additionally, sex ratios were significantly different among ecoregions ($P < 0.001$). The northeast ecoregion had the highest percentage of females (52.8%) and the southeast ecoregion had the lowest percentage of females (44.5%). The ratio of males to females in the west ecoregion was essentially even (50.1% females).

Effects of Freezing on Gonadal Tissue

There was no significant difference between fresh and recently frozen gonad weights for males (paired-$t_{40} = 1.254$, $P = 0.217$) and females (paired-$t_{32} = 0.707$, $P = 0.485$). There was also no significant difference between frozen and thawed ovarian weights (paired-$t_{32} = 0.652$, $P = 0.519$). Although there was a significant difference between frozen and thawed testis weights (paired-$t_{40} = -3.716$, $P = 0.001$), which is likely not biologically significant as the difference was 0.0054g. Therefore, gonads did not need to be thawed prior to measuring monthly weights. Finally, there was no significant difference between fresh and frozen gonad weights for up to six months for males (rmANOVA, $F_{6,41} = 0.80$, P = 0.523) and females (rmANOVA, $F_{6,33} = 1.39$, P = 0.251). As a result, either fresh or frozen gonad weights can be used to calculate accurate GSI values. Mean gonad weight across each time period for male and female gonads are presented in Appendix 2.

Histology

Female Red Lionfish from the nGOM (n = 337) were evaluated histologically. The more developed oocytes were more prevalent in the periphery of the ovary, and were observed on vascularized stalks or peduncles (Figures 13-16). It is important to note that in reproductively active ovaries (Figures 13-16)), many oocyte stages were visible including gonads in the spawning capable phase with multiple stages of vitellogenesis (Figures 14-16), indicating asynchronous oocyte development. Immature phase ovaries (Figure 11) were classified by the presence of tightly packed primary growth (PG) oocytes and an abundance of interstitial tissue (IT) throughout. Additionally, there was an abundance of chromatin nucleolar PG oocytes, which helps to distinguish immature ovaries from regenerating phase ovaries. Early developing sub-phase ovaries (Figure 12) were classified by the presence of cortical alveolar (CA) oocytes, although there are many fewer CA oocytes than PG oocytes. During this phase, the ovaries enter into the reproductive cycle and the ovaries are gonadotropin-dependent; developing oocytes must be spawned or be reabsorbed. Developing phase ovaries (Figure 13) begin to show primary and secondary vitellogenic oocytes (VTG1 and VTG2). The peduncles mentioned earlier can clearly been seen attached to different stages of oocytes in reproductively active ovaries (Figures 13-16). Spawning capable phase ovaries (Figure 14) begin to develop late stage or tertiary vitellogenic oocytes (VTG3) and vitellogenic oocytesare distributed from early to later stages as you move from the center to the periphery of the ovary. Ovaries in the actively spawning sub-phase (Figures 15 and 16) may have easily identified POF visible if spawning has recently occurred (Figure 15), although in many cases transverse sections of peduncles may have a similar appearance to a POF making differentiation difficult. Hydrated oocytes, also seen in the actively spawning sub phase (Figure 16), are not attached to their peduncles but are still in the follicle. Regressing phase ovaries (Figure 17) possess a number of different stage oocytes but also show a great deal of atresia (Alpha, Beta and Gamma stage; Figure 17). Lastly, regenerating phase ovaries (Figure 18) are very similar in structure to immature ovaries (Figure 11). The abundance of PG oocytes is evident although they are not as tightly packed and there are less chromatin nucleolar and more perinucleolar stage PG oocytes (Figure 18).

Chi-square analysis of female macroscopic phase identification accuracy revealed significant deviation from 100% accuracy (Table 12). Overall, there was only 49.7% agreement in phase classification between females identified macroscopically as reproductively inactive when compared to histological identification in the early developing, regressing, and regenerating phases. This represents a significant (P < 0.001) difference between macroscopic and histological classification. Macroscopic identification of females in the developing phase was also significantly different from histological classification (P < 0.001) and also the least accurate, as only 11.9% of the 59 females macroscopically assigned to this phase were verified histologically to be in the developing phase (Table 12). However, 66% of fish macroscopically identified as developing were reproductively active (developing, spawning, capable, and actively spawning phases). The relatively low and significantly different (P < 0.001) percent agreement for the spawning capable phase (65.4%) between macroscopic and histological classifications is likely due to the inability to macroscopically distinguish oocytes undergoing oocyte maturation (OM), which are histologically classified into the actively spawning sub-phase. Similarly, although agreement between the macroscopic and histological actively spawning sub-phase was high (91.7%; Table 12), there was a significant difference between macroscopic and histological classification (P = 0.028). However, when combining the actively spawning sub-phase into the spawning capable phase, agreement between macroscopic and histological assessment increased to 95.6% and showed no significant difference among methods (P = 0.060). Therefore, macroscopic assessments are adequate for the determination of females in the spawning capable phase (including the actively spawning sub-phase) but are unreliable for females in any other reproductive phase. The significant differences between macroscopic and histological classification for many female reproductive phases resulted in redefining macroscopic phases as either reproductively active (combining the developing, spawning capable, and actively spawning macroscopic phases) or reproductively inactive. This reclassification still resulted in a significant difference between macroscopic and histological classifications for both reproductively active ($P < 0.001$) and inactive ($P < 0.001$) females, but improved the overall percent accuracy to 86.2% and 82.2%, respectively. Thus, the broad reproductive categories of reproductively active or inactive appear adequate for macroscopic classification of the reproductive season of female Red Lionfish. Therefore, macroscopic identification of ovaries can accurately be used to determine spawning capable females and also to estimate spawning frequency.

Spawning Seasonality

The GSI was calculated for 2,247 females and 2,280 male Red Lionfish over this three-year study (April 2012 to March 2015). Regression analysis showed a significant positive correlation between female ($r^2 = 0.767$, $F_{1,2246} = 7401.30$, $P < 0.001$, Figure 19A) and male ($r^2 = 0.174$, $F_{1,2279} = 481.41$, $P < 0.001$, Figure 19B) GSI and GFBW. This suggests that GSI does not correct for Red Lionfish GFBW, and implies that larger fish will have higher GSI values regardless of reproductive phase. However, when females were separated into two distinct reproductive groups (inactive vs. active), the GSI values of the reproductive actively females did not show a significant positive correlation with

GFBW ($r^2 = 0.001$, $F_{1,812} = 0.47$, $P = 0.495$) whereas the inactive females still showed a significant positive correlation ($r^2 = 0.291$, $F_{1,1417} = 580.71$, $P < 0.001$).

To further investigate the relationship between GSI and GFBW, female and male GSI values ($\bar{x}$ ± SE) from all ecoregions were plotted with GFBW ($\bar{x}$ ± SE) by month (Figures 19C, D). Peak GSI values for both sexes do not correspond to peak GFBW values, suggesting that these patterns are not the same and thus GSI provides a strong signal of gonadal recrudescence despite the significant relationship between GSI and GFBW. Thus, GSI can be considered to be an accurate indicator of spawning seasonality in northern GOM Red Lionfish.

The spatial patterns of ecoregional mean GSI values were not significantly different for males (Figure 20) or females (Figure 21; K-S test: all $P > 0.05$ for both sexes) and thus, mean GSI values were pooled for each sex, across ecoregions. However, in the case of females, the months of peak GSI values were visually different by ecoregion (Figure 21), with Red Lionfish from the northeast ecoregion exhibiting peak GSI later in the season (August) than the other two ecoregions. However, since there was no significant difference in GSI among ecoregions, GSI values were pooled for all ecoregions by month. Overall, the pattern in mean GSI for both males and females was similar to seasonal patterns in SST, and elevated GSI values (females> 2.0, males >0.053) were observed when SST > 22°C (Figure 22); corresponding to the summer (May through October).

Male and female GSI values were significantly different by month (male: ANOVA, $F_{11,2279} = 19.42$, $P < 0.001$; female: ANOVA, $F_{11,2246} = 19.38$, $P < 0.001$) and a Tukey posthoc test determined six and four homogeneous subsets for females and males, respectively (Table 13). Based on these data, the peak spawning season for female Red Lionfish in the northern GOM was from May to October.

Macroscopic classification of female gonad phases based on classifying females as reproductively active and inactive yielded complementary findings to GSI results (Table 14). Reproductively active females were observed every month of the year, and females in the actively spawning sub-phase were observed in all months except March. The proportion of reproductively active to inactive females was significantly different by season for the northeast and west ecoregions (all $P < 0.001$) but not in the southeast ecoregion ($P = 0.080$) (Table 14A). Additionally, the proportion of reproductively active to inactive females across all ecoregions during the spring and summer was significantly different (both $P < 0.002$) but the fall and winter months were not significantly different ($P = 0.014$ and 0.065) (Table 14A). There was a clear peak in spawning activity (actively spawning sub-phase) during the warmer months (summer) and a clear depression in spawning activity during the cooler months (winter and spring) (Table 14B). Although there was not a significant difference in the proportion of reproductively active to actively spawning females across ecoregions (all $P > 0.02$) or across seasons in the southeast and west ecoregions (both $P > 0.068$), there was a significant difference across seasons in the northeast ecoregion ($P < 0.001$) (Table 14B). In all ecoregions, the highest percentage of females in the actively spawning phase was observed in the same months where GSI peaked (summer, May-October) (Table 14B).

To further investigate spawning seasonality, histological phases of gonads were determined by month and percent in each histological phase was calculated (Table 15). The elevated percentage of regressing phase ovaries in the cooler months (December – March) is in stark contrast to the percentage of actively spawning females from May through November. These data correspond well with the GSI and macroscopic spawning seasonality data that suggests the months with peak spawning activity are May through October. However, spawning capable females were seen in every month, and only in March were no actively spawning females captured. Lastly, although in relatively low percentages, there was a presence of regressing and regenerating phase Red Lionfish throughout the year (except May and July). This suggests an asynchronicity of Red Lionfish spawning in the nGOM on the population level, with individual females likely not spawning during the entire year.

Spawning Frequency

Spawning frequency was not significantly different by ecoregion for each of the four seasons (Chi-square test, all $P > 0.05$; Table 16). Additionally, there was no significant difference by season within the southeast and west ecoregions (Chi-square test, $P > 0.05$) although there was a significant seasonal difference within the northeast ecoregion (Chi-square test, $P < 0.001$; Table 16). Due to the majority of the ecoregions not being different across season, seasonal spawning frequency was pooled for all ecoregions and there were significantly less days between spawns during May through October. This also corresponds with the time of highest female GSI values (Figure 22A), and highest percentage of spawning capable/reproductively active females (Table 14) (Chi-square test, $P < 0.001$; Table 16). Based on these estimates, spawning frequency of a female Red Lionfish in the nGOM varies seasonally, as there are less days between spawns during summer (May through October; every 2.16 days) than in fall, winter and spring (every 5.0, 5.6 and 9.5 days). The months of higher spawning frequency correspond to mean water temperatures > 28°C (Table 16), suggesting spawning frequency is likely influenced by water temperature. To obtain an overall estimate of spawning frequency for the nGOM, seasonal data were combined. Therefore, a female Red Lionfish in the nGOM is capable of spawning every 2.49 days, 11 months out of the year.

Fecundity

Oocyte size frequency distribution revealed that ovaries in the spawning capable phase exhibited a continuous, unimodal size frequency and did not contain oocytes < 500 µm (Figure 23). In contrast, ovaries in the actively spawning sub-phase exhibited a bimodal oocyte size frequency with a second peak occurring > 500 µm (Figure 23), corresponding to hydrated oocytes. Therefore, only oocytes > 500 µm were counted for batch fecundity estimates.

Batch fecundity estimates were determined for 71 Red Lionfish in the nGOM in the actively spawning sub-phase across seasons and ecoregions. Regression analyses showed a significant positive correlation between BF and both TL ($r^2 = 0.237$, $F_{1,70} = 21.41$, $P < 0.001$; Figure 24A) and GFBW ($r^2 = 0.209$, $F_{1,70} = 18.22$, $P < 0.001$; Figure 24B). In contrast, there was a slight positive correlation but the slope was not significantly different from zero between RBF and both TL ($r^2 =0.039$, $F_{1,70} = 2.79$, $P = 0.099$; Figure 25A) and GFBW ($r^2 = 0.016$, $F_{1,70} = 1.11$, $P = 0.297$; Figure 25B). Since there was not a significant correlation between body size and RBF, RBF can be accurately used to compare reproductive output among different sized fish. There was a significant difference in RBF by month (ANOVA, $F_{9,69} = 6.60$, $P < 0.001$; Figure 26 and Table 17). A Tukey post-hoc test determined two homogeneous subsets; RBF was higher in the warmer months (June-Sept) than the rest of the year (Figure 26 and Table 17). March and April were not included in this analysis due to inadequate sample sizes. Mean batch fecundity was 26,904 ± 2,716 eggs and mean RBF was 92.2 ± 7.6 eggs / g GFBW.

Information on both batch fecundity and spawning frequency allows estimation of the number of eggs a single female Red Lionfish could produce during a year. An average size mature female of 188.6g (GFBW) with a RBF of 92.2 eggs/g GFBW, spawning every 2.49 days for 11 months of the year (334 days) is capable of producing 2,332,490 eggs per year.

Discussion

The reproductive life history traits of invasive Red Lionfish in the nGOM are similar to other batch spawners within the family Scorpianidae although vary among other reef fish species. The unique peduncle structure supplies nutrients to the oocytes prior to their release and helps prevent overcrowding (Morris et al. 2011) and is not common in other reef fish species but had been described in other Scorpianidae. A smaller species of scorpionfish (S*corpaena notata*) from the Mediterranean Sea (Muñoz et al. 2005) has similar reproductive traits as they display peak reproductive activity from June through October. In contrast, the closely related black scorpionfish (*Scorpaena porcus*), an oviparous scorpeanid found in the cooler Black Sea and the Mediterranean Sea, exhibits only a four month spawning season (Bilgin and Çelik 2009) although the cooler water temperature may be driving the truncated spawning season compared to other Scorpianidae that are found in warmer waters. Batch fecundity of *S. notata* (5,800 - 33,000, Muñoz et al. 2005) was lower than observed for Red Lionfish from the nGOM although the RBF was much higher. Additionally, this species, native to the Mediterranean, possesses the same ovarian structure observed in lionfish.

