ABSTRACT

MORRIS, JAMES ADIEL, JR. The Biology and Ecology of the Invasive Indo-Pacific Lionfish. (Under the direction of James A. Rice and John J. Govoni.)

The Indo-Pacific lionfishes, *Pterois miles* and *P. volitans*, are now established along the Southeast U.S. and Caribbean and are expected to expand into the Gulf of Mexico and South America. Prior to this invasion little was known regarding the biology and ecology of these lionfishes. I provide a synopsis of lionfish biology and ecology including: invasion chronology, taxonomy, local abundance, reproduction, early life history and dispersal, venomology, feeding ecology, parasitology, potential impacts, and control and management. This information was collected by review of the literature and by direct field and experimental study. I confirm the existence of an unusual supraocular tentacle phenotype and suggest that the high prevalence of this phenotype in the Atlantic is not the result of selection, but likely ontogenetic change. To characterize the trophic impacts of lionfish, I report a comprehensive assessment of diet that describes lionfish as a generalist piscivore that preys on over 40 species of teleost comprising more than 20 families. Next, I use the histology of gonads to describe both oogenesis and reproductive dynamics of lionfish. Lionfish females mature at approximately 170 mm total length and reproduce several times per month throughout the entire calendar year off North Carolina and the Bahamas. To investigate predation, an important component of natural mortality, I assessed the vulnerability of juvenile lionfish to predation by native serranids. Juvenile lionfish were largely avoided as prey suggesting that predation mortality by serranids will not likely be a significant source of mortality for lionfish populations. Last, I used a stage-based, matrix population model to estimate the scale of control that would be
needed to reduce an invading population of lionfish. Together, this research provides the first comprehensive assessment on lionfish biology and ecology and explains a number of life history and ecological interactions that have facilitated the unprecedented and rapid establishment of this invasive finfish. Future research is needed to understand the scale of impacts that lionfish could cause, especially in coral reef ecosystems, which are already heavily stressed. This research further demonstrates the need for lionfish control strategies and more rigorous prevention and early detection and rapid response programs for marine non-native introductions.
The Biology and Ecology of the Invasive Indo-Pacific Lionfish

by

James Adiel Morris, Jr.

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements of the Degree of
Doctor of Philosophy

Zoology

Raleigh, North Carolina

2009

APPROVED BY:

James A. Rice, Ph.D.
Co-chair of Advisory Committee

John J. Govoni, Ph.D.
Co-chair of Advisory Committee

Craig A. Harms, D.V.M, Ph.D.

Kyle W. Shertzer, Ph.D.

Craig V. Sullivan, Ph.D.
BIOGRAPHY

I was raised in Atlantic and Sea Level, North Carolina, two sister commercial fishing communities located on the central coast. I am the eldest son of Jimmy and Robin Morris, and have one younger brother, Julius. Throughout my life, I have been privileged to participate in many of our traditional North Carolina commercial fisheries. I was raised in a fifth generation commercial fishing family and enjoyed a childhood of working in the water alongside my grandfather, Julius Gray, parents, and many extended family members. My earliest memories include playing along the docks and shore of the Atlantic harbor. Later, I enjoyed helping my father pioneer a shellfish aquaculture farm, which involved converting an old fishhouse into a shellfish hatchery. I attended the University of North Carolina at Wilmington and East Carolina University where I earned a Bachelor of Science in Biology in 1999. In 2001, I earned a Master of Science in Biology also from East Carolina University where my thesis applied otolith microchemistry to investigate anadromous striped bass life history in the Neuse and Pamlico river systems of North Carolina and the Stewiacke river in the inner Bay of Fundy, Nova Scotia. Following completion of my Master’s in 2001, I began a career at the NOAA Center for Coastal Fisheries and Habitat Research where I am an Ecologist specializing in marine invasive species and aquaculture research. In 1998, I married a “girl from Harkers Island” Joella Hancock. We now have four children, Jacy, Riley, Zoey, and Charlie.
ACKNOWLEDGEMENTS

I thank my doctoral committee Jim Rice, Jeff Govoni, Craig Sullivan, Craig Harms, and Kyle Shertzer for their assistance and direction during the last five years. Each contributed significantly and provided excellent training in their specialties. I am especially indebted to Jeff Govoni for his mentoring, relentless support and friendship, and for believing in me. The vision for much of this work was stimulated by him and for that I will always be grateful.

I am grateful for the NOAA Aquatic Invasive Species Program, in particular Dorn Carlson and Michelle Harmon, and the NOAA National Centers for Coastal Ocean Science, especially David Johnson and Gary Matlock, for the financial support and foresight of the threat that invasive lionfish pose to our reef communities. None of this would have been possible without their support.

I am indebted to the many divers at the Center for Coastal Fisheries and Habitat Research (CCFHR), especially Paula Whitfield, who collected samples, reported observations, and supported this research even when it was inconvenient for them. I also thank my fellow CCFHR colleagues who tolerated my naïve curiosity and helped shape my research questions. I thank Patti Mararro for her assistance with technical editing and library support. I am especially grateful to Dean Ahrenholz for his counsel, encouragement, and mentoring along the way.

I also thank Lad Akins of the Reef Environmental Education Foundation (REEF). Lad’s support, energy, and practical insights have undoubtedly contributed to and helped
shape many aspects of this work. I particularly would like to thank Bruce Purdy, Stuart Cove, Stephanie Green and the well over 200 REEF volunteers for assistance with specimen collections.

I am also very grateful to my colleagues at the U.S. Geological Survey (USGS), in particular Amy Benson, Pam Fuller, Jackie Langston, and Denise Gregoire for their assistance with countless requests for GIS products and maps and for their integration of lionfish tracking into the USGS NAS database. I am especially grateful to Pam Schofield for her advice, encouragement, and support through much of this work.

I am forever grateful to my family for their encouragement and unconditional support throughout my life. I thank my father for instilling in me an innate work ethic to push forward even when things are hard. I don’t know anyone who works harder and is more selfless than him. I also thank my mother for nurturing my independence and self-worth; she defines what it means to be a mother. I also thank my father-in-law, Joel Hancock, whose love of learning inspires me. Last, I thank my wife, Joella, and my children Jacy, Riley, Zoey, and Charlie for buoying me in the tough times and now celebrating with me in this accomplishment. They are the joy of my life.
# TABLE OF CONTENTS

List of Tables ................................................................................................................... viii

List of Figures .................................................................................................................. ix

**Chapter 1. Introduction** .......................................................................................... 1  
References ...................................................................................................................... 7  
Figures ............................................................................................................................. 10

**Chapter 2. Biology and ecology of the invasive lionfishes, *Pterois miles* and *Pterois volitans*** ....................................................................................................................... 14  
Abstract ......................................................................................................................... 14  
Introduction ..................................................................................................................... 15  
Invasion Chronology ....................................................................................................... 15  
Taxonomy ........................................................................................................................ 16  
Local Abundance ............................................................................................................ 18  
Reproduction .................................................................................................................. 19  
Early Life History And Dispersal .................................................................................... 20  
Venomology .................................................................................................................... 21  
Feeding Ecology ............................................................................................................ 23  
Parasitology ..................................................................................................................... 24  
Potential Impacts ........................................................................................................... 25  
Control and Management ............................................................................................. 26  
Conclusions ..................................................................................................................... 28  
Acknowledgements ......................................................................................................... 28  
References ....................................................................................................................... 29

**Chapter 3. Phenotypic variation of lionfish supraocular tentacles** ......................... 34  
Abstract ......................................................................................................................... 34  
Introduction ..................................................................................................................... 35  
Methods ........................................................................................................................... 36  
Results and Discussion .................................................................................................. 37  
Acknowledgements ......................................................................................................... 40  
References ....................................................................................................................... 41  
Figures ............................................................................................................................. 42

**Chapter 4. Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago** ......................................................................................................................... 46  
Abstract ......................................................................................................................... 46  
Introduction ..................................................................................................................... 47  
Methods ........................................................................................................................... 49
# Results .................................................................................................... 52
# Discussion .............................................................................................. 54
# Conclusion ............................................................................................. 58
# Acknowledgements ............................................................................. 58
# References .............................................................................................. 60
# Tables ..................................................................................................... 64
# Figures .................................................................................................... 67

## Chapter 5. Oogenesis and spawn formation in the lionfishes, *Pterois miles* (Bennet) and *Pterois volitans* (Linnaeus) .............................................................. 71

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>71</td>
</tr>
<tr>
<td>Introduction</td>
<td>71</td>
</tr>
<tr>
<td>Methods</td>
<td>72</td>
</tr>
<tr>
<td>Results</td>
<td>75</td>
</tr>
<tr>
<td>Discussion</td>
<td>81</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>84</td>
</tr>
<tr>
<td>References</td>
<td>85</td>
</tr>
<tr>
<td>Tables</td>
<td>89</td>
</tr>
<tr>
<td>Figures</td>
<td>90</td>
</tr>
</tbody>
</table>

## Chapter 6. Reproductive dynamics of the invasive lionfishes *Pterois miles* and *Pterois volitans* in the Western North Atlantic ........................................... 97

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>97</td>
</tr>
<tr>
<td>Introduction</td>
<td>98</td>
</tr>
<tr>
<td>Methods</td>
<td>99</td>
</tr>
<tr>
<td>Results</td>
<td>102</td>
</tr>
<tr>
<td>Discussion</td>
<td>104</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>107</td>
</tr>
<tr>
<td>References</td>
<td>109</td>
</tr>
<tr>
<td>Tables</td>
<td>111</td>
</tr>
<tr>
<td>Figures</td>
<td>113</td>
</tr>
</tbody>
</table>

## Chapter 7. Investigation of predation on invasive juvenile lionfish in the Atlantic .... .................................................. 118

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>118</td>
</tr>
<tr>
<td>Introduction</td>
<td>119</td>
</tr>
<tr>
<td>Methods</td>
<td>120</td>
</tr>
<tr>
<td>Results</td>
<td>123</td>
</tr>
<tr>
<td>Discussion</td>
<td>125</td>
</tr>
<tr>
<td>Conclusion</td>
<td>128</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>129</td>
</tr>
<tr>
<td>References</td>
<td>130</td>
</tr>
</tbody>
</table>
Tables................................................................................................... 133
Figures.................................................................................................. 134

Chapter 8.  A stage-based matrix population model of invasive lionfish with implications for control ................................................................. 135
  Abstract .............................................................................................. 135
  Introduction ....................................................................................... 136
  Methods ............................................................................................ 137
  Results and discussion ..................................................................... 140
  Acknowledgements ......................................................................... 142
  References ...................................................................................... 143
  Tables ............................................................................................... 145
  Figures ............................................................................................. 147

Chapter 9. Conclusions ........................................................................ 149
  References ....................................................................................... 163
  Table ............................................................................................... 168
LIST OF TABLES

Chapter 4

Table 4.1 Identifiable lionfish prey sorted by taxa…………………………64
Table 4.2 Rankings of importance indices for each fish family for each importance index…………………………………………….…...66

Chapter 5

Table 5.1 Key cytological features characteristic of lionfish oocytes………89

Chapter 6

Table 6.1 Criteria used for histological staging of lionfish ovaries………..111
Table 6.2 Criteria used for histological staging of lionfish testes…………112

Chapter 7

Table 7.1 Summary of predator behavior and number of prey eaten during each period of the predation trials………………………133

Chapter 8

Table 8.1 Values of lower-level parameters, their sources, and the sensitivity of λ to each of them. The matrix element in which the lower-level parameter was used is provided in parentheses. Mortality is expressed as instantaneous rate………………….145
Table 8.2 Matrix element value, computation, and elasticity of λ…………146

Chapter 9

Table 9.1 Summary of main predictors of invasiveness for established non-indigenous freshwater fish species that are relevant to lionfish. The presence (Y) or absence (N) of each predictor is noted for lionfish. Predictor summary was adapted freely from a comprehensive review by Garcia-Berthou (2007)…………….168
LIST OF FIGURES

Chapter 1

Figure 1.1. Summary of marine introductions sorted by taxa for Florida, Caribbean, and Gulf of Mexico regions from 1887 – 2009. Data courtesy of the U.S. Geological Survey Non-indigenous Aquatic Species Database...........................10

Figure 1.2. Progression of the lionfish invasion from 2000 to 2009. Sightings data courtesy of the U.S. Geological Survey Non-indigenous Aquatic Species Database.........................11

Figure 1.3. Composite 9km resolution image of average sea surface temperatures collected by Aqua MODIS in 2008 for North, Central, and South America. Lionfish lethal chronic minimum is estimated to be 10°C (Kimball et al. 2004).............12

Figure 1.4. Potential future range of lionfish based on the lethal thermal minimum of 10°C.................................................................13

Chapter 3

Figure 3.1. Photographs of a lionfish exhibiting the straight (A) and peacock-feather (B) supraocular tentacle phenotype..............42

Figure 3.2. Cumulative frequency plot of lionfish supraocular tentacle type sorted into 20 mm size classes with proportion of each tentacle type displayed for each size class.................................43

Figure 3.3. Box plots comparing supraocular tentacle category and fish size. The boundary of the box closest to zero represents the 25th percentile with the line in the middle representing the median. The boundary of the box farthest from zero indicates the 75th percentile with the whiskers (error bars) indicating the 90th and 10th percentiles. Outliers are indicated by black circles.......44

Figure 3.4. Neighbor-joining tree derived from analyses of absolute distances between 90 western Atlantic lionfish specimens with peacock-feather, straight or no supraocular tentacles. Neighbor-joining analyses were carried out using PAUP (v. 4.0, Swofford 2002)
Chapter 4

Figure 4.1. Sampling locations and number of lionfish collected along the Bahamian archipelago

Figure 4.2. Mean proportion of lionfish diet comprised of teleosts by lionfish 40-mm total length size classes. \( %V = 0.00102TL + 0.602, R^2 = 0.76, P = 0.0050 \), \( %N = 0.00131TL + 0.408, R^2 = 0.55, P = 0.0200 \), \( %F = 0.00109TL + 0.508, R^2 = 0.86, P = 0.003 \)

Figure 4.3. Mean teleost and crustacean prey size consumed by lionfish. Lionfish size displayed in 40-mm total length size classes. Mean teleost prey size = 0.380TL + 14.586, \( R^2 = 0.46, P = 0.010 \). Mean crustacean prey size = 0.045TL + 8.043, \( R^2 = 0.36, P = 0.002 \)

Figure 4.4. Proportion of lionfish stomachs containing prey throughout the day. Mean proportion of lionfish with prey in stomach = -0.044Time + 92.367, \( R^2 = 0.39, P = 0.01 \)

Chapter 5

Figure 5.1. Transverse sections of lionfish ovaries depicting cystovarian morphology and oocyte growth and maturation along the stroma-lumen axis. A) Line drawing, adapted from Koya and Muñoz (2007) and B) electron micrograph (scale bar = 500 \( \mu \)m). BV = blood vessels, MO = mature oocyte, OL = ovarian lumen, OS = ovarian stroma, OW = ovary wall, P = peduncle, PO = primary oocyte

Figure 5.2. Early primary growth stage oocyte (inset) and late primary growth stage oocytes. EPGO = early primary growth stage oocyte, GE = germinal epithelium, GV = germinal vesicle, LPGO = late primary growth oocyte, NU = nucleoli, O = oogonia. Scale bars = 50 \( \mu \)m and 15 \( \mu \)m (inset)
Figure 5.3. A) Early cortical alveolus stage oocyte (Scale bar = 25 µm) and B) mid cortical alveolus stage oocyte (Scale bar = 100 µm). CA = cortical alveoli, GV = germinal vesicle, MCAO = mid cortical alveolus stage oocyte, NU = nucleoli, P = peduncle.

Figure 5.4. A) Early vitellogenic stage oocyte (EVO) (scale bar = 50 µm), B) mid-vitellogenic stage oocyte (MVO) (scale bar = 100 µm), and C) follicular complex of vitellogenic stage oocyte (scale bar = 50 µm). GC = granulosa cells, GV = germinal vesicle, LD = lipid droplets, MVO = mid-vitellogenic stage oocyte, NU = nucleoli, P = peduncle, T = theca, YG = yolk granules, ZR = zona radiata.

Figure 5.5. Early maturation stage oocyte exhibiting germinal vesicle migration and yolk granule and lipid droplet coalescence (scale bar = 100 µm) GV = germinal vesicle, LD = lipid droplets, MO = maturation oocytes, PDP = peduncle detachment (ovulation) point, YG = yolk granules.

Figure 5.6. A) Ovulated eggs and ovary wall depicting production of gelatinous matrix (scale bar = 150 µm) and B) ovary wall complex (scale bar = 50 µm). BM = basement membrane, EC = endothelial cell layer, GM = gelatinous material, HA = hair-like appendages, HO = hydrated oocyte, SC = secretory cells, SM = smooth muscle, O = oil globule, OWC = ovarian wall complex.

Figure 5.7. A) Lionfish ovary with unreleased gelatinous egg mass (scale bar = 10 mm). Anterior end of the ovary is oriented to the left. B) Oocytes in several stages of ovarian atresia. The most advanced-staged atretic oocytes are labeled as AO (scale bar = 100 µm). GE = gelatinous egg mass, OT = ovigerous tissue.

Chapter 6

Figure 6.1 Histological sections of lionfish testes at immature (A), early developing (B), mid developing (C) and late developing (D) stages. Some of the stage-specific histological criteria is noted by arrows. SC = spermatocyte, SG = spermatogonia, ST =
spermatids, SZ = spermatozoa. A) scale bar = 50 µm, B) scale bar = 50 µm, C) scale bar = 200 µm, D) scale bar = 500 µm, arrows indicate lobules filled with spermatozoa………………..113

Figure 6.2. Figure 6.2 Percent of mature female lionfish by 20-mm TL size class. Bar = sample size; line = logistic model ($k = 0.075$, $\gamma = 178.282$, see text for model equation), open circles = number mature in each size class…………………………….114

Figure 6.3. Figure 6.3 Percent of mature male lionfish by 20-mm TL size class. Bar = sample size; line = logistic model ($k = 0.071$, $\gamma = 101.336$, see text for model equation), open circles = number mature in each size class…………………………………………………………115

Figure 6.4. Reproductive status of female lionfish (A) and male lionfish (B) collected from North Carolina, South Carolina, and the Bahamas. ED = early developing; LD = late developing; S = spawning; MD = mid developing……………………………………116

Figure 6.5. Lionfish reproductive activity by season collected from temperate (North Carolina and South Carolina) and tropical (Bahamas). A) NC/SC females; B) Bahamas females; C) NC/SC males; D) Bahamas males………………………….117

Chapter 7

Figure 7.1. Schematic showing the top view and side views of the 1.8-m diameter, 2000-L tank used in the predation trials (not drawn to scale). Top view: Predator, prey, and acrylic tubes depicted with diagonal lines representing the designated 30-cm zone around the tubes. Side view: Predator, prey, acrylic tubes, and tube removal lines depicted. The lines allowed the tubes to be lifted simultaneously, releasing the prey with minimal disturbance…………………………………………………...134

Chapter 8

Figure 8.1. Population growth rate ($\lambda$, solid line) for theoretical values of $P_A$. The threshold is $\lambda=1$, below which population growth is negative……………………………………………………………...147
Figure 8.2. Estimate of the proportion of adult lionfish that would need to be removed from an invading population in order to achieve $\lambda<1$. 

148
CHAPTER 1. INTRODUCTION

Invasive species are capable of causing extinctions of native plants and animals (Clavero and García-Berthou 2005), reducing biodiversity (Olden et al. 2004), competing with native organisms for limited resources, and altering habitats (Mack et al. 2000; Kolar and Lodge 2001; Rahel 2002; Olden et al. 2004). Extreme economic costs have also been observed for many invasions, e.g., Formosan termite, which causes an estimated $300 million in damage annually in New Orleans alone (NISC 2001). Recent estimates suggest that the cost of invasive species to the U.S. economy is $137 billion annually (Pimentel et al. 2000; 2005).

The increase in bioinvasions over the last century is astounding. Bioinvasions have increased significantly since the beginning of the industrial revolution and are strongly correlated with economic growth (Lin et al. 2007). As a result of increased shipping traffic and world travel, the species composition of our diverse ecosystems is being homogenized causing both direct and indirect changes of our natural resources.

The number of introductions of non-indigenous species1 into estuarine and coastal marine environments is small compared to terrestrial and freshwater systems. Owing to

---

1 Invasion ecology is replete with adjectives describing non-indigenous species, some of which have a negative connotation for the local environment (i.e., invasive, weedy) or on humans (i.e., noxious, nuisance) (Colautti and Maclsaac 2004). The term ‘invasive species’ used throughout this dissertation follows the definition provided in the federal register as an organism that is: 1) non-native (or alien) to the ecosystem being considered; and 2) likely to cause economic or environmental harm or harm to human health (Invasive Species Executive Order No. 13112). By this definition, range extensions of native species or non-indigenous species that exhibit no potential for ecological or economic impacts are not considered invasive species.
the rapid increase in shipping over the past several decades, and to the increase in coastal
development, marine introductions are accelerating with more than 400 invasions
reported along the Pacific, Gulf, and Atlantic coasts of the U.S. (Ruiz et al. 1997).
Introductions into nearshore environments of the U.S. East Coast, such as the European
green crab (Behrens Yamada 2001), tunicates (Lambert 2007), and more recently
ornamental finfish (Semmens et al. 2004), are nearly impossible to eradicate once
established, given the expansive habitat, high connectivity and complexity of estuarine
and oceanic currents, and constant propagule pressure (Drake and Lodge 2006). Over the
past century, more than 68 marine introductions have occurred in the Florida, Caribbean,
and Gulf of Mexico regions. The taxonomic diversity of these introductions is high
(Figure 1.1) and few studies have assessed their vectors and potential impacts.

