3-25-2014

Anthropogenic Disturbances in Estuarine Ecosystems: The Effects of Altered Freshwater Inflow, Introduction of Invasive Species, and Habitat Alteration in the Loxahatchee River, FL

Zachary R. Jud

Florida International University, zackjud@gmail.com

DOI: 10.25148/etd.FI14040881

Follow this and additional works at: <https://digitalcommons.fiu.edu/etd>

Part of the [Aquaculture and Fisheries Commons](#), [Biology Commons](#), [Ecology and Evolutionary Biology Commons](#), and the [Marine Biology Commons](#)

Recommended Citation

Jud, Zachary R., "Anthropogenic Disturbances in Estuarine Ecosystems: The Effects of Altered Freshwater Inflow, Introduction of Invasive Species, and Habitat Alteration in the Loxahatchee River, FL" (2014). *FIU Electronic Theses and Dissertations*. 1197.
<https://digitalcommons.fiu.edu/etd/1197>

This work is brought to you for free and open access by the University Graduate School at FIU Digital Commons. It has been accepted for inclusion in FIU Electronic Theses and Dissertations by an authorized administrator of FIU Digital Commons. For more information, please contact dcc@fiu.edu.

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

ANTHROPOGENIC DISTURBANCES IN ESTUARINE ECOSYSTEMS: THE
EFFECTS OF ALTERED FRESHWATER INFLOW, INTRODUCTION OF
INVASIVE SPECIES, AND HABITAT ALTERATION IN THE LOXAHATCHEE
RIVER, FL

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Zachary Robert Jud

2014

To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation, written by Zachary Robert Jud, and entitled Anthropogenic Disturbances in Estuarine Ecosystems: The Effects of Altered Freshwater Inflow, Introduction of Invasive Species, and Habitat Alteration in the Loxahatchee River, FL, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Aaron J. Adams

Joseph N. Boyer

Deron E. Burkepile

Maureen A. Donnelly

Craig A. Layman, Major Professor

Date of Defense: March 25, 2014

The dissertation of Zachary Robert Jud is approved.

Dean Kenneth G. Furton
College of Arts and Sciences

Dean Lakshmi N. Reddi
University Graduate School

Florida International University, 2014

ACKNOWLEDGMENTS

I would like to thank my family, friends, labmates, colleagues, collaborators, and volunteers for their invaluable support throughout the journey that culminated with the creation of this dissertation. I would particularly like to thank my major professor, Dr. Craig Layman, for giving me the opportunity to study in his lab, and providing me with the tools necessary to build a successful career. I would also like to thank my dissertation committee members, Drs. Maureen (Mo) Donnelly, Deron Burkepile, Joe Boyer, and Aaron Adams for their constant support and encouragement over the last few years. My research would not have been possible without the assistance of dedicated staff members and volunteers from the Loxahatchee River District's Wild Pine Laboratory and Loxahatchee River Center, as well as dozens of FIU undergraduate students. This research was partially supported by a Dissertation Year Fellowship from the University Graduate School at Florida International University. Additional support was provided by grants from the Loxahatchee River District, South Florida Water Management District, and Martin County (FL). Finally, I would like to acknowledge Inter-Research for allowing me to reprint a publication that originally appeared in the journal *Aquatic Biology* as Chapter II, Elsevier for allowing me to reprint a publication that originally appeared in the *Journal of Experimental Marine Biology and Ecology* as Chapter III, and Springer for allowing me to reprint a publication that originally appeared in the journal *Environmental Biology of Fishes* as Chapter IV.

ABSTRACT OF THE DISSERTATION

ANTHROPOGENIC DISTURBANCES IN ESTUARINE ECOSYSTEMS: THE
EFFECTS OF ALTERED FRESHWATER INFLOW, INTRODUCTION OF
INVASIVE SPECIES, AND HABITAT ALTERATION IN THE LOXAHATCHEE
RIVER, FL

by

Zachary Robert Jud

Florida International University, 2014

Miami, Florida

Professor Craig A. Layman, Major Professor

With the majority of Earth's population living in coastal areas, estuarine ecosystems have been particularly affected by anthropogenic disturbances. My dissertation research focused on three interrelated types of human disturbance that affect estuaries: Anthropogenic alteration of freshwater inflow, the introduction of invasive species, and habitat alteration. Using the Loxahatchee River (Jupiter, FL) as a model system, my goal was to understand how these disturbances affect estuarine organisms, particularly fishes. One of the most ecologically harmful disturbances affecting estuaries is anthropogenic alteration of freshwater inflow (and resulting changes in salinity patterns). To identify effects of freshwater inflow on the behavior of an ecologically and economically important fish (common snook *Centropomus undecimalis*), I conducted a 19-month acoustic telemetry study. Common snook were more abundant and made more frequent upstream migrations during the wet season, but freshwater inflow did not appear to be the proximate cause for these behaviors. Increased estuarine salinity resulting from

anthropogenic flow alteration may have facilitated the second type of disturbance that I address in this dissertation; the invasion of non-native Indo-Pacific lionfish into estuarine habitats. During the course of my dissertation research, I documented the first ever estuarine invasion by non-native lionfish. Using mark-recapture, I identified high site fidelity in lionfish, a trait that may aid future control efforts. The extremely low minimum salinity tolerance that I identified in lionfish appears to have allowed the species to colonize far upriver in estuaries with anthropogenically modified salinity patterns. Anthropogenic salinity alteration has also led to a severe degradation of oyster reef habitats in the Loxahatchee River. As a foundation species, oysters provide food, shelter, and nursery habitat for a wide variety of estuarine organisms, including many ecologically and economically important fishes. Increasingly, degraded oyster reef habitats have been the focus of restoration efforts. I identified a relatively rapid (< 2 years) convergence between restored and natural oyster reef communities, and documented the importance of vertical relief in restoration success. My dissertation research is critical for the management and conservation of coastal rivers in Florida, while more broadly informing restoration and management decisions in many other estuarine and coastal ecosystems.

TABLE OF CONTENTS

CHAPTER		PAGE
I	INTRODUCTION	1
	References	10
II	UPSTREAM MOVEMENTS OF A DIADROMOUS TOP PREDATOR, COMMON SNOOK <i>CENTROPOMUS UNDECIMALIS</i> , IN AN ANTHROPOGENICALLY ALTERED ESTUARY	13
	Abstract	14
	Introduction	16
	Methods	20
	Results	28
	Discussion	30
	Acknowledgments	40
	References	48
III	RECENT INVASION OF A FLORIDA ESTUARINE SYSTEM BY LIONFISH <i>PTEROIS VOLITANS</i> / <i>P. MILES</i>	53
	Abstract	54
	Introduction	55
	Methods	56
	Results	58
	Discussion	60
	Acknowledgments	64
	References	68
IV	SITE FIDELITY AND MOVEMENT PATTERNS OF INVASIVE LIONFISH <i>PTEROIS</i> SPP. IN A FLORIDA ESTUARY	72
	Abstract	73
	Introduction	75
	Methods	76
	Results	81
	Discussion	83
	Acknowledgments	88
	References	96
V	BROAD SALINITY TOLERANCE IN THE INVASIVE LIONFISH <i>PTEROIS</i> SPP. MAY FACILITATE ESTUARINE COLONIZATION	100
	Abstract	101
	Introduction	102
	Methods	103
	Results	109
	Discussion	113

	Acknowledgments.....	117
	References.....	122
VI	CHANGES IN MOTILE BENTHIC FAUNAL COMMUNITY STRUCTURE FOLLOWING LARGE-SCALE OYSTER REEF RESTORATION IN A SUBTROPICAL ESTUARY	125
	Abstract.....	126
	Introduction.....	127
	Methods.....	129
	Results.....	139
	Discussion.....	147
	Acknowledgments.....	153
	References.....	164
VII	CONCLUSIONS AND FUTURE DIRECTIONS.....	169
	References.....	175
VITA.....		177

LIST OF TABLES

TABLE	PAGE
 CHAPTER II	
2.1 Tagging date, tagging location, and total length (TL) for 86 tagged common snook detected within the Loxahatchee River acoustic array between May 25, 2008 and December 15, 2009	41
 CHAPTER IV	
4.1 Movement and growth data for 55 tagged lionfish. In the total days at large column, values in parentheses reflect the number of days that were used to calculate daily growth rate when a length measurement was not taken at the time of the final sighting. D refers to downstream and U refers to upstream.....	89
 CHAPTER V	
5.1 Lionfish standard length (SL) and mass on day 0, day 16, and day 28 of the study designed to assess effects of long-term exposure to low salinity (7 ‰). Fish in the high-salinity (35 ‰) control treatment are labeled H and fish in the low-salinity (7 ‰) treatment are labeled “L.” Asterisks indicate a fish in the high-salinity control treatment that died on day 20	118
 CHAPTER VI	
6.1 Relative gravimetric abundance of motile benthic organisms collected in sampling trays at natural (upstream, midstream, downstream) and restored oyster reefs in the Loxahatchee River (Florida, USA). Taxa are arranged by total overall gravimetric abundance (natural and restored sites combined). Restored Reef column includes all organisms collected during bimonthly sampling following reef construction, as well as the high/low-relief time series. Asterisks indicate taxa that were identified only at the restored oyster reef. NP = not present	155
6.2 Relative numerical abundance of motile benthic organisms collected in sampling trays at natural (upstream, midstream, downstream) and restored oyster reefs in the Loxahatchee River (Florida, USA). Taxa are arranged by total overall numerical abundance (natural and restored sites combined). Restored Reef column includes all organisms collected during bimonthly sampling following reef construction, as well as the high/low-relief time series. Asterisks indicate taxa that were identified only at the restored oyster reef. NP = not present	157

6.3	Spatial variation in mean biomass of motile benthic oyster reef-associated fauna at three natural reef sites along an upstream-to-downstream gradient (mean $\pm$ standard deviation). Overall mean biomass includes all sampling dates. Annual maximum biomass is the mean of each year's maximum biomass value, which typically occurred at the end of the dry season or the beginning of the wet season. Annual minimum biomass is the mean of each year's minimum biomass value, which usually occurred near the beginning of the dry season. Capital letters in parenthesis represent the results of Tukey HSD post-hoc testing, where different letters indicate significantly different overall mean biomass values at $p < 0.05$	159
-----	--	-----

LIST OF FIGURES

FIGURE		PAGE
 CHAPTER II		
2.1	Map of the Loxahatchee River, near Jupiter, Florida, showing the acoustic telemetry array used to track common snook movements. The location of each acoustic receiver is indicated by a black dot. Our delineation of the riverine/estuarine interface is represented by a black line, and Jupiter Inlet (the location of a large snook spawning aggregation) is outlined by a black polygon. The acoustic receiver at Island Way Bridge was used to determine when tagged common snook had entered the riverine section of the estuary. Two low head dams – Lainhart Dam (the location of South Florida Water Management District’s flow gauge) and Masten Dam – are indicated by stars	42
2.2	Common snook abundance in the Loxahatchee River (black bars) and daily mean freshwater inflow measured at Lainhart Dam (red line) during the study period	43
2.3	Starting time for all round-trip upstream runs that began at (and returned to) Jupiter Inlet, and reached (1) the upper embayment of the NW Fork, (2) the upper reaches of the SW Fork, or (3) the upper reaches of the N Fork	44
2.4	Relative number of common snook in the Loxahatchee River starting upstream runs per day (# of fish starting runs / # of tagged fish in the river on that day: black bars) vs. daily mean freshwater inflow measured at Lainhart Dam (red line)	45
2.5	Freshwater inflow within the wet season (integrated over 2-day time periods) vs. the relative number of snook per day starting upstream runs. The relationship was not significant ($R^2 < 0.001$, $F_{1, 304} = 0.004$, $p = 0.95$)	46
2.6	Duration of upstream runs (from Jupiter Inlet to Island Way Bridge at the start of the riverine section of the NW Fork) vs. duration of downstream runs (Island Way Bridge to Jupiter Inlet)	47
 CHAPTER III		
3.1	Map of the lower Loxahatchee River, near Jupiter, Florida. Crosshatching indicates the section of the north shoreline that was intensively surveyed for lionfish (<i>Pterois volitans</i> / <i>P. miles</i>) every 11-12 weeks. Gray shading indicates the section of the south shoreline that was opportunistically sampled as part of an ongoing mark-recapture study. Width of the survey belt was ~30 m	

	(exaggerated slightly in figure for clarity). Lionfish were found throughout the survey belt. The upstream limit of lionfish capture (dot at A) was ~5.5 km from the ocean, while the downstream limit (dot at B) was ~0.1 km from the ocean. Stars indicate the location of salinity/temperature datasondes.....	65
3.2	<i>Pterois volitans</i> / <i>P. miles</i> utilizing anthropogenically created habitat in the Loxahatchee River (corrugated seawall under a dock).....	66
3.3	<i>Pterois volitans</i> / <i>P. miles</i> . Size distribution of 145 lionfish captured during four primary sampling events along the north shoreline of the Loxahatchee River (Jupiter, FL) in August 2010 ($n = 54$), October 2010 ($n = 24$), January 2011 ($n = 18$), and April 2011 ($n = 49$). An additional 66 lionfish were opportunistically captured along the south shoreline between August 2010 and April 2011, but are not included here because of the temporal variability of this sampling	67

CHAPTER IV

4.1	Map of the Loxahatchee estuary, Jupiter, Florida (center of black box in inset map). The lionfish mark-recapture study was conducted along the south shoreline of the estuary, in the area indicated by hatching. Additional surveys were conducted along the north shoreline (dark gray shading) as part of a concurrent study (Jud et al. 2011). Both survey areas extended ~30 m from shore (not to scale).....	91
4.2	Tagged lionfish (<i>Pterois</i> spp.), showing the oval Floy fingerling tag (FTF-69, Floy Tag & Mfg.) and two color-coded glass beads, sutured into the dorsal musculature between the spinous and soft dorsal fins. A three digit number is printed on the reverse of the Floy tag.....	92
4.3	Distance moved between captures for 73 discrete recapture events. Mean time at liberty (number of days between discrete recaptures) is presented above each distance category.....	93
4.4	Regression of lionfish standard length (SL; mm) versus daily growth rate (mm/day) based on 35 physical recaptures. Linear regression fit is shown. Standard lengths were estimated at the midpoint of each at-large period (initial SL + final SL * 0.5)	94
4.5	Direction of movement versus lionfish standard length (at time of recapture) based on 73 unique recapture events. Standard length was directly measured for physical recaptures, and estimated for visual sightings using a calculated daily growth rate of 0.46 mm/day.....	95

CHAPTER V

- 5.1 Effects of fluctuating salinity on the survival of caged lionfish (*Pterois* spp.) at (a) downstream (2.4 km from ocean), (b) midstream (6.2 km from ocean), and (c) upstream (7.0 km from ocean) sites in the Loxahatchee River estuary. Salinity varied over time as a product of freshwater inflow (long term) and tidal incursion of marine water (twice daily). Estimated time of lionfish death is indicated in the bar across the top of each panel. Note that scale on the x-axis differs among panels. The period of heavy precipitation is indicated by a thick black bar at the top of each panel. Asterisks in (a) represent data gaps due to equipment malfunction119
- 5.2 Kaplan-Meier survival curve for lionfish exposed to salinities of 4 ‰. No mortality was observed at salinities ≥ 5 ‰, the approximate survival salinity minimum (SS_{min}) for lionfish. After 3 hours at 4 ‰, two individuals experienced a complete loss of equilibrium (our predetermined endpoint for SS_{min} determination), combined with a lack of response to tactile stimulation, and a reduction in the frequency of opercular movements. These two individuals were included in the Kaplan-Meier curve, since their condition was an immediate precursor to death. All fish were dead after 94 hours121

CHAPTER VI

- 6.1 Map of the Loxahatchee River estuary (Jupiter, Florida, USA), showing the location of the upstream (Up), midstream (Mid), and downstream (Down) natural reef reference sites, as well as the oyster restoration reef (Rest)160
- 6.2 Non-metric multidimensional scaling (NMDS) ordination showing relative similarity/dissimilarity between natural (Up = upstream reference site, Mid = midstream reference site, and Down = downstream reference site) and restored (Pre = pre restoration, Rest = post restoration) motile oyster reef communities. Each data point represents a single sampling date at a single site (mean of four trays). The relative proximity of two points to one another on the NMDS ordination reflects the relative similarity of the communities represented by those points (i.e., closer points indicate more similar communities). Natural reference reef data were collected between May 2007 and May 2012. Pre-restoration data were collected in March and May 2010, and post-restoration data were collected from September 2010 to May 2012161
- 6.3 Changes in biomass of motile oyster reef-associated organisms following oyster reef restoration. Dashed black lines represent biomass at the restoration reef site, before reef construction (first two data points, March and May 2010), and after reef construction (all points after July 2010). Biomass at three natural reef reference sites is represented by black (upstream site) dark gray (midstream

site) and light gray (downstream site) solid lines. Asterisk = date of restoration reef construction. Error bars have been omitted for clarity162

6.4	Biomass of oyster reef-associated organisms at high- and low-relief experimental plots during the first 16 months following restoration. Throughout the study, mean biomass at high-relief plots was significantly greater than at low-relief plots ($F_{1, 26} = 68.1, p < 0.001$), and there was a significant effect of time since construction on biomass values for both levels of vertical relief ($F_{1, 26} = 24.7, p < 0.001$). Additionally, we observed a significant interaction between effects of relief and time since construction on the biomass of benthic organisms ($F_{6, 26} = 3.20, p = 0.017$). Error bars = standard deviation163
-----	--

CHAPTER I

INTRODUCTION

As the world's population continues to grow, interactions between humans and the environment have begun to play an increasingly important role in the overall health and function of natural systems. With more than half of Earth's population living in coastal areas (Ray 2006), estuarine and coastal ecosystems have been particularly affected by human activities (Lotze et al. 2006). The highly productive habitats associated with coastal systems (e.g., mangrove forests, oyster reefs, salt marshes, seagrass beds, coral reefs) provide some of the most valued ecosystem services at a global scale (Costanza et al. 1997; Granek et al. 2010), which include creating habitat for numerous commercially, recreationally, and ecologically important species, stabilizing shorelines, reducing erosion, and improving water quality, among many others (Beck et al. 2001; Adams et al. 2006; Coen et al. 2007; Courrat et al. 2009; Jud et al. 2011a). Despite their ecological and economic importance, estuaries may be one of the most human-impacted types of ecosystems globally – impacts (e.g., shoreline development, pollution, dam construction, dredging) that have led to precipitous declines in estuarine taxa. The overwhelming cause of these declines has been habitat alteration/destruction and direct over-exploitation of organisms (Lotze et al. 2006; Halpern et al. 2008).

One of the most common anthropogenic habitat modifications in coastal rivers and estuaries is alteration of freshwater inflow patterns (Drinkwater and Frank 1994). The construction of dams for the purpose of hydroelectric power generation, flood control, and water storage for human consumption and agricultural irrigation can greatly affect downstream flows. In coastal rivers, dams can alter the timing, quantity, and quality of freshwater entering estuaries (Chamberlain and Doering 1998; Alber 2002; Bunn and Arthington 2002; Barnes 2005). Although large volumes of water are stored

on the upstream side of dams and flood control structures to facilitate these uses, water is often rapidly discharged during periods of heavy precipitation to prevent upstream flooding. This water management strategy can result in unnaturally high freshwater inflow into estuaries during the wet season, followed by insufficient flows during the dry season (Barnes 2005; Sime 2005). Instead of a gradual increase in flows at the start of the wet season, and a gradual decrease at the end of the wet season, wet season flows on flow-regulated coastal rivers often fluctuate rapidly in response to water management needs (Barnes 2005). Altered seasonal hydrologic patterns brought about by anthropogenic flow modification (Alber 2002) can lead to considerable changes in biotic and abiotic conditions (e.g., primary production, salinity) in estuaries. These changes likely have significant and extremely complex effects on estuarine organisms that have evolved in the presence of unmanipulated water flow patterns.

In many coastal rivers, the reduction of freshwater inflow entering headwater areas through dams and flood control structures, coupled with increased marine water intrusion brought about by dredging and inlet construction, has resulted in a spatial shift in salinity zonation. In flow-controlled estuaries, the upstream intrusion of high-salinity ocean water can lead to considerable changes in community structure (e.g., the replacement of riparian cypress forests with a dense monoculture of red mangroves). In some cases, salinity shifts may alter the forage base available in a particular section of river, potentially leading to behavioral or spatial changes in predatory species. Additionally, in many estuaries, there is now a mismatch between optimal salinity and optimal habitat for certain organisms. For motile organisms (e.g., fishes), altered freshwater inflow patterns may result migrations within estuaries in order to locate areas

of physiologically optimal salinity. In some cases, estuarine salinity changes have resulted in a complete shift in the spatial distribution of sessile organisms (e.g., oysters).

In addition to affecting native estuarine organisms, anthropogenic salinity alteration may facilitate the establishment of non-native invasive organisms. Flow and salinity alteration in estuaries may make available previously inhospitable habitats for colonization by non-native species. While invasive species have long been a threat to terrestrial and freshwater ecosystems, they are increasingly affecting estuaries as well (Ruiz et al. 1997; Cohen and Carlton 1998). Although many invasive species have been documented in estuaries, non-native organisms have rarely been directly implicated in declines of native fauna in coastal systems (Lotze et al. 2006). Yet because most documented coastal marine invasions are by taxa at relatively low trophic positions, e.g., primary producers, planktivores, detritivores, or deposit feeders (Byrnes et al. 2007), an understanding of the impacts of invasive predators in estuarine systems is only starting to emerge.