Accurate reproductive classifications of gonads are important for any life history study. An inexpensive and assumed accurate method for ovary phase identification is macroscopic evaluation. We have shown here that macroscopic evaluation of gonads is only accurate for females in the spawning capable reproductive phase (which includes the actively spawning sub-phase) when macroscopic and histological evaluations were compared. Gardner et al. (2015) reported similar findings for lionfish from Little Cayman, with high accuracy in distinguishing reproductively inactive females from those that were reproductively active, but lower agreement when attempting to distinguish a particular reproductive phase macroscopically. Poor agreement between macroscopic and histological classification is not unique to lionfish. Other species such as Southern Flounder (*Paralichthys lethostigma*; Midway and Scharf 2012) and northern anchovy (*Engmulis mordar*; Hunter and Macewicz 1985) with the same reproductive strategy as lionfish show poor agreement between macroscopic and histological gonadal assessment. Although, Klibansky and Scharf (2015) performed a similar comparison on Black Sea Bass (*Centropristis striata*) and Red Porgy (*Pagrus pagrus*) and showed that macroscopic gonadal assessment can be acceptable for general phase classifications. Our results revealed that following the table provided in Green et al. (2012) to assign reproductive phase will yield inaccurate female phase classifications. However, female Red Lionfish in the spawning capable phase can be accurately identified macroscopically, which allows macroscopic identification of the spawning season of Red Lionfish.

Reproductive information for lionfish in their native range is sparse (Donaldson et al. 2011) although there is some information provided in Priyadharsini et al. (2013) for lionfish from the Indian Ocean. Lionfish collected from the Indian Ocean have GSI values ranging from 0.062 to 3.064

(Priyadharsini et al. 2013), similar to the range of GSI values reported for nGOM Red Lionfish. Mean batch fecundity in the Indian Ocean was reported to be 75,547; however, since all oocytes greater than 80 µm were counted for that study rather than the >500µm size that was counted in this study, it is likely that the batch fecundity reports from the Indian Ocean were grossly overestimated.

In the nGOM, female and male Red Lionfish length at 50% maturity is 167 and 145 mm TL respectively. This information, taken into account with age-at-length data from Chapter II, indicates that Red Lionfish can become mature within the first year of life. This is consistent with findings from the Caribbean (Gardner et al. 2015) and the western Atlantic Ocean (Morris 2009) (Table 18). Length at 50% maturity, fecundity, and spawning seasonality can be affected by differences in thermal regimes associated with differences in latitudinal position (Leggett and Carscadden 1978, Conover 1992). With this information, we would expect the length at 50% maturity from this study would fall in between the Little Cayman Island study and the Carolinas and Bahamas study. This was not the case as Red Lionfish in this study were much smaller than reported in any other study (Table 18); Gardner et al. (2015) reported a size of 50% maturity as 15mm TL larger than what was reported by Morris (2009) (Table 18). The smaller size at 50% maturity observed for Red Lionfish in the nGOM may be due to a reduced availability of food or greater competition as a result of the high densities of Red Lionfish observed in the nGOM (Dahl and Patterson 2014) and can result in maturation at smaller sizes. The smaller size at 50% maturity could also be due to different criteria used to define sexual maturity among the studies; Gardner et al. (2015) used the presence of vitellogenic oocytes to indicate sexual maturity, while our study and that of Morris (2009) considered females mature if CA oocytes were present. Age and growth data from the nGOM, presented in Chapter II, suggests the maximum age of red lionfish in the region is 4.5 years and that the size at sexual maturity occurs at or before age-1. Thus, red lionfish have the potential to reproduce for 4 years in the nGOM although this period is expected to rise as the invasion is still early in the nGOM.

Overall there is not much information on sex ratio across the invaded range although lionfish sex ratio from Little Cayman was reported to be not significantly different from 1:1 (Edwards et al. 2014). These results are similar to what was found in the nGOM, specifically the NE and W ecoregions where there was no significant difference from 1:1. Sex Ratio can be a useful indicator of fish population status. In recreationally and commercially important fish species, often times the larger fish are harvested. In those fish species that exhibit size-specific sexual dimorphism, often times the larger sex will be harvested in greater quantities, thus offsetting the sex ratio. In the case of Red Lionfish, males achieve significantly larger sizes (Chapter II) and thus may be more susceptible to harvest. Therefore, it would be expected that as commercial activity increases, the sex ratio will move further from 1:1.

In the nGOM, the spawning season, or months when GSI values were > 2.0, occurred from May through October. This was less than the 12-month spawning season reported from the warmer waters of the Caribbean Sea where all 12 months exhibited female mean GSI values > 2.0 (Gardner et al. 2015). Priyadharsini et al. (2013) reported GSI values for lionfish in the Indian Ocean as 0.062-3.064 and although monthly resolution was not reported, the range of values is similar to our reported values in the nGOM. Additionally, a truncated spawning season (March-June) for lionfish in their native range was also noted in Morris (2009) per communication with L. Fishelson. GSI values were not reported for the western Atlantic study, although actively spawning lionfish were found during 10 months of the year (Morris 2009) from that area. It is important to note that even though there was a shorter spawning season in the nGOM compared to the Caribbean Sea, actively spawning female Red Lionfish were collected 11 months of the year and spawning capable female Red Lionfish were collected all 12 months of the year. Thermal regimes may be the driving factor in the variation (shortening) of spawning season with latitude (Leggett and Carscadden 1978, Conover 1992) and more protracted spawning seasons are expected in warmer, lower latitude environments. It is not uncommon for native sub-tropical and tropical fish species to be reproductively active throughout the calendar year (Johannes 1978) but what is unique in the case of lionfish in their invaded range is their high reproductive activity for most if not all of the calendar year, even in sub-tropical environments.

Although there was no significant difference in the shapes of the mean GSI curves associated with spawning season among ecoregions, the peak in spawning season is delayed in the cooler NE ecoregion compared to the warmer SE ecoregion. The west ecoregion is unique in that the duration of the peak in spawning activity spans three months as opposed to only one month exhibited by the other two ecoregions. This irregularity may be a result of very different habitat types where these fish were collected. In the west ecoregion, the majority of the samples collected came from oil production structures that span the entire water column and are unique to only the west ecoregion. This vertical structure has the potential to allow Red Lionfish to move vertically along the structure to seek out environmental conditions that are most suitable for reproduction. The majority of Red Lionfish samples collected from the southeast ecoregion were collected on natural bottom habitat. Lastly, the majority of Red Lionfish collected from the northeast ecoregion were collected from small artificial reefs. Future studies to assess differences in life history characteristics between these habitat types should be considered. Further investigation is needed for Red Lionfish that have invaded the oil production structures of the western GOM.

Red Lionfish spawning frequency in the nGOM varied throughout the year (summer: 1.9 to spring:9.5 days between spawning events). On average, Red Lionfish in the nGOM had a spawning frequency of every 2.49 days which was similar but slightly less frequent to the spawning frequency reported for Little Cayman (2.40 days between spawns) but more frequent than the Bahamas (3.6 days) and North Carolina (4.1days). It is important to note that both the Little Cayman study and the Bahamas and North Carolina study calculated spawning frequency over a short period of time (9, 5 and 8 days respectively). Due to the short sampling period in both studies, more detailed, longer-term spawning frequency was not achieved, and the application of one spawning frequency across all months may be inaccurate. In the Red Sea, Fishelson (1975) calculated the spawning frequency of Dwarf Lionfish *Dendrochirus brachypterus* to be less often (every 6-8 days), although these fish were observed in captivity and actual spawning events were observed. Spawning frequency can be affected by a number of factors including temperature, photoperiod (Bapary and Takemura 2010), lunar cycle (Domeier and Colin 1997), prey availability (Tyler and Stanton 1995) and even the size of the female (Claramunt et al. 2007) although the differences in latitudinal temperature regimes are likely a reason for the difference in spawning frequency between the different studies (Leggett and Carscadden 1978).

In the nGOM there appears to be a relationship between SST and spawning frequency, with fewer days between spawns in warmer seasons. Increased frequency of spawning during warmer months can been seen in other batch spawning reef fish such as Red Snapper (*Lutjanus campechanus*) in the nGOM (Collins et al. 1996) and Vermilion Snapper (*Rhomboplites aurorubens*) in the western Atlantic Ocean (Cuellae et al. 1996). Additionally, the high prey consumption of invasive lionfish (Albins and Hixon 2008, Dahl and Patterson 2014) likely facilitates a more rapid spawning frequency. Lionfish, in general, can be classified as income breeders, which are those species whose reproductive activity is related to the amount of prey they consume (Jönsson 1997). Support for this can be seen with spawning frequency and BF of Red Lionfish in the nGOM being suppressed in the winter month when there is less available food. BF has been shown to be significantly affected by food availability (Coward and Bromage 1999), and Gardner (2015) showed that in months following high reproductive activity, lionfish possessed higher mean mass of stomach contents. Recruitment of smaller bodied reef fish (potential prey) to offshore reefs in the GOM primarily occurs during warmer months (Lingo and Szedlmayer 2006, Gallaway et al. 2009), which coincides with increases in spawning frequency, batch fecundity, and peak reproductive activity. Months of reduced prey recruitment may contribute to the reduced, reproductive activity of Red Lionfish in the cooler months.

Methodology for batch fecundity estimates varied among studies in the invaded regions but yielded comparable numbers. The current study counted Gilson’s fluid-preserved oocytes >500 µm diameter based on results from oocyte size frequency analysis; all oocytes counted were hydrated. Gardner et al. (2015) counted 10% NBF- preserved hydrated or oocyte maturation stage oocytes >450 µm in diameter that appeared clear when observed under light. Morris (2009), on the other hand, collected and analyzed egg batches from fish spawned in captivity that were preserved in 95% ethanol, although the size of the eggs counted was not indicated. The present study had a much larger range in BF estimates, but this larger range may be a result of a larger sample size and size range in females analyzed, since BF increases with increasing fish size. Therefore, making comparisons between studies using BF estimates may be inaccurate due to the positively correlated effects associated with Red Lionfish size and BF. Unfortunately, RBF was not calculated for lionfish in any other study. If regional comparisons are to be made in the future, RBF must be calculated to avoid incorrect conclusions based on mean batch fecundity that are influenced by lionfish size.

The RBF values reported here for Red Lionfish are similar to the related oviparous Black Scorpionfish (*Scorpaena porcus*), native to the eastern Atlantic Ocean, (97-258 eggs/ g of body weight, Mokrane and Zerouali-Khodja 2015). Other batch spawning reef fish species found in tropical/sub-tropical regions have RBF values similar to the 39-207 eggs/g GFBW from Red Lionfish in the nGOM. Studies on Red Snapper from various regions in Florida showed variable RBF from 27-235 eggs/g GFBW (Brown-Peterson et al. 2009, Fry et al. 2009). Yellowtail Snapper (*Ocyurus chrysurus*), a smaller bodied snapper, was found to have a RBF of 57-303 eggs/g GFBW (Trejo-Martínez et al. 2011).

The observed monthly variation in BF has several possible explanations. The relationship between temperature and reproductive parameters is evident when considering batch fecundity data. In the nGOM, months of higher temperatures show elevated batch fecundity while in cooler months there is depressed BF.

It is noteworthy that the reproductive life history parameters found in this study from nGOM specimens are similar to those reported for the warmer, tropical environment of Little Cayman Island. Although water temperature may not be a cue for spawning in the warmer tropical environments such as Little Cayman (Gardner et al. 2015), our data shows that spawning activity is related to water temperature in the nGOM. With the results from this study presenting the smallest reported length at 50% maturity, a mean batch fecundity of 26,904 eggs, a mean spawning frequency of 2.49 days, and the ability to spawn11 months out of the year, it is not difficult to understand how the lionfish invasion has been so rapid and successful. The findings from this study will provide important life history metrics that will be used in future management plans and to further understand the potential impacts of this invasive species on the native fisheries of the nGOM.

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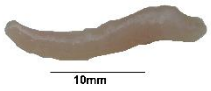
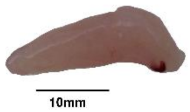
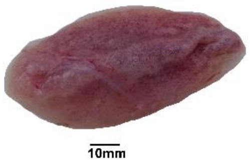
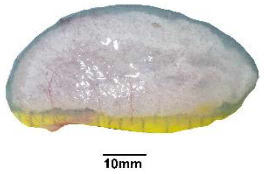
Table 8

Macroscopic gonad stage identification key

Sex	**Phase**	**Description**	**Macroscopic Image**
Unidentified	Immature (Reproductively Inactive)	Threadlike appearance. Individual sex cannot be determined macroscopically.	-----------
Male	Mature	Elongated, more developed gonad. Defined edges. Cream in color.	
Female	Early Developing / Regressing / Regenerating (Reproductively Inactive)	Ovary round and pink colored. No eggs visible. Size dependent on size of fish	
	Developing (Reproductively Active)	Ovary round and pink colored. Folds within the ovary have developed. Small eggs visible.	
	Spawning Capable (Reproductively Active)	Ovary round and pink. Folds that were present in developing phase have filled in. Eggs are large but NO gelatinous mass is present.	

	Actively Spawning (Reproductively Active)	Ovary encased in gelatinous mass and large eggs visible. Care must be taken when removing ovaries in this phase to prevent loss of eggs and subsequent weight.	

Red Lionfish macroscopic gonad stage identification key; adapted from Table 2 in Green et al. (2012). Terminology follows Brown-Peterson et al. (2011).

Table 9

Female Red Lionfish microscopic reproductive phase identification key

Phase	**Description**
Immature	Only oogonia and PG oocytes present. No atresia or muscle bundles. Oocytes tightly packed. Interstitial tissue present and often dominates ovary.
Early Developing	Only PG and CA oocytes present
Developing	PG, CA, Vtg1, and Vtg2 oocytes present. No POF present
Spawning Capable	PG, CA, Vtg1, Vtg2 and Vtg3 oocytes present. POF and atresia can be present.
Actively Spawning	OM, and/or H oocytes present. New POF (<12 h) can be present
Regressing	Most Vtg1, Vtg2 and Vtg3 oocytes undergoing atresia. POF may be present.
Regenerating	Only PG oocytes present. Oocytes loosely packed compared to Immature phase. Interstitial tissue present but not dominant. Gamma and delta atresia can be present.