A recent introduction of the tropical marine reef fishes, the red lionfish (*Pterois
volitan*) and devil firefish (*P. miles*) (Scorpaenidae, order Scorpaeniformes), has resulted
in an unprecedented rate of establishment (Figure 1.2). Lionfish\(^2\) were first reported in
the 1980’s along South Florida and have now spread along the Southeast U.S. and well
into the Caribbean (Figure 1.2). The future distribution of lionfish is likely to be
restricted largely by thermal tolerance (Kimball et al. 2004). The eventual range of
lionfish based on thermal habitat limitations includes the Gulf of Mexico, the entire
Caribbean, and as far south as the temperate regions of the east coast of South America

\(^2\) *Pterois miles* and *P. volitans* are sympatric species distinguishable only by genetics (Hamner et al. 2007). The term “lionfish” in this dissertation refers collectively to both species.
(Figures 1.3 and 1.4). As a venomous scorpionfish native to the Indo-Pacific, lionfish are considered invasive by definition (Invasive Species Executive Order No. 13112) because of their probable impacts to native reef fish communities (Albins and Hixon 2008; Morris et al. 2009) and to human health (Vetrano et al. 2002).

Prior to the introduction of lionfish, little information on their biology and ecology was published. As a result, much of what has been learned about lionfish in their invaded range is new information for the species or genus. An early integrated assessment for lionfish conducted by researchers at the National Oceanic and Atmospheric Administration (Hare and Whitfield 2003) gathered what little life-history information was available and predicted the continued spread of lionfish. Since 2003, a large body of research on lionfish has ensued. Many of the research topics herein were driven by direct requests from coastal managers for information about lionfish and the inherent need to understand the underlying mechanisms that have facilitated this unprecedented invasion.

Chapter two of this dissertation provides a synopsis of the biological and ecological profiles of lionfish. This work was compiled through exhaustive review of the scientific literature and inclusion of results provided from recent and ongoing studies. Requests for accurate information on lionfish from newly invaded regions, specifically the Caribbean, prompted the compilation of this information. Given the plethora of myths and inaccuracies on lionfish available on the internet, provision of correct information to coastal managers was viewed as the first necessary step towards
developing management approaches. Owing to the need for rapid and wide distribution of this information to the Caribbean, this manuscript was published in the *Proceedings of the Gulf and Caribbean Fisheries Institute*, a widely circulated and openly available publication for the Gulf of Mexico and Caribbean regions.

The remaining chapters of this dissertation represent stand-alone studies comprising various investigations of lionfish biology and ecology. Chapter three provides an in-depth study of phenotypic variation of lionfish supraocular tentacles. A previous report suggested that a novel phenotype was rapidly evolving among lionfish populations in their native range. Observations of Atlantic lionfish supraocular tentacles suggested otherwise as genetic analysis found that phenotypic variation of supraocular tentacles is not rapidly evolving, but is likely a natural ontogenetic change. This assessment of phenotypic variation in lionfish is among the first examples of new information provided for the pteroines as a result of this introduction.

Chapter four provides a comprehensive assessment of the feeding ecology of lionfish. A primary concern regarding lionfish impacts is the trophic disruption lionfish may cause to native reef fish communities. This study documents, for the first time, the comprehensive dietary habits of invasive lionfish in coral reef environments of the Bahamian Archipelago. The Bahamas was chosen as a study location because of the rapid invasion of lionfish in the Bahamas (widespread within three years), the high densities of lionfish, the availability of resources for intensive collecting, and the need to assess lionfish impacts in coral reef communities. This study provides a baseline of
dietary habitats during the early years of the lionfish invasion in the Bahamas and will provide a point of reference for future changes in lionfish diet and a basis for forecasting trophic impacts.

Chapter five describes the most fundamental process in female lionfish reproduction, oogenesis. This chapter details the sequential stages of oocyte development and the process by which the ovary produces an enveloping gelatinous matrix to create a bouyant ball of eggs. The results reported in this chapter compare pteroine oogenesis with that of the closely related pigmy lionfish, *Dendrochirus brachypterus* (Fishelson 1975).

Chapter six is an assessment of the reproductive dynamics of invasive lionfish. Analysis of gonads collected over the course of six years revealed various aspects of reproduction at the population scale, including the size at which lionfish become sexually mature, the seasonality of their spawning, how many eggs they release during each spawning event, and how often they release a batch of eggs during their spawning season. Lionfish gonads were collected from both the temperate (North Carolina and South Carolina) and tropical (Bahamas) regions encompassing a wide range of thermal habitats. This assessment provides evidence that lionfish reproduce several times per month throughout the entire calendar year. Annual fecundity of lionfish was estimated to be over two million eggs per year. This work provides, for the first time, a comprehensive assessment of reproductive parameters for the pteroines.
Chapter seven investigates the predation vulnerability of juvenile lionfish by native, top-level predators of the Atlantic. As predation is typically a significant component of natural mortality, the avoidance of lionfish as prey by native predators can have profound impacts on the lionfish’s population growth. Juvenile lionfish were overwhelmingly avoided as prey by native serranids suggesting that their venom defense effectively deters predation outside of their native range.

Chapter eight uses many of the reproductive vital rates measured in Chapter six and some assumed life history vital rates from the literature to derive a stage-based, matrix population model for lionfish. This modeling approach has been used to support management decisions for conservation (Crouse et al. 1987) and for developing control strategies for pests (Brooks and Lebreton 2001). The stage-based matrix model was used to evaluate sensitivities and elasticities of lionfish vital rates and matrix elements. Chapter eight also estimates the proportion of an invading lionfish population that would need to be removed to reduce population growth rate below a sustainable level. This is the first application of a matrix population model that estimates the scale of control measures required to reduce the abundance of a marine invasive finfish.

In Chapter nine, I provide a summary of the findings and their relevance to invasion ecology, understanding of lionfish biology and ecology, and the management of this invasive marine finfish. I also provide recommendations for future research and legislative perspectives on the importation of non-native species.
REFERENCES


Kimball, M.E., J.M. Miller, P.E. Whitfield, and J.A. Hare. 2004. Thermal tolerance and potential distribution of invasive lionfish (*Pterois volitans/miles* complex) on the


Figure 1.1  Summary of marine introductions sorted by taxa for Florida, Caribbean, and Gulf of Mexico regions from 1887 – 2009. Data courtesy of the U.S. Geological Survey Non-indigenous Aquatic Species Database.
Figure 1.2 Progression of the lionfish invasion from 2000 to 2009. Sightings data courtesy of the U.S. Geological Survey Non-indigenous Aquatic Species Database.
Figure 1.3 Composite 9km resolution image of average annual sea surface temperatures collected by Aqua MODIS in 2008 for North, Central, and South America. Lionfish lethal chronic minimum is estimated to be 10°C (Kimball et al. 2004).
Figure 1.4 Potential future range of lionfish based on the lethal thermal minimum of 10°C.
CHAPTER 2. BIOLOGY AND ECOLOGY OF THE INVASIVE LIONFISHES, 
*Pterois miles* AND *Pterois volitans*

James A. Morris, Jr., John L. Akins, Ann Barse, David Cerino, David W. Freshwater, Stephanie J. Green, Roldan C. Muñoz, Claire Paris, and Paula E. Whitfield


ABSTRACT

The Indo-Pacific lionfishes, *Pterois miles* and *P. volitans*, are now established along the U.S. southeast coast, Bermuda, Bahamas, and are becoming established in the Caribbean. While these lionfish are popular in the aquarium trade, their biology and ecology are poorly understood in their native range. Given the rapid establishment and potential adverse impacts of these invaders, comprehensive studies of their biology and ecology are warranted. Here we provide a synopsis of lionfish biology and ecology including invasion chronology, taxonomy, local abundance, reproduction, early life history and dispersal, venomology, feeding ecology, parasitology, potential impacts, and control and management. This information was collected through review of the primary literature and published reports and by summarizing current observations. Suggestions for future research on invasive lionfish in their invaded regions are provided.
INTRODUCTION

The lionfish invasion in the Northwestern Atlantic and the Caribbean represents one of the most rapid marine finfish invasions in history. Despite being a popular member of the marine ornamental aquarium trade, little was known regarding the biology and ecology of these lionfishes prior to this invasion. Information on lionfish abundance, dietary habits, predators, and seasonality of reproduction are scarce. Most of what has been published on lionfish relates largely to lionfish envenomations, which commonly occur during aquarium husbandry or as a result of poor handling by home aquarists.

Invasive lionfish are a concern to coastal managers due to their potential threat to fisheries resources, native fish communities, and human health. Since 2000, National Oceanic and Atmospheric Administration (NOAA) researchers have partnered with non-governmental organizations, academics, and other federal and state agencies to develop a programmatic response to the lionfish invasion. The following provides a synopsis of information on the biology and ecology of the invasive lionfishes that have invaded the Northwestern Atlantic and Caribbean, and a discussion of future research needs and management options.

INVASION CHRONOLOGY

Many non-native marine ornamental fishes have been reported along the U.S. East Coast, with a “hotspot” of introductions occurring in South Florida (Semmens et al. 2004). Lionfish have been documented off Palm Beach, Boca Raton, and Miami, Florida
beginning in 1992; and Bermuda, North Carolina, South Carolina and Georgia beginning in 2000 (Whitfield et al. 2002, Hare and Whitfield 2003, REEF 2008, USGS 2008). Since 2004, lionfish have become widespread in the Bahamas (Whitfield et al. 2007, REEF 2008, USGS 2008). More recently, lionfish were reported in the Turks and Caicos and Cuba in 2007 (Chevalier et al. 2008), and in the Cayman Islands, Jamaica, Dominican Republic (Guerrero and Franco 2008), U.S. Virgin Islands, Belize, and Barbados in 2008 (REEF 2008, USGS 2008). Juvenile lionfishes have also been reported along the U.S. northeast coast including Virginia, New York, Rhode Island, and Massachusetts since 2001. These northeastern specimens are incapable, however, of overwintering due to thermal intolerance (Kimball et al. 2004), and they are not considered established.

It is nearly impossible to determine which introduction event(s) allowed lionfish to become established. Research on the genetic variation of the lionfish populations is providing insight into the minimum number of lionfish and the geographic origin of founder population(s) (Hamner et al. 2007). Interestingly, this is not the first documented invasion of *Pterois* sp. as Golani and Sonin (1992) reported a Mediterranean invasion of *P. miles* from the Red Sea via the Suez Canal.

**TAXONOMY**

*Pterois miles* and *P. volitans* are morphologically similar and distinguishable in their native range by meristics, with *P. volitans* exhibiting one higher count of dorsal and
anal fin rays when compared to *P. miles*. This difference was documented by Schultz (1986) who reported that *P. miles* is found in the Red Sea, Persian Gulf, and Indian Ocean (excluding Western Australia) and *P. volitans* is found in the Western and Central Pacific and Western Australia. Kochzius et al. (2003) used mitochondrial DNA analyses to show that specimens identified as *P. miles* and *P. volitans* were genetically distinct. Their geographic sampling did not allow the determination of whether this distinction was at the species or population level. Hamner et al. (2007) analyzed specimens identified as *P. miles* and *P. volitans* from additional areas of their native range, including Indonesia, where they are sympatric. They found that the two taxa are clearly distinct supporting the designation of two species. Analyses with different molecular markers and additional geographic samples of species of *Pterois* and the out-group comparison with the closely related genus *Dendrochirus*, support the classification of *P. miles* and *P. volitans* as separate species. Recent efforts by Hamner et al. (2007) have confirmed that:

i) Both *P. miles* and *P. volitans* were introduced along the U.S. East Coast,

ii) *P. volitans* comprises approximately 93% of the population, and

iii) A strong founder effect (*i.e.* low genetic diversity) is evident among Atlantic specimens.

The genetic structure of invasive lionfish in the Caribbean is presently unknown. Only one species (*P. volitans*) has been confirmed along the Bahamian archipelago. Documentation of genetic change and adaptation of lionfish populations in their invaded range is warranted (*e.g.*, Morris and Freshwater 2008). Greater understanding of lionfish
genetics could assist with validation of reef fish dispersal and connectivity models in the Northwestern Atlantic, Caribbean, and Gulf of Mexico.

**LOCAL ABUNDANCE**

Whitfield et al. (2007) provided the first assessment of lionfish densities off North Carolina and reported an average of 21 lionfish per hectare across 17 locations in 2004. Lionfish densities off North Carolina have continued to increase. Recent assessments off New Providence, Bahamas indicate lionfish densities are more than 18 times higher than the 2004 North Carolina estimates (Green and Côté 2009). The cryptic nature of lionfish makes them difficult to census. It is likely that estimates of lionfish on complex coral reef habitats under-represent local abundance of juveniles. Thus, these density estimates should be considered conservative. Further, lionfish densities in the Bahamas are more than eight times higher than estimates from their native range (Green and Côté 2009). Few published data are available, however, from the Indo-Pacific region providing high uncertainty for this comparison. In their invaded Atlantic and Caribbean ranges, it is unclear when lionfish densities will reach carrying capacity. Given that many reef fishes along the east coast of the U.S. and Caribbean are overfished (Hare and Whitfield 2003), lionfish might be utilizing vacated niche attributes such as increased availability of forage fishes and reef space.

Monitoring of lionfish densities across habitat types using standardized indices of abundance is needed to determine when lionfish abundances reach carrying capacity.
Lionfish densities are expected to vary depending on such factors as seasonality, local recruitment, local niche availability, and fishing pressure. Studies assessing the drivers controlling lionfish densities in specific habitats are needed to support lionfish control measures and to identify potential pathways for new invaders.

**REPRODUCTION**

The Pteroines, including *P. miles* and *P. volitans*, are gonochoristic; males and females exhibit minor sexual dimorphism only during reproduction (see Fishelson 1975). Lionfish courtship has been well described by Fishelson (1975) who provided a detailed description for the pigmy lionfish, *Dendrochirus brachypterus*, and reported similar courtship behaviors for *Pterois* spp. According to Fishelson, lionfish courtship, which includes circling, side winding, following, and leading, begins shortly before dark and extends well into nighttime hours. Following the courtship phase, the female releases two buoyant egg masses that are fertilized by the male and ascend to the surface. The eggs and later embryos are bound in adhesive mucus that disintegrates within a few days, after which the embryos and/or larvae become free floating.

*P. miles* and *P. volitans* ovarian morphology is similar to that reported for *D. brachypterus* (Fishelson 1978) in that these fishes exhibit cystovarian type ovaries (Hoar 1957) with oocytes developing on stalks or peduncles. The oocytes are terminally positioned near the ovary wall, which secretes the encompassing mucus shortly before spawning. The seasonality of lionfish reproduction throughout their native range is
unknown. Invasive lionfish collected off North Carolina and in the Bahamas suggest that lionfish are reproducing during all seasons of the year.

**EARLY LIFE HISTORY AND DISPERSAL**

Larval stage descriptions for *P. miles* and *P. volitans* are incomplete with only one report by Imamura and Yabe (1996) describing five *P. volitans* larvae collected off northwestern Australia. Scorpaenid larvae exhibit two morphologically distinct groups characterized as “morph A” and “morph B” by Leis and Rennis (2000). Pteroine larvae are grouped among the “morph B” morphotypes, whose traits include: large head, relatively long and triangular snout, long and serrated head spines, robust pelvic spine, and pigment confined to the pectoral fins (Leis and Rennis 2000) and postanal ventral and dorsal midlines (Washington et al. 1984). *Pterois* spp. meristic characters are reported as 12 - 13 dorsal spines, 9 - 12 dorsal rays, three anal spines, 5 - 8 anal rays, 12 - 18 pectoral rays, one pelvic spine, five pelvic rays, and 24 vertebrae (Imamura and Yabe 1996; Leis and Rennis 2000).

The size of *P. miles* or *P. volitans* larvae at hatching is unmeasured, but is likely to be approximately 1.5 mm based on reports for *P. lunulata* (Mito and Uchida 1958; Mito 1963). The specific planktonic larval duration of lionfish is also unknown, although Hare and Whitfield (2003) estimated it to be between 25 to 40 days based on estimates for *Scorpaena* (Laidig and Sakuma 1998).

Dispersal of lionfish presumably occurs during the pelagic larval phase during
which larvae can be dispersed across great distances. For example, lionfish eggs released in the Bahamas are capable of dispersing to New England via the Gulf Stream. Larval connectivity models for reef fishes (e.g., Cowen et al. 2006) provide insight into lionfish larval dispersal and are valuable for predicting the spread of lionfish as evidenced by the recent establishment of lionfish in the Caribbean. Further lionfish dispersal into the lower Caribbean and the Gulf of Mexico seems imminent. Assuming a planktonic larval duration of 25 to 40 days (Hare and Whitfield 2003), the Caribbean and Yucatan currents are capable of dispersing lionfish larvae into the Gulf of Mexico from locations in the Caribbean where lionfish are already resident (i.e., Cuba, Jamaica, Cayman Islands) (Cowen et al. 2006). Based on the rapidity of lionfish establishment along the U.S. East Coast and the Bahamas, lionfish establishment along the southern edges of Central America (Nicaragua, Costa Rica, and Panama), the Yucatan peninsula, and the western Gulf of Mexico is likely within a few years or less. Establishment would also be facilitated by gyres such as the Columbia-Panama Gyre and the Gulf of Mexico loop current, which could provide a mechanism for lionfish to become established in the Florida Keys.

VENOMOLOGY

Lionfish are venomous with their spines containing apocrine-type venom glands. Each spine of the lionfish (except caudal spines) is venomous including 13 dorsal spines, three anal spines, and two pelvic spines. The spines are encased in an integumentary
sheath or skin and contain two grooves of glandular epithelium that comprise the venom
producing tissue. Spine glandular tissue extends approximately three quarters of the
distance from the base of the spine towards the tip (Halstead et al. 1955).

Lionfish envenomation occurs when the spine’s integumentary sheath is
depressed as the spine enters the victim. This process tears the glandular tissue allowing
the venom to diffuse into the puncture wound (Saunders and Taylor 1959). The toxin in
lionfish venom contains acetylcholine and a neurotoxin that affects neuromuscular
transmission (Cohen and Olek 1989). Lionfish venom has been found to cause
cardiovascular, neuromuscular, and cytolytic effects ranging from mild reactions such as
swelling to extreme pain and paralysis in upper and lower extremities of humans (Kizer
et al. 1985). Antivenom of the related stonefish (Synanceia spp.) is highly effective in
neutralizing lionfish venom activity (Shiomi et al. 1989, Church and Hodgson 2002).
The severity of sting reactions in humans is dependent upon such factors as the amount of
venom delivered, the immune system of the victim, and the location of the sting. Records
of home aquarists stung by lionfish provide a comprehensive assessment of how lionfish
stings affect humans (Kizer et al. 1985, Vetrano et al. 2002). The probability of lionfish
envenomation is higher when handling smaller-sized lionfish because the venom
glandular tissue is closer to the tip of the spine and the spine tip is smaller and sharper
(Halstead et al. 1955).

The effectiveness of lionfish venom defense in their invaded range is in question.
Maljković et al. (2008) reported that lionfish were found in the stomachs of groupers;
however, this observation provides no assessment of the frequency of lionfish consumption by grouper. Furthermore, laboratory behavioral experiments suggest that groupers actively avoid lionfish, even during periods of extreme starvation (Chapter 7). Additional research is needed towards understanding predatory interactions between lionfish and native predators.

Work by Sri Balasubashini et al. (2006a, 2006b) indicated that lionfish (P. volitans) venom contains antitumor, hepatoprotective, and antimetastatic effects in mice suggesting a promising application for cancer research. Depending on the outcome of this research and the subsequent demand for lionfish venom, bioprospecting of venom from invasive lionfish could assist with fishery development.

**FEEDING ECOLOGY**

In the Red Sea, lionfish (P. miles) have been reported to feed on assorted taxa of benthic fishes including damselfish, cardinal fish, and anthias (Fishelson 1975, Fishelson 1997). However, in the Pacific Ocean, P. lunulata were observed to feed primarily on invertebrates including penaeid and mysid shrimps (Matsumiya et al. 1980, Williams and Williams 1986). Assessments of invasive lionfish feeding suggest that lionfish are largely piscivorous, but also feed on a number of crustaceans. The particular taxa of highest importance in invasive lionfish diet will likely vary by habitat type and prey availability.

Feeding, growth, and starvation of P. volitans from the Red Sea was investigated
by Fishelson (1997) who reported that lionfish stomachs can expand over 30 times in volume when consuming a large meal. This capability supported Fishelson’s hypothesis that lionfish were capable of longterm fasting, and demonstrated their ability to withstand starvation for periods of over 12 weeks without mortality. Fishelson (1997) also measured daily consumption rates in the laboratory for six size classes of lionfish ranging from 30 - 300g and found that lionfish consumed approximately 2.5 – 6.0% of their body weight per day at 25 - 26 °C. Preliminary observations suggest that lionfish in their invaded range can consume piscine prey at rates greater than reported earlier by Fishelson (1997). Quantification of the feeding ecology of lionfish including consumption rates and prey selectivity will permit better assessment of the impacts of their predation on local reef fish communities.

PARASITOLOGY

Knowledge of the parasites infecting native and non-native lionfish is scant. No comprehensive survey of protozoan or metazoan parasites of either host (P. miles or P. volitans) has been published. There are, however, a few isolated records of single parasite species such as monogeneans from the Red Sea (Paperna 1972, Colorni and Diamant 2005) and Japan (Ogawa et al. 1995), copepods also from Japan (Dojiri and Ho 1988), and leeches from Japan (Paperna 1976) and the Florida coast (Ruiz-Carus et al. 2006). Most published records of lionfish parasites are of ectoparasites; the only record of an endoparasite is of a new myxosporean species, Sphaeromyxa zaharoni which was
found in a lionfish gall bladder from the Red Sea (Diamant et al. 2004). Recent observations of invasive lionfish collected off North Carolina and in the Bahamas have found low prevalence of endo- and ectoparasites when compared to parasites of native reef fishes. Future research describing parasites of invasive lionfish will provide a unique study of opportunistic parasitism by common parasites of marine reef fishes.