Humans are drastically altering estuarine ecosystems in another general way; proactive attempts to restore or recreate particular aspects of ecosystem structure and function that have been lost through previous disturbance. As habitat alteration continues seemingly unabated, restoration projects are becoming an increasingly important tool to combat anthropogenic disturbances. Habitat restorations may be carried out as a specific response to remediate an acute event (e.g., clearcut logging, a ship grounding on a coral reef), or more broadly, to increase the amount of available habitat in systems where chronic disturbance has reduced the overall amount of natural habitat. While restoration efforts frequently have narrow goals (e.g., enhancing a single-species fishery, stabilizing

a section of shoreline), the end result can be the creation of a habitat that closely resembles a natural habitat in terms of structure and function. In estuaries, oyster reefs are an important focus of restoration activities (Coen and Luckenbach 2000; Coen et al. 2007; Taylor and Bushek 2008; Schulte et al. 2009).

The Loxahatchee River in Jupiter, Florida (26°57' N, 80°06' W), provides an excellent opportunity to study human-mediated influences in a flow-managed, subtropical, coastal river and estuary. The river, which flows into the Atlantic Ocean at Jupiter Inlet, drains a 434 km² watershed and receives flow from three major branches and a number of smaller tributaries (VanArman et al. 2005). Much of the freshwater entering the Loxahatchee River is anthropogenically controlled, originating at flood control structures in the river's headwaters (Ridler et al. 2006). The lower Loxahatchee River estuary is comprised of several large embayments, and marks the southern terminus of the Indian River Lagoon, one of the most biodiverse ecosystems in North America (Dybas 2002). A human-made section of the Atlantic Intracoastal Waterway also connects to the estuary. The upper, riverine, section of the Loxahatchee River is one of two federally designated National Wild and Scenic Rivers in Florida (SFWMD 2006). While the upper reaches of the river are largely composed of natural habitats (e.g., cypress-dominated flood plain forests, mangrove-lined shorelines, oyster reefs), lower sections of the river have been highly modified by human activities, including construction of seawalls, docks, and channels.

My Ph.D. research focused on three interrelated types of human disturbance that affect estuarine structure and function – anthropogenic alteration of freshwater inflow, the introduction of invasive species, and habitat alteration – using the Loxahatchee River

as a model flow-controlled system. My goal was to understand how these disturbances affect estuarine organisms, particularly fishes. Although the individual chapters address the effects of several different types of disturbance on different study organisms, each chapter is either directly or indirectly related to the effects of anthropogenic alteration of freshwater inflow on estuarine fishes. This research is critical for the management and conservation of the Loxahatchee River, while more generally informing restoration and management decisions in estuarine and coastal ecosystems. These studies have broad implications, and are relevant to many other estuarine systems in North America, and around the globe

Chapter II, **Upstream movements of a diadromous top predator, common snook *Centropomus undecimalis*, in an anthropogenically altered estuary**, examines the role that freshwater inflow plays in the movement patterns and abundance of common snook *Centropomus undecimalis*, an ecologically and economically important, estuarine-dependent, fish that can move freely between freshwater and saltwater. I utilized acoustic telemetry technology to track movement patterns of individual common snook in response to long-term changes in freshwater inflow (i.e., between the wet season and the dry season), as well as short-term changes in freshwater inflow within the wet season (i.e., flow increase caused by a single precipitation event). Common snook represent a top estuarine predator, and are capable of linking marine ecosystems with freshwater ecosystems through their upstream migrations, so it is important to understand how alteration of natural flow patterns affects their utilization of estuarine habitats.

Increased estuarine salinity resulting from anthropogenic flow alteration and dredging may have facilitated the second type of disturbance that this dissertation

addresses; the invasion of a non-native marine fish species (Indo-Pacific lionfish) into estuarine habitats. My work on the Loxahatchee River was the first to identify lionfish (typically a marine species in their native and invaded ranges) invading an estuary. Chapters III, IV, and V focus on various aspects of the lionfish invasion, the first documented example of a marine fish from the Pacific becoming established in the Atlantic, and perhaps one of the top 15 emerging environmental issues at a global scale (Sutherland et al. 2010). While the initial release of non-native lionfish from the pet trade represents a direct human-mediated disturbance, my findings suggest that the estuarine aspect of the invasion may have been facilitated by anthropogenic alteration of freshwater inflow patterns, and shoreline habitat modification. Because lionfish have no natural predators in the Atlantic, and because native prey species do not recognize them as a threat, this human-mediated invasion has the potential to cause widespread environmental damage, particularly in ecologically important systems like estuaries.

In Chapter III, **Recent invasion of a Florida estuarine system by lionfish *Pterois volitans* / *P. miles***, I documented the first ever estuarine intrusion by invasive lionfish in the Western Hemisphere. Chapter III describes the spatial distribution of lionfish within the Loxahatchee River estuary, and identifies an association with anthropogenically created habitats (e.g., docks, sea walls, submerged debris), suggesting that human-driven changes in habitat availability may facilitate estuarine invasion. Additionally, I describe lionfish diet in the invaded estuary to assess potential interactions with native prey species. This study has been published in *Aquatic Biology* (Jud et al. 2011b).

In Chapter IV, **Site fidelity and movement patterns of invasive lionfish *Pterois* spp. in a Florida estuary**, I conducted a 10-month mark-recapture study in the lower Loxahatchee River estuary to identify movement patterns, site fidelity, and growth rates in invasive lionfish. Understanding movement patterns and site fidelity has important implications for lionfish management and control. This study has been published in the *Journal of Experimental Marine Biology and Ecology* (Jud and Layman 2012).

Chapter V, **Broad salinity tolerance in the invasive lionfish *Pterois* spp. may facilitate estuarine colonization**, examines the role that anthropogenically altered estuarine salinities may have played in the establishment of lionfish in estuarine settings. Using a combination of laboratory and field experiments, I documented minimum salinity tolerance in lionfish. The findings presented in this chapter suggest that increases in estuarine salinity, resulting from reduced freshwater inflow caused by water management policies and increased saltwater intrusion as a result of dredging, may have allowed lionfish to colonize estuarine habitats far from the ocean. This study has been accepted for publication in *Environmental Biology of Fishes* (Jud et al. 2014).

Anthropogenic reductions in freshwater inflow and increased saltwater intrusion have also led to a severe degradation of oyster reef habitats in the Loxahatchee River. As a foundation species, oysters provide food, shelter, and nursery habitat for a wide variety of estuarine organisms, including a number of ecologically and economically important fishes. The main focus of Chapter VI, **Changes in motile benthic faunal community structure following large-scale oyster reef restoration in a subtropical estuary**, is to examine how fish and invertebrate communities that occupy oyster reefs respond to restoration efforts intended to remediate the effects of anthropogenic disturbance. Since

the scale and duration of this project was greater than many other oyster restoration studies, my findings may provide new insight into the design and implementation of future restoration efforts to help facilitate a more rapid convergence between restored and natural reef communities.

References

- Adams AJ, Dahlgren CP, Kellison GT, Kendall MS, Layman CA, Ley JA, Nagelkerken I, Serafy JE (2006) Nursery function of tropical back-reef systems. *Marine Ecology Progress Series* 318:287-301
- Alber M (2002) A conceptual model of estuarine freshwater inflow management. *Estuaries and Coasts* 25:1246-1261
- Barnes T (2005) Caloosahatchee estuary conceptual ecological model. *Wetlands* 25:884-897
- Beck MW, Heck KL, Jr, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* 51:633-641
- Bunn SE, Arthington AH (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management* 30:492-507
- Byrnes JE, Reynolds PL, Stachowicz JJ (2007) Invasions and Extinctions Reshape Coastal Marine Food Webs. *Plos One* 2:1-7.
doi:e29510.1371/journal.pone.0000295
- Chamberlain RH, Doering PH (1998) Preliminary estimate of optimum freshwater inflow to the Caloosahatchee estuary: A resource-based approach. *Proceedings of the Charlotte Harbor Public Conference and Technical Symposium Charlotte Harbor National Estuary Program* 274 p
- Coen LD, Brumbaugh RD, Bushek D, Grizzle R, Luckenbach MW, Posey MH, Powers SP, Tolley SG (2007) Ecosystem services related to oyster restoration. *Marine Ecology-Progress Series* 341:303-307
- Coen LD, Luckenbach MW (2000) Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? *Ecological Engineering* 15:323-343
- Cohen AN, Carlton JT (1998) Accelerating invasion rate in a highly invaded estuary. *Science* 279:555-558
- Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. *Nature* 387:253-260
- Courrat A, Lobry J, Nicolas D, Laffargue P, Amara R, Lepage M, Girardin M, Le Pape O (2009) Anthropogenic disturbance on nursery function of estuarine areas for

- marine species. *Estuarine Coastal and Shelf Science* 81:179-190.
doi:10.1016/j.ecss.2008.10.017
- Drinkwater KF, Frank KT (1994) Effects of river regulation and diversion on marine fish and invertebrates. *Aquatic Conservation: Marine and Freshwater Ecosystems* 4:135-151
- Dybas CL (2002) Florida's Indian River Lagoon: an estuary in transition. *BioScience* 52:554-559
- Granek EF, Polasky S, Kappel CV, Reed DJ, Stoms DM, Koch EW, Kennedy CJ, Cramer LA, Hacker SD, Barbier EB, Aswani S, Ruckelshaus M, Perillo GME, Silliman BR, Muthiga N, Bael D, Wolanski E (2010) Ecosystem services as a common language for coastal ecosystem-based management. *Conservation Biology* 24:207-216. doi:10.1111/j.1523-1739.2009.01355.x
- Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. *Science* 319:948-952.
doi:10.1126/science.1149345
- Jud ZJ, Layman CA, Shenker JM (2011a) Diet of age-0 tarpon (*Megalops atlanticus*) in anthropogenically-modified and natural nursery habitats along the Indian River Lagoon, Florida. *Environmental Biology of Fishes* 90:223-233
- Jud ZR, Layman CA (2012) Site fidelity and movement patterns of invasive lionfish, *Pterois* spp., in a Florida estuary. *Journal of Experimental Marine Biology and Ecology* 414-415:69-74
- Jud ZR, Layman CA, Lee JA, Arrington DA (2011b) Recent invasion of a Florida (USA) estuarine system by lionfish *Pterois volitans* / *P. miles*. *Aquatic Biology* 13:21-26.
doi:10.3354/ab00351
- Jud ZR, Layman CA, Nichols PK (2014) Broad salinity tolerance in the invasive lionfish *Pterois* spp. may facilitate estuarine colonization. doi:10.1007/s10641-014-0242-y
- Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312:1806-1809.
doi:10.1126/science.1128035
- Ray GC (2006) The coastal realm's environmental debt. *Aquatic Conservation: Marine and Freshwater Ecosystems* 16:1-4

- Ridler MS, Dent RC, Arrington DA (2006) Effects of two hurricanes on *Syringodium filiforme*, manatee grass, within the Loxahatchee River Estuary, southeast Florida. *Estuaries and Coasts* 29:1019-1025
- Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: Mechanisms, extent, and consequences. *American Zoologist* 37:621-632
- Schulte DM, Burke RP, Lipcius RN (2009) Unprecedented restoration of a native oyster metapopulation. *Science* 325:1124-1128. doi:10.1126/science.1176516
- SFWMD (2006) Restoration plan for the Northwest Fork of the Loxahatchee River South Florida Water Management District, Watershed Management Department, Coastal Ecosystems Division, Technical Report, 465 pp.,
- Sime P (2005) St. Lucie Estuary and Indian River Lagoon conceptual ecological model. *Wetlands* 25:898-907
- Sutherland WJ, Clout M, Côté IM, Daszak P, Depledge MH, Fellman L, Fleishman E, Garthwaite R, Gibbons DW, De Lurio J, Impey AJ, Lickorish F, Lindenmayer D, Madgwick J, Margerison C, Maynard T, Peck LS, Pretty J, Prior S, Redford KH, Scharlemann JPW, Spalding M, Watkinson AR (2010) A horizon scan of global conservation issues for 2010. *Trends in Ecology & Evolution* 25:1-7
- Taylor J, Bushek D (2008) Intertidal oyster reefs can persist and function in a temperate North American Atlantic estuary. *Marine Ecology Progress Series* 361:301-306. doi:10.3354/meps07429
- VanArman J, Graves GA, Fike D (2005) Loxahatchee watershed conceptual ecological model. *Wetlands* 25:926-942

CHAPTER II

UPSTREAM MOVEMENTS OF A DIADROMOUS TOP PREDATOR, COMMON SNOOK *CENTROPOMUS UNDECIMALIS*, IN AN ANTHROPOGENICALLY ALTERED ESTUARY

Abstract

Anthropogenic alteration of freshwater inflow into coastal rivers and estuaries may affect the behavior of estuarine organisms that evolved under unmanipulated flow patterns. In coastal rivers throughout the Caribbean and the tropical and subtropical Western Atlantic, common snook *Centropomus undecimalis* represents an amphidromous top predator fish that is capable of moving freely between marine and riverine habitats. Because of the economic and ecological importance of common snook, and the widespread alteration of freshwater inflow in coastal systems, it is critical to understand how freshwater inflow in flow-controlled estuaries affects snook behavior. Using acoustic telemetry, we tracked movements of 86 tagged common snook (538-1100 mm total length) in the Loxahatchee River (Jupiter, FL, USA) for 19 months. Our goal was to identify relationships between inflow and common snook movements and behavior at long-term (i.e., flow fluctuation between wet season and dry season) and short-term (i.e., flow fluctuation within wet season) temporal scales. Common snook abundance was more than twice as high during the wet season (late spring through fall) than the dry season. Additionally, common snook made more frequent upstream runs (from Jupiter Inlet to upstream areas in the river) during the wet season than during the dry season. Within the wet season, short-term fluctuations in freshwater inflow could not be used to predict timing of upstream runs. While freshwater inflow does not appear to be the proximate trigger for seasonal fluctuations in snook abundance or upstream habitat use, it may be the ultimate cause on an evolutionary timescale. If common snook behaviors evolved in response to natural seasonal flow patterns (e.g., spawning during higher flows,

which naturally occur during the wet season), anthropogenic alteration of freshwater inflow into estuarine systems may have a significant affect on snook populations.

Introduction

Estuaries and coastal rivers represent critically important ecosystem types, both from an ecological and an economic perspective. Globally, the per-hectare economic value of the ecosystem services provided by estuaries is among the greatest of any ecosystem type (Costanza et al. 1997; Granek et al. 2010). Despite their value, estuaries and coastal ecosystems have been greatly affected by anthropogenic disturbances (Halpern et al. 2008). With habitat alteration/destruction ranked as one of the main causes of population declines among marine and estuarine fauna (Lotze et al. 2006), continued anthropogenic habitat modification in estuaries may come at a significant ecological and economic cost.

One of the most common anthropogenic modifications to coastal systems is alteration of freshwater inflow patterns (Drinkwater and Frank 1994). The construction of dams for the purpose of hydroelectric power generation, flood control, and water storage for human consumption and agricultural irrigation can greatly affect downstream flows. In coastal rivers, dams can alter the timing, quantity, and quality of freshwater entering estuaries (Chamberlain and Doering 1998; Alber 2002; Barnes 2005). Although large volumes of water are stored on the upstream side of dams and flood control structures to facilitate these uses, water is often rapidly discharged during periods of heavy precipitation to prevent upstream flooding. This water management strategy can result in unnaturally high freshwater inflow into estuaries during the wet season, followed by insufficient flows during the dry season (Barnes 2005; Sime 2005). Instead of a gradual increase in flows at the start of the wet season, and a gradual decrease at the end

of the wet season, wet season flows on flow-regulated coastal rivers often fluctuate rapidly in response to the water management needs of humans (Barnes 2005).

The alteration of seasonal hydrologic patterns brought about by anthropogenic modification of freshwater inflow can have negative effects on estuarine-dependent organisms that have evolved in the presence of unmanipulated water flow patterns (Drinkwater and Frank 1994; Alber 2002). For motile organisms such as fishes, which can relocate in response to variable environmental conditions, changes in freshwater inflow patterns can bring about significant behavioral changes (Childs et al. 2008; Sakabe and Lyle 2010). To manage freshwater inflow for the benefit of estuarine and coastal fishes, it is critical to understand how long-term (e.g., between dry season and wet season) and short-term (e.g, during a single precipitation event) changes in inflow may affect fish behavior (Loneragan and Bunn 1999; Alber 2002; Gillson 2011). While riverine inflow has been shown to affect the immigration and emigration of anadromous and catadromous fishes (Alabaster 1970; Drinkwater and Frank 1994; Smith et al. 1994; Milner et al. 2012), the effects of inflow on amphidromous species (fishes that migrate between freshwater and saltwater for non-reproductive purposes) are not as clear. These effects may be particularly important in systems where amphidromous fish species are large-bodied, highly motile, top predators, since these species impact many other components of estuarine food webs (Baum and Worm 2009; Rosenblatt and Heithaus 2011; Andrews and Harvey 2013; Blewett et al. 2013).

Common snook *Centropomus undecimalis* is an ecologically and economically important, estuarine-dependent, amphidromous fish species found in the Caribbean and the tropical and subtropical Western Atlantic (McMichael et al. 1989; Taylor et al. 1998;

Aliaume et al. 2000; Taylor et al. 2000; Andrade et al. 2013; Perera-Garcia et al. 2013). Commercial harvest of common snook occurs throughout the species' range, with the exception of Florida, where the species supports an extensive recreational fishery (Taylor et al. 1998). In Florida's highly modified coastal rivers, the euryhaline common snook is a top predator that is capable of freely moving between freshwater and marine habitats. Spawning occurs primarily in the summer, near inlets, river mouths, passes, and sandy beaches (Peters et al. 1998; Taylor et al. 1998). After a 2.5 week larval phase, juvenile common snook settle into a wide variety of oligohaline, mesohaline, and polyhaline habitats, including mangrove shorelines, salt marshes, and sheltered estuarine basins (McMichael et al. 1989; Peters et al. 1998; Adams et al. 2006; Stevens et al. 2007). Common snook are protandric hermaphrodites, with male-to-female sex change frequently occurring as size increases (Peters et al. 1998; Taylor et al. 2000). For this reason, size-related differences in behavior among adult common snook may be related to gender.

Historically, it was believed that adult common snook primarily used upstream (i.e., riverine) sections of estuaries as thermal refuge during cold weather (Blewett et al. 2009). Recent studies have shown that common snook are found in upstream and downstream sections of estuaries throughout the year (Blewett et al. 2009), and that some individuals appear to migrate up and down coastal rivers at various times of the year (Trotter et al. 2012), suggesting that thermal refuge is not the primary driver of upstream habitat use. However, previous studies have not identified how upstream areas are linked to spawning aggregations further downriver, how individuals or the overall population utilize upstream sections of estuaries, or how environmental drivers such as freshwater

inflow may affect snook movement between downstream spawning habitats and upstream riverine habitats. While anthropogenic alteration of freshwater inflow has been shown to affect the diet of juvenile snook (Adams et al. 2009b), the potential role that flow modification plays on adult snook movement has not been elucidated. Numerous anecdotal observations by recreational anglers suggest that snook move upriver in response to increased freshwater inflow (unpublished data), although this hypothesis has not been tested. Since changes in freshwater inflow may alter snook behavior in ways that ultimately affect energetics, reproduction, trophodynamics, or overall fitness (which in turn may impact Florida's extremely important recreational snook fishery), the relationship between freshwater inflow and snook movements are relevant from a management perspective.

Using the Loxahatchee River (near Jupiter, FL) as a model flow-managed estuarine system, we conducted an acoustic telemetry study to track the movements of individual common snook across two spawning seasons. We were interested in identifying intrapopulation variability in the timing, frequency, and duration of migrations between lower and upper portions of the estuary, with a particular focus on correlating upstream movements with freshwater inflow. Our objectives were to (1) identify whether changes in freshwater inflow at the seasonal scale are related to upstream movement in common snook, (2) determine how short-term fluctuations in freshwater inflow within the wet season affect snook upstream movement, and (3) identify whether patterns of movement in estuaries are related to body size.

Methods

Study system

The Loxahatchee River (26°57' N, 80°06' W) is a coastal river located near Jupiter, Florida, USA (Fig. 2.1) (VanArman et al. 2005). The main stem of the river, the Northwest (NW) Fork, is 27 km long, and flows into the Atlantic Ocean through Jupiter Inlet. For the present study, we refer to the inlet as the section of river starting at the ocean and running 1 km upriver (Fig. 2.1). This narrow (100-150 m across) and relatively deep (up to 8 m) section of the estuary experiences high current velocities during ebb and flood tides. Jupiter Inlet is an important spawning site for common snook on the east coast of Florida (Taylor et al. 1998; Lowerre-Barbieri et al. 2003). During the summer months, a spawning aggregation of >500 adult snook is consistently present in Jupiter Inlet (personal observations).

The lower Loxahatchee River estuary is composed of two main embayments. The lower embayment, which begins ~1 km above the inlet, runs from river kilometer (rkm – kilometers upriver from the ocean) 2-5.2, and is 1,100 m across at the widest point. The upper embayment extends from rkm 5.2-6.5, and is 750 m wide at the widest point. The two embayments average 1.5-2 m in depth, and are separated by a shallow sand spit that extends perpendicular to shore across a narrow section of the river. These two embayments, plus the inlet area, represent the estuarine section of the system. Upstream of rkm 6.5, the estuary rapidly narrows to <100 m in width, and begins to take on riverine characteristics (subsequently referred to as the riverine section). Two smaller branches, the North (N) Fork and the Southwest (SW) Fork, flow into the lower embayment, between rkm 3 and 4. Additionally, the southern terminus of the Indian River Lagoon

connects to the Loxahatchee River just west of the inlet (extending in a northward direction), and the human-made Atlantic Intracoastal Waterway joins the estuary ~2 km upriver from the ocean (extending in a southward direction). Throughout this paper, we refer to the entire system (including estuarine and riverine sections) as the Loxahatchee River.