Female Red Lionfish microscopic reproductive phase identification key. Terminology derived from Table 2 in Brown-Peterson et al. (2011). CA = cortical alveolar; OM = oocyte maturation; PG = primary growth; POF = postovulatory follicle complex; Vtg1 = primary vitellogenic; Vtg2 = secondary vitellogenic; Vtg3 = tertiary vitellogenic; H = Hydrated

Table 10

Length at 50% sexual maturity

Sex	Southeast	Northeast	West	Pooled
Male	155.8 (19.4) N=48	145.4 (15.7) N=265	145.0 (ND) N=30	145.2 (12.4) N=343
Female	168.5 (16.1) N=34	162.6 (14.2) N=163	179.7 (11.9) N=8	166.6 (9.9) N=205

Total length at 50% sexual maturity by sex and ecoregion for Red Lionfish from the northern Gulf of Mexico. The 95% confidence intervals (CI) are shown in parentheses; overlap of 95% CI with adjacent mean values demonstrates non-significance among ecoregions. ND = standard error could not be determined for males in the west ecoregion. Values below total length values represent sample sizes.

Table 11

Sex ratios by ecoregion

	Southeast	**Northeast**	**West**
Sex Ratio (M:F)	1:0.80	1:1.12	1:1.01
Chi-square	$X_{1,3008} = 9.189$ $P = 0.002$	$X_{1,4658} = 3.688$ $P = 0.055$	$X_{1,1388} = 0.003$ $P = 0.957$
All sex ratios significantly different between ecoregions: $X_{2,4527} = 25.457$, $P < 0.001$			

Red Lionfish sex ratios by ecoregion and Chi-square value.

Table 12

Macroscopic compared to microscopic phase identifications

			Microscopic							
		n	Imm	Edev	Dev	SC	AS	Regr	Regn	Percent Agreement
Macroscopic	Edev/Regr/ Regn	163	52	**31**	22	5	3	**22**	**28**	49.7
	Dev	59	1	3	**7**	9	23	16		11.9
	SC	55				**36**	15	4		65.4
	AS	60				4	**55**	1		91.7

Female macroscopic phase identifications compared to microscopic phase identifications for Red Lionfish from the northern Gulf of Mexico. n = total fish in each macroscopic phase. Data represent number of fish based on microscopic identification of phase. All Chi-square P-values were <0.05. Bold numbers represent correct phase classification between macroscopic and histological observations. Imm = Immature, Edev = Early Developing, Dev = Developing, SC = Spawning Capable, AS = Actively Spawning, Regr – Regressing, Regn = Regenerating

Table 13

Monthly gonadosomatic index

A)

Month	GSI	Subset a	Subset b	Subset c	Subset d	Subset e	Subset f
January	1.239 ± 0.084	a	b				
February	1.147 ± 0.101	a					
March	1.170 ± 0.567	a					
April	1.315 ± 0.185	a	b				
May	3.273 ± 0.269				d	e	f
June	2.814 ± 0.124			c	d	e	
July	3.922 ± 0.313					e	f
August	4.180 ± 0.324						f
September	2.694 ± 0.255			c	d		
October	2.399 ± 0.160		b	c	d		
November	1.654 ± 0.209	a	b	c			
December	1.678 ± 0.131	a	b	c			

B)

Month	GSI	Subset a	Subset b	Subset c	Subset d
January	0.046 ± 0.003	a			
February	0.054 ± 0.003	a	b		
March	0.043 ± 0.003	a			
April	0.046 ± 0.003	a			
May	0.070 ± 0.003			c	d
June	0.077 ± 0.002				d
July	0.072 ± 0.004				d
August	0.066 ± 0.003		b	c	d
September	0.053 ± 0.002	a	b		
October	0.055 ± 0.002	a	b	c	
November	0.041 ± 0.003	a			
December	0.047 ± 0.002	a			

Monthly gonadosomatic index (GSI; $\bar{x}$ ± SE) and homogeneous subsets determined by Tukey post-hoc test for Red Lionfish from the northern Gulf of Mexico A) Female, B) Male. SE = standard error of the mean

Table 14

Seasonal macroscopic phases by ecoregion

A)

	Southeast Ecoregion			Northeast Ecoregion			West Ecoregion			
Season	% Inactive	% Active (%AS)	N	% Inactive	% Active (%AS)	N	% Inactive	% Active (% AS)	N	Chi-Square Ecoregion (P)
Spring	48	52 (4)	50	71	29 (0)	95	35	65 (0)	20	12.51 (**0.002)**
Summer	40	60 (13)	565	33	67 (20)	717	21	79 (21)	284	31.43 **(<0.001**)
Fall	55	45 (0)	29	34	66 (9)	198	56	44 (4)	27	8.53 (0.014)
Winter	67	33 (0)	12	46	54 (2)	217	24	76 (18)	17	5.47 (0.065)
Chi-Square Season (P)		6.72 (0.080)			56.20 **(< 0.001**)			17.44 (**0.001)**		

B)

	Southeast Ecoregion			Northeast Ecoregion			West Ecoregion			
Season	% Active (not AS)	% Actively Spawning	N	% Active (not AS)	% Actively Spawning	N	% Active (not AS)	% Actively Spawning	N	Chi-Square Ecoregion (P)
Spring	92	8	26	100	0	28	100	0	13	3.25 (0.197)
Summer	79	21	339	71	29	479	73	27	225	6.93 (0.031)
Fall	100	0	13	86	14	131	92	8	12	2.27 (0.322)

Winter	100	0	4	96	4	118	77	23	13	7.72 (0.021)
Chi-Square Season (P)		7.11 (0.068)			50.71 (**<0.001**)			6.53 (0.089)		

Seasonal macroscopic phases by ecoregion for female Red Lionfish from the northern Gulf of Mexico. Significant differences (Pearson Chi-square) indicated in bold (Bonferronni adjusted significance, P ≤ 0.0167 for seasonal comparisons across ecoregion, P ≤ 0.0125 for ecoregional comparisons across season). A) Comparison of reproductively inactive vs. reproductively active females. AS = actively spawning sub-phase, determined by the presence of hydrated oocytes. B) Comparison of females within the actively spawning sub-phase to reproductively active, non-spawning females. Spring, March – April; Summer, May - October; Fall, November - December; Winter, January - February

Table 15

Histological reproductive phases

Month	N	Immature	Early Developing	Developing	Spawning Capable	Actively Spawning	Regressing	Regenerating
Jan	25	12	8	0	16	16	40	8
Feb	28	28	14	11	4	7	32	4
Mar	25	18	13	17	4	0	22	26
Apr	21	37	28	10	5	5	10	5
May	28	7	11	14	36	28	0	4
Jun	30	3	17	10	21	46	3	0
Jul	23	24	4	0	24	48	0	0
Aug	28	10	0	3	13	61	6	7
Sep	54	28	10	0	7	32	9	14
Oct	44	13	7	22	13	29	11	5
Nov	26	19	4	8	4	53	8	4
Dec	24	0	4	0	58	25	13	0

Monthly percentage of female Red Lionfish from the northern Gulf of Mexico in various histological reproductive phases.

Table 16

Spawning frequency

	Season				
	Spring (Mar-Apr)	Summer (May-Oct)	Fall (Nov-Dec)	Winter (Jan-Feb)	Chi-Square Season (P)
Southeast	5.50	2.44	N/A	N/A	4.194 (0.123)
Northeast	N/A	1.94	4.57	7.20	33.543 (< 0.001)
West	N/A	2.27	8.00	3.00	6.148 (0.105)
Pool	9.50	2.16	5.00	5.63	36.887 (< 0.001)
Chi-Square Ecoregion (P)	0.205 (0.903)	2.487 (0.288)	0.870 (0.647)	0.881 (0.644)	
SST (°C)	20.84	28.14	22.06	18.34	

Spawning frequency (days between spawns) by ecoregion and season for Red Lionfish captured from the northern Gulf of Mexico with seasonal mean sea surface temperature (SST, °C). Chi-square analysis used to determine difference in spawning frequency among months and ecoregion. Southeast (Florida Keys to north 28.25°); Northeast (north 28.25° to west 88°); West (west 88° to Mexican border). All mean SST SE were <0.007 and were not included in the table. SE = standard error of the mean

Table 17

Batch fecundity and relative batch fecundity

Month	n	BF	Mean ± se Relative Batch Fecundity (eggs/g gonad free body weight)RBF	SST (°C)
Jan	2	14,521 ± 6,470	^{a}39.5 ± 11.5	18.44
Feb	2	14,713 ± 6,139	^{a}58.0 ± 12.0	18.18
Mar	0	N/A	N/A	19.50
Apr	1	17,561	50.0	22.19
May	14	12,209 ± 1,362	^{a}41.4 ± 5.0	25.29
Jun	12	33,948 ± 5,322	^{b}102.1 ± 13.7	28.53
Jul	8	29,579 ± 5,470	^{b}110.5 ± 16.4	29.43
Aug	6	70,971 ± 17,652	^{b}207.0 ± 40.4	30.06
Sep	15	27,049 ± 3,720	^{b}111.5 ± 12.8	28.98
Oct	5	13,804 ± 4,471	^{a}68.8 ± 20.3	26.54
Nov	2	19,496 ± 15,498	^{a}55.0 ± 32.0	23.29
Dec	4	19,922 ± 5,329	^{a}61.8 ± 19.7	20.80
Overall	71	26,904 ± 2,717	92.2 ± 7.6	
			ANOVA, $F_{9,69} = 6.60$, $P < 0.001$	

Mean (± SE) monthly batch fecundity (BF, number of eggs) and relative batch fecundity (RBF, eggs/g gonad free body weight) of Red Lionfish collected from the northern Gulf of Mexico. Mean monthly sea surface temperature (SST, °C) were compiled from historic (4 years) NOAA buoy data (11 buoys) in the ecoregions in which Red Lionfish were collected. All SST SE values were <0.007 and were not included in the table. SE = standard error of the mean. Analysis of variance (ANOVA) for relative batch fecundity; homogeneous subsets determined by a Tukey posthoc test are indicated by lower case letters. April (n = 1) and March (n = 0) not included in ANOVA analysis.

Table 18

Comparison of three reproductive life history studies

	Northern Gulf of Mexico (this study)	**Little Cayman (Gardner 2015)**	**NC, SC, and Bahamas (Morris 2009)**
Female Length at 50% Maturity	167mm	190mm	175mm
Peak Spawning Seasonality (GSI > 2.0)	May through October	Year around	-
Actively Spawning Fish (Months)	11	12	10
Spawning Frequency	2.49 days	2.4 days	3.6 days: Bahamas 4.1 days: North Carolina
Batch Fecundity Range	1,684 – 115,838 (n = 71)	1,800 – 41,945 (n = 19)	10,790 – 41,392 (n = 3)

Comparison of three reproductive life history studies from the invaded range of lionfish. For Morris (2009), data presented by state or country except for length at 50% maturity where data are pooled among all three locations

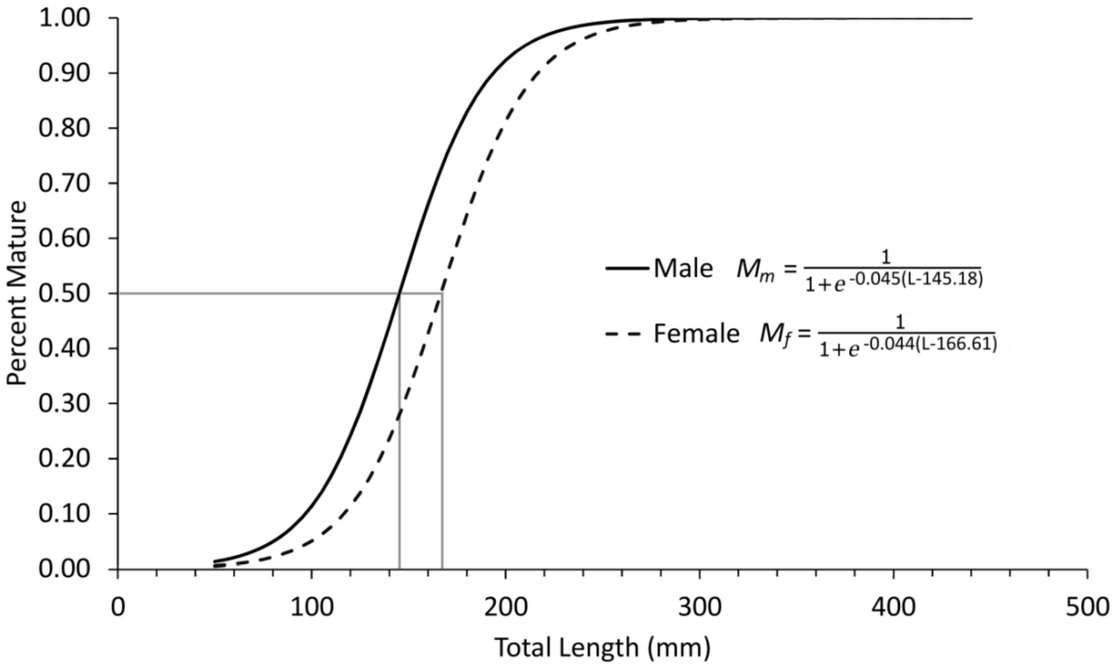


Figure 10. Length at 50% maturity

Percent of mature Red Lionfish by total length (TL) and sex for Red Lionfish in the northern Gulf of Mexico. Two parameter logistic model is plotted by 5 mm TL size bins to determine length at 50% maturity. The vertical lines at 145.18 mm TL and 166.61 mm TL represent the lengths at which males and females (respectively) are 50% mature.