**POTENTIAL IMPACTS**

Potential ecological impacts of lionfish on local reef fish communities will vary depending on the abundance of top level predators, the forage fish community, the density of lionfish, and the geographic location. Local studies providing observations of lionfish impacts on community structure and the abundance of forage fishes are needed. The first evidence of lionfish impacts in their new range was provided by Albins and Hixon (2008) who reported a 79% reduction in forage fish recruitment on experimental patch reefs in the Bahamas during a five week observation period. Analysis of the potential impact of lionfish consumption on whole coral reef fish communities is also being documented in the Bahamas, where data on stomach contents are being combined with abundance estimates of the prey community across various habitat types and seasons. Given the high levels of lionfish biomass found at some locations (Whitfield et al. 2007, Green and Côté 2009), the predatory removal of forage fishes is a growing concern, because many other top level predators (i.e., potential food competitors with lionfish) are overfished or in low abundance (Hare and Whitfield 2003).
It is unclear if lionfish predation on economically important species such as juvenile serranids will harm stock rebuilding efforts. Economically important species were observed in the diet of lionfish in the Bahamas. Research that monitors lionfish predation on economically important species is needed.

Lionfish impacts on tourist recreational activities have been observed. Some locations have posted warning signs advising of the potential for lionfish envenomation. As lionfish densities increase, so too does the risk of envenomations. It is unknown whether increasing lionfish densities will reduce recreational activities and cause economic hardship. This will be dependent on factors such as the prevalence of warning signs, the density of lionfish, the rate of human encounters, and the effectiveness of education and outreach.

CONTROL AND MANAGEMENT

Management of marine finfish invasions is confounded by highly diverse and wide-ranging habitats, swift ocean currents, and jurisdictional constraints. Prevention is the least expensive and most effective management option. There are currently two lionfish management and control efforts in Bermuda and the Bahamas. Bermuda initiated a lionfish culling program in 2008 that included a training program, collecting license, and a special dive flag allowing commercial and recreational fishers to spear lionfish along nearshore reefs. A video description of this program can be seen at http://www.youtube.com/watch?v=LNbKjiUCGRU. Bahamian fisheries officials
instituted a lionfish kill order to fishermen in 2007. They have also actively engaged the public with educational seminars devoted to promoting lionfish as a food fish with the hopes that human consumption will support fishery development. Grassroots “adopt a reef programs” are also being utilized to encourage local citizens to take ownership of small reefs and to protect them from lionfish impacts. Some tourist locations, such as resorts, are physically removing lionfish by spearfishing and handnets to reduce the risk of swimmer interaction. The effectiveness of these approaches is unclear, because too little is known about the rate of lionfish recruitment and movement among the various habitat types. Recently, NOAA researchers have developed techniques to trap lionfish, thus providing a means of removal from deeper waters and larger areas that are impractical for diver removal.

An early detection and rapid response program has been developed (NOAA/REEF/USGS) in south Florida (a hotspot for marine introductions), which utilizes and coordinates resources from over thirty state, federal, and non-governmental organizations in the region. Worshkops utilizing this model are being conducted in regions of the Caribbean to improve local response to marine invasions. Programs such as this represent the first line of defense for marine introductions and should be endorsed and supported by local managers.
CONCLUSIONS

The lionfish introduction provides a reminder of how rapidly a non-native species can become established and potentially compete with native fishes for resources. Early detection and rapid response efforts are of utmost importance in the marine environment due to the complexity and ineffectiveness of eradication measures. Future research on invasive lionfish should focus on understanding and reducing their ecological impacts, the scale of which is yet to be determined.

ACKNOWLEDGEMENTS

We thank the NOAA Aquatic Invasive Species Program, NOAA National Centers for Coastal Ocean Science, the NOAA Undersea Research Program (Grant No. NA030AR4300088), the National Science Foundation (Grants OCE0825625, OCE0550732, PEET No.0328491, DBI-MRI No.0618453), the GEF Coral Reef Targeted Research Program, Connectivity Working Group, and the Natural Sciences and Engineering Research Council of Canada for funding support. We also thank J. Langston and P. Schofield (USGS) for providing lionfish sightings records and D. Ahrenholz, D. Evans, and J. Govoni for their helpful review of this manuscript.
REFERENCES


Fishelson, L. 1997. Experiments and observations on food consumption, growth and
starvation in Dendrochirus brachypterus and Pterois volitans (Pteroinae,

(Scorpaenidae) and Pteragogus pelycus (Labridae) from the eastern

Green, S.J. and I.M. Côté. 2009. Record densities of Indo-Pacific lionfish on Bahamian

Pterois volitans (Linnaeus, 1758) for the Dominican Republic. *Aquatic Invasions*
**3**:255-256.

Halstead, B., M.J. Chitwood, and F.R. Modglin. 1955. The anatomy of the venom
apparatus of the zebrafish, Pterois volitans (Linnaeus). *Anatomical Record:*
**122**:317-333.

analysis reveals two invasive lionfish species with strong founder effects in the
western Atlantic. *Journal of Fish Biology* **71**:214-222

Hare, J. A. and P.E. Whitfield. 2003. An integrated assessment of the introduction of
lionfish (*Pterois volitans/miles* complex) to the Western Atlantic Ocean. *NOAA
Technical Memorandum NOS NCCOS* 2 p 21.


Imamara, H. and M. Yabe. 1996. Larval record of a red firefish, Pterois volitans, from
northwestern Australia (Pisces: Scorpaeniformes). *Bulletin of the Faculty of
Fisheries, Hokkaido University* **47**:41-46.

Kimball M.E., J.M. Miller, P.E. Whitfield, and J.A. Hare. 2004. Thermal tolerance and
potential distribution of invasive lionfish (*Pterois volitans/miles* complex) on the

five-year poison center experience. *Journal of the American Medical Association*
**253**:807-810.


CHAPTER 3. PHENOTYPIC VARIATION OF LIONFISH SUPRAOCULAR TENTACLES

James A. Morris, Jr. and D. Wilson Freshwater

Manuscript published in Environmental Biology of Fishes 83:237-241

ABSTRACT

A previous observation suggested that a novel phenotype of lionfish supraocular tentacle is evolving rapidly in the Red Sea and Indian Ocean. We confirm the existence of this phenotype in high prevalence in invasive populations of lionfish in the Western North Atlantic. Observations of individual lionfish from the Atlantic populations indicate that supraocular tentacles are more prevalent on juvenile and young adult lionfish suggesting this characteristic is size specific and is not associated with a genetic lineage. The high prevalence of this novel phenotype in the Atlantic may be a founder effect rather than continued selection. Genetic analysis further supports this conclusion as this phenotype is present in both Pterois species found in the Atlantic.
INTRODUCTION

The recent introduction of lionfish (*Pterois volitans* and *Pterois miles*) along the southeast coast of the United States and the Bahamas has prompted several ongoing studies investigating their abundance, distribution, and life history (Whitfield et al. 2002; Meister et al. 2005). Lionfish appear to be thriving in this habitat (Whitfield et al. 2007) possibly due to competitive advantages provided by niche availability and venomous defense.

Taxonomic classification within the *Pterois* genus has been a subject of question. *Pterois miles* is considered a synonym of *P. volitans* by some authors (e.g. de Beaufort and Briggs 1962, Fishelson 2006); however, a morphometric study by Schultz (1986) revealed differences between the two species. Kochzius et al. (2003) resolved *P. miles* and *P. volitans* as closely related sibling taxa, but it was not determined if they represented two different populations or species. A more recent analysis by Hamner et al. (2007) suggests that *P. miles* and *P. volitans* are not synonyms and that both species exist in the Atlantic population.

Fishelson (2006) described a novel phenotype of *Pterois miles* [as *P. volitans*, see Schultz 1986; Hamner et al. 2007] with peacock-feather supraocular tentacles (Figure 3.1). Fishelson (2006) reported that this phenotype appears to be evolving rapidly among lionfish in the Indo-Pacific regions. Fishelson (2006) further suggested that this novel phenotype may be providing specific adaptive advantages and offered three possible
advantages: 1) artificial enlargement of the head allowing greater intraspecific or interspecific defense 2) prey attraction and 3) sexual selection.

The objectives of this study were to 1) determine the prevalence of this novel supraocular tentacle in an invasive population of lionfish and 2) investigate the ontogenetic characteristics, species specificity, and genetic relationships of specimens with this novel phenotype.

METHODS

To determine the prevalence of supraocular tentacles in the Atlantic populations of lionfish and if supraocular tentacle phenotypes are specific to genetic lineages, 368 specimens from waters off North Carolina, U.S.A. and the Bahamas were collected during 2006 and 2007. Specimens were measured for total length and weight, and supraocular tentacles were removed and preserved in 10% neutral buffered formalin or 95% ethanol for subsequent examination. Lionfish were categorized as having straight (Figure 3.1a), peacock-feather (Figure 3.1b), or no supraocular tentacles. Variants of the peacock-feather phenotype (Fishelson 2006) were categorized as the straight phenotype. Only lionfish with a true peacock-feather like supraocular tentacle as described by Fishelson (2006) (Figure 3.1b) were characterized as “Peacock”.

To investigate species specificity and the genetic relationships of lionfish with the three tentacle types, mitochondria-encoded cytochrome-\(b\) gene sequences were generated from a subsample of 90 of the Atlantic lionfish (23 with peacock-feather, 32 with
straight, and 35 with no supraocular tentacles). DNA was extracted from muscle tissue preserved in 90% ethanol using a PureGene kit (Gentra Systems, Minneapolis, MN, USA). Amplification and sequencing of the cytochrome-\(b\) locus was carried out as described in Hamner et al. (2007).

Statistical analysis

Lionfish total length was compared among tentacle phenotype categories using a one-way analysis of variance with Tukey’s pairwise comparisons. An alpha less than 0.05 was considered significant. All statistical analyses were performed using SAS statistical software, version 9.1.3, SAS Institute Inc., Cary, North Carolina, U.S.A.

RESULTS AND DISCUSSION

Of the 368 lionfish collected off North Carolina, U.S.A., 129 (35.0%) exhibited peacock-feather supraocular tentacles (Figure 3.1), 93 (25.3%) had straight supraocular tentacles, and 146 (40.7%) had no supraocular tentacles. Tentacle frequency exhibited a size dependent relationship (Figure 3.2) with tentacle prevalence decreasing with increasing fish size. Size was also significantly different among all tentacle phenotypes (\(P < 0.05\)) with the smaller lionfish having predominately straight tentacles and the larger lionfish having no tentacles (Figure 3.3). Peacock-feather like supraocular tentacles were most prevalent in the transitional medium size classes (Figure 3.2, 3.3).
Previous sequence analyses of lionfish in the western Atlantic have found three cytochrome \( b \) haplotypes in \textit{Pterois volitans} specimens and one for specimens of \textit{P. miles} (Hamner et al. 2007). All specimens sequenced in this study had haplotypes identical to those detected by Hamner et al. (2007; GenBank accession numbers EF209676, DQ482583, DQ482585, DQ482587). Four of the specimens expressing the peacock-feather phenotype in the current study were \textit{Pterois miles} and 19 were \textit{Pterois volitans} (Figure 3.4). Two different haplotypes were present among the 19 \textit{P. volitans} with the peacock-feather phenotype. The 30 analyzed specimens expressing the straight phenotype were all \textit{P. volitans} and three haplotypes (including the two found in peacock-feather specimens) were present among these 30 specimens (Figure 3.4).

The supraocular tentacles of lionfish appear to be a characteristic closely associated with body size rather than genetics or adaptive selection as suggested by Fishelson (2006). Peacock-feather like tentacles were found to be most common in juvenile and early adult life stages with prevalence diminishing sharply with specimens >200 mm standard length. The sampling methods used in this study did not allow investigation of change in tentacle morphology through ontogeny on individual lionfish. It is possible that supraocular tentacles exhibit varying phenotypes through juvenile-adult development. Future investigation using laboratory or in situ tagging methods could provide further insight.

Efforts by Fishelson (2006) to assess peacock-feather tentacle prevalence in the Red Sea did not report a lionfish size dependent relationship and relied heavily on
FishBase (Froese and Pauly 2006) photographs, likely biased toward larger specimens. The low prevalence and thus speculation of novelty of the peacock-feather like tentacle reported for lionfish in the Indo-Pacific may be due to this sampling approach.

Further, if peacock-feather like supraocular tentacles are the product of a recent genotypic mutation as suggested by Fishelson (2006), tentacle phenotypes would be restricted to a single species and potentially a single genetic lineage within that species. The expression of the peacock-feather like phenotype in two different lionfish species and in specimens with different cytochrome-\(b\) haplotypes in at least one of these species, suggests this phenotype is not the product of recent mutation. Therefore, we conclude that the genetic potential to develop peacock-feather supraocular tentacles, or conversely straight supraocular tentacles, has been present since before the evolutionary separation of \textit{Pterois miles} and \textit{Pterois volitans} into two species.

In conclusion, we have documented the existence of peacock-feather like supraocular tentacles in the U.S. South Atlantic in high prevalence. Supraocular tentacles appear to be a characteristic of juvenile and early adult stages, suggesting that the presence of the peacock-feather phenotype within the native range of lionfish should be re-examined. Lionfish in the Atlantic are likely undergoing strong founder effects, thus further study of adaptive evolution is warranted. Future research on lionfish supraocular tentacles directed towards developmental variation and function during early adult life stages may provide additional insight.
ACKNOWLEDGEMENTS

The authors thank L. Akins and the Reef Environmental Education Foundation for their gracious support. We are also very grateful to P. Whitfield, D. Kesling, J. Styron, C. Addison, B. Degan, R. Mays, J. Hackney, R. Muñoz, K. Brennan, J. Vander-Pluym, and B. Teer for collection assistance. We thank L. Vitale (Figure 3.1a) and S. Sy (Figure 3.1b) who provided photographs and D. Ahrenholz, D. Evans, J. Govoni, and E. Williams whose reviews significantly improved this manuscript. This work was funded in part by the NOAA Invasive Species Program and Friends of the Center for Marine Science DNA Algal Trust.

Mention of brand names or manufacturer does not imply endorsement by the U. S. Federal Government.
REFERENCES


Figure 3.1 Photographs of a lionfish exhibiting the straight (A) and peacock-feather (B) supraocular tentacle phenotype.
Figure 3.2 Cumulative frequency plot of lionfish supraocular tentacle type sorted into 20 mm size classes with proportion of each tentacle type displayed for each size class.
Figure 3.3  Box plots comparing supraocular tentacle category and fish size. The boundary of the box closest to zero represents the 25th percentile with the line in the middle representing the median. The boundary of the box farthest from zero indicates the 75th percentile with the whiskers (error bars) indicating the 90th and 10th percentiles. Outliers are indicated by black circles.
Figure 3.4 Neighbor-joining tree derived from analyses of absolute distances among 90 western Atlantic lionfish specimens with peacock-feather, straight or no supraocular tentacles. Neighbor-joining analyses were carried out using PAUP (v. 4.0, Swofford 2002) and haplotype designations follow Hamner et al. (2007).
CHAPTER 4. FEEDING ECOLOGY OF INVASIVE LIONFISH (*Pterois volitans*) IN THE BAHAMIAN ARCHIPELAGO

James A. Morris, Jr. and J. Lad Akins

Manuscript in press in *Environmental Biology of Fishes*

ABSTRACT

Feeding ecology of the lionfish (*Pterois volitans*), an invasive species in the Western North Atlantic, was examined by collecting stomach content data from fishes taken throughout the Bahamian archipelago. Three relative metrics of prey quantity, including percent number, percent frequency, and percent volume, were used to compare three indices of dietary importance. Lionfish largely prey upon teleosts (78% volume) and crustaceans (14% volume). Twenty-one families and 41 species of teleosts were represented in the diet of lionfish; the top 10 families of dietary importance were Gobiidae, Labridae, Grammatidae, Apogonidae, Pomacentridae, Serranidae, Blenniidae, Atherinidae, Mullidae, and Monacanthidae. The proportional importance of crustaceans in the diet was inversely related to size with the largest lionfish preying almost exclusively on teleosts. Lionfish stomachs contained the highest levels of prey during the morning sampling period (0800 – 1100 hr).
INTRODUCTION

The lionfishes, *Pterois miles* and *P. volitans*, (Hamner et al. 2007, Morris et al. 2009) are the first non-native marine fishes to become established along the Atlantic coast of the U.S. and the Caribbean. Adult lionfish specimens are now found along the U.S. East Coast from Cape Hatteras, North Carolina, to Florida, and in Bermuda, the Bahamas, and throughout the Caribbean, including the Turks and Caicos, Haiti, Cuba, Dominican Republic, Puerto Rico, St. Croix, Belize, and Mexico (Schofield et al. 2009).

The first documented capture of lionfish in the Atlantic was in 1985 off Dania Beach, Florida (J. Bohnsack, NOAA NMFS, pers. comm.). Additional sightings occurred in 1992 following an accidental release of six lionfishes from a home aquarium into Biscayne Bay, Florida (Courtenay 1995). Many other reports of lionfish were documented in southeast Florida between 1999 and 2003 by Semmens et al. (2004), who attributed many of these sightings to releases by home aquarists.

Recreational divers reported the first sightings of lionfish in the Bahamas in 2004 (REEF 2009). Snyder and Burgess (2007) published the first record of lionfish in the Bahamas, suggesting that lionfish were widely distributed throughout Little Bahama and Grand Bahama Banks. It is uncertain if lionfish invaded the Bahamas via larval transport by ocean currents or if their introduction was the result of additional aquarium releases. Recent genetic studies by Freshwater et al. (2009) suggest that lionfish invaded the Bahamian archipelago via larval dispersal originating from U.S. waters.
Early efforts to assess the density of lionfish off North Carolina by diver surveys and remotely operated vehicles suggested that lionfish populations were rapidly increasing, with trophic interactions with native reef fishes a concern (Whitfield et al. 2002, Hare and Whitfield 2003). Recently, lionfish densities on Bahamian reefs have been found to be in excess of 390 lionfish hectare$^{-1}$, almost five times higher than estimates from the native range (Green and Côté 2009). Albins and Hixon (2008) reported the first evidence of the impacts of lionfish on native fish communities by demonstrating that lionfish reduced recruitment of coral reef fishes on experimental reefs in the Bahamas by nearly 80%.

To date, comprehensive assessments of lionfish diets are lacking in their native and invaded ranges. Preliminary observations of lionfish feeding in their native range suggest that lionfish feed primarily on small fishes and some invertebrates (Fishelson 1975, 1997, Harmelin-Vivien and Bouchon 1976). In the Pacific Ocean, the closely related luna lionfish (*P. lunulata*) was found to feed primarily on invertebrates, including penaeid and mysid shrimps (Matsumiya et al. 1980, Williams and Williams 1986). More recently, Albins and Hixon (2008) reported a list of nine species consumed by invasive lionfish in the Bahamas. While these observations suggest general patterns in lionfish diet, quantitative assessments of lionfish feeding habits in their new range are needed to elucidate the impacts of these predators on invaded reef communities. The overall objectives of this study were to 1) assess dietary habits of lionfish collected from various
habitats in the Bahamian archipelago, 2) determine the relationship between prey and predator size, and 3) document temporal feeding patterns of this invader.

METHODS

Collections

Lionfish were collected from the Bahamian archipelago (Figure 4.1) between January 2007 and May 2008. All specimens were collected by fisheries professionals and trained volunteers while snorkeling or using SCUBA gear at 134 sites comprised of high profile coral reefs, patch reefs, artificial reefs, mangroves, and man-made canals ranging in depth from 1 to 30 m. Sampling sites were chosen opportunistically to optimize sampling success. Most collections utilized hand nets and vinyl collection bags, although some were collected by pole spear. Live captures from nets and bags were euthanized by excess anesthesia in a bath of eugenol (Borski and Hodson 2003). Only two lionfish regurgitated stomach contents during ascension; therefore, stomach content retention measures were unnecessary. Lionfish were placed on ice and dissected the same day as capture.

Lionfish were collected every month of the calendar year ($\bar{X} = 111 \pm 28$ standard error individuals per month), with the smallest sample size collected during June ($n = 10$) and the largest collected during February ($n = 368$). Collections of lionfish were achieved from 0700 – 2100 h; the majority of collections (99.1%) occurred between 0800 and 1700 h.
Cumulative prey curve

A cumulative prey curve was used to assess sample size sufficiency of lionfish stomachs containing identifiable prey. Prey taxa were grouped by family and cumulative numbers of novel prey were determined following 1000 randomizations (Bizzarro et al. 2007). Mean and standard deviation of the cumulative number of novel prey was calculated and sufficiency of sample size was assessed statistically using the linear regression method of Bizzarro et al. (2007) that compares the slope from a regression of the last four stomach samples to a slope of zero using a Student’s t-test of equality of two population regression coefficients (Zar 1999). A p-value > 0.05 was considered to demonstrate sampling sufficiency. To determine the minimum number of stomach samples (with identifiable prey) required to adequately describe lionfish diet, one sample was removed sequentially until the Student’s t-test p-value fell below 0.05 indicating that the asymptote was not achieved.

Stomach content analyses

Stomach contents were identified to lowest possible taxon (without fixation), counted, and measured for total length (TL). No adjustment of prey TL due to partial digestion was performed, thus the estimated prey sizes are potentially underestimated. Volumes of diet items taken from contents were measured by water displacement in a graduated cylinder. The contribution of each prey taxon to the overall diet was assessed using three relative metrics of prey quantity: percent frequency of occurrence (%F),
percent composition by number (%N), and percent composition by volume (%V) (Hyslop 1980, Bowen 1996). Variations in prey size and diet composition across lionfish sizes were examined statistically by conducting a significance test on the slope of a linear regression. An $\alpha$-level $\leq 0.05$ was considered significant.

Dietary importance indices or hybrid diet indices have been widely employed in the study of fish food habits (Bowen 1996), yet their specific use has been criticized (Windell and Bowen 1978) and subject to controversy (Hyslop 1980, Cortés 1997, Hansson 1998). For a robust assessment of prey importance, three indices of importance were calculated:

1. the Index of Relative Importance (IRI) (Pinkas et al. 1971),

$$IRI_a = F_a \cdot (N_a + V_a)$$

2. the Index of Importance (IOI$_a$) (Gray et al. 1997, Hunt et al. 1999),

$$IOI_a = \frac{100 \cdot (F_a + V_a)}{\sum_{a=1}^{s}(F_a + V_a)}$$

3. the Index of Preponderance (IOP) (Natrajan and Jhingran 1962, Sreeraj et al. 2006),

$$IOP_a = \frac{F_a \cdot V_a}{\sum_{a=1}^{s}(F_a + V_a)}$$

where $s$ is the number of prey types, $F_a$ is the frequency of occurrence of species $a$, $V_a$ is the proportion composition by volume of species $a$, and $N_a$ is the proportion composition by number of species $a$. 