Land use in the river's 434 km² watershed is divided between urban/residential development and protected natural areas (VanArman et al. 2005). While the upper, riverine, section of the system contains relatively healthy natural habitats (e.g., cypress forests, mangrove-lined shorelines, oyster reefs), the lower, estuarine section has been highly modified by human activities, including construction of seawalls, docks, and channels (SFWMD 2006; Layman et al. *in press*). Historically, sheet flow emanating from freshwater wetlands during the wet season would slowly flow into the headwaters of the Loxahatchee River (VanArman et al. 2005). However, freshwater inflow into the NW and SW Forks has been highly modified over the past century through the construction of canals, dams, and flood control structures. Today, a network of human-made canals drains residential neighborhoods in the former headwater area, hydrologically isolating the Loxahatchee River from its historical source wetlands (SFWMD 2006). Water from these smaller canals feeds into the C-18 canal, a linear reservoir that feeds both the NW Fork and the SW Fork. Flow of freshwater from the C-18 canal into the upper NW Fork is controlled by the G-92 flood control structure, which is operated by the South Florida Water Management District. The S-46 flood control structure, located at the east end of the C-18 canal, controls the flow of water into the SW Fork; however, the S-46 structure is only opened when the C-18 canal is in danger of

flooding, essentially cutting off all freshwater inflow into the SW Fork during periods of normal precipitation (SFWMD 2006). Two low-head dams are located along the NW Fork, 22 and 23 km upriver from the ocean.

Acoustic monitoring of snook movements

We used acoustic telemetry to track movements of common snook in the Loxahatchee River. Acoustic telemetry has become an increasingly popular tool to identify movement patterns in estuarine fishes (Childs et al. 2008; Hammerschlag-Peyer and Layman 2010; Sakabe and Lyle 2010; Reyier et al. 2011; Walsh et al. 2013). The use of ultrasonic acoustic transmitters (“tags”), in conjunction with arrays of automated, submerged hydrophones (“acoustic receivers”), greatly increases the capacity to continuously monitor activity patterns of marine and estuarine fishes (Almeida 1996; Childs et al. 2008). The acoustic transmitters are typically surgically implanted into the peritoneal cavity of fishes. Each transmitter emits a coded sequence of ultrasonic pings (unique to each fish) at regular intervals, which are then recorded and stored (along with time and date) each time a tagged fish swims within range of an acoustic receiver. In Florida, acoustic telemetry has been used to track the movements of common snook (Adams 2000; Lowerre-Barbieri et al. 2003; Adams et al. 2009a; Adams et al. 2011; Trotter et al. 2012); however, most of these studies focused on snook movements in the lower portions of estuaries, and none specifically examined effects of freshwater inflow patterns on snook behavior.

Between February 12, 2008 and August 31, 2009, 242 common snook were acoustically tagged in estuarine and nearshore waters of east central Florida, from Fort

Pierce to the north, to Juno Beach to the south (a distance of 68 km), as part of several unrelated research projects. A total of 57 common snook were tagged in the Loxahatchee River system (including Jupiter Inlet). However, many of the other common snook that were tagged in the region spent time in the Loxahatchee River, and were included in these analyses. In addition to the Loxahatchee River, snook were tagged in Ft. Pierce Inlet (n = 24), St. Lucie River and Inlet (n = 90), and in nearshore coastal waters (n = 72). Fish were captured using hook-and-line or center-bag seines (183 x 2.5 m, 3.8 cm stretched mesh; 100 x 3 m, 5.1 cm stretched mesh). Captured fish were measured (total length), and briefly (<10 min) held in livewells or coolers containing aerated ambient water while equipment was prepared for surgical implantation of acoustic tags.

To surgically implant acoustic tags, a fish was placed, ventral side up, into a tagging sling. The sling was then positioned at an angle in a cooler filled with aerated ambient water such that the snook's head and gills remained submerged, but the abdomen extended above the water's surface. Since snook exhibit tonic immobility when inverted, chemical anesthesia was not used (Lowerre-Barbieri et al. 2003; Humston et al. 2005). A 2 cm incision was made ~1 cm lateral to the ventral midline, immediately behind the pelvic girdle, and the tag was inserted intraperitoneally (Lowerre-Barbieri et al. 2003). Fish were tagged using Vemco V9 (9 x 24 mm), V13 (13 x 36 mm), and V16 (16 x 64 mm) acoustic transmitters, set to ping on average once every 180 seconds. Incisions were closed with three interrupted sutures, using 3-0 Vicryl braided absorbable suture material. After closure, wounds were sealed with cyanoacrylate glue. All surgical tools and tags were soaked in povidone iodine solution prior to surgery to reduce infection risk. Following surgery, fish were held in the water at the side of the boat until strong caudal

fin movements were observed (generally <5 minutes), at which point they were released. The use of tonic immobility during surgery greatly reduced recovery time compared to surgeries performed under anesthesia (personal observations).

To detect movements of acoustically tagged common snook in the Loxahatchee River, we deployed an array of 44 Vemco omnidirectional underwater acoustic receivers (model VR2 and VR2W) on 5/25/08 (Fig. 2.1). While the underwater receiver array was designed to facilitate multiple ongoing experiments, we placed receivers at several key areas specifically to record movement of snook between the inlet and the riverine portions of the estuary. A series of “acoustic gates” (multiple closely spaced receivers, staggered spatially to identify direction of travel) were deployed at several narrow areas in the system, to allow us to detect passing snook (Heupel et al. 2006). Acoustic gates were installed at Jupiter Inlet, the mouths of the Indian River Lagoon, the Atlantic Intracoastal Waterway, the SW Fork, and the N Fork. Additional gates were placed at the boundary between the upper and lower embayments (rkm 5.2), and the estuarine/riverine transition zone (rkm 6.5). Range testing indicated that most fish passing through each acoustic gate would be detected by at least one receiver, even on days when environmental noise (e.g., wind, rain, wave action) resulted in reduced detection range (personal observations). Receivers were also deployed in the upper reaches of the NW Fork, the SW Fork, and the N Fork to detect snook that utilized these upriver areas. Finally, a receiver was installed at Island Way Bridge (rkm 7.5), 1 km upstream of the estuarine/riverine transition, in a narrow (60 m wide) section of river (Fig. 2.1). For the purpose of this study, snook detected at or above this receiver were considered to have entered the upper, riverine, portion of the system. Depending on substrate type, a number

of different techniques were used to mount acoustic receivers (including inserted into a 10 cm diameter PVC pipe sleeve that was hammered into the river bottom, attached to pilings using cable ties, attached to cinder block bases). Data were downloaded from the receivers every 4 months for the duration of the study. The study was ended on December 15, 2009, in response to a period of record rainfall, followed by an extended period of record cold temperatures, which resulted in extensive snook mortality statewide (Adams et al. 2012; Blewett and Stevens 2013).

Data analysis

Acoustic telemetry data were analyzed using Microsoft Excel and Vemco User Environment (VUE) software. After removing potential false detections for each tagged common snook (i.e., a detection for a given fish that was not followed by a subsequent detection anywhere in the array within 24 hours, or nearly simultaneous detections on two receivers that are separated by > 2 km), we quantified the total number of unique fish that were detected in the river for each day of the study. We then calculated detection period (total number of days from first detection to final detection), as well as detection days (total number of days detected within the acoustic array) for each tagged fish that entered the Loxahatchee River. We compared both of these variables (separately) to fish total length using Pearson's r . Finally, we quantified the number of resident snook (i.e., fish that spent the winter in the Loxahatchee River) versus transient snook (i.e., fish that appeared in the Loxahatchee River between spring and fall, but did not overwinter in the system). A two-sample t-test was used to compare TL between resident and transient individuals.

Daily mean freshwater inflow values at Lainhart Dam in the upper NW Fork were obtained from the South Florida Water Management District's DBHYDRO database. Flow at Lainhart Dam represents a significant portion of the water entering the upper NW Fork (SFWMD 2006). We identified the start of each wet season as the first rapid increase in freshwater inflow in the spring, and the end of each wet season as the final decrease in freshwater inflow in the fall. To compare the mean number of common snook present per day in the Loxahatchee River between the wet season and the dry season, we used a two-sample t-test. Additionally, we compared the mean number of detection days per tagged snook between the wet season and the dry season using a two-sample t-test.

Since fish behavior may have been affected by the capture and surgery process, we did not include acoustic detections that occurred within 72 hours of tagging when analyzing movement patterns. For each fish, we identified all round-trip upstream runs that began at (and returned to) Jupiter Inlet, and reached (1) the upper embayment of the NW Fork, (2) the upper reaches of the SW Fork, or (3) the upper reaches of the N Fork. Since we were primarily interested in movements between Jupiter Inlet and upstream sections of the estuary, we did not include shorter movements that were restricted to the lower estuary, even if they were in an upstream direction. Mean TL was compared between fish that made at least one round-trip upstream run and fish that made no runs using a two-sample t-test. For each upstream run that entered the riverine section of the NW Fork (Island Way Bridge or further upstream), we calculated total run duration, duration of the upstream phase of the run (Jupiter Inlet to Island Way Bridge), and duration of the downstream phase of the run (Island Way Bridge to Jupiter Inlet). The

mean duration of the upstream phase of all runs (between Jupiter Inlet and Island Way Bridge) was compared to the mean duration of the downstream phase of all runs using a two-sample t-test.

In order to identify potential relationships between freshwater inflow and upstream (riverine) habitat use by common snook in the Loxahatchee River, we compared the relative number of snook that began upstream runs on each day of the study ($\#$ of fish starting runs / $\#$ of tagged fish in the river on that day) to mean freshwater inflow levels on that day. We used relative values to account for temporal variation in overall snook abundance in the river (i.e., to cancel out the effect of seasonal variation in snook abundance on the number of fish making upstream runs). The relative number of snook starting upstream runs per day was compared between wet and dry seasons using a Mann-Whitney U test. We used linear regression to determine whether short-term fluctuations in freshwater inflow within the wet season affected the relative number of common snook per day that started upstream runs. For the independent variable in this analysis, we calculated a 2-day integrated daily flow value for each wet season day by averaging that day's flow with the previous day's flow (since there was likely a lag between the time flow levels changed at the Lainhart Dam flow gauge at rkm 23 and the time that flow levels changed at Jupiter Inlet). All average values are presented as a mean $\pm$ standard deviation. Statistical analyses were carried out in SPSS v.16.

Results

Between May 25, 2008 and December 15, 2009 (570 days), 86 unique acoustically tagged common snook were detected by the Loxahatchee River acoustic array (Table 2.1). Nearly 1.2 million detection events were recorded during this time period. Mean total length (TL) of the detected fish was 824 ± 151 mm (range: 538-1100 mm). Of the 57 common snook that were tagged in the Loxahatchee River, two individuals disappeared from the array in <72 hours following tagging, and were not included in subsequent analyses. The additional 31 common snook that were detected by the acoustic array during the study period were initially tagged outside of the Loxahatchee River (Table 2.1). Tagged snook were present within the acoustic array on every day of the study, with an overall mean of 13 ± 8.3 tagged snook present per day (range: 1-42 per day). For all fish that were detected in the acoustic array, the average detection period was 222 ± 203 days (range: 4-553 days). Most individuals either had a detection period of less than 90 days ($n = 44$) or more than 330 days ($n = 36$). There was no correlation between common snook TL and detection period ($r = -0.20, p > 0.05$). The mean number of detection days each tagged fish spent within the acoustic array was 86 ± 105 days (range: 4-515 days). There was a negative correlation between common snook TL and detection days ($r = -0.383, p < 0.001$). We identified 70 snook as transients (i.e., fish that appeared in the Loxahatchee River between spring and fall, but did not overwinter in the system) and 16 as residents (i.e., fish that spent the winter in the Loxahatchee River). Mean TL of resident snook (660 ± 111 mm) was significantly shorter than mean TL of transient snook (860 ± 134 mm; $t_{83} = -5.4, p < 0.001$).

Mean daily freshwater inflow across the entire study period (measured at Lainhart Dam) was $2.2 \pm 1.3 \text{ m}^3/\text{s}$. According to flow data, the 2008 wet season began on June 14 and ended on November 14, and the 2009 wet season began on May 21 and ended on October 19 (Fig. 2.2). Although the wet season started one month later in 2008 than in 2009, both wet seasons lasted ~5 months. The entire study included 306 days classified as wet season and 264 days classified as dry season. Mean daily wet season inflow was $3.3 \pm 1.0 \text{ m}^3/\text{s}$ in 2008 and $3.1 \pm 1.0 \text{ m}^3/\text{s}$ in 2009. Mean daily dry season inflow was $1.2 \pm 0.4 \text{ m}^3/\text{s}$. Mean daily common snook abundance in the acoustic array was more than twice as high during the wet season ($17.5 \pm 8.8 \text{ fish/day}$) as during the dry season ($7.7 \pm 2.1 \text{ fish/day}$; $t_{344} = 18.5, p < 0.001$; Fig. 2.2). Additionally, the mean number of detection days (per tagged fish) was more than 2.5 times greater during the wet season ($62.2 \pm 61.8 \text{ detection days per fish}$) than during the dry season ($23.7 \pm 54.0 \text{ detection days per fish}$; $t_{167} = -4.3, p < 0.001$). Snook abundance remained high throughout the 2009 wet season, but decreased approximately half way through the 2008 wet season (Fig. 2.2).

During the course of the study, we identified 390 round-trip upstream runs (beginning at Jupiter Inlet) by common snook, 284 of which entered the riverine section of the NW Fork. Of the tagged fish that were detected during the study, 54 made at least one round-trip upstream run, and 32 did not make a round-trip run. The mean TL of fish that made at least one round-trip upstream run ($868 \pm 124 \text{ mm}$) was significantly larger than the mean TL of fish that made no runs ($749 \pm 164 \text{ mm}$; $t_{50} = -3.5, p < 0.001$). On average, each fish that was detected during the study made 4.5 ± 6.0 upstream runs (range: 0-26 runs, including fish that made no runs). When we exclude fish that did not

make upstream runs, the mean number of runs per fish was 7.2 ± 6.3 . The majority of upstream runs began in the late night or early morning hours, with few runs commencing during daylight hours (Fig. 2.3). Average time between successive upstream runs was 7.0 ± 8.0 days. The relative number of snook starting runs per day was significantly higher during the wet season than during the dry season (Fig. 2.4: $U = 23028$, $p < 0.001$), with 94% of all upstream runs occurring during the wet season. Within the wet season, the relative number of snook starting upstream runs per day was not affected by freshwater inflow level (Fig. 2.5: $R^2 < 0.001$, $F_{1, 304} = 0.004$, $p = 0.95$).

For fish that made round-trip runs between the Jupiter Inlet and the riverine section of the NW Fork, the mean duration of the upstream runs from the inlet to Island Way Bridge (13.5 ± 16.8 hours, range: 3.1 hours - 6.9 days) was significantly shorter than the mean duration of the runs back downriver to the inlet (35.1 ± 56.5 hours, range: 3.3 hours - 32 days) (Fig. 2.6: $t_{326} = -6.1$, $p < 0.001$). The mean duration of round-trip upstream runs (inlet – riverine section – inlet) was 74.5 ± 102.0 hours (range: 11.9 hours - 55 days).

Discussion

The abundance of tagged common snook in the Loxahatchee River varied seasonally, with the greatest number of fish present during the wet season, which ran from late spring through fall. This time frame corresponds to the spawning season for common snook in Florida, (McMichael et al. 1989; Taylor et al. 1998), where most spawning occurs between May and September (Peters et al. 1998). Although our study did not document common snook spawning, the increased snook abundance we detected

during the wet season coincided with the presence of a large snook spawning aggregation in Jupiter Inlet, suggesting that increased abundance was related to spawning.

For common snook living in subtropical Florida, Taylor et al. (1998) felt that day length and water temperature controlled the timing of spawning, as peak gonadosomatic indices were measured during periods of greatest day length and highest water temperature. However, Taylor et al. (1998) also acknowledged that temporal patterns in spawning may vary slightly among years or locations, and attributed these variations to fluctuations in the physical environment. Since the center of distribution for common snook is in the tropics, where day length and water temperature do not vary considerably throughout the year, the ultimate factor affecting the timing of spawning may be some other seasonal cue, such as precipitation or freshwater inflow level (Andrade et al. 2013). In the Caribbean and Central America, near the center of the common snook's geographical distribution, spawning occurs during the rainy season (Taylor et al. 1998; Aliaume et al. 2000; Perera-Garcia et al. 2011; Andrade et al. 2013), suggesting that the behavioral patterns we detected may not be unique to Florida's temperate/subtropical locale. Although Florida represents the northern extent of the common snook's distribution (range constrained by low winter water temperatures: Shafland and Foote 1983; Howells et al. 1990; Taylor et al. 1998; Adams et al. 2012), the seasonal patterns in snook abundance that we observed may be linked to an evolutionary history in the tropics.

Life history traits for estuarine and riverine species evolved in response to natural flow regimes, before anthropogenic alteration of these system occurred (Bunn and Arthington 2002). For many species of fish that reproduce in estuaries, this includes

spawning during seasonal periods of high freshwater inflow (Drinkwater and Frank 1994). Through much of the common snook's range, the wet season occurs during the warmest time of year, where higher water temperatures, increased precipitation, and greater freshwater runoff, combine to fuel primary and secondary production in estuarine areas (Drinkwater and Frank 1994; Loneragan and Bunn 1999; Gillson 2011), leading to an increase in prey availability and growth rates for newly recruited juvenile snook (Aliaume et al. 2000; Andrade et al. 2013). Spawning during the wet season also allows juvenile common snook to recruit to shallow creeks and flooded riparian areas, habitats that would be inaccessible during dryer periods (Aliaume et al. 2000; Adams et al. 2009b). Additionally, runoff and river flow may play an important role in the dispersal of eggs and larvae, affecting connectivity between systems or self recruitment mechanisms.

If common snook did evolve to reproduce during higher flows that naturally occur during the wet season, anthropogenic alteration of freshwater inflow into estuarine systems may affect spawning or recruitment success. In particular, human-controlled changes that affect the timing of the wet season or flow levels within the wet season could result in a temporal mismatch between snook spawning time and the ideal flow for spawning success (Drinkwater and Frank 1994). Additionally, anthropogenic flow alteration may affect estuarine current patterns that are responsible for transporting snook larvae to appropriate nursery habitats (Drinkwater and Frank 1994). Identifying the role of freshwater inflow on snook spawning success has important management implications, both from the perspective of managing snook stocks, as well as managing freshwater inflow.

By comparing common snook abundance between the 2008 and 2009 wet seasons, which had dramatically different patterns of freshwater inflow, we are able to speculate about the potential relationship between inflow and spawning seasonality, since snook abundance in the Loxahatchee River during the summer months is likely correlated to spawning. Although the wet season began one month earlier in 2009 than in 2008, common snook abundance peaked at roughly the same time during both years. This interannual stability in the timing of peak snook abundance, despite variability in flow, suggests that freshwater inflow is not the proximate cause for seasonal fluctuations in snook abundance (although it may be the ultimate cause on an evolutionary timescale). However, within the spawning season, it appears that fluctuating freshwater inflow may have an affect on snook abundance. In 2008, 3 weeks after the initial onset of the wet season, flows suddenly (over the course of one day) decreased from near season-high levels down to dry-season levels, where they remained for two weeks. This pattern is highly indicative of human management of freshwater inflow, as opposed to a natural decrease in inflow due to reduced precipitation, which would have occurred more slowly. The sudden and dramatic reduction in freshwater inflow that occurred during the summer of 2008 may have led to the decrease in snook abundance we observed during the second half of the wet season (i.e., the sudden flow reduction during the first half of the wet season may have resulted in a truncated spawning season in 2008). In contrast, flows remained relatively high throughout the 2009 spawning season, and we observed a protracted period of high snook abundance compared to 2008. The reduced snook abundance we observed during summer 2008 may have affected spawning output compared to 2009.

In addition to affecting common snook abundance in the Loxahatchee River, variation in freshwater inflow appears to have an influence on within-estuary movements for some portion of the population. While more individual tagged common snook were present per day during the wet season than during the dry season, we also observed a greater percentage of tagged individuals making upstream runs during the wet season. Estuarine and riverine fishes have been shown to move in response to a variety of physical and biological factors (Almeida 1996; Irlandi and Crawford 1997; Hohnsbová et al. 2003; Jaureguizar et al. 2004; Childs et al. 2008); however, the exact stimuli that trigger migrations in many estuarine species are not well understood. Since a number of physical (e.g., salinity, temperature, dissolved oxygen, flow rate, habitat availability) and biological (e.g., primary production, prey availability) factors are directly or indirectly correlated with freshwater inflow, it is possible that anthropogenic alteration of flow may structure the movements of fishes in estuarine systems. Although many estuarine-dependent diadromous fish species move downstream in response to increased freshwater inflow and decreased salinity (Childs et al. 2008; Heupel and Simpfendorfer 2008; Sakabe and Lyle 2010; Walsh et al. 2013), common snook employ the opposite strategy, moving upriver more often during the time of year when flows are greatest.