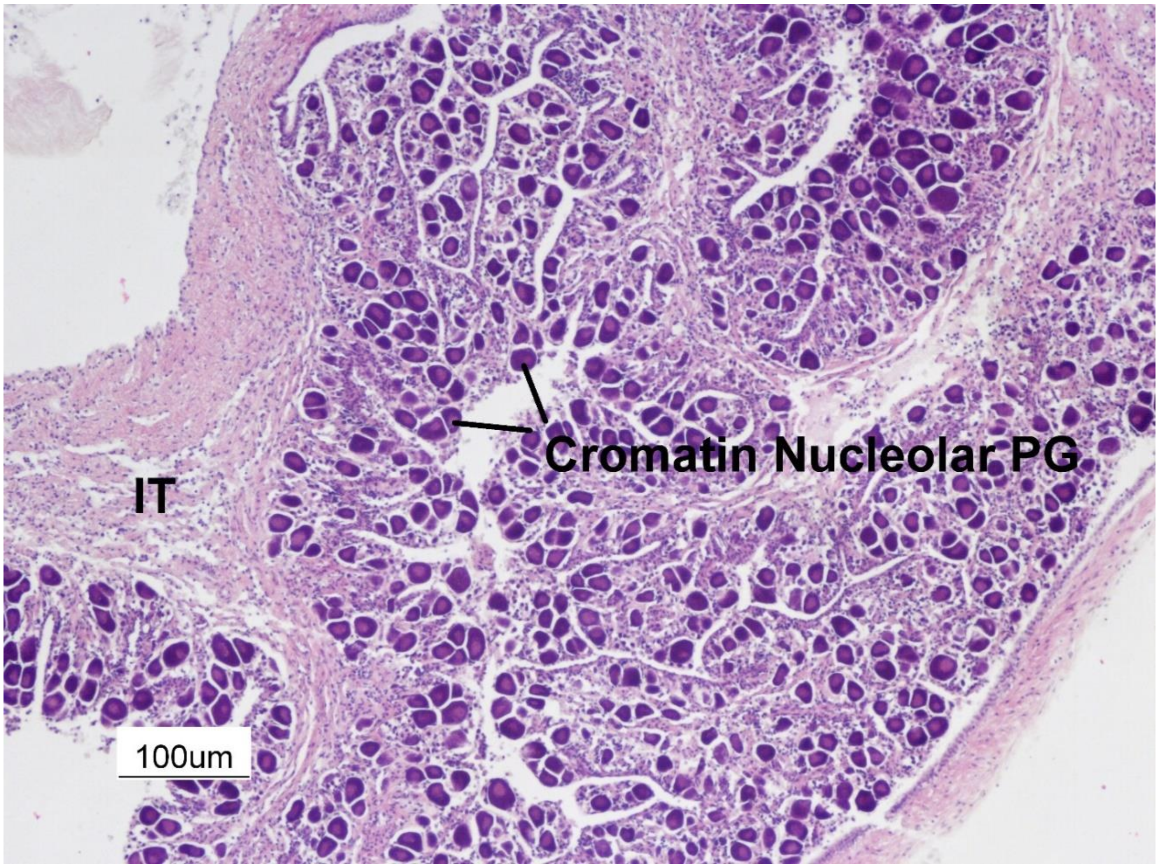


Figure 11. Immature reproductive phase of a female Red Lionfish

Histological micrograph showing the immature reproductive phase of a female Red Lionfish (121 mm TL) from the northern Gulf of Mexico. IT = interstitial tissue; PG = primary growth.

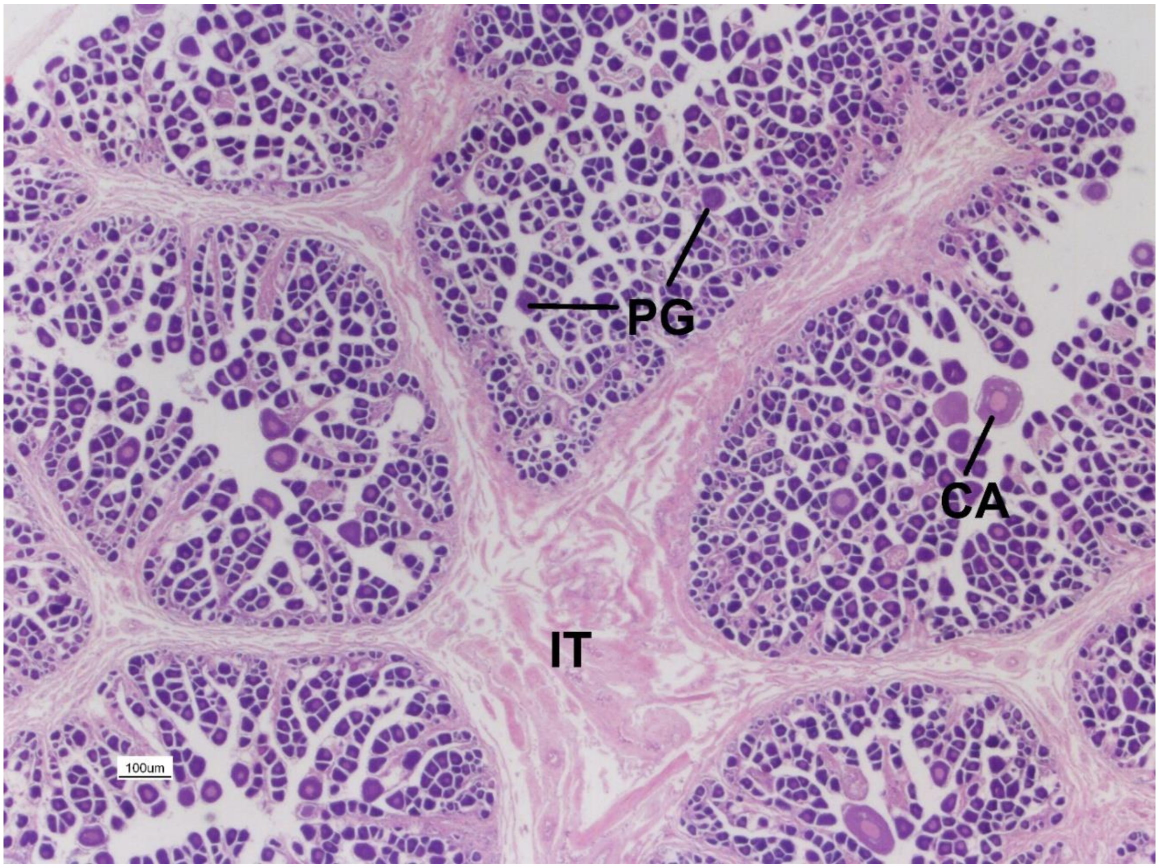


Figure 12. Early developing reproductive subphase of a female Red Lionfish

Histological micrograph showing the early developing reproductive subphase of a female Red Lionfish (211 mm TL) from the northern Gulf of Mexico. IT = interstitial tissue; PG = primary growth; CA = cortical alveolar.

Figure 13. Developing reproductive phase of a female Red Lionfish

Histological micrograph showing the developing reproductive phase of a female Red Lionfish (260 mm TL) from the northern Gulf of Mexico. IT = interstitial tissue; PG = primary growth; CA = cortical alveolar; VTG1 = primary vitellogenic; P = peduncle.

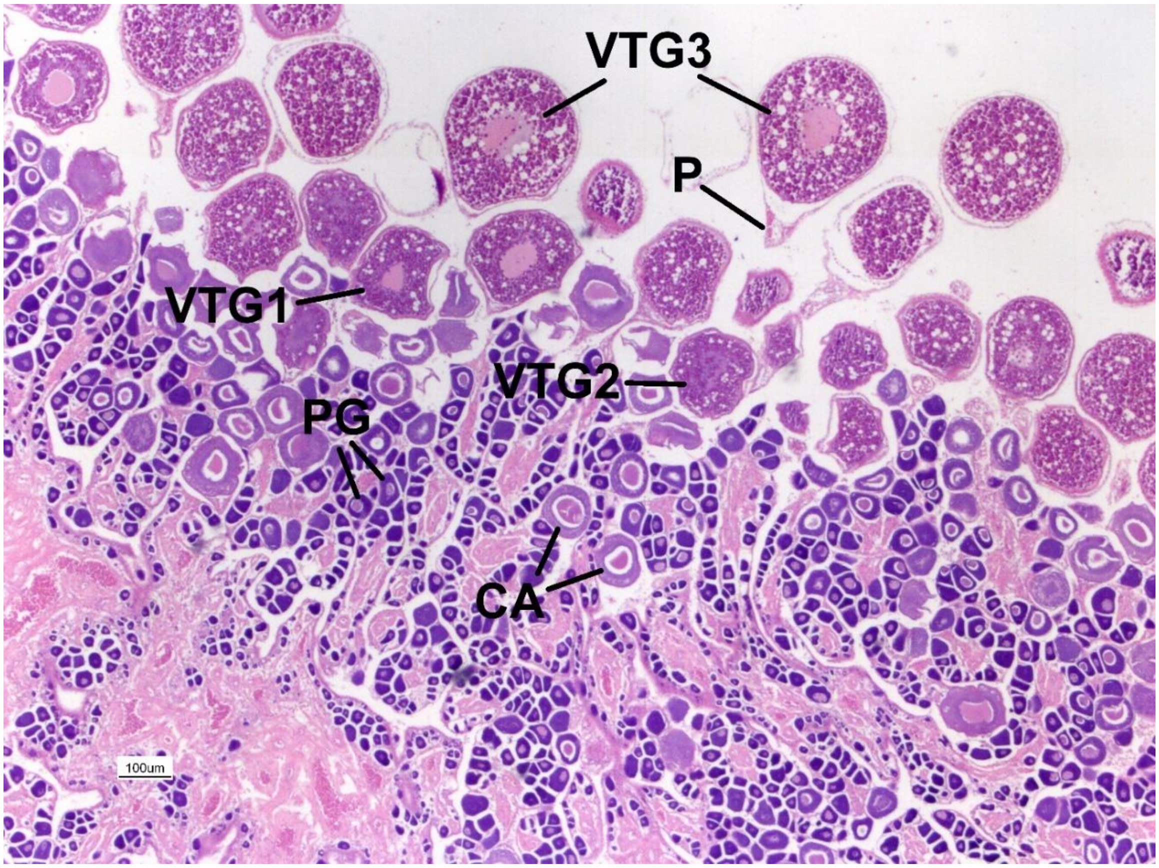


Figure 14. Spawning capable reproductive phase of a female Red Lionfish

Histological micrograph showing the spawning capable reproductive phase of a female Red Lionfish (298 mm TL) from the northern Gulf of Mexico. PG = primary growth; CA = cortical alveolar; VTG1 = primary vitellogenic; VTG2 = secondary vitellogenic; VTG3 = tertiary vitellogenic; P = peduncle. Note that the larger, more developed oocytes are in the periphery of the ovary.

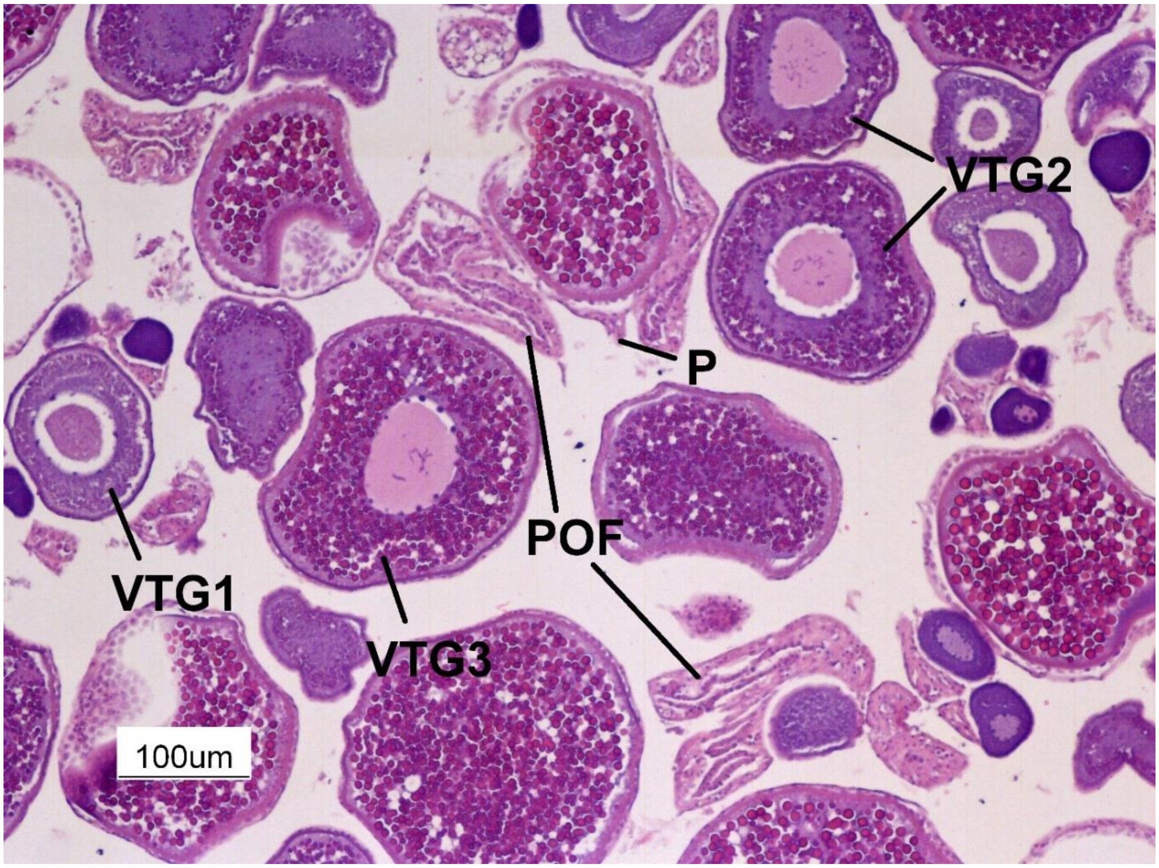


Figure 15. Actively spawning reproductive subphase with POF female Red Lionfish

Histological micrograph showing the actively spawning reproductive subphase with postovulatory follicles <6 h of a female Red Lionfish (274 mm TL) from the northern Gulf of Mexico. VTG1 = primary vitellogenic; VTG2 = secondary vitellogenic; VTG3 = tertiary vitellogenic; POF = postovulatory follicle complex; P = peduncle.