51
RESULTS

The size of lionfish ranged from 62 – 424 mm TL with a mean size (±SE) of 217 ± 7 mm. A total of 1,876 prey items from 1,069 stomachs were assigned to taxa. Volumetric measurements of prey by taxon were determined for 699 stomachs. Lionfish were sampled from diverse habitat types including high profile coral reefs (68%), canals (11%), artificial reefs (9%), other (predominately blue holes) (5%), patch reefs (4%), and mangrove habitats (3%). Cumulative prey curve analysis indicated sample size sufficiency reached the asymptote for stomachs with identifiable prey (p > 0.58). A large number of stomachs were required to attain sufficient sample size as p < 0.05 occurred at sample 706.

Prey composition

Twenty-one families of teleosts, four families of crustaceans, and one family of mollusks were represented in the diets of lionfish (Table 4.1). Teleost fishes dominated lionfish diet comprising 78% by volume (%V), 71.2% by number (%N), and 61.6% by occurrence (%F). Crustaceans were represented at 14.4%V, 28.5% N, and 24.7%F, while mollusks comprised < 0.01%V, %N, and %F. Approximately 21% (n = 225) of the stomachs were empty.

Teleost prey included 41 species and exhibited a wide-range of body shapes and morphological characteristics (Table 4.1). The families with the greatest number of species included Labridae (8), Pomacentridae (6), Gobiidae (5), and Serranidae (4).
Eight families comprised 38% of lionfish diet by volume and 48% of the volume of identifiable teleosts. These included Pomacentridae (7.2%), Labridae (6.7%), Mullidae (5.5%), Grammatidae (5.0%), Serranidae (4.3%), Gobiidae (4.2%), Apogonidae (3.6%), and Blenniidae (1.1%). Unidentified prey accounted for 42.1% N, 38.1% V, and 36.5% F of all food items. The following teleost families had the greatest representation in percent number: Gobiidae (8.4%), Labridae (4.4%), Grammatidae (4.3%), Apogonidae (3.1%), Pomacentridae (1.8%), Serranidae (1.5%), Blenniidae (1%), and Atherinidae (1%). In terms of %F, the same familial order applied with only minor changes in the percentages.

The majority of crustacea prey were identified as shrimps: 25.5% N, 22.1% F, and 12.7% V of the total prey. Of the remaining crustacean prey, 3% V, % F, and % N were represented by four families (Corallanidae, Squillidae, Rhynchocinetridae, Stenopodidae) along with items from the categories of unidentified crab and unidentified crustaceans (Table 4.1).

**Rankings of Importance Indices**

The same ten families of teleosts ranked as the top ten for all three indices (IRI, IOI, IOP) (Table 2). Gobiids, labruids, and grammatids were ranked as the top three in the IRI and IOP lists, whereas the IOI ranked labrids, pomacentrids, and gobiids as most important of the teleost prey.
Diet Composition and Size of Lionfish

The importance of teleosts in the diet of lionfish increased significantly with size in all three dietary metrics (%F $R^2 = 0.86$, $P = 0.0003$; %N $R^2 = 0.55$, $P = 0.02$; %V $R^2 = 0.76$, $P = 0.005$) (Figure 4.2). The mean sizes of teleosts and crustaceans in the diet increased with the size of lionfish (teleost prey $R^2 = 0.46$, $P = 0.01$; crustacean prey $R^2 = 0.36$, $P = 0.002$) (Figure 4.3). The maximum number of crustacean prey per lionfish was 50, whereas the maximum number of teleost prey was 21. The mean ratio of prey size (TL) to lionfish size (TL) was $14.5\% \pm 0.003$ standard error of the mean. The maximum prey size was 48% of the total length of lionfish, whereas the minimum prey size was 0.02%.

Feeding activity

Stomachs of lionfish contained the highest volume of prey during the morning hours of 0800 – 1100 with a significant decrease in mean prey volume towards the evening ($R^2 = 0.39$, $P = 0.01$) (Figure 4.4). Few lionfish were collected at dusk or immediately after dark; therefore the prevalence of feeding at this time is uncertain.

DISCUSSION

In the Bahamian archipelago, invasive lionfish feed predominantly on teleosts and crustaceans. The large number of teleostean families in lionfish diet indicates that lionfish feed upon a wide variety of available prey, but feed primarily on abundant
teleosts and crevice-dwelling species. The proportion of teleosts in the diet was size-dependent, with larger lionfish feeding more heavily on teleosts. Smaller size classes of lionfish had a higher proportion of crustaceans in their diet, primarily shrimps.

The amount of prey in lionfish stomachs over the course of the day suggests that lionfish feeding is highest in the morning (0800 – 1100 h), or the hours prior, with a decrease in feeding activity throughout the day. Diurnal visual observations of lionfish feeding further support this conclusion (L. Akins, S. Green, unpubl. data). Fishelson (1975) reported that lionfish (Pterois volitans) in the Red Sea are primarily nocturnal and become active during crepuscular periods of dawn and dusk. Given the lack of samples in this study from the hours of 2100 to 0700 h, feeding activity during the late night hours (or nocturnal period) is unknown.

Lionfish are suction feeders, a common teleostean feeding technique comprised of rapid expansion of the buccal and opercular cavities coupled with quick forward motion (Van Leeuwen and Muller 1984). Lionfish also use a variety of feeding strategies, including ambush predation and corralling prey with their large, frilly pectoral fins. Lionfish also use their pectoral fins to flush benthic invertebrates from the substrate by palpation (Fishelson 1975). Specialized bilateral swim bladder muscles in lionfish provide novel control of their pitch in the water column, which allows lionfish to alter their center of gravity and provides fine-tuning of position prior to striking prey (Hornstra et al. 2004). Lionfish also use this mechanism to orient and hover; they are frequently observed in an up-side-down position under ledges and on the lateral face of structure.
Hovering behavior, hunting, ambush predation, and the flushing of prey from the benthos enable lionfish to employ a diverse array of feeding strategies well-suited for feeding on benthically-associated and cryptic fauna.

The relative importance of teleost families in the stomachs of lionfish was similar among the three indices of importance, suggesting a high degree of confidence in the rankings of the top ten teleost prey (Table 4.2). Similar rankings of the top two families (gobiids and labrids) among all three indices is evidence that these fishes are of highest importance in the diet of lionfish.

All three indices used here engage at least two of the dietary metrics %F, %N, and %V, but place different weight on the importance of each metric. The IRI, for example, places equal weight on %N and %V, and higher weight on %F. The IOI does not include %N and increases bias towards high volume, but infrequently found prey items. The IOP also does not incorporate %N, but employs a weighted mean approach. The IRI and IOP indices resulted in identical rankings. The IOI reported a different ranking order when compared to the IRI and IOP. The teleost family exhibiting the highest difference in ranking was Mullidae (ninth in the IRI and IOP and fifth in the IOI), probably because of its low %N and %F, but relatively high %V. The IRI and IOP are the more appropriate indices for investigating importance of prey items in lionfish diet because these indices require prey ranked high in importance to be both high in %F and %V.

This study suggests that lionfish feed primarily upon small-bodied teleost fishes, which are an important component of the diet of many economically important fishes of
the tropical and western north Atlantic such as serranids (Lindquist et al. 1994; Eggleston et al. 1998) and lutjanids (Rooker 1995; Duarte and Garcia 1999; Ouzts and Szedlmayer 2003). Direct predation by lionfish on economically-important species, including yellowtail snapper (*Ocyurus chrysurus*) and Nassau grouper (*Epinephelus striatus*), was observed, but these specific species were in relative low frequency.

The scale of ecological or economic impact of lionfish predation is uncertain and multiple scenarios are plausible: 1) prey are abundant because many top-level predators are removed by fishing, thus lionfish could have no direct impact; 2) lionfish will reduce prey communities causing a diminution of prey for native predators; 3) reduced levels of prey will slow, but not inhibit, stock rebuilding efforts for native fishes; and 4) lionfish predation on economically important species will cause direct impacts and possibly cascading effects. Although the likelihood of any of these scenarios occurring is unknown, lionfish appear to be steadily increasing in both abundance and distribution. Recent evidence suggests that lionfish are capable of removing significant proportions (78%) of the prey community on isolated patch reefs (Albins and Hixon 2008). Future studies that quantify the biomass of the prey community and the seasonality of their abundance are needed to clarify direct and indirect impacts of lionfish on native species.

Our sampling did not include quantitative assessments of the prey communities; therefore prey preference cannot be derived from this study. Further, it is possible that lionfish diet may shift over time if predation by lionfish reduces or alters the abundance of the prey fish communities. Seasonal bias could also be present in our sampling as our
sample size did vary among months and tropical reef fish recruitment is known to vary seasonally (Luckhurst and Luckhurst 1977; McFarland et al. 1985). Future assessments of the seasonality of lionfish diet, coupled with assessments of native reef fish recruitment across locales in the Southeast U.S., Caribbean, and Gulf of Mexico are needed to further elucidate the trophic impacts of lionfish. Additional research directed towards understanding the metabolic demands of lionfish coupled with dietary analysis and prey density surveys could quantify consumptive removal of native species by lionfish. These efforts would then allow scaling trophic impacts of lionfish at the individual and population level.

CONCLUSION

This study provides the first comprehensive assessment of feeding habits of the invasive lionfish (*Pterois volitans*) in the tropical Western North Atlantic. Future research is needed to quantify the impacts of lionfish on forage fish communities in various habitats. Given the ecological and economical importance of the higher trophic level predators that will compete with lionfish for dietary resources, increased efforts to remove lionfish through fishery development and/or control strategies are needed to mitigate the impacts of lionfish consumption in their new Atlantic habitats.

ACKNOWLEDGEMENTS

This work was funded in part by the NOAA Aquatic Invasive Species Program, the Elisabeth Ordway Dunn Foundation, and the Reef Environmental Education Foundation.
(REEF). We are grateful to dive operators B. Purdy and S. Cove for their gracious support. All lionfish were collected under a research permit MAF/FIS/12: MAF/FIS/17 to J. Morris. We thank D. Ahrenholz, J. Burke, D. Cerino, I. Côté, S. Green, J. Govoni, P. Schofield, J. Smith, E. Williams, and two anonymous reviewers for their helpful comments on this manuscript. We also thank A. Dehart and the National Aquarium in Washington, D.C., A. Benson, S. Green, K. Sealey, E. Joseph, M. Tucker, N. Smith, C. Rochelle, C. Butler, E. Davenport, and REEF Staff, for their invaluable assistance. A special thank you to the more than two hundred REEF volunteers whose dedicated efforts in the field made this work possible.
REFERENCES


Borski RJ, Hodson RG (2003) Fish research and the institutional animal care and use committee. ILAR J 44:286-294


Fishelson L (1975) Ethology and reproduction of pteroid fishes found in the Gulf of Agaba (Red Sea), especially *Dendrochirus brachypterus* (Cuvier), (Pteroidae, Teleostei) PSZNI Mar Ecol 39:635-656

Fishelson L (1997) Experiments and observations on food consumption, growth and starvation in *Dendrochirus brachypterus* and *Pterois volitans* (Pteroinae, Scorpaenidae). Environ Biol Fish 50:393-401


Hare JA, Whitfield PE (2003) An integrated assessment of the introduction of lionfish (*Pterois volitans/miles* complex) to the Western Atlantic Ocean. NOAA Tech Memo NOS NCCOS 2, p 21


McFarland WN, Brothers EB, Ogden JC, Shulman MJ, Bermingham EL, Kotchian-Prentiss NM (1985) Recruitment patterns in young french grunts, Haemulon flavolineatum (family Haemulidae) at St. Croix, U.S.V.I. Fish Bull 83:413-426


Natrajan AV, Jhingran AG (1962) Index of Preponderance – a method of grading the food elements in the stomach analysis of fishes. Indian J Fish 8:54-59


Pinkas LM, Oliphant S, Iverson ILK (1971) Food habits of albacore, bluefin tuna and bonito in Californian waters. Calif Fish Game 152:1-105


Sreeraj N, Raghavan R, Prasad G (2006) The diet of Horabagrus brachysoma (Gunther), and endangered bagrid catfish from Lake Vembanad (South India). J Fish Biol 69:637-642


Table 4.1 Identifiable lionfish prey sorted by taxa.

<table>
<thead>
<tr>
<th>Taxonomy</th>
<th>Frequency (stomachs)</th>
<th>%F (n=1069)</th>
<th>%N (n=926)</th>
<th>%V (n=699)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mollusca</td>
<td>3</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Unidentified spp.</td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Octopodida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Octopoda</td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Crustacea</td>
<td>264</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified crustacean</td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Unidentified shrimp</td>
<td>236</td>
<td>22.1</td>
<td>25.5</td>
<td>13.8</td>
</tr>
<tr>
<td>Unidentified crab</td>
<td>8</td>
<td>0.7</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Corallanidae</td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Stenopodida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Stenopus hispidus</em></td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Rhynchocinetidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhynchocinetes rigens</em></td>
<td>5</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Squillidae</td>
<td>6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Teleosts</td>
<td>659</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified fish</td>
<td>390</td>
<td>36.5</td>
<td>42.1</td>
<td>41.3</td>
</tr>
<tr>
<td>Atherinidae</td>
<td>9</td>
<td>0.8</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Labridae</td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td><em>Thalassoma bifasciatum</em></td>
<td>13</td>
<td>1.2</td>
<td>1.4</td>
<td>0.6</td>
</tr>
<tr>
<td><em>Halichoeres pictus</em></td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Halichoeres bivittatus</em></td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Clepticus parrae</em></td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
<td>2.6</td>
</tr>
<tr>
<td><em>Halichoeres garnoti</em></td>
<td>13</td>
<td>1.2</td>
<td>1.4</td>
<td>1.9</td>
</tr>
<tr>
<td><em>Halichoeres maculipinna</em></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Bodianus rufus</em></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td><em>Xyrichtys</em> sp.</td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Opistognathidae</td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Gobiidae</td>
<td>20</td>
<td>1.9</td>
<td>2.2</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Coryphopterus personatus/hyalinus</em></td>
<td>39</td>
<td>3.6</td>
<td>4.2</td>
<td>1.6</td>
</tr>
<tr>
<td><em>Coryphopterus eidolon</em></td>
<td>14</td>
<td>1.3</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Coryphopterus dicos</em></td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td><em>Coryphopterus glaucofraenum</em></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td><em>Priolepis hipoliti</em></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Scaridae</td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td><em>Scarus iserti</em></td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>Species</td>
<td>Count</td>
<td>Length</td>
<td>Width</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------------</td>
<td>-------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>Scarus viride</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Blenniidae</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Lucayablennius zingaro</td>
<td></td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Malacocentrus triangulatus</td>
<td></td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Malacocentrus boehlkei</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Tripterygidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enneanectes sp.</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Serranidae</td>
<td></td>
<td>5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Epinephelus striatus</td>
<td></td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Serranus tigrinus</td>
<td></td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Hypoplectrus sp.</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Liopropoma rubre</td>
<td></td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Grammatidae</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Gramma loreto</td>
<td></td>
<td>36</td>
<td>3.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Gramma melacara</td>
<td></td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Synodontidae</td>
<td></td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Pomacentridae</td>
<td></td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Chromis insolata</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Chromis cyanea</td>
<td></td>
<td>7</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Chromis multilineata</td>
<td></td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Stegastes partitus</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Stegastes leucostictus</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Stegastes variabilis</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Apogonidae</td>
<td></td>
<td>21</td>
<td>2.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Apogon townsendi</td>
<td></td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Apogon binotatus</td>
<td></td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Tetradontidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canthigaster rostrata</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Syngnathidae</td>
<td></td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Acanthuridae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthurus bahianus</td>
<td></td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Monacanthidae</td>
<td></td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Monacanthus tuckeri</td>
<td></td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Holocentridae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sargocentron vexillarium</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Cirrhitidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amblycirrhitus pinos</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Aulostomidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aulostomus maculates</td>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mullidea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudupeneus maculatus</td>
<td></td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 4.2 Top 10 ranked fish families for each importance index.

<table>
<thead>
<tr>
<th>Rank</th>
<th>IRI</th>
<th>IOP</th>
<th>IOI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gobiidae</td>
<td>Gobiidae</td>
<td>Labridae</td>
</tr>
<tr>
<td>2</td>
<td>Labridae</td>
<td>Labridae</td>
<td>Pomacentridae</td>
</tr>
<tr>
<td>3</td>
<td>Grammatidae</td>
<td>Grammatidae</td>
<td>Gobiidae</td>
</tr>
<tr>
<td>4</td>
<td>Apogonidae</td>
<td>Apogonidae</td>
<td>Grammatidae</td>
</tr>
<tr>
<td>5</td>
<td>Pomacentridae</td>
<td>Pomacentridae</td>
<td>Mullidea</td>
</tr>
<tr>
<td>6</td>
<td>Serranidae</td>
<td>Serranidae</td>
<td>Serranidae</td>
</tr>
<tr>
<td>7</td>
<td>Blenniidae</td>
<td>Blenniidae</td>
<td>Apogonidae</td>
</tr>
<tr>
<td>8</td>
<td>Atherinidae</td>
<td>Atherinidae</td>
<td>Blenniidae</td>
</tr>
<tr>
<td>9</td>
<td>Mullidea</td>
<td>Mullidea</td>
<td>Atherinidae</td>
</tr>
<tr>
<td>10</td>
<td>Monacanthidae</td>
<td>Monacanthidae</td>
<td>Monacanthidae</td>
</tr>
</tbody>
</table>
Figure 4.1 Sampling locations and number of lionfish collected along the Bahamian archipelago.
Figure 4.2 Mean proportion of lionfish diet comprised of teleosts by lionfish 40-mm total length size classes. %V = 0.00102TL + 0.602, R² = 0.76, P = 0.0050, %N = 0.00131TL + 0.408, R² = 0.55, P = 0.0200, %F = 0.00109TL + 0.508, R² = 0.86, P = 0.003.
Figure 4.3  Mean teleost and crustacean prey size consumed by lionfish. Lionfish size displayed in 40-mm total length size classes. Mean teleost prey size = 0.380TL + 14.586, $R^2 = 0.46$, $P = 0.010$. Mean crustacean prey size = 0.045TL + 8.043, $R^2 = 0.36$, $P = 0.002$. 
Figure 4.4 Proportion of lionfish stomachs containing prey throughout the day. Mean proportion of lionfish with prey in stomach $= -0.044 \text{Time} + 92.367$, $R^2 = 0.39$, $P = 0.01$. 
CHAPTER 5. OOGENESIS AND SPAWN FORMATION IN THE LIONFISHES, 
*Pterois miles* (BENNET) AND *Pterois volitans* (LINNAEUS)

James A. Morris, Jr., Craig. V. Sullivan, and John J. Govoni

Manuscript in review at *Transactions of the American Fisheries Society*

**ABSTRACT**

The Indo-Pacific lionfishes, *Pterois miles* and *P. volitans*, have invaded the U.S. East Coast and the Caribbean and pose significant threat to native reef fish communities. Few studies have documented reproduction in pteroines from the Indo-Pacific. This study provides a description of oogenesis and spawn formation in *P. miles* and *P. volitans* collected from offshore waters of North Carolina, U.S.A. Using histological and laboratory observations, we found no differences in reproductive biology between *P. miles* and *P. volitans*. We report that these lionfish species spawn buoyant eggs encased in a hollow mass of mucus produced by specialized secretory cells of the ovarian wall complex. *P. miles* and *P. volitans* oocytes develop on vascularized peduncles with all oocyte stages present in the ovary of spawning females and the most mature oocytes placed terminally, near the ovary lumen. Given these reproductive characteristics, these species of lionfish are indeterminate batch spawners and are thus capable of sustained reproduction throughout the year when environmental conditions are suitable. This mode
of reproduction partly accounts for the rapid establishment of these lionfishes in the Northwestern Atlantic and Caribbean.

INTRODUCTION

Two species of non-native lionfish, *Pterois miles* (Bennet, 1828) and *P. volitans* (Linnaeus, 1758), are now established along the Southeast coast of the United States and in parts of the Caribbean (Hamner et al. 2007; USGS 2008; Morris et al. 2009). Recent assessments of lionfish ecological impacts suggest that lionfish are capable of trophic disruption (Albins and Hixon 2008); however, the scale of these impacts on reef fishes is uncertain.

*P. miles* and *P. volitans* are two of nine recognized species in the genus *Pterois* and can be distinguished from one another only by meristics (Schultz 1986) or by analysis of mitochondrial DNA sequences (Hamner et al. 2007; Morris and Freshwater 2008). In the United States, lionfish are one of the most heavily imported ornamental reef fishes (Semmens et al. 2004; Ruiz-Carus et al. 2006) and they were likely introduced into Atlantic waters from the Indo-Pacific by recreational or commercial aquarists (Hare and Whitfield 2003; Ruiz-Carus et al. 2006). Lionfish densities have increased annually in offshore waters of North Carolina, U.S.A. (Whitfield et al. 2002; 2007) and in the Bahamas (Green and Côté 2009), with higher densities observed in the Atlantic than ever reported in their native range.

Lionfish are scorpaeniforms, which comprise a diverse order of fishes encompassing a broad spectrum of reproductive strategies and adaptations (Kendall 1991;
In general, the morphological and histological structure of the scorpaeniform ovary is poorly understood, and this has led to a lack of understanding of their reproductive evolution (Wourms 1991). Some scorpaenids are known to spawn gelatinous, buoyant egg masses including *P. lunulata*, Temminck and Schlegel (Mito and Uchida 1958), *Sebastolobus macrochir* (Günther) (Masuda et al. 1984), *Scorpaena guttata*, Girard (Oron 1955), *Dendrochirus spp.* (Fishelson 1975; Moyer and Zaiser 1981), and *Helicolenus dactylopterus* (Delaroche) (Krefft 1961; Sanchez and Acha 1988). Descriptions of ovarian structure have only been reported for *D. brachypterus* (Cuvier) (Fishelson 1975; 1977; 1978), *H. d. dactylopterus* (Delaroche) (White et al. 1998), and *S. alascanus*, Bean (Erickson and Pikitch 1993). Detailed descriptions of ovarian morphology and cytology are unavailable for any pteroines including the Atlantic invaders, *P. miles* and *P. volitans*.