It appears that the round-trip upstream runs that we observed (most often during the wet/spawning season) may be an ingrained behavior in common snook, rather than an acute response to changing environmental stimuli. Within the wet season, short-term fluctuations in freshwater inflow did not affect the relative number of fish making upstream movements, suggesting that freshwater inflow was not the proximate trigger for these runs. Upstream runs occurred throughout both wet/spawning seasons, regardless of

flow level or snook abundance (e.g., runs occurred during an unusually low-flow period in the 2008 wet season, and runs continued to occur during the second half of the 2008 wet season, despite decreased snook abundance). Although the ultimate cause of these upstream runs is unknown, movements to the riverine section of the estuary may serve to maximize snook fitness by reducing competition or predation, increasing prey availability or prey capture ability, or some combination of these. Alternatively, for snook that are located in high-current areas like an inlet, the energetic cost of an upstream run may be no greater than the cost associated with holding position in the current.

This work expands our knowledge on the ecology of common snook, building upon previous studies that examined riverine habitat use by the species. There exists a long-standing paradigm among biologists, anglers, and managers that adult common snook primarily utilize upstream sections of rivers as thermal refuge during cold weather, and occupy high-salinity areas near the ocean-estuary interface during the summer spawning season. Adult snook were known to periodically use riverine sections of estuaries, but these areas were not historically considered important habitats (Blewett et al. 2009). Recent studies have shown that common snook are present in upstream sections of estuaries throughout the year (Blewett et al. 2009; Trotter et al. 2012), suggesting that the importance of these habitats extends beyond thermal refuge.

Blewett et al. (2009) and Trotter et al. (2012) assumed that snook present in riverine areas during the spawning season were primarily river residents, fish that either periodically migrated to the lower estuary to spawn, or spent their entire life in riverine areas without ever moving downriver. Our findings, however, show that common snook abundance in upstream riverine areas during the spawning season is at least partially

driven by continuous immigration and emigration of individuals to and from spawning areas in the lower estuary. These observations support previous findings that showed individual common snook regularly appearing and disappearing from a spawning aggregation, even though overall aggregation size remained relatively consistent throughout the spawning season (Lowerre-Barbieri et al. 2003). Although Lowerre-Barbieri et al. (2003) did not identify where individuals went when they left the spawning aggregation (one tagged snook was recaptured by an angler at an upstream site 15 km from the spawning area, 2 days after it had disappeared from the spawning aggregation), we now know that many of the fish that disappear and reappear at spawning sites are making upstream runs. The link between riverine areas and spawning areas is important from a management perspective, since population estimates based on spawning aggregation size will fail to account for individuals that are utilizing habitats away from inlets. Additionally, since sexually mature fish appear to be spending considerable time away from spawning aggregations during the spawning season, individual spawning output may be lower than the values used in current population models.

The majority of the common snook in this study appeared in the array in late spring or early summer, and disappeared in the fall. Many of these transient individuals exhibited strong spawning site fidelity, returning to Jupiter Inlet during the 2008 and 2009 spawning seasons (similar to snook in other parts of Florida: Adams et al. 2009a; Adams et al. 2011). While some transient common snook were only detected at the inlet section of the estuary ($n = 15$), the majority of transient fish ($n = 43$) made at least one round-trip upstream run (while still spending the majority of their time in the inlet section of the system). During these runs, most snook followed a relatively direct path while

swimming upriver. When returning to the inlet from upstream areas, many snook took a less direct path, often meandering through the estuary for a period of time before reaching the inlet. This meandering behavior explains why upstream run times were typically shorter than downstream run times. The remaining 12 transient individuals appeared to exhibit high site fidelity to specific sections of the Loxahatchee River, away from the inlet. These individuals only made occasional movements to the inlet or riverine areas during the spawning season.

Only 19% ($n = 16$) of the fish we detected in the Loxahatchee River were considered residents, i.e., fish that remained in the Loxahatchee River year-round, including winter 2008-2009. All but one of these resident snook were initially tagged in the Loxahatchee River; however, many other transient snook were also tagged within the system. Most resident snook appeared to exhibit high site fidelity within a specific section of the estuary (often near the initial tagging site), and only rarely (or never) visited the inlet. These fish occasionally made movements within the estuary, but almost always returned to their original location.

The size of resident snook, which were smaller on average than transient fish that left the system after the spawning season, may relate to the movement patterns we observed. Common snook are protandric hermaphrodites, with many males undergoing sex change as size increases (Peters et al. 1998; Taylor et al. 2000). It is likely that many of the resident snook in this study (mean TL = 660 ± 111 mm) were males, as only ~25% of the population would be female by this size (Taylor et al. 2000). However, even the smallest resident fish (555 mm TL) was likely sexually mature since males begin to reach sexual maturity at <200 mm fork length (Taylor et al. 2000), and all males sampled by

Peters et al. (1998) were mature by 500-522 mm standard length. Only two resident snook (the largest residents: 864 and 890 mm TL) spent considerable time at Jupiter Inlet during the spawning season. Although likely mature, the remaining resident fish rarely spent time at the inlet. Lowerre-Barbieri et al. (2003) found that male common snook collected directly from spawning aggregations on the east coast of Florida were 690-1038 mm TL, much larger than the size at which males reach maturity (and larger than all but four of the resident snook in our study). The movement patterns we observed among resident fish, combined with the size range of males found in spawning aggregations, suggests that smaller (although sexually mature) male common snook may rarely participate in spawning aggregations at inlets on Florida's east coast. In contrast, much smaller common snook (average size ~400 mm standard length) have been observed at spawning sites on the west coast of Florida (Adams et al. 2009a). The size difference that we observed between snook that made upstream runs and those that did not was likely driven by smaller resident fish that rarely ran upriver. Additionally, smaller resident fish, which spent a greater portion of their total detection period within the acoustic array, likely contributed to the negative relationship between TL and detection days.

Contrary to the thermal refuge paradigm, most resident snook in the Loxahatchee River spent the winter in the lower embayment ($n = 12$). Riverine habitat use was less common in winter, with three individuals overwintering at the estuarine/riverine interface in the NW Fork, and one individual overwintering in the upper reaches of the N Fork. While these findings appear to further support the assertion by Blewett et al. (2009) that upstream habitat use by common snook is not limited to thermal refuge during the winter, the decline in total abundance that we observed during the winter months suggests that

the majority of the snook that spawn at the mouth of the Loxahatchee River overwinter elsewhere. Trotter et al. (2012) observed that snook tagged in the upstream portion of an estuary would occasionally leave the system during the spawning season, returning to the same general upstream area during late summer or early fall. This behavior may explain why a number of snook that were originally tagged during winter or spring in the St. Lucie Estuary, or during winter in a dredged basin in the Indian River Lagoon near Ft. Pierce Inlet, appeared in the Loxahatchee River during spawning season. Based on the findings of Trotter et al. (2012), it is likely that the transient snook found in Loxahatchee River from spring through fall, overwinter in the St. Lucie Estuary or near Ft. Pierce Inlet. These areas may represent important winter habitats for snook on the east coast of Florida. Since snook tagging in the Loxahatchee River was restricted to the summer months, we are unable to determine whether a different contingent of transient snook (which, hypothetically, may spawn in another estuary) utilize the system in the winter.

Our findings underscore the complex nature of common snook management and conservation. We have demonstrated considerable connectivity among estuaries on the east coast of Florida. In addition to the interestuarine movements discussed above, snook tagged in the Loxahatchee River have been detected as far away as Cape Canaveral (170 km north of Jupiter Inlet), and snook tagged at Cape Canaveral and Sebastian Inlet (110 km north of Jupiter Inlet) have been detected in the Loxahatchee River (after the conclusion of this study), suggesting that localized disturbance events have the potential to affect snook populations across a broad geographical area. Future efforts should be made to identify and protect spawning and overwintering habitats, as these areas are critical to maintaining stable common snook populations. In addition to preserving the

physical habitats that common snook utilize, it is equally important to restore natural flow patterns in estuaries that snook use for spawning. While efforts have been made to establish ecologically relevant minimum freshwater inflow thresholds for many estuaries in Florida, these thresholds focus on dry season inflow, and largely ignore flow patterns that occur during the wet season. Minimum flow during the dry season should not be the only factor considered by water managers. For the benefit of organisms that utilize estuaries outside of the dry season, future management objectives should also include duplicating historical temporal flow patterns, and stabilizing flows to reduce unnaturally rapid fluctuations.

Acknowledgments

This project was made possible by the logistical and financial support of the Loxahatchee River District. We thank Dave Sabin and Dave Porter for their invaluable assistance in the field. We appreciate the support of members of the Florida Atlantic Coast Telemetry (FACT) working group. Research protocols were approved by Florida International University's Institutional Animal Care and Use Committee (IACUC-07-015), and a Florida Fish and Wildlife Conservation Commission Special Activities License (SAL-09-1151-SR).

Table 2.1. Tagging date, tagging location, and total length (TL) for 86 tagged common snook detected within the Loxahatchee River acoustic array between May 25, 2008 and December 15, 2009.

Tagging Date	TL (mm)	Tagging Location	Tagging Date	TL (mm)	Tagging Location
2/12/2008	999	St. Lucie River	7/8/2008	925	Loxahatchee River
2/14/2008	752	St. Lucie River	7/8/2008	845	Loxahatchee River
2/15/2008	909	St. Lucie River	7/8/2008	848	Loxahatchee River
2/20/2008	N/A	Loxahatchee River	7/8/2008	891	Loxahatchee River
2/28/2008	905	St. Lucie River	7/8/2008	832	Loxahatchee River
2/28/2008	1100	St. Lucie River	7/8/2008	958	Loxahatchee River
2/28/2008	1020	St. Lucie River	7/8/2008	890	Loxahatchee River
2/28/2008	844	St. Lucie River	7/8/2008	961	Loxahatchee River
2/28/2008	762	St. Lucie River	7/8/2008	850	Loxahatchee River
4/30/2008	878	St. Lucie River	7/8/2008	935	Loxahatchee River
4/30/2008	756	St. Lucie River	7/8/2008	808	Loxahatchee River
4/30/2008	629	St. Lucie River	7/8/2008	825	Loxahatchee River
4/30/2008	800	St. Lucie River	7/9/2008	853	St. Lucie River
5/2/2008	833	St. Lucie River	7/9/2008	856	St. Lucie River
5/5/2008	647	St. Lucie River	7/13/2008	631	Loxahatchee River
5/5/2008	903	St. Lucie River	7/14/2008	590	Loxahatchee River
5/6/2008	892	St. Lucie River	12/15/2008	893	Ft. Pierce Inlet
6/10/2008	778	St. Lucie River	12/15/2008	798	Ft. Pierce Inlet
6/11/2008	538	Loxahatchee River	12/15/2008	822	Ft. Pierce Inlet
6/11/2008	573	Loxahatchee River	12/16/2008	859	Ft. Pierce Inlet
6/11/2008	608	Loxahatchee River	12/16/2008	884	Ft. Pierce Inlet
6/11/2008	593	Loxahatchee River	4/28/2009	950	St. Lucie River
6/11/2008	614	Loxahatchee River	5/19/2009	612	St. Lucie River
6/11/2008	649	Loxahatchee River	6/30/2009	573	Loxahatchee River
6/17/2008	573	Loxahatchee River	6/30/2009	1033	Loxahatchee River
6/18/2008	555	Loxahatchee River	6/30/2009	952	Loxahatchee River
6/18/2008	590	Loxahatchee River	7/3/2009	748	Loxahatchee River
6/19/2008	590	Loxahatchee River	7/3/2009	684	Loxahatchee River
6/30/2008	596	Loxahatchee River	7/3/2009	800	Loxahatchee River
6/30/2008	637	Loxahatchee River	7/3/2009	1056	Loxahatchee River
7/1/2008	561	Loxahatchee River	7/3/2009	998	Loxahatchee River
7/2/2008	567	Loxahatchee River	7/7/2009	1086	Loxahatchee River
7/8/2008	870	Loxahatchee River	7/7/2009	753	Loxahatchee River
7/8/2008	875	Loxahatchee River	7/10/2009	800	Nearshore
7/8/2008	1052	Loxahatchee River	8/4/2009	1045	Loxahatchee River
7/8/2008	970	Loxahatchee River	8/4/2009	845	Loxahatchee River
7/8/2008	980	Loxahatchee River	8/4/2009	1065	Loxahatchee River
7/8/2008	855	Loxahatchee River	8/4/2009	850	Loxahatchee River
7/8/2008	896	Loxahatchee River	8/4/2009	892	Loxahatchee River
7/8/2008	913	Loxahatchee River	8/31/2009	946	Nearshore
7/8/2008	935	Loxahatchee River	8/31/2009	922	Nearshore
7/8/2008	864	Loxahatchee River	8/31/2009	980	Nearshore
7/8/2008	961	Loxahatchee River	8/31/2009	912	Nearshore

Fig. 2.1. Map of the Loxahatchee River, near Jupiter, Florida, showing the acoustic telemetry array used to track common snook movements. The location of each acoustic receiver is indicated by a black dot. Our delineation of the riverine/estuarine interface is represented by a black line, and Jupiter Inlet (the location of a large snook spawning aggregation) is outlined by a black polygon. The acoustic receiver at Island Way Bridge was used to determine when tagged common snook had entered the riverine section of the estuary. Two low head dams – Lainhart Dam (the location of South Florida Water Management District’s flow gauge) and Masten Dam – are indicated by stars.

Fig. 2.2. Common snook abundance in the Loxahatchee River (black bars) and daily mean freshwater inflow measured at Lainhart Dam (red line) during the study period.

Fig. 2.3. Starting time for all round-trip upstream runs that began at (and returned to) Jupiter Inlet, and reached (1) the upper embayment of the NW Fork, (2) the upper reaches of the SW Fork, or (3) the upper reaches of the N Fork.

Fig. 2.4. Relative number of common snook in the Loxahatchee River starting upstream runs per day (# of fish starting runs / # of tagged fish in the river on that day: black bars) vs. daily mean freshwater inflow measured at Lainhart Dam (red line).

Fig. 2.5. Freshwater inflow within the wet season (integrated over 2-day time periods) vs. the relative number of snook per day starting upstream runs. The relationship was not significant ($R^2 < 0.001$, $F_{1,304} = 0.004$, $p = 0.95$).

Fig. 2.6. Duration of upstream runs (from Jupiter Inlet to Island Way Bridge at the start of the riverine section of the NW Fork) vs. duration of downstream runs (Island Way Bridge to Jupiter Inlet).

References

- Adams AJ, Hill JE, Kurth BN, Barbour AB (2012) Effects of a severe cold event on the subtropical, estuarine-dependent common snook, *Centropomus undecimalis*. Gulf and Caribbean Research 24:13-21
- Adams AJ, Hill JE, Samoray C (2011) Characteristics of spawning ground fidelity by a diadromous fish: a multi-year perspective. Environmental Biology of Fishes 92:403-411
- Adams AJ, Wolfe RK, Barkowski N, Overcash D (2009a) Fidelity to spawning grounds by a catadromous fish, *Centropomus undecimalis*. Marine Ecology Progress Series 389:213-222. doi:10.3354/meps08198
- Adams AJ, Wolfe RK, Layman CA (2009b) Preliminary examination of how human-driven freshwater flow alteration affects trophic ecology of juvenile snook (*Centropomus undecimalis*) in estuarine creeks. Estuaries and Coasts 32:819-828. doi:10.1007/s12237-009-9156-x
- Adams AJ, Wolfe RK, Pine WE, Thornton BL (2006) Efficacy of PIT tags and an autonomous antenna system to study the juvenile life stage of an estuarine-dependent fish. Estuaries and Coasts 29:311-317. doi:10.1007/bf02781999
- Adams MJ (2000) Pond Permanence and the Effects of Exotic Vertebrates on Anurans. Ecological Applications 10:559-568
- Alabaster JS (1970) River flow and upstream movement and catch of migratory salmonids. Journal of Fish Biology 2:1-13
- Alber M (2002) A conceptual model of estuarine freshwater inflow management. Estuaries and Coasts 25:1246-1261
- Aliaume C, Zerbi A, Joyeux JC, Miller JM (2000) Growth of juvenile *Centropomus undecimalis* in a tropical island. Environmental Biology of Fishes 59:299-308. doi:10.1023/a:1007662611839
- Almeida PR (1996) Estuarine movement patterns of adult thin-lipped grey mullet, *Liza ramada* (Risso) (Pisces, Mugilidae), observed by ultrasonic tracking. Journal of Experimental Marine Biology and Ecology 202:137-150
- Andrade H, Santos J, Taylor R (2013) Life-history traits of the common snook *Centropomus undecimalis* in a Caribbean estuary and large-scale biogeographic patterns relevant to management. Journal of Fish Biology 82:1951-1974
- Andrews KS, Harvey CJ (2013) Ecosystem-level consequences of movement: seasonal variation in the trophic impact of a top predator. Marine Ecology Progress Series 473:247-260. doi:10.3354/meps10095

- Barnes T (2005) Caloosahatchee estuary conceptual ecological model. *Wetlands* 25:884-897
- Baum JK, Worm B (2009) Cascading top-down effects of changing oceanic predator abundances. *Journal of Animal Ecology* 78:699-714
- Blewett DA, Stevens PW (2013) The effects of environmental disturbance on the abundance of two recreationally important fishes in a subtropical floodplain river. *Florida Scientist* 76:191-197
- Blewett DA, Stevens PW, Call ME (2013) Comparative ecology of euryhaline and freshwater predators in a subtropical floodplain river. *Florida Scientist* 76
- Blewett DA, Stevens PW, Champeau TR, Taylor RG (2009) Use of rivers by common snook *Centropomus undecimalis* in southwest Florida: A first step in addressing the overwintering paradigm. *Florida Science* 72:310-324
- Bunn SE, Arthington AH (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management* 30:492-507
- Chamberlain RH, Doering PH (1998) Preliminary estimate of optimum freshwater inflow to the Caloosahatchee estuary: A resource-based approach. *Proceedings of the Charlotte Harbor Public Conference and Technical Symposium Charlotte Harbor National Estuary Program* 274 p
- Childs AR, Cowley PD, Næsje TF, Booth AJ, Potts WM, Thorstad EB, Økland F (2008) Do environmental factors influence the movement of estuarine fish? A case study using acoustic telemetry. *Estuarine Coastal and Shelf Science* 78:227-236
- Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. *Nature* 387:253-260
- Drinkwater KF, Frank KT (1994) Effects of river regulation and diversion on marine fish and invertebrates. *Aquatic Conservation: Marine and Freshwater Ecosystems* 4:135-151
- Gillson J (2011) Freshwater flow and fisheries production in estuarine and coastal systems: Where a drop of rain is not lost. *Reviews in Fisheries Science* 19:168-186
- Granek EF, Polasky S, Kappel CV, Reed DJ, Stoms DM, Koch EW, Kennedy CJ, Cramer LA, Hacker SD, Barbier EB, Aswani S, Ruckelshaus M, Perillo GME, Silliman BR, Muthiga N, Bael D, Wolanski E (2010) Ecosystem services as a common language for coastal ecosystem-based management. *Conservation Biology* 24:207-216. doi:10.1111/j.1523-1739.2009.01355.x

- Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. *Science* 319:948-952. doi:10.1126/science.1149345
- Hammerschlag-Peyer CM, Layman CA (2010) Intrapopulation variation in habitat use by two abundant coastal fish species. *Marine Ecology Progress Series* 415:211-220
- Heupel MR, Semmens JM, Hobday AJ (2006) Automated acoustic tracking of aquatic animals: scales, design and deployment of listening station arrays. *Marine and Freshwater Research* 57:1-13. doi:10.1071/mf05091
- Heupel MR, Simpfendorfer CA (2008) Movement and distribution of young bull sharks *Carcharhinus leucas* in a variable estuarine environment. *Aquatic Biology* 1:277-289. doi:10.3354/ab00030
- Hohausová E, Copp GH, Jankovský P (2003) Movement of fish between a river and its backwater: diel activity and relation to environmental gradients. *Ecology Of Freshwater Fish* 12:107-117
- Howells RG, Sonski AJ, Shafland PL, Hilton BD (1990) Lower temperature tolerance of snook, *Centropomus undecimalis*. *Northeast Gulf Science* 11:155-158
- Humston R, Ault JS, Larkin MF, Luo JG (2005) Movements and site fidelity of the bonefish *Albula vulpes* in the northern Florida Keys determined by acoustic telemetry. *Marine Ecology Progress Series* 291:237-248. doi:10.3354/meps291237
- Irlandi EA, Crawford MK (1997) Habitat linkages: the effect of intertidal saltmarshes and adjacent subtidal habitats on abundance, movement, and growth of an estuarine fish. *Oecologia* 110:222-230
- Jaureguizar AJ, Menni R, Guerrero R, Lasta C (2004) Environmental factors structuring fish communities of the Río de la Plata estuary. *Fisheries Research* 66:195-211
- Layman CA, Jud ZR, Arrington DA, Sabin D (*in press*) Fish behavior as a means to assess oyster reef restoration. *Ecological Restoration*
- Loneragan NR, Bunn SE (1999) River flows and estuarine ecosystems: Implications for coastal fisheries from a review and a case study of the Logan River, southeast Queensland. *Australian Journal of Ecology* 24:431-440. doi:10.1046/j.1442-9993.1999.00975.x
- Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation, and