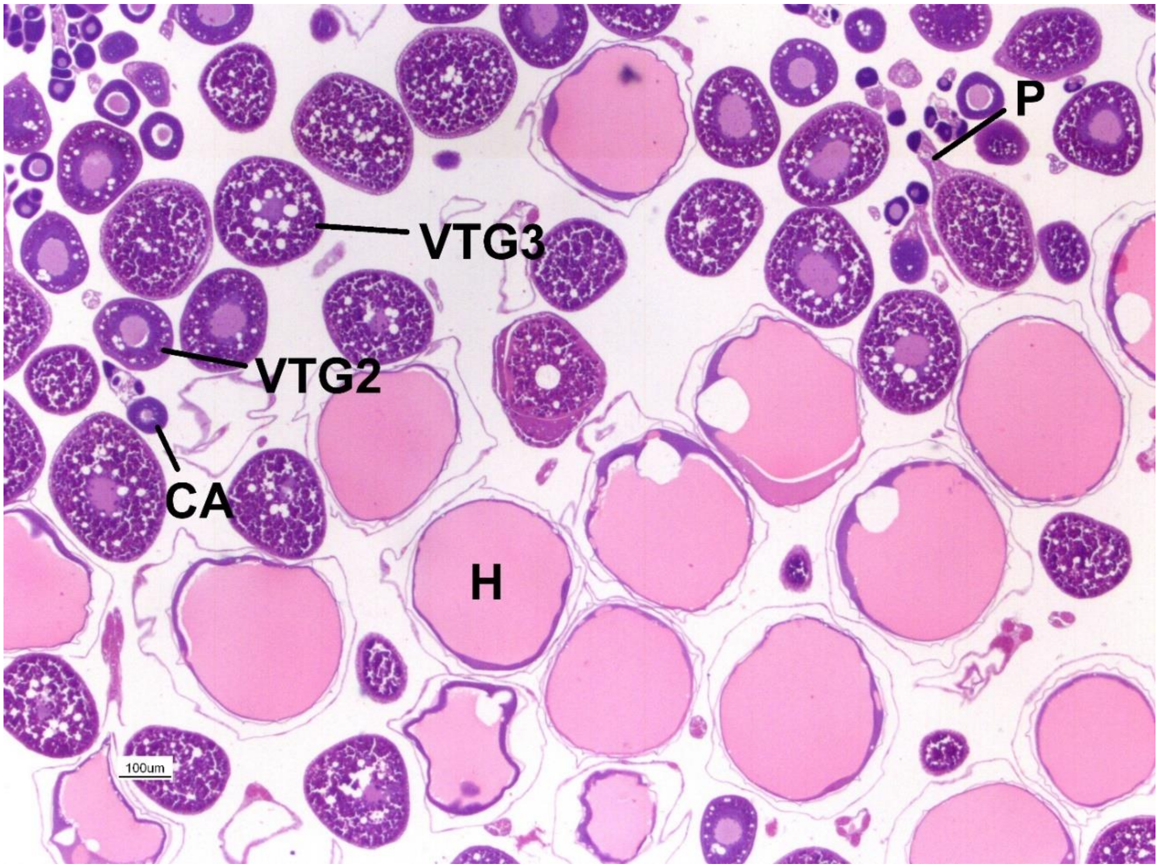


Figure 16. Actively spawning reproductive subphase with hydrated oocytes female Red Lionfish

Histological micrograph showing the actively spawning reproductive subphase with hydrated oocytes of a female Red Lionfish (252 mm TL) from the northern Gulf of Mexico. VTG2 = secondary vitellogenic; VTG3 = tertiary vitellogenic; CA = cortical alveolar; H = Hydrated; P = peduncle.

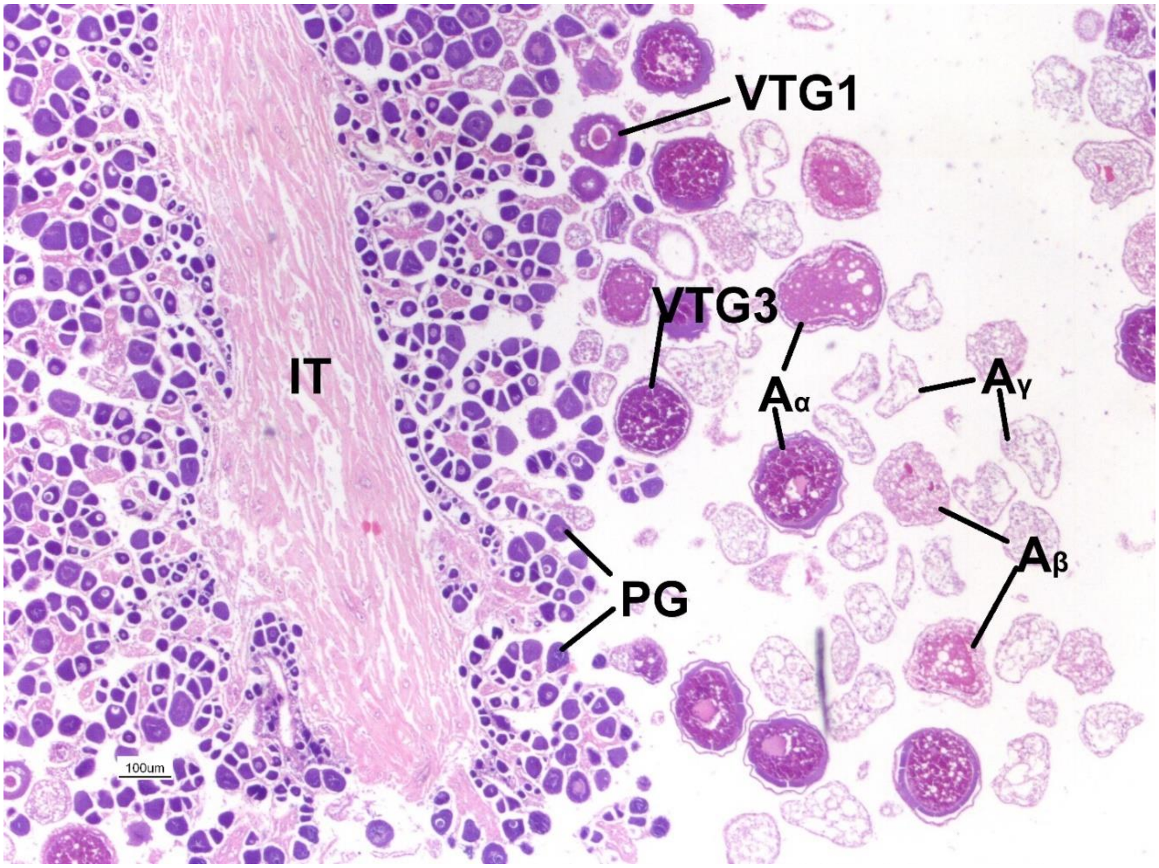


Figure 17. Regressing reproductive phase of a female Red Lionfish

Histological micrograph showing the regressing reproductive phase of a female Red Lionfish (307 mm TL) from the northern Gulf of Mexico. IT = interstitial tissue; PG = primary growth; VTG1 = primary vitellogenic; VTG3 = tertiary vitellogenic; Aα = Atretic (Alpha); Aβ = Atretic (Beta); Aγ = Atretic (Gamma).

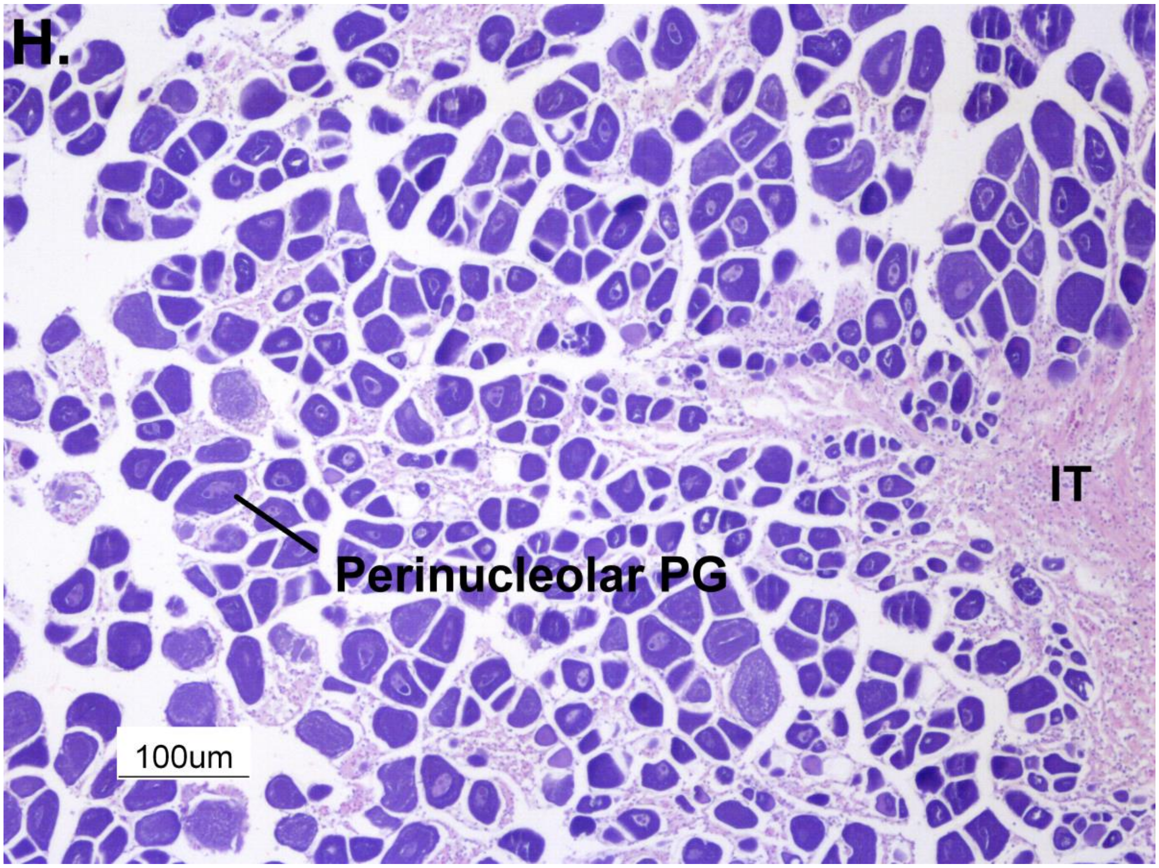


Figure 18. Regenerating reproductive phase of a female Red Lionfish

Histological micrograph showing the regenerating reproductive phase of a female Red Lionfish (228 mm TL) from the northern Gulf of Mexico. IT = interstitial tissue; PG = primary growth.

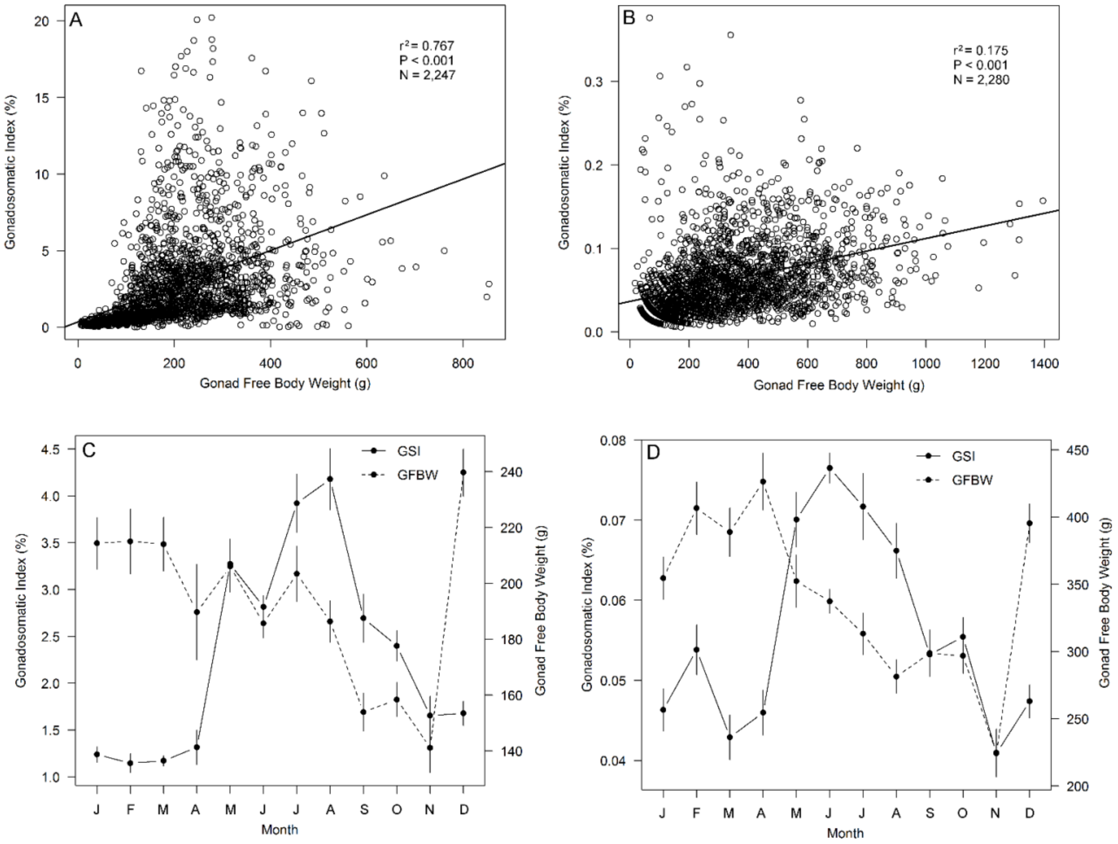


Figure 19. Gonadosomatic index

Relationship between gonadosomatic index (GSI) and body weight for female and male Red Lionfish from the northern Gulf of Mexico. A) Female GSI by gonad free body weight (GFBW). Regression equation: GSI = 0.0117(GFBW) + 0.3544 B) Male GSI by GFBW. Regression equation: GSI = 8E-05(GFBW) + 0.0365 C) Mean (± SE) female GSI and GFBW by month. D) Mean (± SE) male GSI and GFBW by month. SE = standard error of the mean.