The present study provides a description of the morphological and cytological structure of the ovary and of oogenesis in the lionfishes *P. miles* and *P. volitans*. This description provides a foundation for understanding the reproductive biology of these non-native species and explains, in part, their rapid establishment in their new range.

**METHODS**

Over 280 female lionfish (\( \bar{x} = 241 \pm 3.6 \) SE mm total length; size range = 84 - 388 mm total length) were collected by spearfishing or hand nets throughout the calendar year from the offshore waters of North Carolina, U.S.A. and euthanized by excess anesthesia.
in a bath of tricaine methane sulfonate or by cervical transection. Ovaries were immediately removed and fixed in 10% neutral buffered formalin (NBF) for up to 30 days before being processed histologically. For small ovaries, the entire lobe was fixed in 10% NBF. For large ovaries, samples of tissue were excised from the mid-ovary and placed in 10% NBF. All fixed tissues were rinsed in phosphate buffered saline, dehydrated through a graded ethanol series, and embedded in paraffin using standard histological techniques. The paraffin blocks were sectioned at 5-6 µm and the sections were stained with a mixture of Mayer's/Harris hematoxylin and alcoholic Eosin Y (Sheehan and Hrapchak 1980).

In preparation for scanning electron microscopy (SEM), lionfish ovarian sections were fixed in 10% NBF for 24 h. Tissue samples were washed twice for approximately 15 min in 0.1 M phosphate buffer (pH 7.2 - 7.4) before dehydration in a graded ethanol series. After drying, the samples were attached to aluminum SEM stubs with carbon tape and then sputter-coated with approximately 20 nm of gold-palladium.

Photomicrographs of histological sections were taken for the ovaries of seven representative lionfish of a mean total length (mm) of 288.3 ± 14.5 standard error of the mean. All photomicrographs were taken with a Leitz-Wetzlar Dialux 22 microscope equipped with a Leica DFC320 R2 digital camera and stage-specific maximum oocyte diameters (n = 20 per oocyte stage) were measured using a calibrated ocular micrometer. Adult female lionfish were held in the laboratory and eggs (n = 10) were taken from a recently released egg mass. Diameters of the eggs were measured under a Zeiss 475052-
9901 dissecting stereomicroscope fitted with a calibrated ocular micrometer. SEM images were taken with a JEOL JSM-6360 LV scanning electron microscope operated at 5 kV accelerating voltage.

Ovarian morphology and oogenesis was compared between *P. miles* and *P. volitans* using five similar-sized specimens of each species. The mean total length (mm) and standard error of the mean was $\bar{x} = 279 \pm 20.52$ for *P. miles* and $\bar{x} = 278.2 \pm 20.72$ for *P. volitans*. Species identification was accomplished by analyses of mitochondrial genes for cytochrome b (mtCytb). DNA was extracted from muscle tissue preserved in 90% ethanol with a PureGene kit (Gentra Systems, Minneapolis, MN, USA) and used for amplification and sequencing of the mtCytb locus with the genotyping conducted as described in Hamner et al. (2007). Oogenesis was compared between species by assessing general ovarian and oocyte morphology based on conventional understanding of oocyte growth in teleosts (Selman et al. 1989; 1993; Le Menn et al. 2007).

**RESULTS**

No differences in oogenesis or ovarian morphology were found between *P. miles* and *P. volitans*. Given this finding, the term “lionfish” is used hereafter to describe both species. We found lionfish ovaries to be paired, fusiform organs located in the postero-dorsal region of the body cavity. The ovarian circulatory system is comprised of an ovarian artery and veins entering anteriorly and running centrally through each ovarian lobe. The central stroma of each ovarian lobe develops radially around this vascular
system (Figure 5.1) and is overlain by a germinal epithelium, which gives rise to oogonia. Immature oocytes are found near the central stroma and mature oocytes are positioned adjacent to the ovarian lumen, which lies beneath the peripheral ovary wall (Figure 5.1). The lumen of each ovarian lobe fuses caudally to form the oviduct. This cystovarian structure, wherein the central ovarian stroma develops radially around the blood circulatory system and is surrounded by a peripheral ovarian cavity, was found to be typical of scorpaenids that spawn a buoyant egg mass encompassed by gelatinous material (Koya and Muñoz 2007).

Lionfish oogenesis can be categorized by four sequential oocyte stages: primary growth, cortical alveolus, vitellogenesis, and maturation. With this categorization, we describe the cytological features of lionfish oocyte and follicle development, ovulation, and spawn formation using the key developmental stage-specific criteria summarized in Table 5.1.

**Primary Growth Stage**

Early primary growth-stage oocytes (dia. = 20 - 60 µm) of lionfish exhibit a strongly basophilic ooplasm and a prominent germinal vesicle with vesicular nucleoplasm containing visible chromatin and single or several prominent basophilic nucleoli (Figure 5.2). Oocytes are positioned near the central stroma with oogonia visible in adjacent tissue of the germinal epithelium (Figure 5.2). As primary growth proceeds (oocyte dia. = 40 - 100 µm), multiple nucleoli become evident in the oocyte germinal
vesicle (Figure 5.2). Later, in the cortical alveolus stage, the nucleoli eventually assume a peripheral position in concavities of the nuclear envelope (Figure 5.3A). Oocytes in late primary growth stage are positioned farther from the central stroma, towards the ovary lumen and the cytoplasm around their germinal vesicle becomes granulated.

**Cortical Alveolus Stage**

Oocytes in the cortical alveolus stage (dia. = 80 - 165 µm) are surrounded by a well-developed follicular complex, which consist of a zona radiata overlain by a monolayer of granulosa cells, a basement membrane, and a well-vascularized, multicellular outer layer of theca cells. The nuclear chromatin is progressively more granulated in appearance and the numerous nucleoli move to a peripheral position just under the nuclear membrane as the ooplasm becomes less basophilic (Figure 5.3A). The appearance of nascent cortical alveoli within the oocyte marks the beginning of the cortical alveolus stage. The cortical alveoli are distinguished from the ooplasm as opaque granules (Figure 5.3B). The alveoli appear initially in the cortical ooplasm and form a ring around the germinal vesicle; they later proliferate and are displaced peripherally as a dark granulated ring of ooplasm expands around the germinal vesicle.

**Vitellogenic Stage**

Oocytes in the vitellogenic stage (dia. = 130 - 500 µm) are suspended on individual peduncles, or stalks, that originate from the central ovarian stroma and extend
towards the ovary lumen (Figure 5.4ABC). Multiple oocytes in the primary growth stage are visible along the base of the peduncle and are more concentrated closer to the stroma. The follicular complex, including the zona radiata, granulosa cells, basal lamina, and theca cells, appears thicker and more developed than at earlier oocyte stages (Figure 5.4C). The prominent germinal vesicle is centrally located and contains abundant heterochromatin with a granulated appearance and multiple peripheral nucleoli (Figure 5.4A). Oocytes in the early vitellogenic stage exhibit yolk granules that stain well with acidophilic dyes. The granules first appear in the peripheral ooplasm, and later form a ring around the oocyte cortex in the same region occupied by the cortical alveoli (Figure 5.4A). As the vitellogenic stage progresses, the yolk granules increase in number and size until they are distributed throughout the ooplasm (Figure 5.4B). As yolk granules accumulate within the oocyte, the cortical alveoli are displaced progressively toward the peripheral ooplasm. Rings of homogeneous, basophilic ooplasm are displaced to the oocyte periphery and to a position adjacent to the germinal vesicle.

Coincident with deposition of yolk granules within the ooplasm is the deposition of lipid droplets. These are removed during histological processing and appear as empty spaces located among the yolk granules in the oocyte sections (Figure 5.4B,C). While considerable deposition of lipid droplets occurs as early as the cortical alveolus stage in some teleosts, most deposition of lipids into lionfish oocytes occurs during mid- to late-vitellogenesis (Figures 5.4 and 5.5).
Maturation Stage and Ovulation

During the oocyte maturation stage (dia. $\geq 500$ µm), the germinal vesicle rapidly migrates peripherally and then disintegrates. As the oocytes mature, the large, distinct, and highly acidophilic yolk granules coalesce simultaneously with the accumulation of homogeneous yolk throughout the ooplasm (Figure 5.5). This newly formed homogeneous yolk is less acidophilic and more translucent than the rings of homogeneous ooplasm formed earlier at the oocyte periphery and around the germinal vesicle. Up to the onset of ovulation, the lipid droplets fuse together, forming progressively larger droplets of various sizes within the ooplasm. As shown in Figure 5.5, some coalescence of the lipid droplets can precede obvious migration of the germinal vesicle toward the oocyte periphery. The ooplasm eventually consists of one or a few large lipid droplets and several masses of homogeneous, translucent, and slightly acidophilic yolk, apparent against a background of more opaque yolk as shown for ovulated eggs. Prior to ovulation, the stalk of the peduncle (bearing the maturing oocyte on its terminus) extends from its origin at the central ovarian stroma to the ovary lumen. Maturing oocytes are sequestered near the opposite ovary wall.

At ovulation, the oocytes detach from peduncles and are ovulated from their follicles at the point where the peduncular epithelium joins the follicular epithelium immediately below the oocyte. Simultaneously, oocyte hydration occurs and a gelatinous matrix surrounds the new batch of ova (Figure 5.6A). A single layer of specialized secretory cells located below the inner epithelium of the ovarian wall produces the
encasing gelatinous matrix (Figure 5.6B). These secretory cells are underlain by a basement membrane, an endothelial cell layer, a layer of smooth muscle, and a fibrous layer of connective tissue, which collectively form the ovary wall complex (Figure 5.6B). During production of the gelatinous matrix, the secretory cells are columnar and spindle-like with hair-like appendages extending from their apical surface (Figure 5.6B).

*Spawn Formation*

Before release, the gelatinous egg masses slough off the ovigerous tissue from anterior to posterior and pass into the oviduct leaving an opening at the anterior end of each gelatinous egg mass. Each ovarian lobe produces a single gelatinous egg mass, which is released individually during spawning (Figure 5.7A). A subsample of ovulated eggs (n = 10) extracted from egg masses shortly after spawning were slightly ovoid with a mean diameter of 804 ± 25 µm. Each ovum contained one large oil globule (dia. approximately 160 µm).

*Atresia*

After spawning, or when environmental conditions are not favorable for oogenesis and spawning, vitellogenic and maturation stage oocytes undergo preovulatory atresia. Atretic oocytes appear vacuolated and disorganized. They also are characterized by disintegrating yolk granules, diminishing homogeneous yolk, small lipid droplets that coalesce into larger droplets, and, when atresia is advanced, the absence of a germinal
vesicle. The apical segment of the peduncle that bore the oocyte is reabsorbed. Oocytes in the primary and cortical alveolus stage may remain in "resting" status until conditions are favorable for further development (Figure 5.7B).

**DISCUSSION**

This study provides the first description of oocyte maturation, ovulation, and atresia, and of spawn formation in the lionfishes, *P. miles* and *P. volitans*. All stages of oocyte growth and maturation were simultaneously observed in mature females, indicating that non-native lionfish in the Atlantic are asynchronous spawners. This reproductive mode can support continuous production of eggs when environmental conditions are favorable.

This description of lionfish oogenesis will support future assessments of reproduction in the invaded habitats of lionfish. For example, lionfish ovaries exhibiting only primary growth and cortical alveolus stage oocytes provide evidence that the local lionfish population is at a reproductively quiescent stage or very early in its spawning season. However, the presence of oocytes at all stages of growth and maturation provides evidence that the local population is actively spawning. Conversely, the presence in many females of highly atretic vitellogenic and maturation stage oocytes signals that the reproductive season is ending or has ended in a given locale. This information will be essential to forecast reproductive potential in the lionfish's new range, where seasonal decrease in water temperature could limit spawning to discrete periods.
Lionfish ovarian morphology, while similar to that in some other scorpaenids, is uncommon among teleosts. Lionfish ovaries represent the most advanced of the cystovarian morphotypes (type II-3) as described by Koya and Muñoz (2007). In lionfish ovaries, the vascular system is central, originates in the anterior end of the ovary, and runs longitudinally through the center of each lobe. The ovarian cavity is located between the ovarian wall and the central stroma. This ovary type is specialized for production of gelatinous secretions by the ovary wall complex and utilizes specialized peduncular structures to support individual ovarian follicles during oocyte development.

Ovarian peduncles, also termed pedicles, stems, branches, delle, or stalks, (Erickson and Pikitch 1993) are seen in both viviparous (Hoar 1969) and oviparous (Brummett et al. 1982) fishes. Peduncles also are described in other vertebrates, including birds and reptiles (Franchi 1962). Peduncles facilitate ovarian growth by preventing egg crowding (Fishelson 1975), by providing direct nutrient delivery (Hoar 1969), and by facilitating internal fertilization (Nagahama 1983). Given that lionfish are asynchronous spawners capable of serial production of multiple batches of eggs, the vascularized peduncle might enhance oocyte development via more direct oxygen and nutrient delivery to the follicles. Batch frequency is presently unknown for *P. miles* or *P. volitans*, although Fishelson (1975) observed captive pigmy lionfish *D. brachyopterus* spawning every six to eight days for eight months. Additional ultra-structural and biochemical study of the ovarian peduncle could provide insights into its nutritive role.
Fishelson (1975) reported 15,000 eggs in an egg mass of pigmy lionfish (\textit{D. brachypterus}). Given the larger body size of \textit{P. miles} and \textit{P. volitans} when compared to \textit{D. brachypterus}, higher fecundity might be expected. When comparing lionfishes to oviparous reef dwelling fishes of the Atlantic and Caribbean (e.g., serranids, lutjanids), it is apparent that lionfish batch fecundity is much lower than these other fishes, however, the year-round spawning season and frequent spawning events ultimately result in high fecundity (Chapter 6).

The egg mass morphology of lionfish provides a potential mechanism for optimizing fertilization rate. The eggs are embedded within a gelatinous matrix, which sloughs off the ovary from anterior to posterior creating a hollow open-ended mass. As the courtship phase of reproduction ends, the female lionfish ascends towards the surface releasing the hollow gelatinous egg masses, which are then fertilized externally by the male (Fishelson 1975). We hypothesize that the hollow construct of the egg mass is capable of sperm entrapment and concentration, thus facilitating fertilization. This adaptation might partly account for the relatively low batch fecundity observed in the lionfishes.

The reproductive characteristics of \textit{P. miles} and \textit{P. volitans} such as asynchronous mode, cystovarian morphology, vascularized peduncles, and the production of hollow buoyant gelatinous egg masses might confer reproductive advantages, thus explaining, in part, their rapid establishment in the Atlantic and Caribbean. The similarity of reproductive biology between these two closely related lionfish species is not surprising
given their meristic and genetic similarities (Schultz 1986; Kochzius et al. 2003; Hamner et al. 2007).

Future studies of reproduction in *P. miles* and *P. volitans* should focus on assessments at the population level including estimates of batch fecundity and periodicity, spawning seasonality, and reproductive demographics including size at sexual maturity. This information will be critical for elucidating the mechanisms of rapid establishment and expansion of these invaders in the Northwestern Atlantic and Caribbean.

**ACKNOWLEDGMENTS**

We thank P. Whitfield and the many scientific divers from the NOAA Center for Coastal Fisheries and Habitat Research for sampling assistance. We also thank M. Dykstra (NCSU LAELOM) for assistance with SEM imagery, D. Freshwater for undertaking the genetic identification of lionfish samples, and S. Horton for providing histological support. We thank D. Wyanski, D. Ahrenholz, and J. Smith for their helpful comments on an early draft of this manuscript. This work was funded in part by the NOAA Aquatic Invasive Species Program. Mention of brand names or manufacturers does not imply endorsement by the U.S. Federal Government.
REFERENCES


Table 5.1 Key cytological features characteristic of lionfish oocytes.

<table>
<thead>
<tr>
<th>Oocyte stage (diameter)</th>
<th>Histological features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary growth (20 – 60 µm)</td>
<td>Basophilic ooplasm, prominent germinal vesicle, multiple nucleoli appearing during late stage</td>
</tr>
<tr>
<td>Cortical alveolus (80 – 165 µm)</td>
<td>Cortical alveoli (yolk vesicles) appear in the ooplasm around the germinal vesicle, numerous nucleoli peripherally located around the germinal vesicle, nuclear chromatin more granulated, ooplasm less basophilic</td>
</tr>
<tr>
<td>Vitellogenic (130 – 500 µm)</td>
<td>Oocytes individually suspended on peduncles, germinal vesicle centrally located with multiple peripheral nucleoli, follicle elements thicker and more developed, yolk granules form a ring around the oocyte cortex and eventually occupy entire ooplasm</td>
</tr>
<tr>
<td>Maturation and ovulation (≥ 500 µm)</td>
<td>Germinal vesicle migrates peripherally and its membrane disintegrates, yolk granules coalesce, lipid droplets coalesce, egg detaches from peduncle and is ovulated from the follicle, gelatinous mucus produced by ovarian wall complex encompasses the ova</td>
</tr>
<tr>
<td>Atresia</td>
<td>Oocytes disorganized and appear highly vacuolated, yolk disintegrates, lipid droplets coalesce into numerous larger droplets, germinal vesicle disintegrates, apical segment of peduncle involutes and is reabsorbed</td>
</tr>
</tbody>
</table>
Figure 5.1 Transverse sections of lionfish ovaries depicting cystovarian morphology and oocyte growth and maturation along the stroma-lumen axis. A) Line drawing, adapted from Koya and Muñoz (2007) and B) electron micrograph (scale bar = 500 µm). BV = blood vessels, MO = mature oocyte, OL = ovarian lumen, OS = ovarian stroma, OW = ovary wall, P = peduncle, PO = primary oocyte.
Figure 5.2 Early primary growth stage oocyte (inset) and late primary growth stage oocytes. EPGO = early primary growth stage oocyte, GE = germinal epithelium, GV = germinal vesicle, LPGO = late primary growth oocyte, NU = nucleoli, O = oogonia. Scale bars = 50 µm and 15 µm (inset).
Figure 5.3  A) Early cortical alveolus stage oocyte (Scale bar = 25 µm) and B) mid cortical alveolus stage oocyte (Scale bar = 100 µm). CA = cortical alveoli, GV = germinal vesicle, MCAO = mid cortical alveolus stage oocyte, NU = nucleoli, P = peduncle.
Figure 5.4  A) Early vitellogenic stage oocyte (EVO) (scale bar = 50 µm), B) mid-vitellogenic stage oocyte (MVO) (scale bar = 100 µm), and C) follicular complex of vitellogenic stage oocyte (scale bar = 50 µm). GC = granulosa cells, GV = germinal vesicle, LD = lipid droplets, MVO = mid-vitellogenic stage oocyte, NU = nucleoli, P = peduncle, T = theca, YG = yolk granules, ZR = zona radiata.
Figure 5.5  Early maturation stage oocyte exhibiting germinal vesicle migration and yolk granule and lipid droplet coalescence (scale bar = 100 µm) GV = germinal vesicle, LD = lipid droplets, MO = maturation oocytes, PDP = peduncle detachment (ovulation) point, YG = yolk granules.
Figure 5.6 A) Ovulated eggs and ovary wall depicting production of gelatinous matrix (scale bar = 150 µm) and B) ovary wall complex (scale bar = 50 µm). BM = basement membrane, EC = endothelial cell layer, GM = gelatinous material, HA = hair-like appendages, HO = hydrated oocyte, SC = secretory cells, SM = smooth muscle, O = oil globule, OWC = ovarian wall complex.
Figure 5.7  A) Lionfish ovary with unreleased gelatinous egg mass (scale bar = 10 mm). Anterior end of the ovary is oriented to the left. B) Oocytes in several stages of ovarian atresia. The most advanced-staged atretic oocytes are labeled as AO (scale bar = 100 µm). GE = gelatinous egg mass, OT = ovigerous tissue.
CHAPTER 6. REPRODUCTIVE DYNAMICS OF THE INVASIVE LIONFISHES
_Pterois miles_ AND _P. volitans_ IN THE WESTERN NORTH ATLANTIC

James A. Morris, Jr.

ABSTRACT

The Indo-Pacific lionfishes, _Pterois miles_ and _P. volitans_, are now established along the Southeast U.S. and parts of the Caribbean. The reproductive mechanisms underlying this unprecedented invasion are largely unknown. For this reason, the characteristics of lionfish reproduction, including size at maturity, spawning seasonality, batch frequency, and fecundity were estimated from lionfish specimens collected from the temperate (North Carolina and South Carolina) and tropical (Bahamas) regions of their new Atlantic range. Fifty percent of male lionfish were found to be sexually mature at ~ 100 mm total length (TL), while 50% of female lionfish were mature at ~180 mm TL. Lionfish spawn throughout the entire calendar year with peak spawning during the summer months. Lionfish batch frequency estimates were 3.6 d for North Carolina specimens and 4.1 d for Bahamian collected specimens. Fecundity estimates revealed that lionfish are capable of releasing over two million eggs per year. This work provides the first comprehensive assessment of reproductive parameters in the pteroines and provides valuable insight into how invasive lionfish have become so rapidly established.
INTRODUCTION

The Indo-Pacific lionfishes, *Pterois miles* (Bennet, 1828) and *P. volitans* (Linnaeus, 1758) are now considered established along the Southeast U.S. coast, the Bahamas, and parts of the Caribbean (Hamner and Freshwater 2007; Morris et al. 2009; Schofield et al. 2009). The release of lionfish into Atlantic waters was first reported in the literature by Courtenay (1995) who documented the release of six lionfish from a waterfront porch on Biscayne Bay, Dade County, Florida in 1992 during Hurricane Andrew. Several reports of lionfish sightings occurred, however, prior to 1992 with the earliest known report occurring in 1985 (Schofield et al. 2009). The rate of lionfish sightings increased substantially from 1993 to 2002 with widespread establishment from North Carolina to northern Florida reported by Whitfield et al (2002). Presently, lionfish abundance and geographic range continues to increase remarkably with lionfish reaching much higher densities than reported in their native environments (Whitfield et al. 2007; Green and Côté 2009).