- recovery potential of estuaries and coastal seas. *Science* 312:1806-1809.
doi:10.1126/science.1128035
- Lowerre-Barbieri SK, Vose FE, Whittington JA (2003) Catch-and-release fishing on a spawning aggregation of common snook: does it affect reproductive output? *Transactions of the American Fisheries Society* 132:940–952
- McMichael RH, Jr, Peters KM, Parsons GR (1989) Early life history of the snook, *Centropomus undecimalis*, in Tampa Bay, Florida. *Northeast Gulf Science* 10:113-125
- Milner NJ, Solomon DJ, Smith GW (2012) The role of river flow in the migration of adult Atlantic salmon, *Salmo salar*, through estuaries and rivers. *Fisheries Management and Ecology* 19:537-547. doi:10.1111/fme.12011
- Perera-Garcia MA, Mendoza-Carranza M, Contreras-Sanchez W, Ferrara A, Huerta-Ortiz M, Hernandez-Gomez RE (2013) Comparative age and growth of common snook *Centropomus undecimalis* (Pisces: Centropomidae) from coastal and riverine areas in Southern Mexico. *Revista de Biología Tropical* 61:807-819
- Perera-Garcia MA, Mendoza-Carranza M, Contreras-Sanchez WM, Huerta-Ortiz M, Perez-Sanchez E (2011) Reproductive biology of common snook *Centropomus undecimalis* (Perciformes: Centropomidae) in two tropical habitats. *Revista de Biología Tropical* 59:669-681
- Peters KM, Matheson RE, Taylor RC (1998) Reproduction and early life history of common snook, *Centropomus undecimalis* (Bloch), in Florida. *Bulletin of Marine Science* 62:509-529
- Reyier EA, Lowers RH, Scheidt DM, Adams DH (2011) Movement patterns of adult red drum, *Sciaenops ocellatus*, in shallow Florida lagoons as inferred through autonomous acoustic telemetry. *Environmental Biology of Fishes* 90:343-360. doi:10.1007/s10641-010-9745-3
- Rosenblatt AE, Heithaus MR (2011) Does variation in movement tactics and trophic interactions among American alligators create habitat linkages? *Journal of Animal Ecology* 80:786-798. doi:10.1111/j.1365-2656.2011.01830.x
- Sakabe R, Lyle JM (2010) The influence of tidal cycles and freshwater inflow on the distribution and movement of an estuarine resident fish *Acanthopagrus butcheri*. *Journal of Fish Biology* 77:643-660. doi:10.1111/j.1095-8649.2010.02703.x
- SFWMD (2006) Restoration plan for the Northwest Fork of the Loxahatchee River South Florida Water Management District, Watershed Management Department, Coastal Ecosystems Division, Technical Report, 465 pp.,

- Shafland PL, Foote KJ (1983) A lower lethal temperature for fingerling snook (*Centropomus undecimalis*). *Northeast Gulf Science* 6:175-177
- Sime P (2005) St. Lucie Estuary and Indian River Lagoon conceptual ecological model. *Wetlands* 25:898-907
- Smith GW, Smith IP, Armstrong SM (1994) The relationship between river flow and entry to the Aberdeenshire Dee by returning adult Atlantic salmon. *Journal of Fish Biology* 45:953-960
- Stevens PW, Blewett DA, Poulakis GR (2007) Variable habitat use by juvenile common snook, *Centropomus undecimalis* (Pisces: Centropomidae): applying a life history model in a southwest Florida estuary. *Bulletin of Marine Science* 80:93-108
- Taylor RG, Grier HJ, Whittington JA (1998) Spawning rhythms of common snook in Florida. *Journal of Fish Biology* 53:502-520
- Taylor RG, Whittington JA, Grier HJ, Crabtree RE (2000) Age, growth, maturation, and protandric sex reversal in common snook, *Centropomus undecimalis*, from the east and west coasts of South Florida. *Fishery Bulletin* 98:612-624
- Trotter AA, Blewett DA, Taylor RG, Stevens PW (2012) Migrations of common snook from a tidal river with implications for skipped spawning. *Transactions of the American Fisheries Society* 141:1016-1025. doi:10.1080/00028487.2012.675903
- VanArman J, Graves GA, Fike D (2005) Loxahatchee watershed conceptual ecological model. *Wetlands* 25:926-942
- Walsh CT, Reinfelds IV, Ives MC, Gray CA, West RJ, van der Meulen DE (2013) Environmental influences on the spatial ecology and spawning behaviour of an estuarine-resident fish, *Macquaria colonorum*. *Estuarine Coastal and Shelf Science* 118:60-71. doi:10.1016/j.ecss.2012.12.009

CHAPTER III

RECENT INVASION OF A FLORIDA ESTUARINE SYSTEM BY LIONFISH

PTEROIS VOLITANS / P. MILES

Jud ZR, Layman CA, Lee JA, Arrington DA (2011) Recent invasion of a Florida (USA) estuarine system by lionfish *Pterois volitans* / *P. miles*. Aquatic Biology 13:21-26

Abstract

The invasion of lionfish (*Pterois volitans* and *P. miles*) throughout the western Atlantic and Caribbean is emerging as a serious ecological problem. While lionfish have been identified on coral reefs and other marine systems, additional ecosystems may be impacted as the invasion spreads. Here we identify the first estuarine intrusion of lionfish in their invasive range. Two hundred and eleven lionfish were captured in the Loxahatchee River estuary (Florida) between August 2010 and April 2011, with some individuals located as far as ~5.5 km from the ocean. Multiple size classes were documented (standard lengths ranged from 23 to 185 mm), and post-settlement juveniles were present throughout the sampling period. All individuals were found in close association with anthropogenically created habitats (e.g., docks, sea walls, submerged debris), suggesting that human-driven changes in habitat availability may facilitate estuarine invasion. Fifteen prey taxa were found in lionfish stomachs, with diets dominated by small shrimp. Since estuaries are already highly threatened by human impacts, and provide critical habitat for numerous commercially, recreationally, and ecologically important species, establishment of lionfish in these ecosystems is of particular concern.

Introduction

Estuaries are highly productive systems that provide some of the most valued ecosystem services at a global scale (Costanza et al. 1997; Granek et al. 2010), including habitat for numerous commercially, recreationally, and ecologically important species (Beck et al. 2001; Adams et al. 2006; Courrat et al. 2009; Jud et al. 2011). Despite their importance, estuaries may be one of the most impacted types of ecosystems – impacts (e.g., shoreline development, pollution, dam construction, dredging, etc.) that have led to precipitous declines in marine and estuarine fauna. The overwhelming cause of these declines has been habitat alteration/destruction and direct over-exploitation of organisms (Lotze et al. 2006; Halpern et al. 2008). Although estuaries are also affected by invasive species (Ruiz et al. 1997; Cohen and Carlton 1998; Byrnes et al. 2007), non-native organisms have rarely been directly implicated in declines of native fauna in coastal systems (Lotze et al. 2006). Yet because most documented coastal marine invasions are by taxa at relatively low trophic positions, e.g., primary producers, planktivores, detritivores, or deposit feeders (Byrnes et al. 2007), an understanding of the impacts of invasive predators in estuarine systems is only starting to emerge.

Here, we identify a new threat to estuarine ecosystems in the western Atlantic and Caribbean, the invasive Indo-Pacific lionfish (*Pterois volitans* and/or *P. miles*, hereafter referred to as lionfish). The spread of invasive lionfish in the western Atlantic and Caribbean has been well documented (Whitfield et al. 2002; Hamner et al. 2007; Freshwater et al. 2009; Schofield 2009), with the invasion considered one of the top 15 emerging environmental issues at a global scale (Sutherland et al. 2010). To date, most lionfish research has focused on invaded coral reefs and other marine habitats. While

lionfish have been identified in the lower 1 km of a mangrove-lined creek in The Bahamas (Barbour et al. 2010), the hydrology and ecology of this system are substantially different from true riverine estuaries that receive considerable freshwater input and experience fluctuating salinities (Layman et al. 2007; Valentine-Rose et al. 2007). We have recently identified lionfish utilizing estuarine habitats in the Loxahatchee River, near Jupiter, Florida. This is the first documented intrusion of lionfish into an estuarine system in their invasive range.

Herein we provide information on the lionfish invasion of the Loxahatchee River, FL, including (1) a description and characteristics of lionfish capture locations, (2) size structure of sampled fish, and (3) basic diet information. These data provide a first step toward exploring future invasions of lionfish into estuarine systems in the region.

Methods

The Loxahatchee River (26°57' N, 80°06' W), located near Jupiter, Florida, receives flow from three major branches and a number of smaller tributaries (Fig. 3.1). The river drains a 434 km² watershed and flows into the Atlantic Ocean at Jupiter Inlet (VanArman et al. 2005). While the upper reaches of the river are largely composed of natural habitats (e.g., cypress forests, mangrove-lined shorelines, oyster reefs), lower sections of the river have been highly modified by human activities, including construction of seawalls, docks, and channels. The river bottom in the lower section of the estuary is largely composed of sand, without any high-relief features (e.g., rocks, ledges, etc.). In this part of the estuary, structurally complex habitats that are favored by lionfish (both natural and human made) are restricted to shoreline areas.

Despite periodic underwater surveys during the previous three years (for unrelated research projects), we did not document lionfish in the Loxahatchee River until August 2010. This initial sighting prompted a more thorough search of the system. We identified and captured lionfish by visually surveying (while snorkeling) a belt extending out ~30 m from the shoreline, running parallel to the river's edge. Sampling frequency and spatial extent differed between the north and south shorelines of the river. On the north shoreline, our primary sampling location, we surveyed a continuous belt extending from the river mouth to an area ~5.5 km upstream from the ocean (upstream limit of clear water needed for visual surveys) every 11-12 weeks (Fig. 3.1). During each of these intensive sampling events (carried out in August 2010, October 2010, January 2011, and April 2011), 100% of the shoreline in this section of estuary was visually surveyed, including all natural (e.g., mangroves, sandy bottom, seagrass) and human-impacted (e.g., docks, seawalls, rock rip-rap piles, debris, etc.) habitats. We attempted to capture and kill all lionfish present along the north shoreline during the course of each survey. Fish were captured using pole spears and hand nets, and all sampling was conducted during daytime incoming tides to maximize visibility. Sampling was also carried out along a shorter (~1.5 km) section of the south shoreline as part of an ongoing mark-recapture study (Fig. 3.1). Opportunistic sampling of the south shoreline was conducted throughout the study period (August 2010-April 2011), rather than at fixed time intervals.

Standard lengths (SL) were measured for all collected lionfish (north and south shorelines). We conducted preliminary stomach content analyses on individuals captured along the north shoreline during our surveys in August and October 2010 (see Jud et al. 2011 for methods). To obtain an overall description of lionfish diet, we calculated the

following values for each prey taxon present in the sampled stomachs: percent frequency of occurrence (%O), percent composition by number (%N), and percent composition by wet mass (%M) (Morris and Akins 2009). Based on these values, an Index of Relative Importance (IRI) was calculated for each prey taxon i , where $IRI_i = \%O_i (\%N_i + \%M_i)$. The IRI is a compound index that incorporates quantity, mass, and frequency of occurrence into a single numerical measure, facilitating dietary comparisons and providing a more accurate estimate of “dietary importance” of prey items (Hynes 1950; Hyslop 1980; Cortes 1997).

Temperature and salinity measurements for the period January 2010 to April 2011 were obtained from a pair of datasondes (600XLM, YSI Hydrodata Ltd.) located ~1 m below the surface, within the section of river where lionfish were collected (Fig. 3.1). This timeframe roughly corresponds to the potential period of lionfish occupation in the estuary.

Results

A total of 211 lionfish were captured in the Loxahatchee River between August 2010 and April 2011. Collection sites were ~0.1 to 5.5 km from the ocean (Fig. 3.1). All fish were found in close association with human-made structures along the river’s shoreline (Fig. 3.2). Lionfish were frequently observed hovering around or resting on debris under docks (e.g., cinder blocks, concrete slabs, discarded fish traps, etc.) or near the base of rock rip-rap piles. Additional individuals were found resting in a vertical orientation against dock pilings or corrugated sea walls. All fish were captured ~0.5-2 m below the surface. Although we surveyed natural shoreline habitats (mangroves,

seagrass, sand bottom), no lionfish were identified in these areas. Additionally, no lionfish were observed at more than 1,700 natural sites throughout the estuary that were surveyed during summer 2010 as part of an unrelated study (Loxahatchee River District, unpubl. data), further emphasizing the species' affinity for human-made structures within the system.

Mean standard length (SL) $\pm$ standard deviation of all 211 captured lionfish was 92.1 ± 33.5 mm, with a range of 23-185 mm. All individuals were likely ≤ 12 months of age at time of capture (J. Morris, unpubl. data). Lengths of the 145 individuals captured along the north shoreline during primary sampling events varied by month (Fig. 3.3). At the time of our first sampling of the north shoreline in August 2010, mean lionfish SL was 96.7 ± 21.7 mm ($n = 54$). Mean SL along the north shoreline increased to 118.7 ± 34.2 mm in October 2010 ($n = 24$), decreased to 66.4 ± 38.5 mm in January 2011 ($n = 18$), and finally increased to 88.4 ± 25.5 mm in April 2011 ($n = 49$).

Preliminary stomach content analyses were performed on 71 lionfish captured along the north shoreline in August and October 2010, 66 of which (93%) contained prey items. A total of 15 prey taxa were identified. The prey taxa found in the greatest proportion of sampled lionfish stomachs (excluding empty stomachs) were unidentified (i.e., digested) teleosts (59% of sampled stomachs), followed by palaemonid shrimp (58% of stomachs), and penaeid shrimp (58% of stomachs). The remaining 12 prey taxa (Blenniidae, Gerreidae, Lutjanidae, Gobiidae, Panopeidae, Portunidae, Porcellanidae, Paguroidea, Alpheidae, *Lysmata* sp., Amphipoda, and unidentified crabs) were each found in less than 23% of the sampled stomachs. Overall, 88% of lionfish stomachs contained shrimp, 79% contained fishes and 23% contained crabs. Palaemonids and

penaeids were the numerically dominant prey groups found in lionfish stomachs, while penaeids and two teleost taxa (Gerreidae, Blenniidae) were the gravimetrically dominant prey items in lionfish stomachs (based on mass in stomachs). The three most important prey taxa based on Index of Relative Importance values were Penaeidae, Palaemonidae, and unidentified (i.e., digested) teleosts.

From January 2010 to April 2011, water temperatures in the section of river where lionfish were collected ranged from 12.2 – 34.4°C, and salinities (~1 m below surface) varied from 5.8 – 38.6‰. Lower salinities were common during the wet season (June-October), concurrent with the first third of our lionfish sampling period. Extreme low salinities (i.e., <10‰) were limited both temporally (hours, to <1 day) and spatially (the more upstream datasonde only). During the wet season, the estuary exhibited stratified conditions, with a thin (~0.25-0.5 m) layer of turbid freshwater floating over a layer of clear, higher-salinity water.

Discussion

Our initial findings suggest that the presence of lionfish in the Loxahatchee River estuary is more than just a short-term phenomenon. Based on observed size distributions (Fig. 3.3), it appears that successful recruitment may have occurred multiple times throughout 2010 and early 2011. Small post-settlement juveniles were captured on each sampling date, suggesting that recruitment may occur year round. We initially predicted that recruiting lionfish would settle in the lower reaches of the estuary, closer to the ocean; however, several of the smallest individuals we captured ($SL \leq 28$ mm) were

located >4 km upriver, indicating that small juveniles may possess the ability to settle well into estuarine systems.

While there is no published record for salinity tolerance in lionfish, their presence in the Loxahatchee River suggests that the species may be able to behaviorally (or physiologically) handle fluctuating estuarine salinities. We believe a salt wedge and associated salinity stratification, common in estuaries (Simpson et al. 1990), may have provided a stable high-salinity benthic refuge for lionfish when surface salinities were reduced. All lionfish were captured at ≥ 0.5 m in depth, suggesting they may avoid lower-salinity surface waters. Even during a period of extremely high freshwater inflow associated with a passing tropical storm, we continued to observe lionfish in the Loxahatchee River.

Despite record cold water temperatures during the winter of 2010 (Loxahatchee River District, unpubl. data), water temperatures in the section of river inhabited by lionfish remained above the species' lethal minimum temperature, 10°C (Kimball et al. 2004). As such, wintertime low temperatures appear to be an insufficient barrier to the permanent establishment of lionfish in South Florida and Caribbean estuaries. Additional laboratory experiments are needed to determine physiological tolerances (salinity, temperature) in estuarine lionfish.

Human activities may facilitate the successful invasion of estuaries by lionfish through the creation of structurally complex artificial habitats that the species appear to favor, particularly in systems that lack natural high-relief habitats. With a unique set of muscles attached to the swim bladder that allows them to assume a vertical orientation (Hornstra et al. 2004), lionfish are highly adapted to exploit vertical surfaces, including

numerous anthropogenically created habitats found in estuaries (e.g., sea walls, pilings). Rapid establishment of lionfish in the Loxahatchee River estuary may represent another example of artificial habitats facilitating the spread of invasive species (Sheehy and Vik 2010).

Although no significant predation of lionfish has been documented in the Atlantic, the large predators (e.g., serranids) that may occasionally consume lionfish (Maljković et al. 2008) are typically rare in estuarine systems compared to coral reefs (Dorenbosch et al. 2009). The most abundant estuarine predators (e.g., juvenile lutjanids and carangids) are gape limited and would only be able to potentially consume the smallest lionfish. Larger estuarine predators (e.g., centropomids) have not yet been shown to feed on lionfish. We observed one instance of a green moray eel (*Gymnothorax funebris*) consuming a wounded lionfish in the Loxahatchee River, but moray eels are likely far less abundant in estuaries than on coral reefs.

Without additional research, it is difficult to predict the future impacts of lionfish in estuaries. The species' rapid rate of prey consumption (Fishelson 1997) may alter prey communities, particularly since feeding rates in the lionfish's invasive range appear to be even greater than in their native range (Côté and Maljković 2010). Although lionfish in their native range frequently have empty stomachs (Fishelson 1997), a common pattern among piscivorous fishes (Arrington et al. 2002), the fish we sampled almost always had prey in their stomachs. This low percentage of empty stomachs has been observed across the lionfish's invasive range (Albins and Hixon 2008; Morris and Akins 2009; Barbour et al. 2010), suggesting that these invasive predators feed frequently, perhaps in response to prey naïveté and a consequent increase in prey capture success rates. Additionally,

release from predation in their invaded range may allow lionfish to spend more time foraging and less time sheltering from predators.

On coral reefs, invasive lionfish have been shown to reduce recruitment of native fishes by nearly 80% over a five week period (Albins and Hixon 2008); similar predation rates in estuaries could have major, yet undocumented, impacts, particularly for species that rely on estuarine systems as nursery habitat. The continued presence of lionfish in estuarine nursery habitats may threaten the early life history stages of a number of commercially, recreationally, and ecologically valuable fish species, either through indirect interactions (e.g., prey depletion), or as a result of direct predation (Morris and Akins 2009). Although preliminary, our diet data have already revealed some consumption of commercially and recreationally important lutjanid species by lionfish in the Loxahatchee River.

Colonization of an estuary by lionfish provides an example of the rapidly expanding range (and potential ecological impacts) of the species in the region. Although the invasion of lionfish will undoubtedly have broad-reaching effects, the impacts are of particular concern for highly threatened ecosystems like estuaries. Since lionfish are often found in turbid bays in their native range (A. Anton, unpubl. data), estuaries may become another major site of invasion as regional populations continue to grow. At this point in the invasion, efforts to control lionfish populations should remain focused on the most critical or threatened ecosystems (e.g., nursery habitats) – those systems where direct removal of lionfish would have the greatest ecological benefits. Since lionfish are less likely to be observed and reported in estuaries than on coral reefs, it is possible that estuarine invasions may go undetected for considerable periods of time. Early detection

and control of lionfish in estuaries may be crucial to offset their long-term ecological impacts in these critical ecosystems.

Acknowledgments

The field work carried out during this study would not have been possible without the assistance of Matt Wittenrich, Dave Porter, Robin Abbey-Lee, Matt Pedersen, Lorene Bachman, and Jerry Metz. We thank Dave Sabin for creating GIS maps of the Loxahatchee River. Additionally, we appreciate the support and cooperation of numerous homeowners along the Loxahatchee River. Lionfish were collected pursuant to Florida Fish and Wildlife Conservation Commission Permit # SAL-09-1118A-SR. Partial funding was provided by NSF OCE #0746164 and OCE #0940019.

Fig. 3.1. Map of the lower Loxahatchee River, near Jupiter, Florida. Crosshatching indicates the section of the north shoreline that was intensively surveyed for lionfish (*Pterois volitans* / *P. miles*) every 11-12 weeks. Gray shading indicates the section of the south shoreline what was opportunistically sampled as part of an ongoing mark-recapture study. Width of the survey belt was ~30 m (exaggerated slightly in figure for clarity). Lionfish were found throughout the survey belt. The upstream limit of lionfish capture (dot at A) was ~5.5 km from the ocean, while the downstream limit (dot at B) was ~0.1 km from the ocean. Stars indicate the location of salinity/temperature datasondes.

Fig. 3.2. *Pterois volitans* / *P. miles* utilizing anthropogenically created habitat in the Loxahatchee River (corrugated seawall under a dock).

Fig. 3.3. *Pterois volitans* / *P. miles*. Size distribution of 145 lionfish captured during four primary sampling events along the north shoreline of the Loxahatchee River (Jupiter, FL) in August 2010 ($n = 54$), October 2010 ($n = 24$), January 2011 ($n = 18$), and April 2011 ($n = 49$). An additional 66 lionfish were opportunistically captured along the south shoreline between August 2010 and April 2011, but are not included here due to the temporal variability of this sampling.