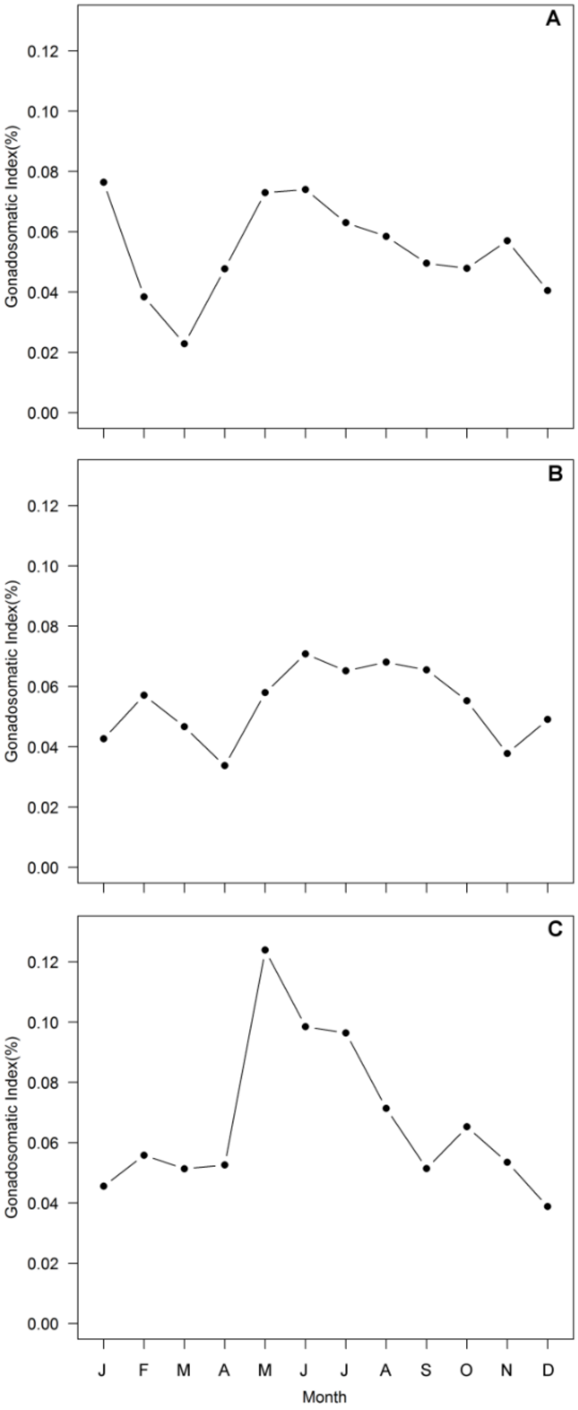


Figure 20. Male gonadosomatic index by ecoregion

Mean monthly male gonadosomatic index (GSI) values for Red Lionfish captured from three ecoregions in the northern Gulf of Mexico. A) Southeast (Florida Keys to north 28.25°), B) northeast (north 28.25° to west 88°), and C) west (west of 88°).

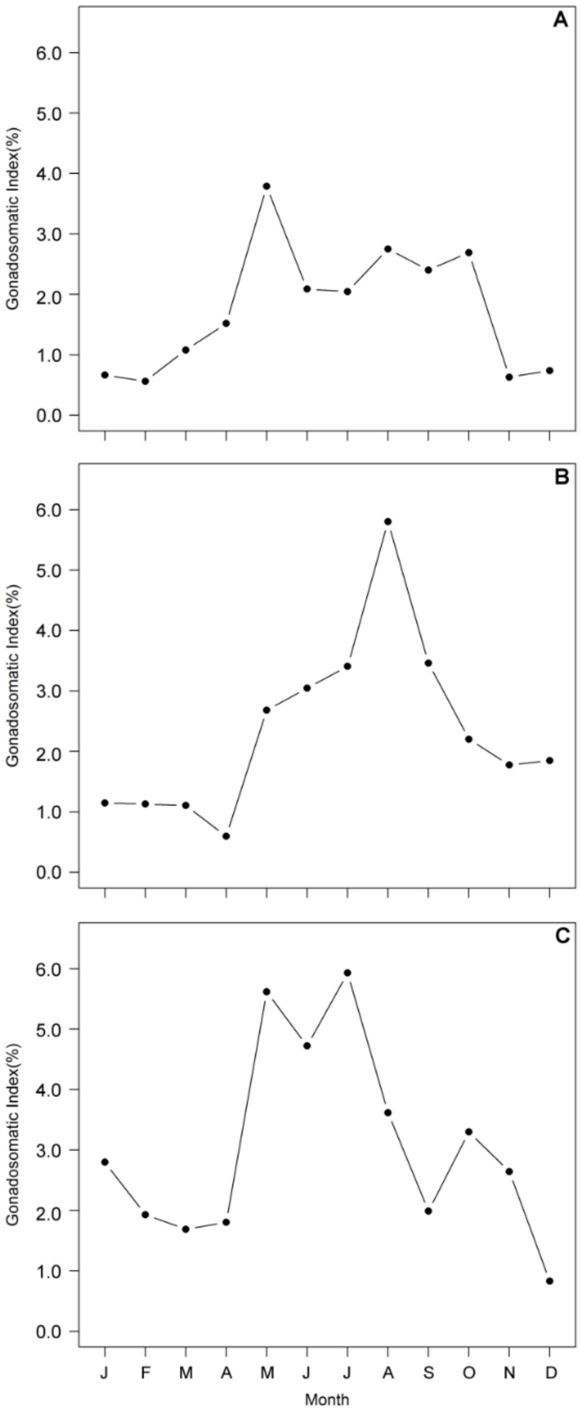


Figure 21. Female gonadosomatic index by ecoregion

Mean monthly female gonadosomatic index (GSI) values for Red Lionfish captured from three ecoregions in the northern Gulf of Mexico. A) Southeast (Florida Keys to north 28.25°), B) northeast (north 28.25° to west 88°), and C) west (west of 88°).

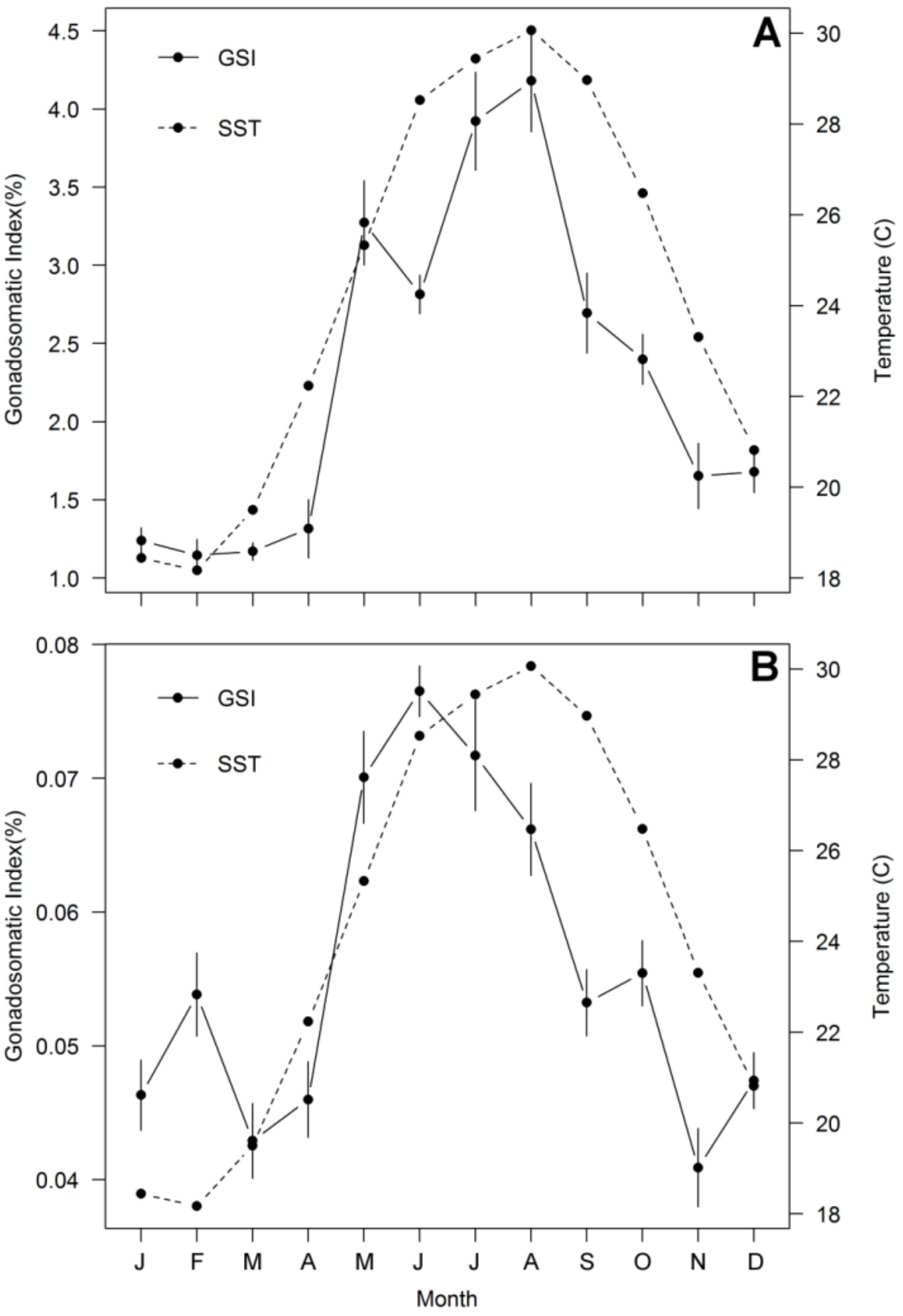


Figure 22. Gonadosomatic index by sex

Relationship between gonadosomatic index (GSI) and sea surface temperature (SST) for Red Lionfish captured from the northern Gulf of Mexico. A) Mean (± SE) monthly female GSI and SST. B) Mean (± SE) monthly male GSI and SST. All mean SST SE were <0.007 and were not included in the table. SE = standard error of the mean.

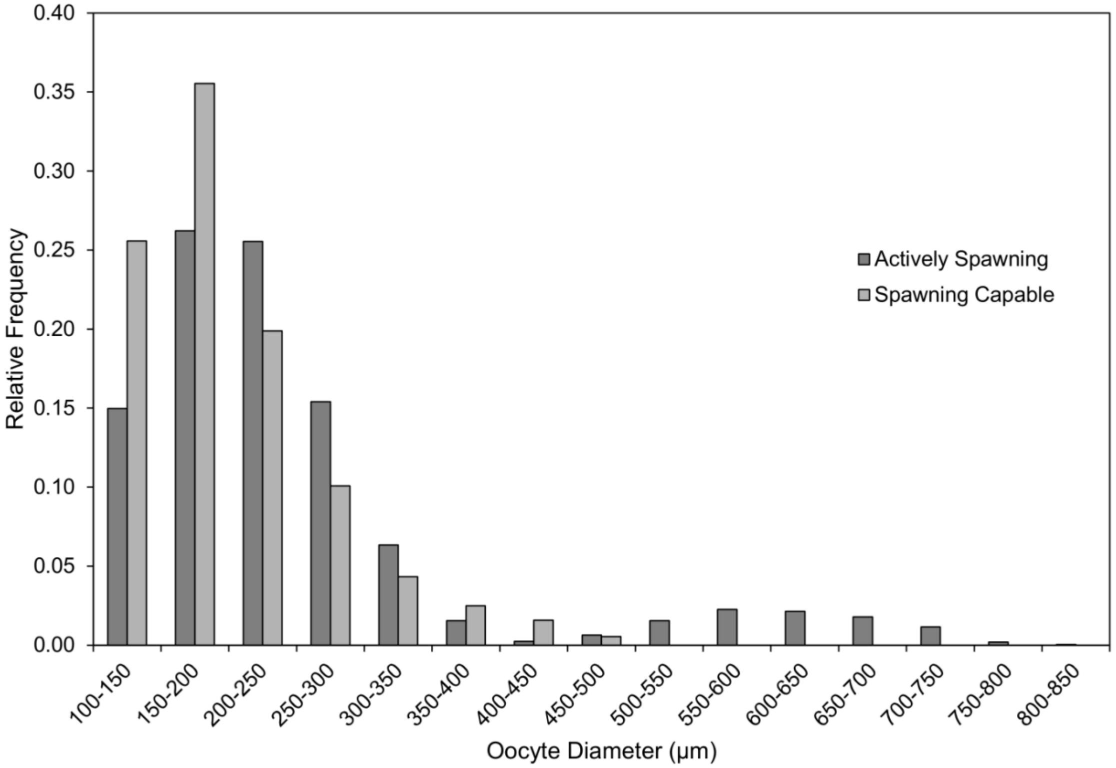


Figure 23. Oocyte size frequency distribution

Relative oocyte size-frequency distribution for Red Lionfish in the spawning capable phase and actively spawning sub-phase.