Lionfish are of the order scorpaeiniformes, a large and diverse order of fishes comprising 35 families, 250 genera, and over 1,000 species (Nelson 1994; Muñoz et al. 1999). Scorpaenids exhibit varied strategies of reproduction including all three of the reproductive modes known for teleosts (oviparity, ovoviviparity, and viviparity). Lionfish are oviparous, asynchronous, batch spawners (see Chapter 5) and are thus capable of releasing serial batches of eggs as environmental conditions allow. This
reproductive strategy may be one of the enabling life-history characteristics that has contributed to lionfish invasiveness.

No detailed study of the reproductive dynamics of pteroines is available from either their native (Indo-Pacific) or invaded range. This assessment of lionfish reproduction spans across the calendar year in both temperate and tropical regions. The specific objectives of this study were to assess size at sexual maturity, spawning seasonality, spawning frequency, and fecundity.

METHODS

Sample collections

Lionfish were collected from both temperate (North Carolina and South Carolina) and tropical (Bahamas) regions of the Western North Atlantic by spearfishing, netting, hook and line, and trapping. Ninety-nine percent of the collections from North Carolina (n=600) and South Carolina (n=20) were taken from offshore at locations between 27 - 45 m depth from 2001-2007. These locations are characterized by hard bottom habitat, water temperature regimes above the critical thermal minimum (10°C) for lionfish (Kimball et al. 2004), and are areas that hold lionfish throughout the winter (P. Whitfield, pers. comm.). Collections from the Bahamian Archipelago (n=1,039) were from 2006-2008 in both shallow (<1 m) and deep waters (up to 30 m) encompassing a wide-variety of habitat types including artificial reefs, mangroves, high profile coral reefs, patch reefs, and man-made canals. Juvenile lionfish were also collected from the Quezon Province,
Philippines (n=83). It is assumed that no reproductive differences between *P. miles* and *P. volitans* are detectable (see Chapter 5).

**Reproductive assessment**

All lionfish were euthanized by either lowering temperature below the level of hypothermia or applying lethal doses of MS-222 or eugenol. Gonadal tissue, either preserved whole or excised from the center of the gonad, was preserved in 9-10% neutral buffered formalin, rinsed in phosphate buffered saline, dehydrated through a graded ethanol series, and embedded in paraffin according to conventional histological technique. Sections were cut at 5-6 µm and stained with a mixture of Mayer's/Harris hematoxylin and alcoholic Eosin Y (Sheehan and Hrapchak 1980). Each histological sample of lionfish gonadal tissue was staged according to the criteria provided in Tables 6.1 and 6.2 (Brown-Peterson et al. 2007).

The smallest size at maturity was determined by gender from all three regions (North Carolina and South Carolina, Bahamas, and Philippines). A two-parameter logistic model for maturity (Quinn and Deriso 1999) was used to assess the relationship between size and maturation status

\[
m_x = \frac{1}{1 + e^{-k(L-\gamma)}}
\]

where \(m_x\) is maturity of gender \(x\), \(k\) is the slope, \(L\) is lionfish total length, and \(\gamma\) is the size at which 50% of the sample population is mature. Spawning seasonality of lionfish from the North Carolina and South Carolina region and the Bahamian archipelago was
assessed by collecting lionfish specimens throughout the calendar year. Lionfish collections from various years, pooled by the calendar month collected, provided adequate monthly sample size. I assume no inter-annual variability for lionfish spawning seasonality. Reproductive activity was also compared by season (winter, spring, summer, and fall) between the temperate (North Carolina and South Carolina) and tropical (Bahamas) regions.

Spawning frequency of lionfish was determined by assessing the proportion of lionfish ovaries with hydrated oocytes sampled over a consecutive number of days using the equation \(1/(\text{sum of fish with hydrated oocytes/total number of sexually mature females})\) provided by DeMartini and Fountain (1981) and Hunter et al. (1986). Two sampling events were analyzed for spawning frequency: 45 sexually mature lionfish collected off North Carolina over an 8-d sampling period and 75 sexually mature lionfish collected in the Bahamas over a 5-d sampling period. These samples constitute the northern (temperate) and southern (tropical) ranges of invasive lionfish and were taken during months when lionfish spawning has been observed. A second method for assessing spawning frequency using postovulatory follicles (Hunter et al. 1986) was attempted, but was found unreliable because of the uncertainty of postovulatory follicle persistence in the ovary.

Batch fecundity of lionfish was assessed by counting the number of eggs in a lionfish egg ball. Egg balls (n=6) were collected during lionfish (250 – 350 mm total length) spawning events in captivity. Egg balls, which were typically produced the first
or second morning following lionfish capture from the wild, were collected from the surface water of holding tanks and preserved in 95% ETOH. Because lionfish eggs are tightly bound in a gelatinous matrix (Chapter 5), we assumed no egg loss as a result of collection. To accurately assess batch fecundity, the preserved egg balls were agitated using a magnetic stirring device to break apart the egg ball creating loose eggs and gelatinous matrix fragments that still contained some eggs. The free eggs and gelatinous matrix containing eggs were then separated using a 1-mm sieve. Egg count per volume was then obtained for the material that passed through the sieve (eggs and small fragments of gelatinous matrix) and the material retained on the sieve (gelatinous matrix containing eggs). Approximately 10% of the total volume of both the separated eggs and eggs bound in the gelatinous matrix was counted for each egg ball.

RESULTS

Size at sexual maturity

The size of the smallest sexually mature female lionfish was 98 mm TL for the Bahamas, 158 mm TL for North Carolina and South Carolina, and 172 mm TL for the Philippines. The size of the smallest mature male lionfish was 100 mm TL for the Bahamas, 105 mm TL for the Philippines, and 132 mm TL for North Carolina and South Carolina. For pooled samples from all locations, length at 50% sexual maturity was approximately 175 mm TL for females (n=718) and 100 mm TL for males (n=927) (Figure 6.2 and Figure 6.3).
**Spawning seasonality**

Female lionfish were actively reproducing throughout the entire calendar year in both the North Carolina and South Carolina region and the Bahamas as indicated by the presence of spawning capable stage (SC) ovaries in every month and spawning stage (S) ovaries in all but two months (September and October) (Figure 6.3). Male lionfish exhibited late developing stage testes for all months of the year (Figure 6.3).

Seasonal differences in spawning activity were detected for lionfish collected from both the North Carolina and South Carolina and Bahamas regions with the highest proportion of lionfish with SC- and S-stage ovaries found during the summer months (June, July, and August) (Figure 6.4). Male lionfish exhibited a more consistent level of reproductive activity throughout the calendar year (Figure 6.4).

**Spawning frequency**

The estimated frequency of lionfish spawning was every 3.6 days for North Carolina and 4.1 d for the Bahamas.

**Fecundity**

The mean ± S.D. number of eggs per egg ball was 12,315 ± 5,939 equating to a mean batch fecundity of 24,630 eggs (two egg balls per spawning event). The range of egg counts per egg ball was 5,395 - 20,696 eggs. Using the average of the two spawning
frequency observations (spawning every 3.85d) and the mean number of eggs per egg ball, the mean monthly fecundity is estimated to be 194,577 eggs with a mean annual fecundity (assuming year round spawning) of 2,335,052 eggs per lionfish.

**DISCUSSION**

Assessment of lionfish reproduction in the waters off North Carolina and South Carolina and the Bahamas support the notion that lionfish are prolific and capable of rapid progamento. The size of lionfish at sexual maturity suggests early maturation in their life history. The exact age of maturity is unknown as there are no published ageing studies for lionfish. Preliminary estimates of lionfish age at 180 mm TL using daily increments on otoliths indicate that both male and female lionfish can become sexually mature in well under one year of age (Ahrenholz and Morris, unpub. data).

The smallest mature female lionfish in the present collections were from the Atlantic collections. Population bottlenecks, such as invasion events and overfishing, can result in a decreased size at maturity and higher reproductive output (Hutchings 2002). Continued monitoring of lionfish size at maturity could detect an increase which could indicate that population(s) are reaching carrying capacity or are no longer undergoing rapid expansion.

The spawning seasonality of lionfish in the Western North Atlantic is alarming from an ecological standpoint because it suggests year-round reproduction and thus a consistent and continuous supply of propagules. The probability of successful
establishment in a new habitat could be higher when larval output is distributed across environmental variables such as temperature and food availability. This characteristic of lionfish reproduction may have contributed to its rapid establishment.

This is the first published assessment of spawning seasonality in the pteroines in both its new and native ranges. One preliminary report of spawning seasonality is provided for the closely related pigmy lionfish, *Dendrochirus brachypterus*, by Fishelson (1975) who observed ripe females in the Red Sea from June till December. More recent observations by Fishelson (L. Fishelson, pers. comm.) on spawning of *P. volitans* and *P. radiata* in the Gulf of Aqaba revealed a cycle typical to numerous coral reef fishes of this region: spawning beginning at the second half of winter, beginning in March, and continuing until the end of June, with some sporadic single spawning later. No spawning was observed in the summer during the highest water temperature, 26-27°C. Contrary to the earlier report, *D. brachypterus* are now also found to spawn year-round (L. Fishelson, pers. comm.).

Many tropical marine fishes are reproductively active throughout the year (Lowe-McConnell 1979). The detection of peak reproductive activity of lionfish during the summer months could indicate that spawning is influenced by seasonal changes in water temperature, even at tropical locations such as the Bahamas. Future efforts to assess lionfish spawning seasonality in the lower Caribbean, where water temperatures are much warmer throughout the year, could yield different results (i.e., perhaps higher reproductive output).
The spawning frequency of every 3-4 d is also similar to that reported for the pigmy lionfish. Fishelson (1975) reported captive spawning of the pigmy lionfish every 6-8 d under stable thermal conditions and good feeding. Our estimates of lionfish spawning frequency from field collected specimens indicate a higher frequency and could be due to a number of scenarios including: higher reproductive output resulting from the invasion, error due to estimating spawning frequency using final oocyte maturation, differences between the *Pterois* and *Dendrochirus* genera, or a more accurate assessment of spawning frequency in wild versus captive species. Future efforts to assess the persistence of postovulatory follicles in ovaries of lionfish could aid in validating spawning frequency. As lionfish are a tropical marine fish, it is likely that post ovulatory follicles are absorbed rapidly. The sampling of lionfish throughout the day, including the pre-dawn hours when lionfish are spawning (Fishelson 1975), is needed to further elucidate the rate of postovulatory follicle absorption.

This observation of lionfish fecundity is also higher than reported by Fishelson (1975) for the pigmy lionfish (2,000 - 15,000 eggs per ball verses 5,395 – 20,696). This finding is not surprising given the significant increase in body size of *Pterois* spp. when compared to *Dendrochirus* spp. Fecundity, similar to size at maturity, is phenotypically plastic and capable of increasing or decreasing as fish populations become stressed (Rochet et al. 2000). Given the small sample size of counted egg balls in this study, continued efforts to assess size-specific fecundity are warranted. Collection of lionfish
during the mid-night to early morning periods could be useful for assessing batch fecundity histologically.

Lionfish reproductive characteristics observed in this study provide insight into the biological mechanisms that have supported the rapid expansion of lionfish populations in waters along the Southeast U.S. coast, the Bahamas, and presently the Caribbean. Lionfish mature early, reproduce often and throughout the calendar year, and are capable of producing millions of eggs annually. This reproductive profile describes the most rapidly established non-indigenous marine finfish of the Western North Atlantic, Caribbean, and likely the Gulf of Mexico and could be used as a baseline from which to predict the potential for new invaders with similar reproductive biology. Future efforts are needed to assess the potential risk of other non-native fishes exhibiting similar characteristics as lionfish and the likelihood of additional introductions.

ACKNOWLEDGEMENTS

I thank the NOAA Aquatic Invasive Species Program and the NOAA National Centers for Coastal Ocean Science for funding support. I am also grateful to Paula Whitfield (NOAA/NCCOS), David Wyanski (SCDNR), Bruce Purdy (Blackbeards Cruises), Stuart Cove (Stuart Cove Dive Bahamas), Stephanie Green (Simon Frasier University), the Shedd Aquarium of Chicago, IL, and the many NOAA CCFHR divers who collected lionfish specimens. This work would not have been possible without the support of L. Akins and the Reef Environmental Education Foundation volunteers. I am grateful to Sandra Horton (NCSU School of Veterinary Medicine) for histological preparations. I
also thank Lakeisha Anderson and Michael Braynen for their support of Bahamian lionfish collections. Sampling of lionfish in the Bahamas was accomplished under research permit MAF/FIS/12: MAF/FIS/17 to J. Morris. An early version of this manuscript was greatly improved by suggestions from Dean Ahrenholz and Joseph Smith.
REFERENCES


Table 6.1 Criteria used for histological staging of lionfish ovaries.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature (virgin)</td>
<td>Oogonia and primary oocytes only. No evidence of atresia. Oogonia abundant along margin of lamellae, ovarian wall is thin. (Figure 5.2).</td>
</tr>
<tr>
<td>Early developing</td>
<td>Most-advanced oocytes in cortical-alveoli stage. (Figure 5.3).</td>
</tr>
<tr>
<td>Spawning capable</td>
<td>Most-advanced oocytes in migratory-nucleus stage. Partial coalescence of yolk globules. (Figure 5.5).</td>
</tr>
<tr>
<td>Spawning</td>
<td>Completion of yolk coalescence and hydration in most-advanced oocytes. Zona radiata becomes thinner. (Figure 5.6).</td>
</tr>
</tbody>
</table>
Table 6.2  Criteria used for histological staging of lionfish testes.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td>Small transverse section compared to resting male. Spermatogonia present and little or no spermatocyte development. Lobules not evident. (Figure 6.1A).</td>
</tr>
<tr>
<td>Early developing</td>
<td>Continuous germinal epithelium in some lobules. Spermatocytes and spermatids present. Few spermatozoa present in lobules; no spermatozoa in ducts. (Figure 6.1B).</td>
</tr>
<tr>
<td>Mid developing</td>
<td>Continue germinal epithelium at periphery. Discontinuous at ducts. All stages of spermatogenesis present. Spermatozoa in lumen of lobules and ducts (&lt;20% area of testes contains spermatozoa). (Figure 6.1C).</td>
</tr>
<tr>
<td>Late developing</td>
<td>Discontinuous germinal epithelium in lobules at ducts and periphery. Numerous spermatocytes throughout. Mostly spermatocytes and spermatids. Reduced number of spermatogonia. Lobules can begin anastomosing. Spermatozoa fill lobules and ducts (&gt;20% area of testes contains spermatozoa). (Figure 6.1D).</td>
</tr>
</tbody>
</table>
Figure 6.1 Histological sections of lionfish testes at immature (A), early developing (B), mid developing (C) and late developing (D) stages. Some of the stage-specific histological criteria is noted by arrows. SC = spermatocyte, SG = spermatogonia, ST = spermatids, SZ = spermatozoa. A) scale bar = 50 µm, B) scale bar = 50 µm, C) scale bar = 200 µm, D) scale bar = 500 µm, arrows indicate lobules filled with spermatozoa.
Figure 6.2 Percent of mature female lionfish by 20-mm TL size class. Bar = sample size; line = logistic model \( (k = 0.075, \gamma = 178.282, \text{see text for model equation}) \), open circles = number mature in each size class.
Figure 6.3  Percent of mature male lionfish by 20-mm TL size class. Bar = sample size; line = logistic model \( (k = 0.071, \gamma = 101.336, \text{see text for model equation}) \), open circles = number mature in each size class.
Figure 6.4  Reproductive status of female lionfish (A) and male lionfish (B) collected from North Carolina, South Carolina, and the Bahamas. ED = early developing; LD = late developing; S = spawning; MD = mid developing.
Figure 6.5  Lionfish reproductive activity by season collected from temperate (North Carolina and South Carolina) and tropical (Bahamas) regions. A) NC/SC females; B) Bahamas females; C) NC/SC males; D) Bahamas males.
CHAPTER 7. INVESTIGATION OF PREDATION ON INVASIVE JUVENILE LIONFISH IN THE ATLANTIC

James A. Morris, Jr. and James A. Rice

Manuscript in review at the Journal of Experimental Marine Biology and Ecology

ABSTRACT

The Indo-Pacific lionfishes (*Pterois miles* and *P. volitans*) have recently become established in the Northwestern Atlantic and parts of the Caribbean. To investigate the vulnerability of juvenile lionfish to predation by native reef fishes, replicated laboratory trials were conducted using four serranid (grouper) species of the Northwestern Atlantic. Serranids clearly exhibited avoidance of juvenile lionfish and preference for an alternative prey, even during periods of starvation. Furthermore, lionfish did not display a flight response to large predators, potentially because of their venom defense. These results suggest that predation on juvenile lionfish may be low relative to predation on native juvenile reef fishes and may partly explain their rapid establishment in the Atlantic.
INTRODUCTION

The Indo-Pacific lionfishes (*Pterois miles* and *P. volitans*) are now established along the east coast of the United States (Whitfield et al. 2002, Hamner et al. 2007) and in the Bahamas (Snyder and Burgess 2007). Lionfish are presently invading the Caribbean (Schofield et al. 2009) and could impact reef fish communities via competition or direct predation (Albins and Hixon 2008; Morris et al. 2009). The first record of lionfish introductions was noted by Courtnay (1995) who documented the release of six lionfish from an aquarium in south Florida. The number of lionfish sightings increased substantially by early 2000 (Whitfield et al. 2002) with densities approaching that of some native species within five years at locations off North Carolina (Whitfield et al. 2007a; 2007b).

Lionfish, like many of the scorpaenids, are venomous possessing a highly developed venom apparatus consisting of 13 dorsal spines, three anal spines, and two pelvic spines. Venom glands are located along two anterior-lateral grooves of each spine and extend three quarters of the distance from the base of the spine towards the tip (Halstead et al. 1955). Lionfish venom is delivered immediately after entry of the spine tears the integumentary sheath rupturing the glandular tissue and releasing venom into the puncture wound (Saunders and Taylor 1959). The toxin in lionfish venom contains acetylcholine, which affects neuromuscular transmission (Nair et al. 1985; Cohen and Olek 1989), and has been found to cause cardiovascular, neuromuscular, and cytolytic effects in animals and humans. There are 29 native scorpaenids distributed in the
Atlantic from Cape Hatteras, North Carolina to the Caribbean, 14 of which have similar venom delivery systems as those seen in lionfish (Smith and Wheeler 2006; Froese and Pauly 2008). In addition to being venomous, lionfish are similar to native scorpaenids in their quasi-cryptic behavior, but can be easily distinguished by their large pectoral fins, contrasting color bands, and skin protrusions on the head (Fishelson 2006; Morris and Freshwater 2007).

It is uncertain how native fishes of the Atlantic and Caribbean will interact with the lionfishes and to what degree native predators will avoid lionfish as prey. It is plausible that reduced predation on lionfish during the initial phases of the introduction may have resulted in low natural mortality and thus contributed to the rapid establishment of lionfish. To investigate this possibility, we designed a laboratory experiment capable of assessing predation on juvenile lionfish by native serranid (grouper) species. In this study, we set out to answer two questions: 1) do serranids exhibit avoidance of juvenile lionfish? And if so, 2) will starved serranids feed on juvenile lionfish?

METHODS

Laboratory predation trials were performed using four species of serranids: black sea bass (*Centropristis striata*; n=16, 333 ± 15.6 mm total length), red grouper (*Epinephelus morio*; n=2; 661 mm and 372 mm total length), gag grouper (*Mycteroperca microlepis*; n=1; 762 mm total length), and goliath grouper (*Epinephelus itajara*; n=1; 304 mm total length). All grouper species except the goliath grouper were collected from
offshore reefs (known to have high densities of lionfish) of North Carolina approximately two weeks before the feeding trials. The goliath grouper was collected in nearshore waters of North Carolina and reared in captivity for approximately two years. The majority of the trials were performed using black sea bass as they are an aggressive and common reef fish predator of the Northwestern Atlantic. Black sea bass are well known for their ravenous feeding with a diet consisting primarily of teleosts and crustaceans (Lindquist et al. 1994; Steimle and Figley 1996).

All feeding trials were conducted in 1.8-m diameter, 2000-L seawater tanks at the Center for Coastal Fisheries and Habitat Research in Beaufort, North Carolina, USA. At least four days before each trial, the predator was placed in the trial tank for acclimation. During the acclimation period, predators were fed cut squid and shrimp. Immediately prior to the trials, all predators were starved a minimum of 36 hours. During the acclimation and trial periods, the tanks were enclosed with black curtains and the laboratory noise level was reduced to a minimum. Following the initial acclimation and starvation periods, two 76 cm x 15 cm transparent, open-ended, hollow acrylic tubes (internal diameter = 14.75 cm) were placed on opposite sides of the tank approximately 1 m apart (Figure 7.1). These tubes served as prey protection chambers allowing behavioral observations of predation attempts with a barrier preventing immediate consumption of the prey. The predator was given an additional 30 minutes to acclimate to the presence of the tubes.
Two prey types were used in each feeding trial: a pinfish (*Lagodon rhomboides*) or a spottail pinfish (*Diplodus holbrooki*) of 97.9 ± 2.0 mm mean total length (TL) and a juvenile lionfish of 94.6 ± 2.2 mm mean (TL). After the 30-minute period of predator acclimation to the tubes, the two prey types were randomly assigned to the two tubes. Prey were then released simultaneously from dipnets into the top of their assigned tube. The mean size difference between the two individual prey fish used in each trial was 3.0 ± 0.7 mm with the largest size difference being 13 mm.

**Behavioral observations**

Following the release of prey into the tubes, the behavior of the predator was recorded for ten minutes using a Samsung® SDC-415 video camera mounted above the tank. In some cases, this observation period ended prematurely when the predator knocked over the tube and released the prey. Following this period, the tubes were lifted remotely (Figure 7.1) and the prey were released into the tank. Video of predation attempts on each prey type was recorded for an additional ten minutes. After this predation period any remaining prey were left in the tank with the predator for an additional forty-eight hours. Following this extended period, final prey survival was assessed.
Behavioral video analysis

Prey preference of the predator was assessed during the first predation period by quantifying the amount of time the predator was positioned near each prey type. To assess this behavior, a 30-cm zone was designated around each tube (Figure 7.1) and the proportion of time the predator spent inside the zone of each tube was quantified (each 30 cm zone constituted approximately 17% of the total area of the tank). The data were transformed by taking the arcsin square root of the proportion of time and the means for each prey type were compared statistically using a Student’s t-Test (SAS version 9.1.3) with $\alpha = 0.05$ considered to be significant.