References

- Adams AJ, Dahlgren CP, Kellison GT, Kendall MS, Layman CA, Ley JA, Nagelkerken I, Serafy JE (2006) Nursery function of tropical back-reef systems. *Marine Ecology Progress Series* 318:287-301
- Albins MA, Hixon MA (2008) Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. *Marine Ecology Progress Series* 367:233-238
- Arrington DA, Winemiller KO, Loftus WF, Akin S (2002) How often do fishes “run on empty”? *Ecology* 83:2145–2151
- Barbour AB, Montgomery ML, Adamson AA, Diaz-Ferguson E, Silliman BR (2010) Mangrove use by the invasive lionfish *Pterois volitans*. *Marine Ecology Progress Series* 401:291-294
- Beck MW, Heck KL, Jr, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* 51:633-641
- Byrnes JE, Reynolds PL, Stachowicz JJ (2007) Invasions and Extinctions Reshape Coastal Marine Food Webs. *Plos One* 2:1-7. doi:e29510.1371/journal.pone.0000295
- Cohen AN, Carlton JT (1998) Accelerating invasion rate in a highly invaded estuary. *Science* 279:555-558
- Cortes E (1997) A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Canadian Journal of Fisheries and Aquatic Sciences* 54:726-738
- Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. *Nature* 387:253-260
- Côté IM, Maljković A (2010) Predation rates of Indo-Pacific lionfish on Bahamian coral reefs. *Marine Ecology Progress Series* 404:219-225. doi:10.3354/meps08458
- Courrat A, Lobry J, Nicolas D, Laffargue P, Amara R, Lepage M, Girardin M, Le Pape O (2009) Anthropogenic disturbance on nursery function of estuarine areas for marine species. *Estuarine Coastal and Shelf Science* 81:179-190. doi:10.1016/j.ecss.2008.10.017

- Dorenbosch M, Grol MGG, de Groene A, van der Velde G, Nagelkerken I (2009) Piscivore assemblages and predation pressure affect relative safety of some back-reef habitats for juvenile fish in a Caribbean bay. *Marine Ecology Progress Series* 379:181-196. doi:10.3354/meps07896
- Fishelson L (1997) Experiments and observations on food consumption, growth and starvation in *Dendrochirus brachypterus* and *Pterois volitans* (Pteroinae, Scorpaenidae). *Environmental Biology of Fishes* 50:391-403
- Freshwater DW, Hines A, Parham S, Wilbur A, Sabaoun M, Woodhead J, Akins L, Purdy B, Whitfield PE, Paris CB (2009) Mitochondrial control region sequence analyses indicate dispersal from the US East Coast as the source of the invasive Indo-Pacific lionfish *Pterois volitans* in the Bahamas. *Marine Biology* 156:1213-1221. doi:10.1007/s00227-009-1163-8
- Granek EF, Polasky S, Kappel CV, Reed DJ, Stoms DM, Koch EW, Kennedy CJ, Cramer LA, Hacker SD, Barbier EB, Aswani S, Ruckelshaus M, Perillo GME, Silliman BR, Muthiga N, Bael D, Wolanski E (2010) Ecosystem services as a common language for coastal ecosystem-based management. *Conservation Biology* 24:207-216. doi:10.1111/j.1523-1739.2009.01355.x
- Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. *Science* 319:948-952. doi:10.1126/science.1149345
- Hamner RM, Freshwater DW, Whitfield PE (2007) Mitochondrial cytochrome b analysis reveals two invasive lionfish species with strong founder effects in the western Atlantic. *Journal of Fish Biology* 71:214-222. doi:10.1111/j.1095-8649.2007.01575.x
- Hornstra HM, Herrel A, Montgomery WL (2004) Gas bladder movement in lionfishes: A novel mechanism for control of pitch. *Journal of Morphology* 260:299-300
- Hynes HBN (1950) The food of fresh-water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*) with a review of methods used in the studies of the food of fishes. *Journal of Animal Ecology* 19:36-58
- Hyslop EJ (1980) Stomach contents analysis - a review of methods and their application. *Journal of Fish Biology* 17:411-429
- Jud ZJ, Layman CA, Shenker JM (2011) Diet of age-0 tarpon (*Megalops atlanticus*) in anthropogenically-modified and natural nursery habitats along the Indian River Lagoon, Florida. *Environmental Biology of Fishes* 90:223-233

- Kimball ME, Miller JM, Whitfield PE, Hare JA (2004) Thermal tolerance and potential distribution of invasive lionfish (*Pterois volitans/miles* complex) on the east coast of the United States. *Marine Ecology Progress Series* 283:269-278
- Layman CA, Quattrochi JP, Peyer CM, Allgeier JE (2007) Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecology Letters* 10:937-944. doi:10.1111/j.1461-0248.2007.01087.x
- Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312:1806-1809. doi:10.1126/science.1128035
- Maljković A, van Leeuwen TE, Cove SN (2008) Predation on the invasive red lionfish, *Pterois volitans* (Pisces: Scorpaenidae), by native groupers in the Bahamas. *Coral Reefs* 27:501. doi:10.1007/s00338-008-0372-9
- Morris JA, Jr, Akins JL (2009) Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago. *Environmental Biology of Fishes* 86:389-398. doi:10.1007/s10641-009-9538-8
- Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: Mechanisms, extent, and consequences. *American Zoologist* 37:621-632
- Schofield PJ (2009) Geographic extent and chronology of the invasion of non-native lionfish (*Pterois volitans* [Linnaeus 1758] and *P. miles* [Bennett 1828]) in the Western North Atlantic and Caribbean Sea. *Aquatic Invasions* 4:473-479
- Sheehy DJ, Vik SF (2010) The role of constructed reefs in non-indigenous species introductions and range expansions. *Ecological Engineering* 36:1-11. doi:10.1016/j.ecoleng.2009.09.012
- Simpson J, Brown J, Matthews J, Allen G (1990) Tidal straining, density currents, and stirring in the control of estuarine stratification. *Estuaries and Coasts* 13:125-132
- Sutherland WJ, Clout M, Côté IM, Daszak P, Depledge MH, Fellman L, Fleishman E, Garthwaite R, Gibbons DW, De Lurio J, Impey AJ, Lickorish F, Lindenmayer D, Madgwick J, Margerison C, Maynard T, Peck LS, Pretty J, Prior S, Redford KH, Scharlemann JPW, Spalding M, Watkinson AR (2010) A horizon scan of global conservation issues for 2010. *Trends in Ecology & Evolution* 25:1-7
- Valentine-Rose L, Layman CA, Arrington DA, Rypel AL (2007) Habitat fragmentation decreases fish secondary production in Bahamian tidal creeks. *Bulletin of Marine Science* 80:863-877

VanArman J, Graves GA, Fike D (2005) Loxahatchee watershed conceptual ecological model. *Wetlands* 25:926-942

Whitfield PE, Gardner T, Vives SP, Gilligan MR, Courtenay WR, Jr, Ray GC, Hare JA (2002) Biological invasion of the Indo-Pacific lionfish *Pterois volitans* along the Atlantic coast of North America. *Marine Ecology Progress Series* 235:289-297

CHAPTER IV

SITE FIDELITY AND MOVEMENT PATTERNS OF INVASIVE LIONFISH

***PTEROIS* SPP. IN A FLORIDA ESTUARY**

Jud ZR, Layman CA (2012) Site fidelity and movement patterns of invasive lionfish, *Pterois* spp., in a Florida estuary. Journal of Experimental Marine Biology and Ecology 414-415:69-74

Abstract

Understanding how individuals within a population of invasive organisms disperse during various life history stages has obvious implications for long term population dynamics in the invaded range. With the rapid expansion of the invasive Indo-Pacific lionfish (*Pterois volitans* and *P. miles*) in the western Atlantic and Caribbean, it has become increasingly important to understand how individuals move following initial recruitment as this may have critical implications for population control and management. We conducted a 10-month mark-recapture study in the lower Loxahatchee River estuary (Florida, USA) to identify movement patterns and site fidelity in juvenile and young adult lionfish. We tagged 55 lionfish, ranging in size from 45-185 mm standard length (66-256 mm total length). Eighty percent of the tagged fish were recaptured at least one time during the course of the study. Lionfish in this system exhibited extremely high site fidelity over extended periods of time and across multiple size classes. Maximum range occupied by individuals along the shoreline of the estuary was small (mean = 28 m, asymmetrical 95% CI: 10 to 51 m), and did not vary with lionfish size. The majority of lionfish recaptures (74%) occurred at or near (0-10 m) the previous capture site, even after weeks or months at liberty. In systems where lionfish exhibit extremely high site fidelity and small maximum ranges, localized population control may be feasible, since lionfish removed from a given habitat would be replaced largely through larval recruitment rather than migration of older individuals. However, since lionfish grow extremely rapidly (averaging 0.46 mm/day, but reaching as high as 0.78 mm/day in one individual), localized control efforts would need to be carried out frequently in order to maintain a younger, smaller population. Localized control may be

less effective if lionfish exhibit greater movement and lower site fidelity in other invaded systems.

Introduction

Patterns of dispersal through ontogeny play an important role in the establishment and spread of invasive organisms (Carlton 1989; Kolar and Lodge 2001; Wilson et al. 2009). In marine systems, factors associated with reproduction and early life history (e.g., spawning frequency, egg and larval dispersal, larval survival, settlement behavior), combined with some type of anthropogenic dispersal vector, are typically believed to drive the initial distribution of invasive organisms (Carlton and Geller 1993; Carlton 1996; Ruiz et al. 1997). However, movements that occur during later life history stages can also influence the distribution and population structure of invasive species over time. Understanding how individuals within a population of invasive organisms move following initial recruitment has a number of implications related to long term dispersal, as well as control and eradication (Brown et al. 2006; Cookingham and Ruetz 2008; Lapointe et al. 2010; Vrieze et al. 2011).

The Indo-Pacific lionfish *Pterois volitans* and *P. miles* (morphologically indistinguishable species, hereafter referred to as lionfish) have spread rapidly throughout the western Atlantic and Caribbean (Whitfield et al. 2002; Hamner et al. 2007; Freshwater et al. 2009; Schofield 2009). Several parameters associated with lionfish dispersal during early life history have been documented, including spawning frequency, gamete production, and pelagic larval duration (Ahrenholz and Morris 2010; Morris et al. 2011). High fecundity, combined with an approximately 26-day pelagic larval phase, likely led to the rapid and widespread dispersal of the species throughout their invaded range. However, little is known about post-recruitment movement patterns in lionfish, despite the fact that these movements may affect population dynamics. While existing

lionfish population models include parameters related to recruitment and early life history processes, they do not account for movement of individuals following recruitment (Barbour et al. 2010a; Morris et al. 2010). Several short-term foraging studies (Albins and Hixon 2008; Côté and Maljković 2010; Green et al. 2011) have inferred that lionfish do not typically undertake large movements during or between foraging bouts. However, these studies were not designed to track lionfish movement over extended periods of time (weeks to months).

The goal of this study was to examine movement patterns of post-recruitment lionfish at a temporal scale of weeks to months. Our specific objectives were to (1) identify level of site fidelity among lionfish, and (2) determine whether maximum range occupied was a function of body size. Additionally, we used recapture data to identify daily growth rates across a range of lionfish sizes. Such data may play a significant role in the design and implementation of future management and eradication plans, as lionfish site fidelity and movement patterns may ultimately drive the success of localized control efforts. Although lionfish movements may differ among invaded systems, our findings provide a starting point for similar studies in other more complex habitats.

Methods

To identify lionfish movement patterns, we conducted a mark-recapture study in the lower Loxahatchee River estuary (26°57' N, 80°06' W), near Jupiter, Florida (VanArman et al. 2005). The lower portion of this estuary is heavily marine influenced, with semidiurnal tides pushing ocean water into the system through Jupiter Inlet. Substrate in the lower Loxahatchee estuary is primarily sand, with structurally complex

habitats (e.g., seagrass, mangroves, human-made structures) restricted to shoreline areas (Jud et al. 2011). Along the section of shoreline that we utilized for this study, structurally complex habitats (mostly small artificial reefs and docks) were discrete and patchy, rather than continuous, and were separated by an average of 30 m (range: 6-97 m) of bare sand bottom. Because of the linear nature of the system, habitats could be classified along an estuarine (i.e., further upstream) to marine (i.e., further downstream) gradient. Compared to other frequently invaded habitats (e.g., coral reefs), the patchy nature and linear arrangement of habitats, shallow water depth, and proximity to shore made a tagging study in the Loxahatchee estuary logistically easier to conduct. Although lionfish are typically considered reef fish, their presence in an estuary is not surprising given that they are commonly found in nonreef habitats (e.g., turbid bays) in their native range (A. Anton, Unpublished results).

We tagged lionfish along a section of the south shoreline of the Loxahatchee estuary located between 2.0 and 3.7 km from the ocean (Fig 4.1). Although lionfish have been found further upriver in the Loxahatchee estuary, we chose this section because it was similar to some other habitats (e.g., mangroves, coastal rock jetties, shallow artificial reefs, canals) that have been invaded by lionfish (Morris and Akins 2009; Barbour et al. 2010b; Biggs and Olden 2011). We sampled this section of shoreline at least one time per month from September 2010 to July 2011 (except February and March 2011). During each daytime sampling event, we visually surveyed an ~30 m wide belt along the entire section of shoreline while snorkeling. We attempted to capture and tag all untagged lionfish that were observed during the visual surveys.

Lionfish were captured using hand nets and anesthetized using tricaine methanesulfonate (MS-222) mixed with aerated seawater (100 mg/l). Standard length (SL) and total length (TL) were measured. Fish were then tagged using Floy fingerling tags (FTF-69, Floy Tag & Mfg.). These 6.4 x 3.2 mm plastic tags were sutured into the dorsal musculature between the spinous and soft dorsal fins and secured with a single overhand knot (Fig. 4.2). Slack was left in the loop of suture to allow for growth. Each tag contained a unique three digit number. Additionally, to facilitate underwater visual identification of individual tagged fish, color-coded glass beads (~3 mm diameter) were added to the loop of suture material. Depending on body size, each fish received between zero and three glass beads. The arrangement of bead colors was unique to each fish and could be readily identified while snorkeling. Tag retention rates using this method were not directly tested, but no tag shed was observed in two caged individuals over a period of ~3 months, and only two untagged individuals captured in the field had scars that were indicative of tag loss. Following tagging, fish were placed into aerated seawater until fully recovered, and then returned to their exact capture location. GPS was used to record the location of each tagging site. To document the exact position of a fish within the tagging site, we precisely described various habitat characteristics (e.g., specific rocks, sponges, human-made items) that were immediately adjacent to the individual at the time of capture.

Recaptures of tagged lionfish during visual surveys were divided into two categories; (1) visual sightings and (2) physical recaptures. Visual sightings occurred when the identity and exact location of a previously tagged lionfish could be determined while snorkeling, without physically handling or removing the fish from the water.

Physical recapture of lionfish with hand nets was necessary to acquire positive identification when tags became obscured by the growth of fouling organisms. These individuals were briefly removed from the water to verify identity, and then returned to their exact capture site. We used this opportunity to clean the tags and measure individuals to calculate growth rates. For the remainder of the paper, these two categories will be referred to jointly as “recaptures” (i.e., including both visual sightings and physical recaptures). By lumping visual sightings and physical recaptures for analysis purposes, we provide more fine-scale spatial and temporal detail regarding movement patterns.

In addition to surveying the study area along the south shoreline, we concurrently conducted extensive visual surveys for lionfish in other sections of the estuary (Fig. 4.1) as part of a separate study (Jud et al. 2011). These surveys were conducted every 11-12 weeks and would have allowed us to identify tagged lionfish that had migrated out of the core study area and into other parts of the estuary. At the conclusion of the tagging study in July 2011, all remaining tagged lionfish were collected.

For each lionfish recapture, we calculated the number of days that had passed since the previous capture. Since some fish were recaptured multiple times, we examined (1) movements that occurred during each discrete at-large period, and (2) total observed maximum range occupied between initial tagging and final recapture. We used digitalized aerial imagery to measure the straight-line distance moved between each recapture. All movements were categorized as upstream or downstream. Maximum range occupied was calculated by measuring the straight-line distance between the most upstream and the most downstream capture locations for each fish. While range

measurements (like home range) are typically reported as two-dimensional area values (Burt 1943; Hammerschlag-Peyer and Layman 2010), we report one-dimensional values (i.e., distance) for maximum range occupied because structurally complex habitats are arrayed in a relatively narrow band along the shoreline in the Loxahatchee estuary. Since all of our documented lionfish movements were along this linear shoreline, a one-dimensional interpretation of maximum range occupied simplifies comparison of habitat use among tagged individuals.

We calculated daily growth rates for all individuals that were physically recaptured and measured (change in SL / days at liberty). Regression analysis was used to quantify the relationship between lionfish size and daily growth rate. For this analysis, we chose to use estimated length at the midpoint of each at-large period ($\text{initial SL} + \text{final SL} * 0.5$) rather than using initial or final length. This allowed us to account for variability in time at liberty among individuals. Mean daily growth rate (calculated from all physical recaptures) was used to estimate SL at the time of each visual sighting, where exact measurements were lacking. Actual and estimated lengths at the time of each discrete recapture were used to compare direction of movement among 25 mm size classes. To relate body size to overall maximum range occupied, we first calculated SL at the midpoint of each individual's total at-large period (from initial tagging to final recapture). We then used these midpoint SL values to compare maximum range occupied across 25 mm size classes.

Results

Between September and November 2010, and April and May 2011, we tagged 55 lionfish in the Loxahatchee estuary (Table 4.1). Tagged fish ranged in size from 45 to 185 mm SL (66 to 256 mm TL), with a mean $\pm$ standard deviation of 102 ± 26 mm SL (144 ± 35 mm TL). Forty-four individuals were recaptured at least one time, representing an 80% recapture rate. Of the 44 recaptured individuals, 27 (61%) were recaptured once and 10 (23%) were captured twice. The remaining seven individuals (16%) were recaptured 3-5 times each. In total, 73 discrete recapture events were recorded during the course of the study. Thirty-eight of these were visual sightings, and 35 were physical recaptures. The mean total time at liberty ($\pm$ standard deviation) for the 44 recaptured individuals was 56 ± 44 days (from initial tagging to final recapture). Two individuals were recaptured 197 days after they were tagged, the longest period at liberty.

The majority of lionfish did not move between captures. Out of 73 discrete recapture events, 41 (56%) represented fish that had remained in the exact location (± 0.5 m) since their previous capture, and an additional 13 (18%) were fish that had moved less than 10 m (Fig. 4.3). Only two recaptures (3%) represented movements of more than 100 m. The greatest distance moved during any single at-liberty period was 420 m in 67 days by a 126 mm SL individual. All recaptured lionfish were located along the south shoreline of the estuary, where tagging had been carried out, and all were found in structurally complex habitats. Concurrent with this study, we thoroughly surveyed a 5.5 km section of the north shoreline of the estuary on multiple occasions, killing >200 lionfish (Jud et al. 2011). We did not detect any tagged lionfish during these surveys.

Regression analysis was conducted to establish the relationship between lionfish length (SL) in mm, and daily growth rate (G) in mm/day, for all lionfish that were physically recaptured and measured, resulting in the equation:

$$G = -0.0019SL + 0.6587 \quad (n = 35, R^2 = 0.14, P < 0.05: \text{Fig. 4.4})$$

The mean daily growth rate ($\pm$ standard deviation) based on 35 physical recaptures (representing 28 individuals) was 0.46 ± 0.13 mm/day. The most rapid growth rate was 0.78 mm/day in an individual that grew from 68 to 86 mm SL in 23 days (Fig. 4.4).

We used the mean daily growth rate value to estimate SL for each of the 38 visual sightings, allowing us to examine movement patterns based on size for all 73 discrete recaptures. For most size classes, the greatest proportion of individuals remained stationary between captures (Fig. 4.5). When movements did occur, downstream movements were more frequent than upstream; however, the magnitude of most movements was small. Of the ten longest discrete movements that we observed (≥ 30 m), nine were in an upstream-to-downstream direction. The individuals that made these longer movements ranged in size from 80-146 mm (mean $\pm$ SD: 116 ± 24 mm), and were tagged throughout the study area. Direction of movement appeared to vary between seasons. While the frequency of downstream movements (including the small number of longer movements) was similar between fall/early winter and spring/early summer, there were fewer upstream movements during the spring/early summer period.

The mean observed maximum range occupied for the 44 individuals that were recaptured was 28 m (asymmetrical 95% confidence interval: 10 to 51 m). Twenty-one

individuals (48%) had maximum ranges of ≤ 0.5 m (i.e., ~ 0 m) during their entire at-large period. Another nine fish (20%) had maximum ranges of ≤ 6 m. Only one individual had a maximum range of > 150 m. Maximum range occupied did not vary among 25 mm size classes (Kruskal-Wallis; $H=1.54$, $P=0.67$). For six (35%) of the 17 individuals that were recaptured more than once, total observed maximum range occupied was a product of multiple downstream movements. Four individuals (24%) made a combination of upstream and downstream movements while at liberty, four (24%) remained stationary, and three (17%) made multiple upstream movements.

Discussion

Lionfish in the Loxahatchee estuary appear to exhibit extremely high site fidelity over extended periods of time and across multiple size classes. We found that a large percentage of tagged individuals were recaptured at the same location (often within a few cm) as their previous capture, even after weeks or months at liberty. Based on our exceptionally high recapture rate (80%), these movement patterns are likely representative of the local estuarine lionfish population. While tagged lionfish were observed moving from one patch of suitable habitat to another along the south shoreline of the estuary, we did not document any individuals moving across the estuary to the north shoreline. To do so would require crossing 300-700 m of featureless sand bottom.