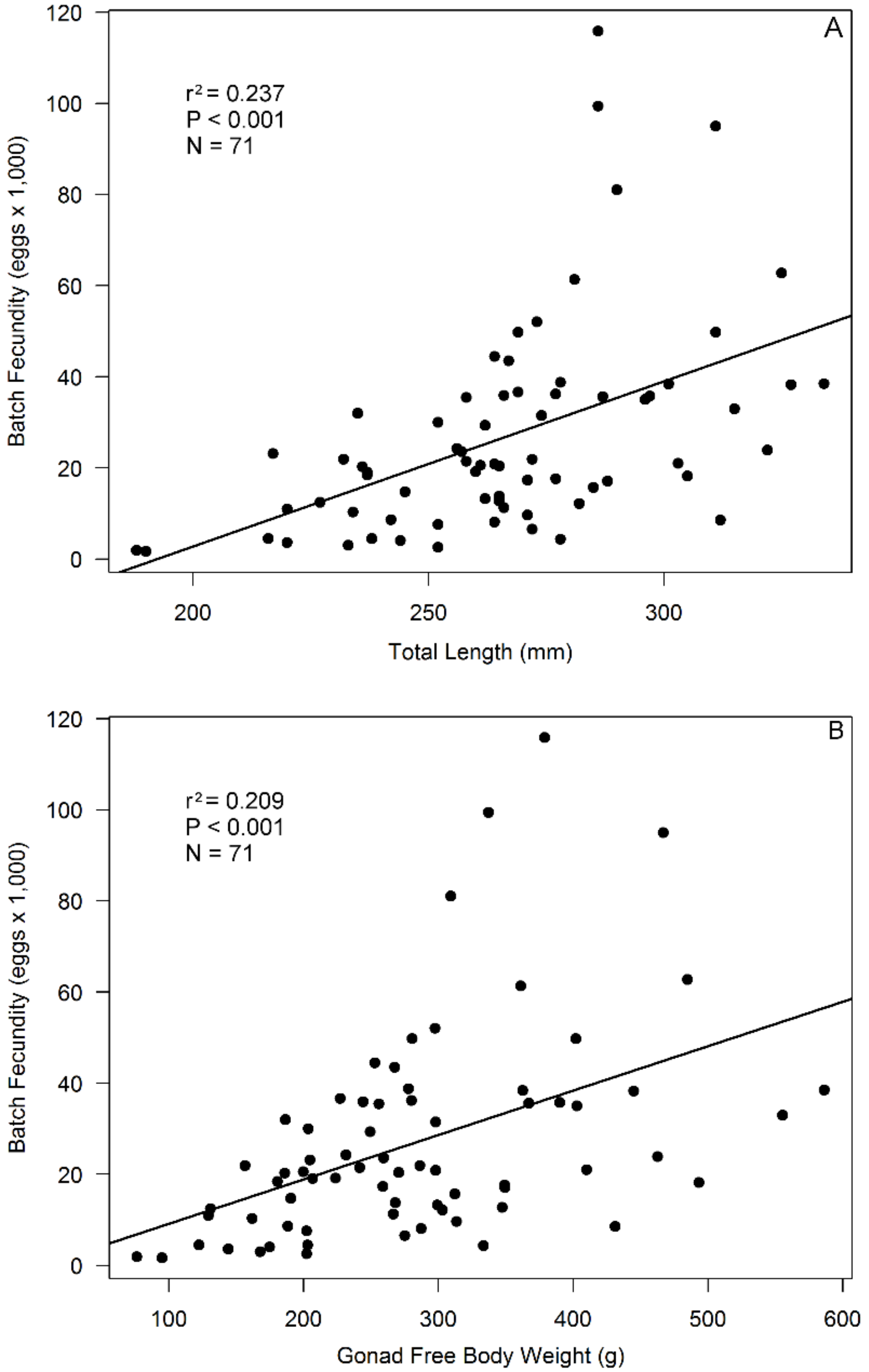


Figure 24. Batch fecundity and fish size

Relationship between batch fecundity (BF) and fish size for Red Lionfish captured from the northern Gulf of Mexico. A) Batch fecundity versus total length. Regression equation: BF = 362.7(TL) – 69,810 B) Batch Fecundity versus gonad free body weight. Regression equation: BF = 97.475(GFBW) - 636.62.

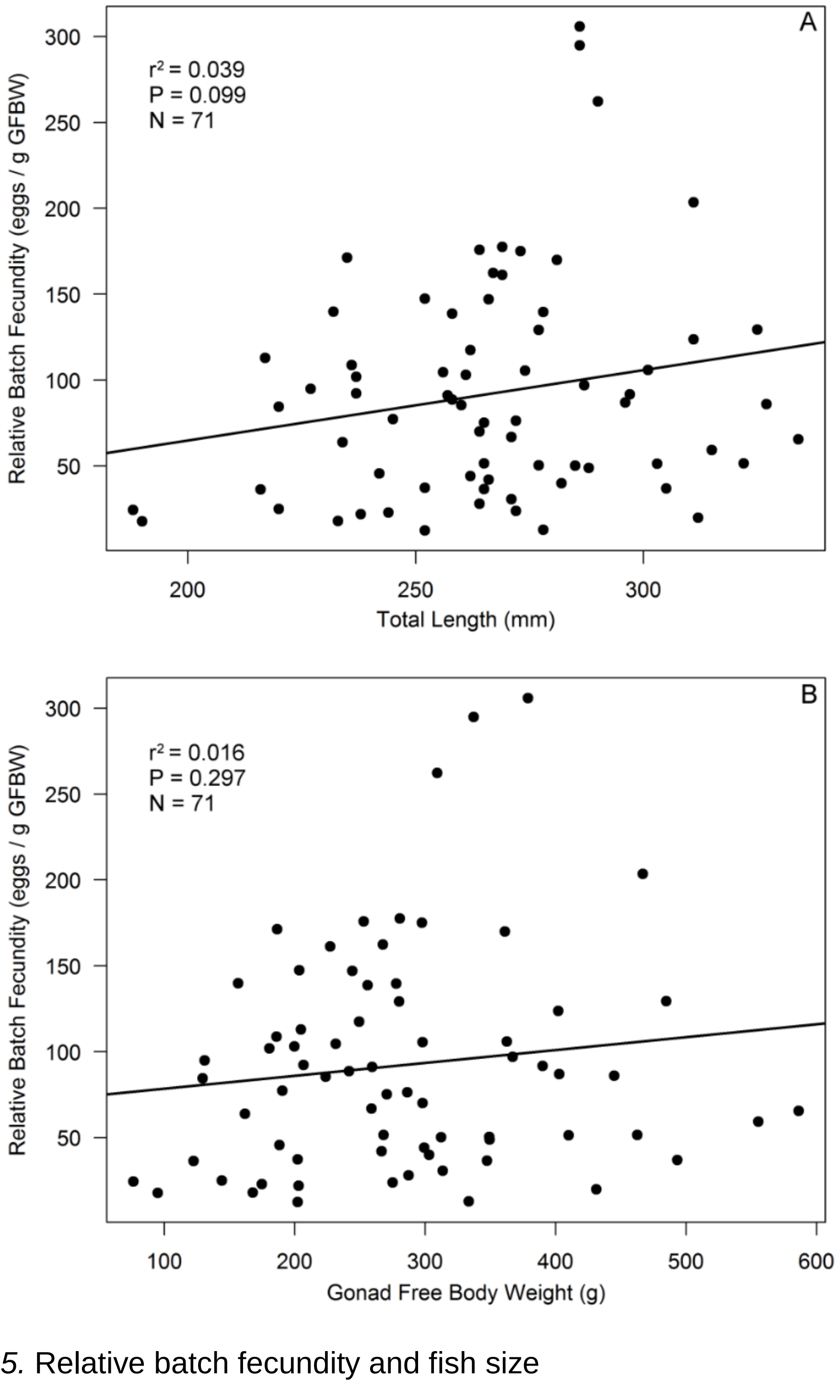


Figure 25. Relative batch fecundity and fish size

Relationship between relative batch fecundity (RBF) and fish length or gonad free body weight (GFBW) for Red Lionfish captured from the northern Gulf of Mexico. A) Relative batch fecundity versus total length. Regression equation:

RBF = 0.4106TL - 17.257 B) Relative batch fecundity versus gonad free body weight. Regression equation: RBF = 0.0748 (GFBW) + 71.092.

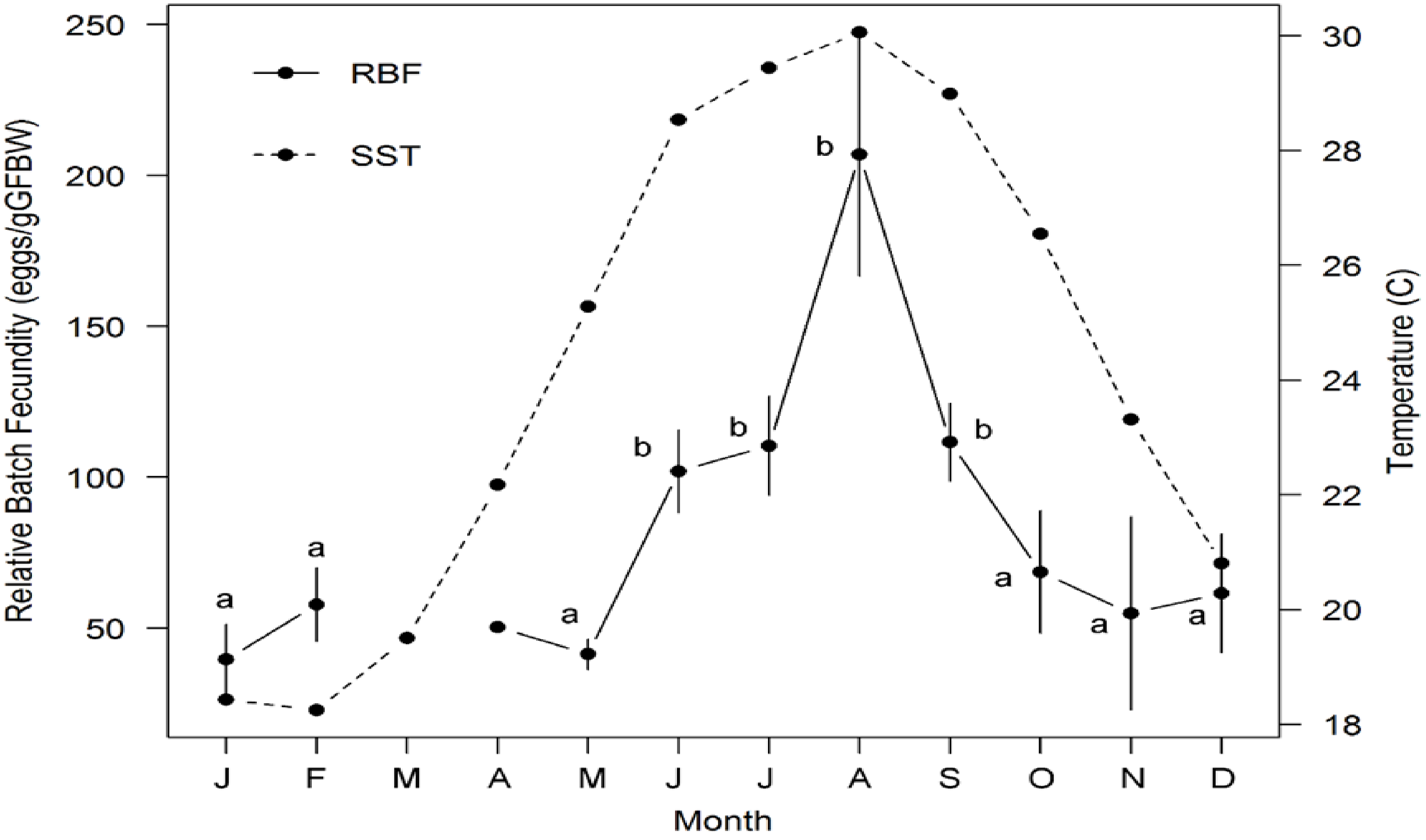


Figure 26. Relative batch fecundity and sea surface temperature

Relationship between mean relative batch fecundity (RBF) and sea surface temperature (SST) for Red Lionfish captured from the northern Gulf of Mexico. Mean monthly sea surface temperature (SST, °C) were compiled from historic (4 years) NOAA buoy data (11 buoys) in the ecoregions in which Red Lionfish were collected. Homogeneous subsets determined by a Tukey posthoc test following a significant ANOVA ($P < 0.001$) are indicated by lower case letters. April ($n = 1$) and March ($n = 0$) not included in ANOVA analysis. All mean SST SE were <0.007 and were not included in the table. SE = standard error of the mean.

CHAPTER IV – SYNTHESIS

This study focused on invasive Red Lionfish (*Pterois volitans*) life history in the northern Gulf of Mexico (nGOM). As discussed in the previous chapters, invasive Red Lionfish were first observed in the nGOM in 2010. Since their detection, their numbers have increased as well as the number of studies focusing on their biology and effects on the native ecosystem have increased. The results of this study will provide much-needed life history metrics for this species in the nGOM and throughout their invaded range. These data will hopefully be used to update and develop new management plans, conduct future detailed comparisons to other invaded regions, and help further assess and understand the effects Red Lionfish are having on native ecosystems.

In Chapter I, collection methods and justification for breaking the nGOM into three distinct ecoregions [southeast (Florida Keys to north 28.25°), northeast (north 28.25° to west 88°), and west (west of 88° to the Mexico border)] was outlined. More than 15,000 Red Lionfish were collected from the nGOM for this study, although the majority of the fish collected came from the northeast region. This ecoregion hosted the majority of the lionfish-specific derby's during the time of this study and the northeast region is where the greatest density of lionfish have been observed (Dahl and Patterson 2014). Seasonal classification was also addressed by evaluating mean monthly sea surface temperature from NOAA's National Data Buoy Center database and months were pooled with similar thermal regimes; spring (March – April), summer (May – October), fall (November – December) and winter (January - February). These four seasons were used for analysis by ecoregion and/or sex depending on statistical or biological significance.

In Chapter II, Red Lionfish age, growth and total length – total weight (TL-TW) relationships using otoliths sectioned and aged in the laboratory and TL-TW data collected from field specimens were evaluated. Estimated Marginal Means (EMM), or mean response of total weight adjusted for total length, were calculated on the raw data and showed that although TL-TW relationships were significantly different by ecoregion, the differences were minimal (females differences = 18.21 g; males differences = 22.75 g) and male Red Lionfish achieve a greater mean TW (333.62 ± 3.58) compared to females (195.13 ± 3.69). Sexual dimorphism has been documented for lionfish in other parts of their invaded region (Little Cayman; Edwards et al. 2014) as well as for other related scorpaenid species (Blackbelly Rosefish; White et al. 1998), although this is the first time this has been documented for invasive Red Lionfish in the nGOM. Red Lionfish ages in the nGOM ranged from 0-4.5 years old; a single back-calculated age of a specimen placed them in the nGOM in 2008 prior to their first detection in the region. Age and growth was significantly different by sex separately and pooled sexes among all three ecoregions. Male and female Red Lionfish in the southeast ecoregion exhibited the highest growth rate (K) and asymptotic maximum length (L_{inf}) while the western ecoregion had the lowest growth parameters. Red Lionfish age and growth parameters were also significantly different by sex in each of the three ecoregions as well as all ecoregions pooled; males exhibiting greater 'K' and 'L_{inf}' values compared to females which further strengthens the existence of sexual dimorphism in nGOM Red Lionfish.