RESULTS

During the sixteen predation trials with black sea bass, predation behavior was almost always towards the pinfish and not the lionfish (Table 7.1). Only a single black sea bass approached the lionfish in a predatory manner. During the initial observation period when the prey were still in the tubes, black sea bass were positioned within 30 cm of the pinfish tube for an average of 47% of the time, compared to only 11% of the time for the lionfish tube. While this difference was significant ($t$-statistic = 3.12, df = 15, $p = 0.005$), the time spent near the lionfish came largely from two trials when the black sea bass was in a stationary resting position close to the lionfish tube. During this time, the black sea bass was not oriented toward the lionfish but was technically within 30 cm of the lionfish tube. Excluding these two trials, black sea bass spent 53% of the time near
the pinfish tube and only 3% near the lionfish tube. Only one black sea bass attacked the tube containing the lionfish, attempting only one strike, while 13 out of 16 (81%) black sea bass repeatedly attacked the acrylic tube containing the pinfish. Attacking behavior involved rapid strikes varying from seconds to several minutes (up to ten minutes during one trial). During five of the sixteen trials, the black sea bass prematurely released the pinfish by knocking over the tube resulting in an immediate chase and ultimately consumption of the pinfish. In contrast, no lionfish tubes were moved or disturbed and thus no lionfish were released prematurely by the black sea bass.

After the initial behavioral assessment, the lionfish and pinfish (if not already released by the black sea bass) were released simultaneously from the tubes and predation behavior was observed for ten minutes. In eight of the 11 trials in which the pinfish was not released prematurely, the black sea bass exhibited repeated predatory behavior towards the pinfish. This behavior included stalking, chasing, striking, and in one case, consuming the pinfish. In contrast, no such predatory behavior was observed towards the lionfish in any of the sixteen trials. Interestingly, the lionfish exhibited no flight response when released from the protective tube or when the black sea bass came within striking distance, whereas all of the pinfish exhibited immediate flight response. During several trials the black sea bass backed away or swam away when the lionfish swam nearby.

Following these observations, the lionfish and pinfish (if uneaten) were left in the tank with the black sea bass for an additional 48 hours to observe if prolonged starvation
increased the predation on lionfish. Of the sixteen trials, three were disrupted due to a water tank system failure that removed the lionfish from the tank. In the remaining trials (n=13) only one lionfish (7.6%) was eaten. In contrast, 12 of the 16 trials (75%) ultimately resulted in an eaten pinfish.

During the additional trials with other serranid species, no predatory behavior was exhibited toward the lionfish. Predatory behavior towards the pinfish was similar to that observed in black sea bass. Furthermore, despite the much larger size of several of the other serranids, lionfish still exhibited no flight response, and predators moved away from the lionfish whenever it approached.

**DISCUSSION**

The predation trials performed in this study provide the first evidence of avoidance of lionfish and preference for alternative prey (in this case pinfish) by grouper species of the Atlantic Ocean. In addition, lionfish exhibited no flight response when approached by the predator. Interestingly, the serranids in this study typically exhibited avoidance of lionfish rather than attempting to attack. This avoidance is likely visually stimulated, as avoidance behavior was observed immediately after the introduction of the prey. Given the overwhelming contrast observed in the behavior of the serranids toward pinfish and lionfish during this study, predation on juvenile lionfish is likely a small contribution to their natural mortality.

Lionfish periodically expanded their pectoral fins when in the vicinity of the predator. Measurements of lionfish pectoral fins indicate that full extension increases the
frontal width of lionfish to twice their total body length, suggesting that the display of pectoral fins may be a mechanism for deterring predators. During this experiment, however, it was apparent that serranids treated lionfish as non-palatable prey even when the lionfish was not displaying its pectoral fins (i.e., while in the acrylic tube). In addition, the predators were observed backing up and avoiding the lionfish when it was swimming towards them without pectoral fins fully displayed.

Lionfish have few known natural predators even in their native range. Bernadsky and Goulet (1991) documented a lionfish (Pterois miles) in the stomach of a cornetfish, Fistularia commersonii, captured in the Red Sea. The authors suggested that the cornetfish purposefully consumed the lionfish caudal fin first to avoid envenomation. The authors also state that lionfish fin rays (spines) pivot freely at their base and suggested that a rear attack would prevent the injection of venom during consumption. However, our examination of spine rotation range in live lionfish discounts the assertion by Bernadsky and Goulet (1991), as the lionfish spines are not capable of full basal rotation. Furthermore, lionfish dorsal and anal spines are positioned to discourage a caudal attack and thus, caudal consumption of a lionfish would likely require the breaking of the spines and consequent envenomation of the predator. While the single observation of Bernadsky and Goulet (1991) provides a hint of natural predation, there is no comprehensive stomach content analysis available to evaluate whether lionfish occur rarely or frequently in the diet of cornetfish.
One potential source of predation on juvenile lionfish is cannibalism (Allen and Eschmeyer 1973). We have observed one instance of lionfish cannibalism in captivity with no ill effects of envenomation observed on the larger lionfish. Cannibalism in teleost fishes while in captivity, however, is common and may not reflect a natural event (Smith and Reay 1991). Furthermore, diet analyses of >1,000 adult lionfish from the Atlantic have provided no evidence of cannibalism (Morris and Akins, in press). Fishelson (1997) also reported laboratory observations of predation on juvenile lionfish by larger lionfish and suggested that in the Red Sea, lionfish juveniles are frequently isolated from adults, a possible strategy for reducing predation. No comprehensive stomach content analysis is available for lionfish in their native habitat, so the relative frequency of cannibalism in lionfish from this region remains unknown.

A recent observation in the Bahamas has documented juvenile lionfish in the stomachs of a tiger grouper, *Mycteroperca tigris*, and two Nassau grouper, *Epinephelus striatus* (Maljković et al. 2008). These observations suggest that serranids are indeed capable of feeding on lionfish; however, the results reported here suggest that serranids do not readily prey on lionfish.

Given the existence of 14 venomous native scorpaenids to this region, native reef fishes may have evolved an avoidance of venomous scorpaenids. Our observations suggest that avoidance may be intrinsic to native reef fishes. Lionfish densities (and biomass) are much higher than native scorpaenids in the western North Atlantic, which suggests a higher probability of interaction (and competition) between lionfish and native
reef fishes than may have previously occurred between native scorpaenids and native reef fishes.

Further evidence supporting low predation on lionfishes is derived from observations of the Indo-Malayan octopus, which has been known to exhibit Batesian mimicry of lionfish. Norman et al. (2001) documented this mimicry that also included mimicry of animals such as venomous sea snakes and sand anemones. The evolution of lionfish mimicry in the Indo-Malayan octopus implies that predation on lionfish is relatively low in their native habitat and suggests the potential for lower predation rates in their invaded regions.

While this study provides observations of serranid predatory behavior, other species of reef fishes and elasmobranches remain untested. Additional trials and field observations of stomach contents of fishes in close proximity to large densities of lionfish may provide more conclusive information regarding predation on lionfish. Species that frequently feed on spiny and/or venomous prey, e.g., cobia (*Rachycentron canadum*) (Smith 1995), are possible candidates as predators of lionfish. We cannot rule out the possibility that native reef fishes will develop the capability to feed on juvenile lionfish, especially at locations where lionfish are highly abundant.

**CONCLUSION**

The observations of this study provide the first documentation of predatory interactions between juvenile lionfish and serranid species of the Atlantic. The high level
of avoidance demonstrated by serranids in this study, coupled with the diet analyses and behavioral observations mentioned above, suggest it is unlikely that lionfish will contribute significantly to the diet of common predatory fish, or that predation on lionfish will be sufficient to limit growth of lionfish populations. Relatively low predation mortality of lionfish may partly explain their rapid establishment in the Atlantic.

Further experiments with diverse predatory species are warranted. If natural predation is low, fishing mortality may be especially important to management strategies for this invader. In their native range, lionfish are considered to be a popular table fish (Froese and Pauly 2008). In addition, recent medical research suggests that lionfish venom may be of value for reducing cancer growth in humans (Sri Balasubashini et al. 2006a; 2006b). Thus, harvest of lionfish for food and pharmacological research could provide incentives supporting a control strategy based on fishing pressure. This appears to be the best management option for mitigating the ecological impacts of this species introduction in the Western North Atlantic and Caribbean and future invaded regions.

ACKNOWLEDGEMENTS

We thank D. Cerino for technical support and D. Ahrenholz, L. Akins, J. Govoni, D. Johnson, and E. Williams for their review of this manuscript. We also thank T. Jackson for insights regarding mimicry of lionfish by octopus. This work was funded in part by the NOAA Aquatic Invasive Species Program. Mention of brand names or manufacturer does not imply endorsement by the U.S. Federal Government.
REFERENCES


131


Table 7.1  Summary of predator (black sea bass) behavior and number of prey eaten during each period of the predation trials.

<table>
<thead>
<tr>
<th></th>
<th>Pinfish</th>
<th>Lionfish</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial 10 min, prey in tube</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predators attempting attacks</td>
<td>15/16 (94%)</td>
<td>1/16 (6.3%)</td>
</tr>
<tr>
<td>Mean time predator spent near prey</td>
<td>47%</td>
<td>11%</td>
</tr>
<tr>
<td>Prey consumed after premature release</td>
<td>5/5 (100%)</td>
<td>0/0 (0%)</td>
</tr>
<tr>
<td><strong>Second 10 min, prey released</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predators attempting attacks</td>
<td>8/11 (73%)</td>
<td>0/16 (0%)</td>
</tr>
<tr>
<td>Prey consumed</td>
<td>1/11 (9%)</td>
<td>0/16 (0%)</td>
</tr>
<tr>
<td><strong>48-hr starvation period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prey consumed</td>
<td>6/10 (60%)</td>
<td>1/13 (8%)*</td>
</tr>
<tr>
<td><strong>Total prey consumed</strong></td>
<td>12/16 (75%)</td>
<td>1/13 (8%)*</td>
</tr>
</tbody>
</table>

* In three trials a water system failure inadvertently removed the lionfish from the tank sometime during the 48-h starvation period.
Figure 7.1 Schematic showing the top view and side views of the 1.8-m diameter, 2000-L tank used in the predation trials (not drawn to scale). Top view: Predator, prey, and acrylic tubes depicted with diagonal lines representing the designated 30-cm zone around the tubes. Side view: Predator, prey, acrylic tubes, and tube removal lines depicted. The lines allowed the tubes to be lifted simultaneously, releasing the prey with minimal disturbance.
ABSTRACT
The rapid invasion of lionfish into the Western North Atlantic and Caribbean will undoubtedly affect native reef fishes via such mechanisms as trophic disruption and niche takeover. The dynamics of this invasion are poorly understood. We constructed a stage-based, matrix population model in which matrix elements were comprised of lower-level parameters. Parameters of lionfish vital rates were estimated from the literature, field studies, and new laboratory experiments. Sensitivity and elasticity analyses revealed that population growth rate is most sensitive to the lower-level parameter larval mortality and to the matrix element adult survival. Based on this model, approximately 85% of the adult lionfish population would have to be removed annually for population growth rate to approach unsustainable levels.
INTRODUCTION

Invasive lionfish, *Pterois miles* and *P. volitans*, are now established along the Southeast U.S. and parts of the Caribbean (Morris et al. 2009; Schofield et al. 2009). Lionfish represent the first marine reef fish invader to this region and are thought to have been released purposefully by aquarists and/or unintentionally by a hurricane (Courtney 1995). The rapidity of the lionfish invasion in the Western North Atlantic and Caribbean is unprecedented among marine fishes. Not only are invasive lionfish now widely distributed geographically, they are in some locations, one of the most abundant reef fishes (Green and Côté 2009). Furthermore, lionfish have the potential to significantly affect the trophic structure of native reef fish populations (Albins and Hixon 2008; Chapter 9).

Lionfish are top-level reef predators (Morris and Akins, in press) and will likely compete with native reef fishes of the snapper-grouper complex. This competition is potentially problematic as many of the fishes of this complex are being heavily exploited (Coleman et al. 1999). Lionfish could hamper stock rebuilding efforts for many of these species if control or management actions are not taken.

To better understand this invasion, we have constructed a stage-based, matrix population model of lionfish. Elements of the matrix were constructed using lower-level parameters comprised of life-history characteristics such as fecundity and instantaneous mortality rates. Our specific objectives were to assess the sensitivity of lionfish population growth rate ($\lambda$) to matrix elements and to lower-level parameters. We also
assess the scale of control efforts required to reduce $\lambda$ below a sustainable level for an invading population of lionfish.

**METHODS**

A stage-based, matrix model (Lefkovitch 1965; Crouse et al. 1987; Caswell 2001; Jiao et al. 2009) was constructed for female lionfish using three life stages: larvae (L), juveniles (J), and adults (A). Lionfish vital rates were assessed by stage rather than age as no age determination study has been conducted for *Pterois* sp. The matrix model accounts for survival, growth, and reproduction to describe monthly transitions among life stages:

\[
\begin{bmatrix}
L_{t+1} \\
J_{t+1} \\
A_{t+1}
\end{bmatrix} =
\begin{bmatrix}
0 & 0 & F_A \\
G_L & P_J & 0 \\
0 & G_J & P_A
\end{bmatrix}
\begin{bmatrix}
L_t \\
J_t \\
A_t
\end{bmatrix}
\]

where $G$ is the probability of surviving and growing to the next stage; $P$ is the probability of surviving and remaining in the same stage; and $F$ is fertility.

To calculate the matrix elements (Table 8.1), we obtained estimates of vital rates from literature, field studies, and laboratory experiments. The median larval mortality rate from McGurk (1987) for pelagic fish larvae at mean water temperature $\geq 10^\circ$C was used to estimate instantaneous larval mortality ($M_L$). The value of $10^\circ$C was chosen as the cutoff as it represents the chronic lethal minimum for lionfish (Kimball et al. 2004).
Larval duration (D_L) was determined through analysis of settlement marks and daily increments in the otoliths of juvenile lionfish collected in the Bahamas (D. Ahrenholz and J. Morris, unpub. data). Juvenile mortality (M_J) was derived from the weight-mortality function provided by Lorenzen (1996). To derive a typical juvenile mortality estimate, the mid-point of estimated weight values from a length-weight linear regression was determined from field collected specimens from 20 mm to 174 mm total length. The size at 50% maturity (174 mm total length) was used as the upper range of juvenile lionfish size (Chapter 6). The median egg mortality rate reported by McGurk (1987) for pelagic eggs of fishes with a mean water temperature \( \geq 10^\circ \text{C} \) was used as an estimate of instantaneous egg mortality (M_E). Egg duration (D_E) was assumed to be similar to that of most teleosts with pelagic eggs (J. Morris, pers. obs.). Batch fecundity estimate (24,630 eggs per batch) was derived by counting the number of eggs in newly released egg balls from lionfish in captivity (Chapter 6). Based on gonadal histology of specimens collected from the Atlantic (n = 1,674), lionfish spawn every month of the year, spawning frequency is every 3.85 d, and the proportion of female lionfish (\( \rho \)) is 46% (Chapter 6). Monthly fecundity (f) was derived by multiplying batch fecundity by the number of spawning events per month (7.9). Adult mortality (M_A) was also estimated using the Lorenzen (1996) weight-mortality function. The mid-point of extrapolated weight values from a length-weight linear regression of field collected specimens from 175 mm to 390 mm total length was used to obtain a typical adult mortality value. All model runs were performed using a one-month time-step based on the shortest stage duration (larval).
Population growth rate ($\lambda$) was represented by the dominant eigenvalue of the matrix model (Caswell 2001). Sensitivities of $\lambda$ to matrix elements ($a_{ij}$) were computed as partial derivatives (Caswell 2001):

$$\frac{\partial \lambda}{\partial a_{ij}} = \frac{v_i w_j}{(w,v)}$$

where $v$ is the left eigenvector associated with $\lambda$, $w$ is the right eigenvector associated with $\lambda$, and $(w,v)$ is the scalar product. These sensitivities were subsequently used to compute elasticities of matrix elements and sensitivities of lower-level parameters.

We used standard methods (Caswell 2001) to compute elasticities ($e_{ij}$) of $\lambda$ with respect to matrix elements:

$$e_{ij} = a_{ij} \frac{\partial \lambda}{\lambda} \frac{\partial a_{ij}}{\partial a_{ij}}.$$ 

The $e_{ij}$ represent proportional contributions to $\lambda$, and thus can be easier to interpret than sensitivities, particularly when matrix elements are measured in different units or operate on different scales (e.g., survival and fecundity).

Sensitivities of $\lambda$ to lower-level parameters ($x$) were also investigated using the equation

$$\frac{\partial \lambda}{\partial x} = \sum_{ij} \frac{\partial \lambda}{\partial a_{ij}} \frac{\partial a_{ij}}{\partial x}$$

as described by Caswell (2001).

To examine the possibility of controlling lionfish population growth in its new environment, adult survivorship ($P_A$) was systematically reduced and $\lambda$ recomputed. The relationship between $P_A$ and $\lambda$ was used to identify a threshold adult survivorship, below
which the population could not sustain itself ($\lambda<1$). The Baranov catch equation (Baranov 1918) was used to estimate additional mortality needed to achieve $\lambda<1$. This approach to eradication could represent, e.g., increased adult mortality imposed by fishing.

**RESULTS AND DISCUSSION**

Lionfish $\lambda$ was 1.12 with the matrix elements $P_A$ and $P_J$ exhibiting the highest values of elasticity (Table 8.2). Sensitivity of lower-level parameters indicated that $\lambda$ was most sensitive to $M_L$ (Table 8.1). Values of $P_A < 0.68$ were required before reaching $\lambda<1$ (Figure 8.1). Assuming that an additional source of mortality could be fishing or removal efforts, approximately 85% of a lionfish population would have to be harvested annually in order to reduce lionfish $\lambda$ below a sustainable level (Figure 8.2).

It is intuitive that the adult mortality matrix element ($P_A$) yields the highest value of elasticity. Given that lionfish reproduce monthly throughout the year, the time that they survive as adults substantially governs $\lambda$. This modeling exercise demonstrates the scale of removal efforts (i.e., fishing mortality or other method of removal) required to reduce lionfish population growth during the invasion when the population(s) are density independent. For large scale lionfish populations that are now resident in the Atlantic, removal of 85% of adult lionfish is likely not feasible. For small scale populations, however, with low connectivity to a larger population(s) (i.e., some Caribbean islands), lionfish control using fishing mortality and targeted removal might be a management
approach. Exploiting lionfish as food for humans, as in its native range, could provide a significant source of mortality needed to reduce lionfish populations, especially in protected areas.

Larval mortality was the most sensitive lower-level parameter. Unfortunately, natural mortality is not reported for any larval tropical scorpaenid. To parameterize the model, it is assumed that lionfish larval mortality is similar to that reported for many other pelagic marine fishes. This assumption may be an overestimate, however, given that predation is a major component of natural mortality and that scorpaenid larvae have large cranial spines, which likely evolved for predation defense. Similarly, juvenile and adult natural mortality estimates are likely conservative for lionfish, as these estimates were derived from the Lorenzen weight-mortality function (Lorenzen 1996). Lorenzen (1996) applied a mean approach to estimating mortality based on weight for many different fish species. The prevalence of predation, the principle component of natural mortality, on both juvenile and adult lionfish is an open question. Given that lionfish are novel prey to native predators of the Atlantic and Caribbean and are equipped with venomous spines for defense, predation on lionfish may be low compared with native reef fishes (Chapter 7). For these reasons, the estimated fishing effort required to curb lionfish $\lambda$ during the invasion should be viewed as optimistic.

Modeling lionfish demographics and vital rates does provide a unique perspective on the lionfish invasion. Collective consideration of lionfish life stages and the ecological influences on survival rates provides an assessment of how lionfish have
become so rapidly established. Future research should assess changes in lionfish abundance and recruitment rates, movement of lionfish among reef habitats, the natural morality at all life stages, and utility of management options.

The high sensitivity of $\lambda$ to the mortality lower-level parameters ($M_L$, $M_J$, and $M_A$) suggests that small adjustments in mortality can result in large changes in $\lambda$. Estimates of mortality rates specific to lionfish are needed to improve this modeling approach. The relatively low sensitivity of $\lambda$ to the other lower-level parameters ($\rho$, $D_L$, $M_E$, $F$, and $D_E$) suggests that error in these parameter estimates would have little effect on model results. Future research that incorporates stochasticity and the potential range of lower-level parameter estimates could provide further insight into the sensitivity of $\lambda$.

The lionfish invasion provides a stark example of the ongoing need for prevention, early detection, and rapid response to marine invaders. The results of this study highlight the importance of quick removal of marine non-native fish, as extraordinary resources are required to reduce or eradicate invasive fishes once they are established.