A number of factors may contribute to the high site fidelity that we observed in lionfish. Extreme prey naïveté and enemy release, which often occur following the introduction of a novel predator (Sih et al. 2010), could influence lionfish movement patterns. Native prey species may not recognize lionfish as predators, allowing the

invaders to successfully hunt from a fixed location without having to actively forage for elusive prey. Furthermore, lionfish may not be recognized as prey by native predators, reducing the frequency of movements associated with predator avoidance. Alternatively, lionfish may forage at night, returning to a fixed resting spot during daylight hours, when all of our observations were made. While lionfish on invaded coral reefs are most active around sunrise and sunset, they do not appear to travel far during most foraging bouts (Côté and Maljković 2010; Green et al. 2011), suggesting that our daytime observations provide an accurate estimate of long-term habitat use.

Invasive lionfish have been shown to occupy a very wide variety of habitats, including the sea floor at depths of 300 m, offshore and nearshore coral reefs, inshore seagrass, mangrove, and human-made habitats, and even estuarine habitats up to 5.5 km from the ocean (Barbour et al. 2010b; Albins and Hixon 2011; Biggs and Olden 2011; Jud et al. 2011). While ontogenetic shifts in habitat use have been documented in many species of reef fishes, with the most common shifts occurring between inshore nursery habitats (e.g., estuaries, mangrove forests, sea grass beds) and offshore adult habitats (e.g., coral reefs) (de la Moriniere et al. 2002; Gillanders et al. 2003; Mumby et al. 2004; Adams et al. 2006; Verweij et al. 2007; Grol et al. 2011), it is not presently known how lionfish use different habitats through ontogeny. Although the spatial and temporal scale of this study prevented any definitive conclusions from being drawn regarding ontogenetic habitat shifts in lionfish, we were able to provide some initial observations about habitat use in the Loxahatchee estuary across the range of sizes that we tagged. Because we did not observe a positive relationship between maximum range occupied and lionfish body size, nor did we see strong evidence of incremental downstream

movements with increasing size, it seems likely that juvenile and young adult lionfish that initially settle in estuaries do not necessarily experience an inshore-to-offshore migration like many other marine fishes. However, since most of the larger movements we observed were in a downstream direction (regardless of fish size), it is possible that some individuals do eventually leave the estuary and enter the ocean.

As with any passive tagging study, we were only able to positively confirm the presence of individuals that were recaptured; the ultimate fate of tagged fish that were not recaptured was unknown (e.g., mortality, tag shed, long-distance migration). Tagging studies often underestimate (or completely fail to detect) long-distance movements of fishes, since recapture efforts usually occur at or near the initial tagging location (Gillanders et al. 2003). Since we did not search for tagged lionfish in the myriad offshore habitats adjacent to the Loxahatchee estuary, we are unable to reject the possibility that some individuals did move out of the system.

It is possible that lionfish larger than those tagged in this study may exhibit a different set of movement behaviors. The size range we tagged was a product of the relatively recent nature of the invasion in the Loxahatchee estuary (Jud et al. 2011). We tagged the largest lionfish that we observed in the newly invaded system (185 mm SL, 256 mm TL), but this was smaller than the maximum size obtained by lionfish in the western Atlantic and Caribbean (483 mm TL; R. Straney, Unpublished results). However, since lionfish begin to reach sexual maturity at approximately 100 mm TL (~70 mm SL), our sample included both juveniles and young adults (Morris 2009). This suggests that sexual maturity alone does not trigger a shift towards offshore (i.e., coral reef) habitats for lionfish that initially recruit to inshore habitats.

The smallest lionfish we observed were almost always solitary. However, larger individuals were frequently found in groups of 2-10. Lionfish that were tagged as small solitary juveniles were often later recaptured at another location in the presence of several other individuals. It is not clear whether some specific habitat characteristic is causing these aggregations, or whether they are of a social origin. During the course of this study, we observed that lionfish are capable of making an audible noise when disturbed. Although sound production has not previously been documented in lionfish, other members of Scorpaenidae are known to be soniferous (Kasumyan 2008). It is possible that vocalization plays a role in the social behavior of lionfish (including aggregating behaviors), as is the case with other soniferous reef fishes (Tricas et al. 2006; Mann et al. 2009; Nelson et al. 2011).

The Loxahatchee estuary represents a simple linear system in which to develop an initial understanding of how lionfish move through their environment. We feel that the same tagging effort in a more complex three-dimensional habitat (e.g., a continuous tract of coral reef) would have resulted in much lower recapture rates due to the difficulties associated with thoroughly surveying such systems. Although habitats in the Loxahatchee estuary are similar to some other nearshore habitats that have been invaded by lionfish (mangroves, canals, small artificial reefs, etc.), the structural arrangement of these habitats is quite different from the continuous coral reefs where lionfish are frequently found. It is unclear how the movement patterns we documented in an estuarine system will compare to other invaded habitats, especially coral reefs. If the patterns we observed hold true in other systems, it seems likely that lionfish would

readily move between closely situated habitat patches (or within continuous habitats), but would be less likely to move across large open expanses between habitats patches.

Salinity variation is one factor that differentiates estuarine lionfish habitats from most other invaded systems. Although the upper portion of the Loxahatchee estuary does experience fluctuating salinity due to freshwater inflow, we do not believe that this influenced lionfish movement patterns in the lower portion of the estuary where tagging was carried out. A strong salt wedge was consistently present at our study site (Jud et al. 2011), and salinity in the lower portion of the water column was almost always the same as seawater (~35‰). Since we observed some upstream movements during the wettest part of the year (late summer to fall) as well as a reduction in upstream movements during the driest part of the year (spring to early summer), it seems unlikely that freshwater inflow alone was responsible for the downstream movements we documented.

While complete eradication of lionfish in the western Atlantic and Caribbean is unlikely (Barbour et al. 2010a; Morris et al. 2010), the post-recruitment movement patterns we identified may play an important role in the effectiveness of future lionfish management and control efforts in certain habitats. If the high site fidelity and small maximum ranges that we observed in the Loxahatchee estuary also occur in other invaded systems, these behavioral traits would likely increase the effectiveness of localized control measures, since lionfish removed from a given habitat would largely be replaced through larval recruitment alone, rather than a combination of recruitment and direct migration of older individuals. In systems where lionfish exhibit high site fidelity and small post-recruitment movements, intensive local removal over time could lead to populations of lionfish that are dominated by younger individuals, resulting in a smaller

ecological impact through reduced prey consumption and diminished reproductive capacity. However, at this time, lionfish movement patterns are not well understood in other invaded habitats so it isn't clear whether our findings will apply outside of estuarine systems. Based on our observations, successful localized control through continuous removal seems more likely for discrete or patchy habitats that are similar to those found in the Loxahatchee estuary (e.g., small and isolated natural or artificial reefs) as opposed to continuous and complex habitats like expansive fringing or barrier coral reefs. Because of the extremely rapid growth rate exhibited by lionfish, localized control efforts would need to be carried out frequently in order to maintain a younger, smaller population. For this reason, future management goals must maintain a realistic balance between the cost and effort needed to locally control lionfish populations and the actual benefit (ecological, economic, aesthetic, etc.) associated with reduced lionfish abundance.

Acknowledgments

We would like to thank J. Lee for assistance in the field and D. Sabin for creating maps of the study sites. Additionally, we appreciate the continued support and cooperation of numerous homeowners along the Loxahatchee estuary as well as the Loxahatchee River District. Lionfish were collected pursuant to Florida Fish and Wildlife Conservation Commission Permit # SAL-09-1118A-SR. Partial funding was provided by NSF OCE #0746164 and OCE #0940019.

Table 4.1. Movement and growth data for 55 tagged lionfish. In the “total days at large” column, values in parentheses reflect the number of days that were used to calculate daily growth rate when a length measurement was not taken at the time of the final sighting. D refers to downstream and U refers to upstream.

Tag #	Tagging date	# of recaptures	Initial SL (mm)	Total growth (mm)	Total days at large	Daily growth rate (mm/d)	Home range (m)	Direction of travel
700	9/2/2010	1	75	28	57	0.49	6	D
706	9/2/2010	5	110	37	87	0.43	1.2	U and D
707	9/2/2010	5	112	42	87	0.48	1.2	U and D
708	9/2/2010	3	116		55		0	
709	9/8/2010	0	118					
710	9/8/2010	1	112	20	49	0.41	25	U
711	9/8/2010	2	118		23		0	
712	9/8/2010	0	118					
701	9/8/2010	1	90	24	49	0.49	18	D
702	9/8/2010	1	83	21	49	0.43	0	
713	9/8/2010	1	121	21	49	0.43	1.5	U
714	9/26/2010	2	115	16	31	0.52	1.5	D
715	9/26/2010	3	100	38	120	0.32	12	U and D
716	9/26/2010	2	104	42	197	0.21	135	D
717	9/26/2010	0	123					
718	9/26/2010	2	125	16	31	0.52	18	U
719	9/26/2010	2	118		31		18	U
703	9/26/2010	4	78	14	88 (31)	0.45	147	D
720	9/26/2010	1	131		5		0	
721	9/26/2010	3	54	77	197	0.39	102	U and D
722	10/1/2010	0	126					
724	10/1/2010	2	50	30	58	0.52	45	D
723	10/1/2010	0	118					
726	10/27/2010	2	131	15	57	0.26	142	D
727	10/27/2010	0	127					
728	11/28/2010	3	126	23	158 (134)	0.17	4.5	U
750	4/11/2011	1	68	18	23	0.78	0.9	U
761	4/11/2011	2	99	43	91	0.47	0	
762	4/11/2011	2	85	44	91	0.48	0	
755	4/11/2011	1	73	13	23	0.57	13	D
756	4/11/2011	1	83		23		0	
751	4/11/2011	1	81	9	24	0.38	0	
764	4/11/2011	0	185					
765	4/11/2011	1	106		24		0	
757	4/11/2011	2	89	37	91	0.41	423	D

Table 4.1. Continued

Tag #	Tagging date	# of recaptures	Initial SL (mm)	Total growth (mm)	Total days at large	Daily growth rate (mm/d)	Home range (m)	Direction of travel
758	4/11/2011	1	97		24		0	
766	4/11/2011	1	118	12	24	0.50	0	
767	4/11/2011	1	122		24		0	
763	4/11/2011	1	118		24		0	
768	4/11/2011	1	125		24		0	
769	4/11/2011	1	107		24		0	
770	4/11/2011	1	97		24		0	
759	4/11/2011	1	78		24		0	
771	4/11/2011	1	114		24		1.8	D
772	4/11/2011	1	93		24		1.8	D
773	4/11/2011	1	119		24		0	
760	5/4/2011	1	93	40	68	0.59	96	D
774	5/4/2011	0	98					
775	5/4/2011	1	102	32	68	0.47	0	
776	5/4/2011	1	129	33	68	0.49	12	D
777	5/4/2011	0	124					
752	5/4/2011	0	50					
753	5/4/2011	0	52					
783	5/5/2011	1	76	45	68	0.66	0	
754	5/5/2011	1	45	40	68	0.59	0

Figure 4.1. Map of the Loxahatchee estuary, Jupiter, Florida (center of black box in inset map). The lionfish mark-recapture study was conducted along the south shoreline of the estuary, in the area indicated by hatching. Additional surveys were conducted along the north shoreline (dark gray shading) as part of a concurrent study (Jud et al. 2011). Both survey areas extended ~30 m from shore (not to scale).

Figure 4.2. Tagged lionfish (*Pterois* spp.), showing the oval Floy fingerling tag (FTF-69, Floy Tag & Mfg.) and two color-coded glass beads, sutured into the dorsal musculature between the spinous and soft dorsal fins. A three digit number is printed on the reverse of the Floy tag.

Figure 4.3. Distance moved between captures for 73 discrete recapture events. Mean time at liberty (number of days between discrete recaptures) is presented above each distance category.

Figure 4.4. Regression of lionfish standard length (SL; mm) versus daily growth rate (mm/day) based on 35 physical recaptures. Linear regression fit is shown. Standard lengths were estimated at the midpoint of each at-large period (initial SL + final SL * 0.5).

Figure 4.5. Direction of movement versus lionfish standard length (at time of recapture) based on 73 unique recapture events. Standard length was directly measured for physical recaptures, and estimated for visual sightings using a calculated daily growth rate of 0.46 mm/day.

References

- Adams AJ, Dahlgren CP, Kellison GT, Kendall MS, Layman CA, Ley JA, Nagelkerken I, Serafy JE (2006) Nursery function of tropical back-reef systems. *Marine Ecology Progress Series* 318:287-301
- Ahrenholz DW, Morris JA, Jr (2010) Larval duration of the lionfish, *Pterois volitans* along the Bahamian Archipelago. *Environmental Biology of Fishes* 88:305-309
- Albins M, Hixon M (2011) Worst case scenario: potential long-term effects of invasive predatory lionfish (*Pterois volitans*) on Atlantic and Caribbean coral-reef communities. *Environmental Biology of Fishes*:1-7
- Albins MA, Hixon MA (2008) Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. *Marine Ecology Progress Series* 367:233-238
- Barbour AB, Allen MS, Frazer TK, Sherman KD (2010a) Evaluating the potential efficacy of invasive lionfish (*Pterois volitans*) removals. *Plos One* 6:1-7
- Barbour AB, Montgomery ML, Adamson AA, Diaz-Ferguson E, Silliman BR (2010b) Mangrove use by the invasive lionfish *Pterois volitans*. *Marine Ecology Progress Series* 401:291-294
- Biggs CR, Olden JD (2011) Multi-scale habitat occupancy of invasive lionfish (*Pterois volitans*) in coral reef environments of Roatan, Honduras. *Aquatic Invasions* 6:447-453
- Brown GP, Phillips BL, Webb JK, Shine R (2006) Toad on the road: Use of roads as dispersal corridors by cane toads (*Bufo marinus*) at an invasion front in tropical Australia. *Biological Conservation* 133:88-94
- Burt WH (1943) Territoriality and home range concepts as applied to mammals. *Journal of Mammalogy* 24:346-352
- Carlton JT (1989) Man's role in changing the face of the ocean: Biological invasions and implications for conservation of near-shore environments. *Conservation Biology* 3:265-273
- Carlton JT (1996) Pattern, process, and prediction in marine invasion ecology. *Biological Conservation* 78:97-106
- Carlton JT, Geller JB (1993) Ecological roulette - the global transport of nonindigenous marine organisms. *Science* 261:78-82
- Cookingham MN, Ruetz CR, III (2008) Evaluating passive integrated transponder tags for tracking movements of round gobies. *Ecology Of Freshwater Fish* 17:303-311

- Côté IM, Maljković A (2010) Predation rates of Indo-Pacific lionfish on Bahamian coral reefs. *Marine Ecology Progress Series* 404:219-225. doi:10.3354/meps08458
- de la Moriniere EC, Pollux BJA, Nagelkerken I, van der Velde G (2002) Post-settlement life cycle migration patterns and habitat preference of coral reef fish that use seagrass and mangrove habitats as nurseries. *Estuarine Coastal and Shelf Science* 55:309-321. doi:10.1006/ecss.2001.0907
- Freshwater DW, Hines A, Parham S, Wilbur A, Sabaoun M, Woodhead J, Akins L, Purdy B, Whitfield PE, Paris CB (2009) Mitochondrial control region sequence analyses indicate dispersal from the US East Coast as the source of the invasive Indo-Pacific lionfish *Pterois volitans* in the Bahamas. *Marine Biology* 156:1213-1221. doi:10.1007/s00227-009-1163-8
- Gillanders BM, Able KW, Brown JA, Eggleston DB, Sheridan PF (2003) Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries. *Marine Ecology Progress Series* 247:281-295. doi:10.3354/meps247281
- Green S, Akins J, Côté I (2011) Foraging behaviour and prey consumption in the Indo-Pacific lionfish on Bahamian coral reefs. *Marine Ecology Progress Series* 433:159-167
- Grol M, Nagelkerken I, Rypel A, Layman C (2011) Simple ecological trade-offs give rise to emergent cross-ecosystem distributions of a coral reef fish. *Oecologia* 165:79-88
- Hammerschlag-Peyer CM, Layman CA (2010) Intrapopulation variation in habitat use by two abundant coastal fish species. *Marine Ecology Progress Series* 415:211-220
- Hamner RM, Freshwater DW, Whitfield PE (2007) Mitochondrial cytochrome b analysis reveals two invasive lionfish species with strong founder effects in the western Atlantic. *Journal of Fish Biology* 71:214-222. doi:10.1111/j.1095-8649.2007.01575.x
- Jud ZR, Layman CA, Lee JA, Arrington DA (2011) Recent invasion of a Florida (USA) estuarine system by lionfish *Pterois volitans* / *P. miles*. *Aquatic Biology* 13:21-26. doi:10.3354/ab00351
- Kasumyan A (2008) Sounds and sound production in fishes. *Journal of Ichthyology* 48:981-1030
- Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16:199-204

- Lapointe NWR, Thorson JT, Angermeier PL (2010) Seasonal meso- and microhabitat selection by the northern snakehead (*Channa argus*) in the Potomac river system. *Ecology Of Freshwater Fish* 19:566-577
- Mann D, Locascio J, Coleman F, Koenig C (2009) Goliath grouper *Epinephelus itajara* sound production and movement patterns on aggregation sites. *Endangered Species Research* 7:229-236
- Morris JA, Jr (2009) The biology and ecology of the invasive Indo-Pacific lionfish. Ph.D. dissertation, North Carolina State University, Raleigh, NC
- Morris JA, Jr, Akins JL (2009) Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago. *Environmental Biology of Fishes* 86:389-398. doi:10.1007/s10641-009-9538-8
- Morris JA, Jr, Shertzer KW, Rice JA (2010) A stage-based matrix population model of invasive lionfish with implications for control. *Biological Invasions* 13:7-12
- Morris JA, Jr, Sullivan CV, Govoni JJ (2011) Oogenesis and spawn formation in the invasive lionfish, *Pterois miles* and *Pterois volitans*. *Scientia Marina* 75:147-154. doi:10.3989/scimar.2011.75n1147
- Mumby PJ, Edwards AJ, Arias-Gonzalez JE, Lindeman KC, Blackwell PG, Gall A, Gorczynska MI, Harborne AR, Pescod CL, Renken H, Wabnitz CCC, Llewellyn G (2004) Mangroves enhance the biomass of coral reef fish communities in the Caribbean. *Nature* 427:533-536. doi:10.1038/nature02286
- Nelson MD, Koenig CC, Coleman FC, Mann DA (2011) Sound production of red grouper *Epinephelus morio* on the West Florida Shelf. *Aquatic Biology* 12:97-108. doi:10.3354/ab00325
- Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: Mechanisms, extent, and consequences. *American Zoologist* 37:621-632
- Schofield PJ (2009) Geographic extent and chronology of the invasion of non-native lionfish (*Pterois volitans* [Linnaeus 1758] and *P. miles* [Bennett 1828]) in the Western North Atlantic and Caribbean Sea. *Aquatic Invasions* 4:473-479
- Sih A, Bolnick DI, Luttbeg B, Orrock JL, Peacor SD, Pintor LM, Preisser E, Rehage JS, Vonesh JR (2010) Predator-prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos* 119:610-621
- Tricas TC, Kajiura SM, Kosaki RK (2006) Acoustic communication in territorial butterflyfish: test of the sound production hypothesis. *Journal of Experimental Biology* 209:4994-5004. doi:10.1242/jeb.02609

- VanArman J, Graves GA, Fike D (2005) Loxahatchee watershed conceptual ecological model. *Wetlands* 25:926-942
- Verweij MC, Nagelkerken I, Hol KEM, van den Beld A, van der Velde G (2007) Space use of *Lutjanus apodus* including movement between a putative nursery and a coral reef. *Bulletin of Marine Science* 81:127-138
- Vrieze L, Bergstedt R, Sorensen P (2011) Olfactory-mediated stream-finding behavior of migratory adult sea lamprey (*Petromyzon marinus*). *Canadian Journal of Fisheries and Aquatic Sciences* 68:523-533
- Whitfield PE, Gardner T, Vives SP, Gilligan MR, Courtenay WR, Jr, Ray GC, Hare JA (2002) Biological invasion of the Indo-Pacific lionfish *Pterois volitans* along the Atlantic coast of North America. *Marine Ecology Progress Series* 235:289-297
- Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM (2009) Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology & Evolution* 24:136-144. doi:10.1016/j.tree.2008.10.007

CHAPTER V

BROAD SALINITY TOLERANCE IN THE INVASIVE LIONFISH *PTEROIS* SPP. MAY FACILITATE ESTUARINE COLONIZATION

Jud ZR, Nichols PK, Layman CA (2014) Broad salinity tolerance in the invasive lionfish *Pterois* spp. may facilitate estuarine colonization. Environmental Biology of Fishes

Abstract

The ongoing invasion of non-native Indo-Pacific lionfish (*Pterois* spp.) represents a significant ecological threat throughout the Western Atlantic and Caribbean. As a generalist species, lionfish have been able to rapidly colonize a wide variety of ecosystems, including coral reefs, seagrass beds, mangroves, the sea floor at depths as great as 300 meters, and even brackish estuaries. While lionfish have been encountered in a number of estuarine systems, the spatial distribution of lionfish in estuaries is likely limited by the species' ability to tolerate low salinities. Here, we experimentally identify minimum salinity tolerance in lionfish by measuring survival salinity minimum – the lowest salinity at which all individuals survive for 48 hours. Additionally, we examine whether long-term exposure to low (but sub-lethal) salinities has negative effects on lionfish. Field observations in the Loxahatchee River estuary (Jupiter, FL) showed that lionfish can survive brief exposure to salinities as low as 1 ‰. At one estuarine location, fish survived exposure to salinity fluctuations of ~28 ‰ every 6 hours for several days. In laboratory trials, survival salinity minimum for lionfish was 5‰; however, some individuals survived at 4 ‰ for up to 94 hours before dying. Lionfish that were held at 7 ‰ for 28 days showed no differences in mortality, behavior or growth, when compared to control fish held at 35‰ (typical ocean salinity). This broad salinity tolerance may allow lionfish to colonize estuaries throughout their invaded range, and may facilitate dispersal across the Amazon-Orinoco plume. Because of the ecological and economic importance of estuaries, this facet of the lionfish invasion warrants further study.