Chapter III provided an in-depth analysis and description of invasive Red Lionfish reproduction, although female reproductive characteristics were analyzed in more depth. Invasive Red Lionfish reproduction has been studied in other parts of their invaded range (Morris 2009, Morris et al. 2011a, Gardner et al. 2015) but appears to differ among the different regions. In this study, male and female Red Lionfish total lengths at 50% maturity for all ecoregions were not significantly different, therefore ecoregions were pooled by sex. Male and female length at 50% maturity was not significantly different although males achieved maturity at a smaller size than females (145.18 and 166.61 mm TL, respectively). Male and female sex ratios were not significantly different from 1:1 for all ecoregions pooled. However, sex ratios among the three ecoregions were significantly different from each other. While this is similar to findings in one other study (Edwards et al. 2014), there is not much comparative information regarding sex ratio across their invaded range. Spawning seasonality has also been reported for lionfish from their native and invaded range and like the nGOM, spawning seasonality was related to water temperature (Gardner et al. 2015, Morris 2009). A similar trend was evident in the nGOM, as GSI followed the seasonal rise and fall of mean SST seasonally. Compared to warmer waters of the Caribbean Sea, spawning season was slightly shorter in the nGOM. Within the nGOM, there was no significant difference in spawning season by ecoregion although peak spawning season was different and was likely due to the delay in warmer temperatures or habitat type. On average, Red Lionfish in the nGOM had a spawning frequency of every 2.49 days which is similar to what was reported in other invaded regions (Gardner et al. 2015, Morris 2009) although, for this study, higher resolution spawning frequency information was also calculated on a seasonal basis. Differences in spawning frequency can be the result of a number of biotic and abiotic factors (Bapary and Takemura 2010, Domeier and Colin 1997, Tyler and Stanton 1995, Claramunt et al. 2007). In cooler seasons (fall, winter, and spring), there were less days between spawns (every 5.0, 5.6 and 9.5 days) compared to the summer (May through October; every 2.16 days) when spawning seasonality was at its peak. This is the first study to report relative batch fecundity (RBF) of invasive Red Lionfish. Batch fecundity and RBF were calculated and resulted in a clear seasonal peak in values that coincided with seasons with warmer water temperatures; A more accurate metric for calculating reproductive output is RBF as it takes fish size out of the calculation and allows for one to make clear comparisons on reproductive output between ecoregions. While there were some clear differences in reproductive life history across the invaded range of the GOM and Caribbean Sea, reproductive parameters found in nGOM specimens were most similar to those reported for the warmer, tropical environment of Little Cayman Island.

Management of invasive species is a difficult task; for example, when trying to reduce the abundance of an invasive species, native species may also be affected by removal efforts (Rinella et al. 2009). Therefore, it is important to understand the life history of invaders to maximize removal success and minimize the effect of those removal techniques on the native species. Management and response plans to the lionfish invasion are only as accurate and timely as the data that is used to inform them. A recent publication by Chagaris et al. (2015) modeled potential lionfish management strategies and how they are predicted to impact a number of native recreationally and commercially important species on the West Florida Shelf. Lionfish life history information, including age, growth, and reproduction, was used in the model, although most of the life history parameters were from other regions. Thus, the results may not have been as accurate (underestimated). There have also been a number of other management plans drafted from around the invaded region (Morris 2012, ANSTF 2014, Johnston et al. 2015) that cite age, growth and reproduction research and parameters. While Morris (2012) and Johnston et al. (2015) specifically mention the need for age and growth, ongoing research is only mentioned in Johnston et al. (2015), and no mention of current age, growth or reproductive parameters are reported from Red Lionfish invaded range. Information reported in ANSTF (2014) highlights a number of invasive lionfish life history studies and their reported parameters. While the information presented in these plans is helpful, updates will need to be made so that current research and findings are being used in future work. Length-weight, age and growth metrics provided in this study may be used for developing region-specific age-structured population models that can be used to evaluate potential effects of targeted removals on the lionfish population such as in Barbour et al. (2011). Further, reproductive life history parameters reported in this study can also be used in stage-based matrix population models (Morris et al. 2011b). These data can also be coupled with other life history data to inform management decisions that will help mitigate the effects Red Lionfish are having on the native fishes and their ecosystems in the nGOM. These data may also be used to predict future impacts of invasive Red Lionfish to the native ecosystem as well as provide insights to managing other potential marine invasive fishes that pose a similar threat to the region. Identifying locations and times of year that larger, older, and thus more fecund, and gluttonous individuals are more vulnerable to harvest has proven to be a valid management technique for native species as those areas or times of year are more heavily protected or regulated (Zhou et al. 2010, Tobin et al. 2013). The opposite management technique can be employed for lionfish if these locations or times of year can be identified. Andradi-Brown et al. (2016) demonstrated that due to the ability of lionfish to inhabit the mesophotic zone (30-150 m), management or removal of the species can be greatly hindered as those depths are beyond recreational SCUBA limits. It has also been shown that lionfish may exhibit ontogenetic habitat migrations as smaller lionfish were found in shallow nursery habitat compared to offshore waters where larger lionfish were present (Claydon et al. 2012). Lionfish from the deeper depths may be moving back up the continental slope into shallower waters following their removal from shallower waters. Information on age, growth, and reproduction may help inform when and where lionfish removals may be most effective. The movement patterns reported can be coupled with these life history characteristics to target the oldest, most fecund or vulnerable individuals in the population resulting in the control or reduction of the lionfish population. Size, sex and reproductive stage of invasive lionfish have been used to inform management relating to targeted removals in Belize (Mizrahi et al. 2017). It was shown that the smaller sized lionfish have a more specialized diet and therefore have a more dramatic impact on those species they are preying on compared to the medium and larger sized lionfish that have a more generalist diet and their impacts to any one species may be less severe (Mizrahi et al. 2017). To better manage the impact to those species that may be more affected by smaller lionfish, effort should be made to target the smaller lionfish in areas where those threatened species live. Additionally, reproductively active female lionfish in Belize (Mizrahi et al. 2017) and in months of greater reproductive activity in Little Cayman Gardner (2015) there was significantly more prey in their stomachs. This suggests that removal efforts should focus around times of increased reproductive activity (warmer months), which is from May-October for the nGOM.

Future work should focus on incorporation of updated life history characteristics into current models and management plans. Further, more detailed comparisons of life history characteristics between the native and invaded range of lionfish as well as the across the invaded range will provide valuable input into which areas are being more impacted by the invasion and thus should receive more attention.

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APPENDIX A – Age and Growth

Table A1.

Summary of weight and length relationships reported by region

Region	Regression Equation (Pooled)	R^2	N	Source
Yucatan, Mexico	$TW = 7.95x10^{-3} (TL)^{3.18}$	0.99	2,143	Sabido-Itzá et al. 2015
Little Cayman	$TW = 3.00x10^{-6} (TL)^{3.24}$	0.97	1,887	Edwards et al. 2014
North Carolina	$TW = 2.89x10^{-5} (TL)^{2.89}$	-	774	Barbour et al. 2011
Northwest Florida	$TW = 2.07x10^{-6} (TL)^{3.34}$	0.98	934	Dahl and Patterson 2014
Northern Gulf of Mexico	$TW = 1.00x10^{-6} (TL)^{3.44}$	0.99	582	Fogg et al. 2013
Northern Gulf of Mexico (Males)	$TW = 3.00x10^{-6} (TL)^{3.29}$	0.97	2,406	This Study
Northern Gulf of Mexico (Females)	$TW=2.00x10^{-6} (TL)^{3.37}$	0.97	2,264	This Study

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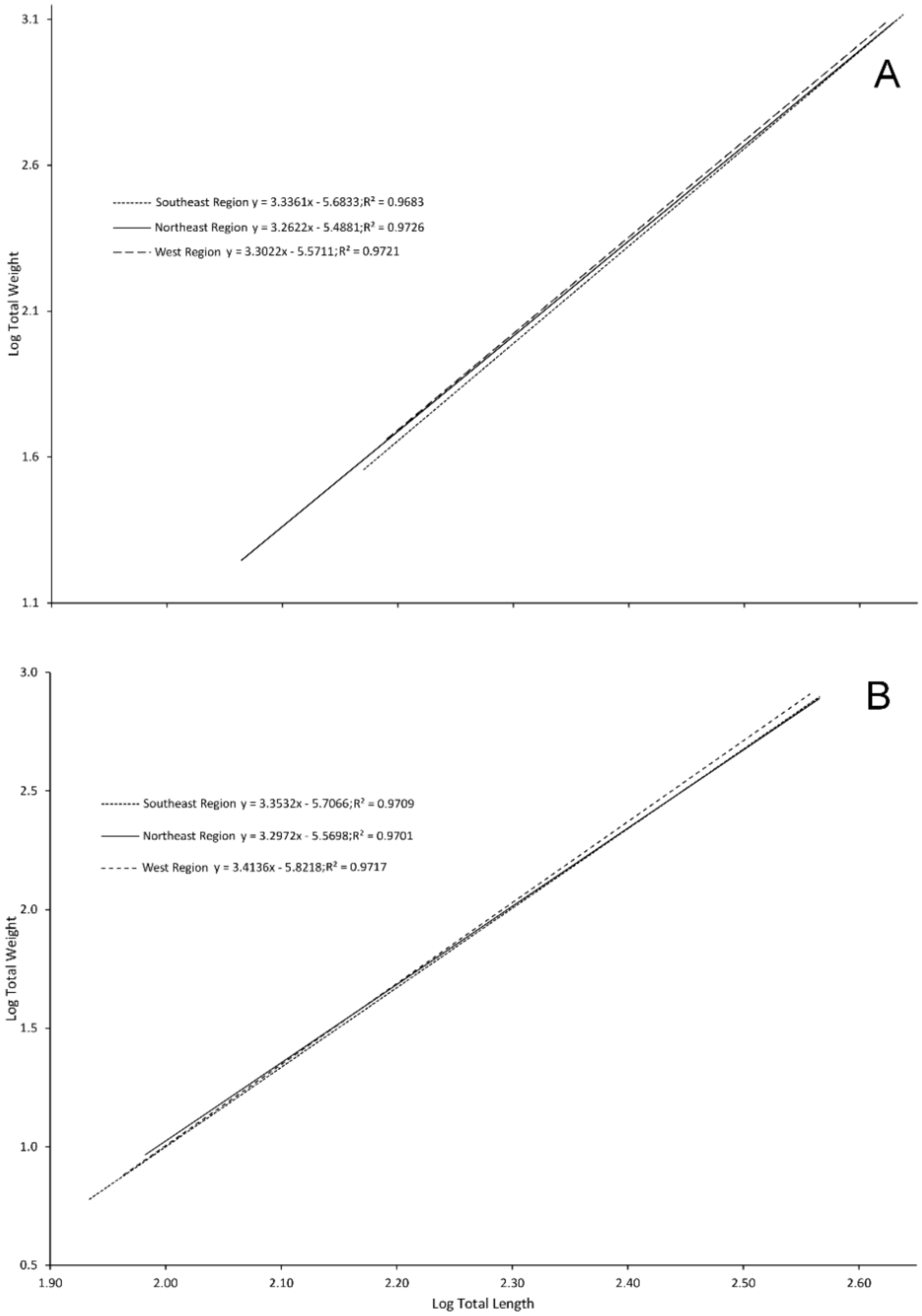

Figure A1. Linear regression lines by ecoregion

Linear regression lines by ecoregion for A) male and B) female Red Lionfish based on log10 total length and log10 total weight.

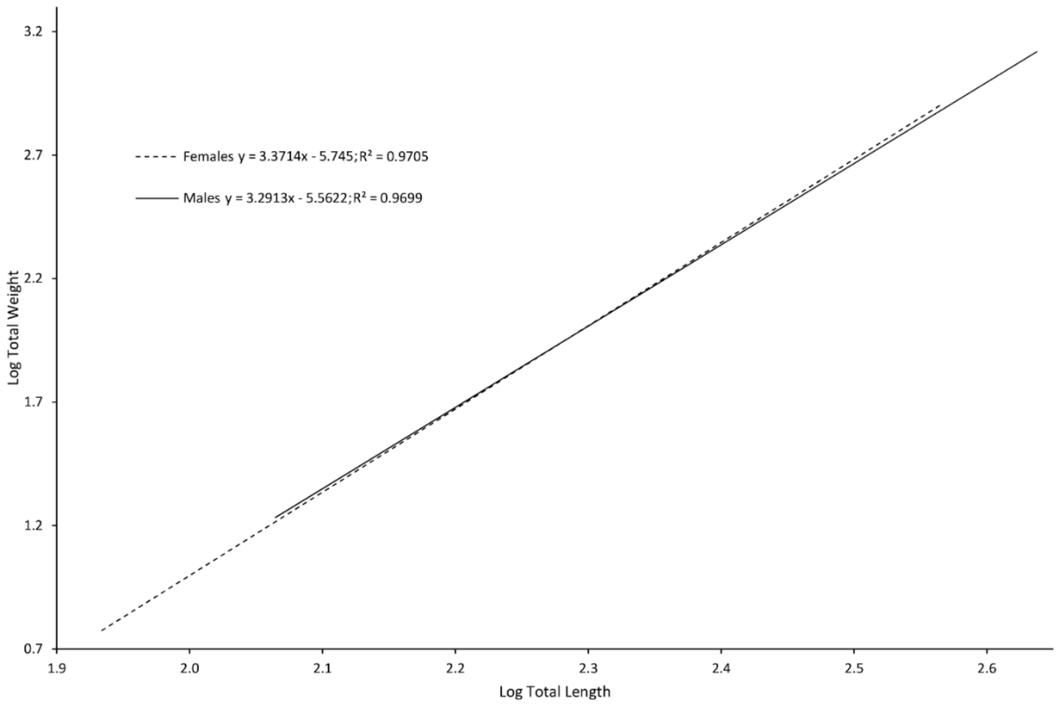

Figure A2. Linear regression lines by sex

Linear regression lines by sex for pooled ecoregion data based on $\log_{10}$ total length (mm) and $\log_{10}$ total weight (g).

APPENDIX B – Reproduction

Table A2.

Mean Gonad weight by month

	Fresh	Frozen	Thawed	1mo	2mo	3mo	4mo	5mo	6mo
Females	12.0 ± 2.4	12.0 ± 2.4	12.0 ± 2.4	12.0 ± 2.4	12.0 ± 2.4	12.0 ± 2.4	12.0 ± 2.4	12.0 ± 2.4	12.0 ± 2.4
Males	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1

Mean Gonad weight (g ± SE) for males ($n = 41$) and females ($n = 33$) across 6 months. All $P > 0.05$. SE = standard error of the mean.