**ACKNOWLEDGEMENTS**

We thank the NOAA Aquatic Invasive Species Program, the NOAA National Centers for Coastal Ocean Science, and the NOAA Southeast Fisheries Science Center for their support of this research. We also thank Jeff Govoni and Erik Williams for their valuable insights during model development.
REFERENCES


Table 8.1 Values of lower-level parameters, their sources, and the sensitivity of $\lambda$ to each of them. The matrix element in which the lower-level parameter was used is provided in parentheses. Mortality is expressed as instantaneous rate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Reference</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval mortality $M_L$ ($G_L$)</td>
<td>0.350</td>
<td>month$^{-1}$</td>
<td>McGurk 1987</td>
<td>-3.17</td>
</tr>
<tr>
<td>Adult mortality $M_A$ ($P_A$)</td>
<td>0.052</td>
<td>month$^{-1}$</td>
<td>Lorenzen 1996</td>
<td>-0.57</td>
</tr>
<tr>
<td>Juvenile mortality $M_J$ ($P_J, G_J$)</td>
<td>0.165</td>
<td>month$^{-1}$</td>
<td>Lorenzen 1996</td>
<td>-0.34</td>
</tr>
<tr>
<td>Proportion female $\rho$ ($F_A$)</td>
<td>46%</td>
<td>---</td>
<td>Morris, unpub. data</td>
<td>0.23</td>
</tr>
<tr>
<td>Larval duration $D_L$ ($G_L$)</td>
<td>30</td>
<td>days</td>
<td>Morris, unpub. data</td>
<td>-0.04</td>
</tr>
<tr>
<td>Egg mortality $M_E$ ($F_A$)</td>
<td>0.310</td>
<td>day$^{-1}$</td>
<td>McGurk 1987</td>
<td>-7.50x10$^{-7}$</td>
</tr>
<tr>
<td>Fecundity $f$ ($F_A$)</td>
<td>194,577</td>
<td>month$^{-1}$</td>
<td>Morris, unpub. data</td>
<td>5.43x10$^{-7}$</td>
</tr>
<tr>
<td>Egg duration $D_E$ ($F_A$)</td>
<td>3</td>
<td>days</td>
<td>Morris, unpub. data</td>
<td>-7.75x10$^{-8}$</td>
</tr>
</tbody>
</table>
Table 8.2  Matrix element value, computation, and elasticity of $\lambda$.

<table>
<thead>
<tr>
<th>Element</th>
<th>Value</th>
<th>Computation</th>
<th>Elasticity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_A$</td>
<td>0.951</td>
<td>$e^{-MA}$</td>
<td>0.508</td>
</tr>
<tr>
<td>$P_J$</td>
<td>0.787</td>
<td>$11/12 \ e^{-MJ}$</td>
<td>0.210</td>
</tr>
<tr>
<td>$G_L$</td>
<td>0.00003</td>
<td>$e^{-MDL}$</td>
<td>0.094</td>
</tr>
<tr>
<td>$G_J$</td>
<td>0.072</td>
<td>$1/12 \ e^{-MJ}$</td>
<td>0.094</td>
</tr>
<tr>
<td>$F_A$</td>
<td>11,837</td>
<td>$\rho f e^{-MEDE}$</td>
<td>0.094</td>
</tr>
</tbody>
</table>
Figure 8.1  Population growth rate (λ, solid line) for theoretical values of P_A. The threshold is λ=1, below which population growth is negative.
Figure 8.2 Estimate of the proportion of adult lionfish that would need to be removed from an invading population in order to achieve $\lambda<1$. 


CHAPTER 9. CONCLUSIONS

Much has been learned from biological invasions since the mid-1800’s. Our modern understanding of ecology and evolution owes largely to natural experiments involving non-indigenous species. Some of the fundamental principles of ecology (e.g., evolution, speciation, dispersal) that limit species distributions and structure communities and ultimately ecosystems were developed from observations on biological invasions by Charles Darwin, Joseph Grinnel, Charles Elton, and others (Sax et al. 2007).

The lionfish introduction likewise represents “a natural experiment” capable of providing new information on fundamental ecological processes of the Southeast U.S., Caribbean, and Gulf of Mexico including dispersal, competition, and ultimately community structure. For example, the growing distribution of lionfish provides a model of dispersal as lionfish larvae are utilizing oceanographic currents and islands as stepping-stones throughout the Caribbean and ultimately into the Gulf of Mexico (Morris et al. 2009; Schofield et al. 2009). This information could benefit future management of native reef fisheries through improved understanding of dispersal and connectivity (Cowen et al. 2006). Critical chokepoints could also be investigated for preventing new introductions or controlling the spread of established invaders (see Hare and Whitfield 2003).
Marine fish invasions

While few marine fish introductions have occurred relative to freshwater systems, marine fish invasions are considered highly consequential and capable of displacing native species and altering biodiversity and community structure (e.g., Grozholz et al. 2000; Streftaris et al. 2005). Baltz (1991) provides a review of non-native marine fish introductions and reports that well over 100 species have been introduced worldwide. Many of these introductions occurred because of transplantations for fisheries enhancement, canal construction, or ballast water releases.

Since Baltz’s report, a number of studies have examined the economic and ecological consequences of past and recent introductions. A few examples of reef fish introductions relevant to this study of invasive lionfish are available from the south Pacific (Hawaii) and the Mediterranean. The peacock grouper (*Cephalopholis argus*), bluestripe snapper (*Lutjanus kasmira*), and blacktail snapper (*Lutjanus fulvus*) are now established in Hawaii following their intentional introduction in the 1950’s (Randall 1987). Two of these species are causing economic or ecological harm. Bluestripe snapper are considered a nuisance by commercial fishermen who attribute the reduction in catches of valuable goatfishes (*Parupeneus porphyreus* and *P. multifasciatus*) to the overabundance of bluestripe snapper (Randall 1987). Since Randall’s (1987) assessment, the peacock grouper has undergone significant population growth where it is now one of the most dominant near shore reef predators in the main Hawaiian Islands (Dierking 2007). Consumption by peacock grouper now exceeds 11% of the standing stock.
biomass of the reef fish community; thus, they are likely altering community structure (Dierking 2007). No negative impacts of the blacktail snapper have been reported, likely a result of their low abundance (Randall 1987).

The Mediterranean provides abundant examples of the profound impacts of non-natives. Over 60 Red Sea fish species have entered the eastern Mediterranean via the Suez Canal and are either established or undergoing rapid colonization (Golani 1993; Goren and Galil 2005). The extent of the changes in marine fish assemblages resulting from these invasions is wide-reaching and well documented (Goren and Galil 2005). The most alarming impact is the irreversible domination of community structure (50-90% of fish biomass) and function (alteration of the food web) (Goren and Galil 2005). Species specific examples include the non-native rabbitfishes (*Siganus rivulatus* and *S. luridus*), which have replaced the native herbivores and drastically changed the dynamics of energy flow through the food web (Galil 2007). The goatfish (*Upeneus moluccensis*) has replaced the native red mullet (*Mullus barbatus*) in commercial fisheries providing a classic example of niche takeover by a non-native species following a failed year class of a similarly trophic-positioned native species (i.e., occupation of a vacant niche) (Galil 2007).

In North and South America, anadromous salmonids have been introduced either intentionally or as a result of aquaculture releases. One highly problematic example is the Chinook salmon (*Oncorhynchus tshawytscha*), which was intentionally introduced into Chile from 1978-1989. This species is now spreading across a large part of South
America and poses an ecological concern during both the freshwater (increased nutrient release in headwater streams) and marine (trophic imbalance) components of its life history (Correa and Gross 2008).

These examples summarize the known impacts of finfish invaders that have impacted a diverse range of marine environments. The impacts of marine fish invaders are specific to the health of the local community and niche availability. The interactions of invasive species with known stressors, such as global climate change, have long been a concern. Perhaps the most poignant example of how small changes in water temperature can impact the invasiveness of a non-native is the lizardfish (*Saurida undosquamis*). After being introduced into the Mediterranean via the Suez canal, the lizardfish exhibited a rapid increase in abundance in 1955, which has been attributed largely to a 1-1.5°C rise in seawater temperature (Galil 2007). The high abundance of lizardfish in the eastern Mediterranean displaced the native hake (*Merluccius merluccius*) and became so abundant that it constituted more than one fifth of the total landings along the Mediterranean coast of Israel (Galil 2007). Given the forecasted increases in seawater temperature because of global climate change, much concern should be given to how small changes in water temperature could influence both the abundance and scale of impacts of invasive species. It is without question that understanding the invasiveness of invaders will require an integrated approach encompassing many aspects of biology, ecology, and their interactions with abiotic influences.
Invasiveness and lionfish

Past efforts have attempted to understand and even predict the likelihood for a species to become established outside of its native range. Using approaches such as ‘species profiling’ (Moyle and Light 1996; Ricciardi and Rasmussen 1998; Kolar and Lodge 2002) and quantitative analysis (Kolar and Lodge 2001; Marchetti et al. 2004a), risk assessments of invasiveness for many freshwater fish species have been conducted (Ricciardi and Rasmussen 1998; Kolar and Lodge 2002). Given the high number of freshwater fish introductions over the past century, quantitative approaches are becoming possible and are providing valuable insights into both abiotic and biotic characteristics that have enabled the establishment of past invaders.

There is, however, an inherent uncertainty in distinguishing between the abiotic and biotic characteristics that drive invasions (Kolar and Lodge 2001). Given the high variability of introductions among habitat types, the taxonomic diversity of invaders, and the lack of information on unsuccessful introductions (many of which are unreported), much can be gained through meta-analysis across many introductions and taxa of fishes. This approach increases the detectability of invasive characteristics and highlights the most invasive characteristics by virtue of their repeated occurrence (García-Berthou 2007).

Lionfish possess multiple life history and ecological traits that together (Marchetti et al. 2004a), have enabled their rapid establishment along the Southeastern U.S. and Caribbean. It is unclear whether any individual component of lionfish life history has
contributed more than others towards invasiveness. Interestingly, lionfish have many of the same life-history traits that are known to be main predictors of invasiveness for freshwater fish (Table 9.1).

**Lionfish ecological impacts**

The future expansion of lionfish into the coastal waters of the southern Caribbean, Gulf of Mexico, and eastern South America is probable and troublesome. Coral reef environments in the Caribbean basin are already stressed because of coral bleaching, fishing pressure, and disruptive algal growth. The addition of a non-indigenous predatory reef fish to the present coral reef stressors could cause irreversible changes in these systems. Probable scenarios include a reduction of forage fish biomass (Albins and Hixon 2008), possible increase in algal growth owing to herbivore removal by lionfish (Chapter 4), and direct competition with native top-level predators. Cascading trophic impacts on economically important species and niche takeover by lionfish are not outside of the realm of possibilities.

Lionfish are largely piscivorous, but also feed on crustaceans when available (Chapter 4). The daily biomass of prey consumed by lionfish was reported by Fishelson (1997) for ambient temperatures of the Red Sea (25 – 26°C). According to this report, a lionfish population of 80 individuals along a 1-km reef could consume over 50,000 prey year⁻¹. The influence of lionfish predation on community structure was also noted by
Fishelson (1997) who reported that lionfish are among the influential top-level reef predators known to impact prey community structure.

Lionfish densities in the Atlantic are much higher than reported for their native range (Whitfield et al 2007; Green and Côté 2009; Grubich et al. 2009). As a result, recent visual census surveys indicate that lionfish are capable of removing all of the forage fish biomass being produced in some reef systems at their present densities (S. Green, pers. comm.). Future monitoring of lionfish dietary habits could reveal prey switching to include more crustaceans in their diet as forage fish abundance declines. An increase in crustacean consumption by lionfish could directly impact some economically important species as crustaceans are a known staple in the diet of some juvenile and adult serranids (Eggleston et al. 1998).

Lionfish are a top-level predator and thus compete with other native top-level predators (i.e., the snapper-grouper complex) for resources. The snapper-grouper complex is heavily exploited by commercial and recreational fisheries resulting in niche vacancy in the reef fish community. The occupation of this vacated niche by lionfish could be problematic for stock rebuilding programs presently underway for the snapper-grouper complex of the Southeast U.S. and Caribbean. Alarmingly, there are classic examples of niche takeover by one fish species following the removal of another (Botsford et al. 1997). It is unclear if niche takeover by lionfish will impact stock recovery of threatened species such as Nassau grouper. Reduction of lionfish densities via control measures is needed to ensure adequate protection of native stocks.
The Management of Invasive Lionfish

The development of lionfish control strategies for the Southeast U.S., Gulf of Mexico, and Caribbean is an obvious next step. Lionfish management plans are presently needed for protected areas such as National Marine Sanctuaries and National and State Parks. The development of lionfish control strategies that employ existing snapper-grouper fisheries, many of which are being closed, could help offset economic impacts of these closures, while simultaneously helping with lionfish control measures. The creation of a direct fishery for lionfish is problematic, because the term “fishery” by definition invokes species protection under the Magnusson-Stevens Fishery Conservation and Management Act (16 USC 1801 et seq). An economic dependence on lionfish is not desirable, because the objective is to reduce lionfish populations to levels where their presence no longer impacts native fisheries (Chapter 8). When developing lionfish control strategies, which could include commercial harvest, it would be prudent to exempt lionfish from protection under the Magnusson-Stevens Fishery Conservation and Management Act, at the onset. This would remove the legislative mandate to develop a fishery management plan for lionfish, therefore superseding any attempt to protect a lionfish fishery.

The creation of lionfish control strategies for protected areas and easily accessible habitats is a realistic goal that should be implemented immediately. Lionfish are fit for human consumption and would bring value especially in markets that supply other reef fishes. Lionfish meat is mild and firm, which are two necessary qualities for edible fish.
Interestingly, scorpionfishes are considered a delicacy in French and Mediterranean
cuisine and are the basis for common dishes such as rascasse and bouillabaisse.
Development of markets, both locally and regionally, could create a demand for lionfish
capable of reducing local populations. This approach will likely be most successful in
locations such as the Florida Keys and the Caribbean where lionfish are found in shallow
and near shore waters.

Lionfish capture methods such as spear fishing and trapping are the most
promising harvest techniques given their relative low bycatch. Hook and line catches of
lionfish have been reported along the Southeast U.S. (K. Brennan, National Marine
Fisheries Service, pers. comm.) and Bermuda (C. Flook, Bermuda Aquarium Museum
and Zoo, unpub. data), however, these catches have been low in number relative to the
densities of lionfish reported in some of these locations. Bermuda fishermen are
reporting regular catches of lionfish, however, in lobster traps, suggesting that lionfish
could be harvested as a bycatch.

The creation of lionfish control measures will require population-level abundance
assessments. While some studies have documented the increase in lionfish densities at a
few sites off North Carolina (Whitfield et al. 2007) and in the Bahamas (Green and Côté
2009), there are presently no efforts underway to assess the size or growth rate of lionfish
population(s). Without population assessment, it will be difficult to ascertain when
lionfish reach carrying capacity, an inevitable event for most invasions.
The existing indices of abundance for reef fishes of the Southeast U.S. and Gulf of Mexico are not likely to provide adequate data for lionfish population assessment. Reef fishes are assessed in these regions largely using fisheries-dependent (headboat sampling, logbooks, etc.) and some fisheries independent sampling by the Marine Resources Monitoring, Assessment, and Prediction program (MARMAP). MARMAP surveys include the use of chevron fish traps and short longlines. Headboat sampling has resulted in only a few lionfish catches (n<40) in the Southeast U.S. since 2004 (K. Brennan, National Marine Fisheries Service, pers. comm.). The size of lionfish collected by hook and line suggests high sampling bias for the larger (male) lionfish (J. Morris, unpub. data). MARMAP chevron trapping surveys have not captured lionfish to date (M. Reichart, South Carolina Department of Natural Resources, pers. comm.), however, recent efforts to develop trapping methods for lionfish using live bait have been successful (J. Morris, unpub. data). The integration of lionfish sampling into existing MARMAP trapping efforts could result in a reliable index of abundance.

Considerations for import limitations

The unprecedented invasion of lionfish demonstrates the urgency for reviewing the present importation requirements for marine ornamental fishes. As of January 2009, the U.S. Congress is reviewing the Non-native Wildlife Invasion Prevention Act (H.R. 669), an Act that will require the Secretary of the Interior to develop a process for assessing the risk of all non-native wildlife species proposed for importation into the
United States. This Act would expand upon the existing mandates aimed at preventing non-native introductions (e.g., Invasive Species Executive order (No. 13112), the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (16 USC 4701-4751), and the National Invasive Species Act of 1996 (P.L. 104-332)) to include a screening process based on invasiveness and establishment likelihood. This new legislation is different in that it takes a “guilty until proven innocent” rather than an “innocent until proven guilty” approach. In the present state, a species must be declared “injurious” under the Lacey Act of 1998 (18 USC §42), a classification that can take years to achieve before importation and interstate commerce is banned (see Lodge et al. 2006). H.R. 669 works to prevent harmful introductions at the onset and could provide much needed protection for regions with high numbers of intentional releases of marine fishes such as south Florida (Semmens et al. 2004).

While it will be difficult, predicting the invasiveness of marine ornamental finfish is possible. For example, the life history characteristics exhibited by lionfish (Table 9.1) are commonly found among other non-natives being imported in high volume into coastal regions of the U.S. and could be used as a starting point for risk assessment. At a minimum, those species that are expected to rapidly outgrow the living space provided by aquaria should be considered a high risk for intentional release (Duggan et al. 2006).
Early Detection and Rapid Response

Prevention, early detection, and rapid response are the least expensive options for managing invasive species (Simberloff 2009). As observed with lionfish, invaders often exhibit a lag time before establishment, sometimes consisting of years to decades (Crooks 2005). It is during this lag time, that early detection and rapid response (ED/RR) programs can be most effective, removing the invader before it has reached critical mass and exponential growth rate (Drake and Lodge 2006).

ED/RR programs for coastal marine environments are perhaps more challenging than terrestrial and freshwater systems given accessibility and expansiveness of habitat challenges (Locke and Hanson 2009). In the case of marine ornamental introductions, however, ED/RR is a viable option because past introductions have been closely correlated with highly developed coastlines (Semmens et al. 2004). These locations often have intensive recreational, dive tourism, and fishing activities all of which are capable of providing early detection. Education and outreach to local coastal resource managers and the public is important in establishing rigorous early detection.

Protected areas, such as national and state parks, National Marine Sanctuaries, and the National Estuarine Research Reserves are robust resources for early detection and should be viewed as sentinel locations. These areas typically have ongoing volunteer-based monitoring programs, locally trained staff or volunteers capable of detecting non-native species, and legislative mandates ensuring protection of the resource, e.g. Marine Protected Areas Executive Order (No. 13158), Marine Protection, Research, and
Sanctuaries Act (16 U.S.C. 1431 et seq., 1447 et seq.; 33 U.S.C. 1401 et seq., 2801 et seq), National Marine Sanctuaries Act (Title III 16 USC 1431-14445c-1), Estuary (Estuarine) Protection Act (16 USC 1221-1226), Coral Reef Protection Executive Order (No. 13089), and the Coral Reef Conservation Act (16 USC 6401-6409). These mandates require managers of protected areas to work towards maintaining the biotic integrity of the resources they manage.

The threat of invasive species to these resources in the Southeast U.S. and Caribbean in the past has not been an immediate concern. The lionfish invasion, however, has provided a stark example of the looming threat of marine invasives. Protected area managers of National Parks and National Marine Sanctuaries, in the regions impacted by lionfish, are presently working to develop management plans capable of mitigating the impacts of lionfish. These plans will require the use of volunteer and park staff to monitor and detect early the arrival and impacts of invasive lionfish.

Perhaps the most promising example of early detection and rapid response is seen in South Florida, a region that has been inundated by marine ornamental introductions over the past decade (Semmens et al. 2004). In 2008, a team of researchers from the National Oceanic and Atmospheric Administration, the U.S. Geological Survey, and the Reef Environmental Education Foundation, organized a workshop to coordinate ED/RR among over 30 stakeholders and government agencies in South Florida. In just
one year’s time, this program has resulted in over 39 marine non-native fish sighting reports, six of which were confirmed and recovered within a few days of detection.

In summary, this dissertation provides a comprehensive assessment of the biology and ecology of the invasive Indo-Pacific lionfish that have invaded the waters along the Southeast U.S. and Caribbean. Lionfish undoubtedly possess many biological traits that have enabled them to become a model invader. The unprecedented nature of this invasion requires fisheries and coastal managers to reconsider existing legislation and approaches to managing marine invasive finfish. The support and timeliness of future work focused on detecting and mitigating the impacts of lionfish will ultimately determine the scale of lionfish impacts.
REFERENCES


Table 9.1 Summary of main predictors of invasiveness for established non-indigenous freshwater fish species that is relevant to lionfish. The presence (Y) or absence (N) of each predictor is noted for lionfish. Predictor summary was adapted from a comprehensive review by Garcia-Berthou (2007).

<table>
<thead>
<tr>
<th>Main predictor</th>
<th>Reference</th>
<th>Lionfish</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad diet</td>
<td>1,7</td>
<td>Y</td>
<td>Chapter 4</td>
</tr>
<tr>
<td>High physical tolerance</td>
<td>1,2,3,8</td>
<td>Y</td>
<td>Kimball et al. 2004</td>
</tr>
<tr>
<td>Prior invader</td>
<td>1,2,3,10</td>
<td>Y</td>
<td>Golani and Sonin 1992</td>
</tr>
<tr>
<td>Fast growth</td>
<td>1</td>
<td>Y</td>
<td>Morris, unpub. data</td>
</tr>
<tr>
<td>Large native range</td>
<td>2,3</td>
<td>Y</td>
<td>Schultz 1986</td>
</tr>
<tr>
<td>High adult trophic status</td>
<td>2</td>
<td>Y</td>
<td>Chapter 4</td>
</tr>
<tr>
<td>High propagule pressure</td>
<td>2,3,5,6</td>
<td>Y</td>
<td>Ruiz-Carus et al. 2006</td>
</tr>
<tr>
<td>Long life span</td>
<td>3</td>
<td>Y</td>
<td>Morris, unpub. data</td>
</tr>
<tr>
<td>High fecundity</td>
<td>6,8</td>
<td>Y</td>
<td>Chapter 6</td>
</tr>
<tr>
<td>Large egg diameter</td>
<td>6</td>
<td>Y</td>
<td>Chapter 5</td>
</tr>
<tr>
<td>Long reproductive season</td>
<td>4</td>
<td>Y</td>
<td>Chapter 6</td>
</tr>
<tr>
<td>Young age at maturity</td>
<td>8</td>
<td>Y</td>
<td>Chapter 6</td>
</tr>
<tr>
<td>Large body size</td>
<td>2,9,10,5</td>
<td>Y</td>
<td>Chapter 2</td>
</tr>
<tr>
<td>Short distance to native source</td>
<td>2,10</td>
<td>N</td>
<td>Schultz 1986</td>
</tr>
<tr>
<td>Parental care</td>
<td>2,3,6</td>
<td>N</td>
<td>Chapter 5</td>
</tr>
</tbody>
</table>