Introduction

The rapid invasion of the Western Atlantic and Caribbean by the Indo-Pacific lionfish *Pterois volitans* and *P. miles* (morphologically indistinguishable species, hereafter referred to as lionfish) was likely facilitated by a number of behavioral and physiological traits possessed by the species (reviewed in: Albins and Hixon 2011). Lionfish are habitat generalists, having been found to occupy a variety of habitats in the invaded range, including coral reefs, seagrass beds, mangroves, human-created habitats, and the ocean floor at depths as great as 300 m (Barbour et al. 2010; Biggs and Olden 2011; Claydon et al. 2012; Côté et al. 2013a). Additionally, as dietary generalists possessing feeding behaviors novel to the region, lionfish have proven to be very efficient predators of native species (Morris and Akins 2009; Green et al. 2011; Albins and Lyons 2012; Lonnstedt and McCormick 2013). Since the presence of invasive lionfish has been linked to severe declines in fish abundance in coral reef ecosystems (Albins and Hixon 2008; Green et al. 2012), the potential ecological and economic effects of lionfish in other invaded ecosystems is of great concern.

In 2010, we identified lionfish utilizing estuarine habitats in the Loxahatchee River, near Jupiter, FL (Jud et al. 2011; Jud and Layman 2012). Since estuaries provide critical nursery habitats for numerous ecologically and economically important species (Beck et al. 2001; Courrat et al. 2009), the presence of an invasive and highly successful generalist predator in these systems is troubling. To date, we have observed lionfish as far as 6.6 km from the ocean in the Loxahatchee River, in salinities as low as 8 ‰ (Z. Jud, unpubl. data). However, based on limited field observations, we were unable to speculate about the range of salinities that lionfish can tolerate. Additionally, the long-

term effects of low (but sub-lethal) salinity on lionfish behavior, growth, and survival may ultimately determine the distribution of lionfish in estuarine systems. While predictions of future range expansion in lionfish have been based primarily on thermal tolerance (Kimball et al. 2004; Morris and Whitfield 2009), salinity tolerance may also be an important factor controlling the eventual distribution of the species. Of particular importance, salinity tolerance may determine whether the Amazon-Orinoco plume will act as a barrier to the southward spread of lionfish along the Atlantic coast of South America.

Herein, we utilize a series of laboratory experiments to determine how reduced salinities in estuarine ecosystems may affect invasive lionfish. Our objectives were twofold: First, we wanted to determine how long-term exposure to low (but sub-lethal) salinity may affect lionfish survival, growth, and behavior. Second, we wanted to identify the minimum salinity at which lionfish can survive for at least 48 hours. Preliminary field observations suggested that lionfish were able to survive in low salinities, and provided an estimate of minimum salinity tolerance. We then used these preliminary values as a starting point to more thoroughly test long- and short-term salinity tolerance in the laboratory.

Methods

Field Observations

Our initial observations of *in situ* salinity tolerance in lionfish occurred opportunistically during an unrelated caging experiment intended to assess lionfish trophic interactions (hereafter referred to as the *in situ* cage study). Although that study

was not specifically designed to test lionfish salinity tolerance, an unexpected period of heavy precipitation provided an opportunity to document the reaction of lionfish to varying salinities in a natural setting. These observations also allowed us to choose an appropriate sub-lethal salinity level to utilize during a subsequent laboratory trial aimed at addressing our first objective.

For the *in situ* cage study, we selected three sites in the Loxahatchee River, located 2.4 km (downstream site), 6.2 km (midstream site), and 7.0 km (upstream site) upriver from the ocean. At each site, eight cylindrical plastic mesh cages were deployed (55 cm diameter, 45 cm tall, 13 mm mesh). We added 20 l of limestone gravel (~20 mm diameter) and one small brick to each cage to provide shelter for lionfish prey (e.g., small crabs, shrimp, and fishes, which began to colonize cages immediately following deployment). Since this study was not originally intended to assess the effects of salinity on lionfish, cage design and site location were selected based on the objectives of the trophic interaction experiment mentioned above.

Twenty-four lionfish (76-155 mm standard length) were captured in Jupiter Inlet and the lower Loxahatchee River (Jupiter, FL). Salinities at the capture sites ranged from 24-36 ‰. Fish were divided into three groups, such that each group contained approximately the same size distribution of individuals. The groups were then placed into three temporary holding cages in the river. To allow fish to acclimate to the ambient salinity of each study site, we moved the temporary holding cages upriver in a series of incremental steps. This acclimation process took 3 days for the upstream site (where salinities were lowest), 2 days for the midstream site (with intermediate salinities), and 1 day for the downstream site (with highest salinities). We staggered the start dates of the

upstream movement/acclimation process by 1 day per site, so each set of fish would arrive at their respective study site on the same day. Following an additional 24 hours of acclimation at the study sites, fish were added individually to the experimental cages.

At each study site, we deployed a datasonde (Hydrolab DataSonde 5X, Hach Hydromet Inc.) that recorded salinity every 15 minutes. Since the Loxahatchee River frequently exhibits stratified conditions, such that highest salinities occur immediately above the benthos, datasondes were mounted ~4 cm above the river bottom, among the lionfish cages. All 24 caged lionfish were visually observed one time per day using mask and snorkel (~ 1 minute/cage), with observations occurring near high tide, when water clarity was greatest. Because extremely low water clarity made it difficult to see into cages, only simple behavioral observations could be made. We noted whether fish were alive and maintaining equilibrium, alive but lacking equilibrium, or dead. Although the ultimate cause of lionfish mortality during the *in situ* cage study was not known, we made the assumption that deaths, which were always preceded by loss of equilibrium, were a result of reduced salinity following the precipitation event. After 40 days, living lionfish were euthanized using MS-222 (tricaine methanesulfonate, 400 mg/l), weighed, and measured.

Laboratory Trials

To address our first objective, identifying the long-term effects of reduced salinity on lionfish survival, growth, and behavior, we exposed fish to a salinity of 7 ‰ for 28 days in a laboratory setting. We chose this salinity based on our findings from the *in situ* cage study (above), *in situ* observations of wild lionfish at 8 ‰ (Z. Jud, unpubl. data), as

well as the results of a small pilot study that showed lionfish could survive and feed at 6 ‰ for short periods of time (L. Arrington, unpubl. data). During the 28-day study, we looked for changes in behavior or mortality (compared to control fish housed at 35 ‰) that may have been caused by long-term exposure to low salinity. Additionally, we used growth rate (mm/day for standard length, g/day for mass) to assess potential physiological costs associated with living at low salinities.

In the laboratory, we set up eight pairs of 38 l glass aquaria. Each aquarium contained a sponge filter, and lighting was provided by banks of fluorescent tubes running on a 12:12 light cycle. Ambient room temperature was maintained at 25°C. Within each pair of aquaria, one tank (the control) was filled with 35 ‰ saltwater (obtained from a saltwater well), and the other with 7 ‰ saltwater (35 ‰ water, diluted with tap water and aerated for 24 hours to remove chlorine). Salinity was measured using a calibrated refractometer, and verified at the start of the study with a calibrated YSI Pro2030 (YSI Inc.).

Sixteen lionfish were captured in Jupiter Inlet and the lower Loxahatchee River estuary using hand nets. Salinities at the time of capture were 27-35 ‰. These fish were transported in 32 ‰ water from the field to the laboratory, where they were divided into two groups based on approximate body length, such that both groups contained approximately the same distribution of fish sizes. The two groups (which would become the control group and the low-salinity group) were temporarily placed into separate 140 l coolers equipped with electric aerators, where they were housed for a 72-hour period. During this time, food was withheld from all fish. Additionally, salinity in one of the two coolers was slowly lowered from 32 ‰ to 7 ‰ in ~4 ‰ increments through the addition

of dechlorinated tap water every 12 hours. After 72 hours of fasting (and salinity acclimation for the eventual low-salinity group), fish were sedated using MS-222 mixed with aerated seawater (100 mg/l), weighed (blotted wet weight), and measured for standard length (SL) and total length (TL). Withholding food from fish prior to weighing minimized the effects of stomach contents on body mass.

Lionfish used in the long-term laboratory salinity trials ranged in size from 54 to 142 mm SL, with TL ranging from 77 to 188 mm (Table 5.1). There was no difference in mean SL ($\pm$ standard deviation) between the control group (95 ± 31 mm) and the group that had been acclimated to a salinity of 7 ‰ (96 ± 34 mm) (2-sample t-test: $t_{(12)} = 0.08$, $p = 0.94$; SPSS v.16). From these two groups of fish, we created eight approximately size-matched pairs. Within each pair, one fish was placed into a tank containing the high-salinity control treatment (35 ‰) and the other was placed into an adjacent tank containing the low-salinity treatment (7 ‰). Each set of paired tanks was randomly assigned a location on a bank of aquarium racks to minimize location-based effects.

Lionfish were observed three times per day (morning, midday, evening – 5 minutes per observation), and all behavioral changes that may have been an indication of stress were documented (e.g., decreased feeding compared to control fish, cessation of fin movements, loss of equilibrium, death). To maintain water quality, 40 % water changes were conducted every other day. Ammonia and nitrite levels were tested daily. During the first half of the study (day 1-16), fish were fed every 3 days. Due to high ammonia and nitrite levels, feeding frequency was reduced to every 4 days during the second half of the study (day 16-28) to improve water quality. Although several different types of food were offered to lionfish during this study (e.g., feeder guppies, feeder goldfish,

feeder ghost shrimp), only one type of food was provided on each feeding day. Within lionfish pairs, both individuals were given approximately the same size prey item at each feeding to assure equal food intake. Since the lionfish we utilized encompassed a wide range of body sizes, we provided prey items that were $\sim 1/4$ to $1/3$ of lionfish TL. Lionfish were weighed and measured (using above protocol) on day 16 and day 28. Fish were fasted for 72 hours before being weighed. Two-sample t-tests were used to compare mean growth rates (changes in length and mass per day) between treatments (SPSS v.16).

At the culmination of the initial phase of the experiment (day 28), we began to slowly lower the salinity in each of the 7 ‰ treatment tanks in order to address objective 2. Our goal was to identify the survival salinity minimum (SS_{min} – the lowest salinity at which all individuals survive for 48 hours) for lionfish that had already been acclimated to low salinities (7 ‰) for an extended period of time (Jian et al. 2003; Cheng et al. 2013). Salinity was lowered by 1 ‰ (over a 10 minute period) every 48 hours (Woo and Chung 1995) through the addition of deionized water buffered to a pH of 8.3 (Marine Buffer, Seachem Laboratories Inc. 0.02 g/l). All lionfish were observed three times per day (morning, midday, evening – 1 minute per observation), in order to identify when an endpoint had been reached. The endpoint we originally planned to use for SS_{min} determination was complete loss of equilibrium in individual fish, as equilibrium loss has been observed to occur immediately before lionfish death during exposure to lethal salinities (Z. Jud, unpubl. data). Fish that had completely lost equilibrium were considered to be on the verge of death, at which point they were removed from the SS_{min} determination trial, and placed back into water with a salinity of 7 ‰, in order to

determine whether fish in this condition would recover if salinities rapidly rose (as would occur during an incoming tide in a natural system). Once SS_{min} had been exceeded (i.e., at least one fish had lost equilibrium or died), we stopped reducing salinities in the treatment aquaria in order to determine how long the remaining lionfish could survive at a salinity just below SS_{min} . While equilibrium loss was our intended endpoint, since five out of seven fish were found dead during daily observation periods, we used death as an endpoint in all but two cases. Upon completion of the study, all remaining fish were euthanized using MS-222 in aerated tank water (400 mg/l).

Results

Field Observations

At all three study sites, salinities fluctuated with each tidal cycle, rising with the flood tide, and falling with the ebb tide (the estuary experiences semi-diurnal tides). Approximately 3 days after we initiated the *in situ* lionfish caging study, the Loxahatchee River watershed experienced a 2-day period of heavy precipitation, causing salinities in the estuary to suddenly decrease. At the downstream site, salinity at low tide briefly dropped to 8-10 ‰ on four occasions immediately following the precipitation event (Fig. 5.1a). However, high-tide salinities during this period were 32-33 ‰. By day 10 of the study (~7 days after the start of the precipitation event) salinities had risen back to pre-rainfall levels. For the remainder of the 40-day study, salinities at the downstream site fluctuated between 20-36 ‰. We did not observe any mortality or loss of equilibrium in the fish caged at this site, even during the initial period of reduced salinity (Fig. 5.1a).

Compared to the downstream site, the midstream site exhibited considerable salinity variation within each tidal cycle. For the first 3 days of the study (through the first day of the precipitation event), the daily salinity range at this site was 4-30 ‰ depending on tidal phase (Fig. 5.1b). No lionfish mortality or loss of equilibrium was observed at these salinities, but most individuals gravitated towards the bottom of the cages. On days 4 and 5 of the study (during and shortly after the precipitation event), low-tide salinities fell below 2 ‰; however, high-tide salinities were 25-30 ‰ (Fig. 5.1b). We observed no mortality or loss of equilibrium, despite brief exposure to salinities below 2 ‰. On day 6 of the study, low-tide salinities dropped below 1 ‰, and all lionfish lost equilibrium (Fig. 5.1b). On the following day, all lionfish were dead.

While the upstream site was in relatively close proximity to the midstream site, freshwater inflow had a greater effect at this location due to the nature of the river channel. Prior to the precipitation event (days 1 and 2 of the study), daily salinities at this site ranged from 2-16 ‰ depending on tidal phase (Fig. 5.1c). There was no lionfish mortality or loss of equilibrium observed during this period, although fish were primarily found in the lower portion of their cage. Salinities dropped rapidly during the third day of the study (the first day of the rain event), ranging from 6 ‰ at high tide to 1 ‰ at low tide. No mortality or equilibrium loss was observed during this 24-hour period, despite salinities consistently below 6 ‰. By day 4 of the study, when low tide salinities fell below 1 ‰, all lionfish had lost equilibrium (Fig. 5.1c). All lionfish were dead by day 5.

Laboratory Trials

During laboratory trials aimed at addressing objective 1, we demonstrated that lionfish were able to survive for extended periods of time at low salinities (7 ‰). Fifteen of 16 lionfish lived for the full 28-day duration of the study. One fish in the high-salinity (35 ‰) control treatment died on day 20. With this exception, we did not observe any behavioral changes that may have been an indication of stress in either the high-salinity (35 ‰) control treatment or the low-salinity (7 ‰) treatment (through day 25 – see below). During daily observations, all fish in both salinity treatments appeared active, either swimming around their tank, or resting on the bottom (or against the side glass) while exhibiting steady rhythmic movements of the caudal, anal, and soft dorsal fins (two behavioral patterns that we considered “normal behavior” based on numerous observations of unstressed lionfish in the wild and non-experimental aquarium settings).

Until day 25 of 28, all fish in both salinity treatments ate immediately when offered food. Prey items were typically consumed within ~5 seconds of being placed into the water. On day 25 (the final time food was offered during the experiment), one fish in the high-salinity control treatment, and one fish in the low-salinity treatment, did not feed. Both of these fish exhibited a reduced level of activity, and increased gill ventilation rates. Upon microscopic examination of skin smears and gill biopsies, we determined that both fish were infected by the parasitic dinoflagellate *Amyloodinium ocellatum*. It is possible that the observed changes in behavior and the failure to feed were a result of this infection. With the exception of the two fish with *A. ocellatum* infections, no other behaviors indicative of stress were observed through the culmination of the experiment on day 28. Both infected fish were euthanized on day 28.

For the first 16 days of the study, mean daily growth rate ($\pm$ standard deviation) for length (SL) was identical between the high- and low-salinity treatment groups (0.13 ± 0.06 mm/day, range 0.06-0.25 mm/day for both treatments). Additionally, there was no significant difference in mean daily growth rate for mass between the low-salinity treatment (0.10 ± 0.15 g/day) and the high-salinity control treatment (0.03 ± 0.06 g/day) during this time period (2-sample t-test: $t_{(9)} = 1.33$, $p = 0.22$).

Between day 16 and day 28, lionfish in both salinity treatments showed little change in length. Only three of eight fish in the low-salinity treatment and one of seven fish in the high-salinity control treatment increased in length during this period, but these length increases were very small (Table 5.1). The four fish that increased in length were among the smallest individuals in the study. Length did not increase for the remaining 11 individuals. During this same period, 14 of the 15 remaining fish experienced a loss in mass (Table 5.1). There was no significant difference in mean daily mass loss between the low-salinity treatment (-0.13 ± 0.11 g/day) and the high-salinity control treatment (-0.14 ± 0.26 g/day) during the final 12 days of the study (2-sample t-test: $t_{(12)} = 0.08$, $p = 0.94$). Fish continued to feed normally during this period (with the exception of two fish on day 25 – see above), but were fed less frequently as a means of improving water quality.

Beginning on day 28, we began to slowly reduce salinities (in 1 ‰ increments every 48 hours) in the tanks holding the seven remaining low-salinity treatment fish (fish that had been exposed to 7 ‰ for the previous 28 days) in order to identify the minimum salinity at which lionfish can survive for at least 48 hours (objective 2). We did not observe any loss of equilibrium or death at salinities greater than 4 ‰. However, within

3 hours of lowering salinities to 4 ‰, two lionfish began to exhibit a sudden and severe loss of equilibrium, a lack of response to tactile stimulation, and a reduction in the frequency of opercular movements (Fig. 5.2). Since these two fish had reached our predetermined endpoint for the study (i.e., they lost equilibrium), we culminated their trials, and returned them to a salinity of 7 ‰. Within 3 hours, these fish had regained equilibrium, and were not exhibiting any behaviors indicative of stress. The remaining five lionfish did not exhibit acute signs of severe distress when salinities were lowered to 4 ‰. Three of these fish gradually became less active, eventually dying (without observed equilibrium loss) after 27-48 hours of exposure to salinities of 4 ‰ (Fig. 5.2). The final two fish survived for 78 and 94 hours at 4 ‰ before dying (without observed equilibrium loss). Since no loss of equilibrium or death was observed at salinities of 5 ‰ or greater, and all individuals reached an endpoint at 4 ‰, SS_{min} for lionfish appears to be ~ 5 ‰. Salinity tolerance did not appear to be affected by lionfish length within the size range we examined, as individuals of all lengths (56-146 mm SL) survived in salinities ≥ 5 ‰, but lost equilibrium or died at 4 ‰.

Discussion

In addition to being habitat (Whitfield et al. 2002; Biggs and Olden 2011; Claydon et al. 2012) and dietary generalists (Albins and Hixon 2008; Morris and Akins 2009; Layman and Allgeier 2012; Valdez-Moreno et al. 2012; Côté et al. 2013b), lionfish appear to be able to tolerate a broad range of salinities. Although not typically considered a euryhaline species, our data suggest lionfish can survive at low salinities (7 ‰) for at least one month without exhibiting any obvious changes in behavior, feeding,

or growth rate. While $SS_{\min}$ for lionfish appears to be ~ 5 ‰, ~ 70 % of the fish we tested in the laboratory survived at 4 ‰ for >24 hours (up to 94 hours for one individual). This, combined with our field observations, demonstrates the ability of lionfish to survive brief exposure to very low salinity conditions (i.e., ≤ 4 ‰). Fish that lost equilibrium at 4 ‰ in the laboratory recovered quickly when salinities were returned to 7 ‰ (similar to the salinity increases that can occur during incoming tides). In the wild, the influx of high-salinity water during the flood tide appears to allow lionfish to survive brief exposure to salinities as low as ~ 1 ‰ at low tide. At one of the estuarine sites in this study, lionfish experienced salinity fluctuations of ~ 28 ‰ every 6 hours without any short-term (i.e., over several days) loss of equilibrium or mortality. These findings suggest that lionfish may be able to colonize all but the lowest-salinity sections of estuaries throughout the invaded range.

While the ability of lionfish to survive in low-salinity environments is a novel discovery, a number of marine species typically regarded as stenohaline have been shown to be able to tolerate relatively low salinities (Wu and Woo 1983; Lambert et al. 1994; Woo and Chung 1995; Jian et al. 2003; Lee et al. 2005; Cheng et al. 2013; García et al. 2013). In a study examining salinity tolerance in marine fishes, including taxa from seven coral reef-associated families, Wu and Woo (1983) found that 12 of 13 species could survive at salinities ≤ 10 ‰ for two weeks, with six of those species tolerating salinities ≤ 5 ‰. The emperor angelfish (*Pomacanthus imperator*), a reef-associated species that co-occurs with lionfish through much of the Indo-Pacific, can survive for a month at 7 ‰, and has a survival salinity minimum of 6 ‰ (Woo and Chung 1995), similar to our findings with lionfish in the Western Atlantic.

During the laboratory portion of this study, we failed to detect differences in growth between lionfish that had been exposed to high salinities (35 ‰) and those that had been exposed to low salinities (7 ‰), suggesting that any physiological costs associated with osmoregulation at 7 ‰ are insufficient to result in reduced growth (compared to 35 ‰). However, our ability to accurately compare growth rates between high-salinity and low-salinity treatments may have been hindered by our feeding regime in the laboratory. In particular, we were unable to feed lionfish to satiation, as this would have caused water quality in the relatively small aquaria to degrade due to excess waste production. Our feeding regime during the first two weeks of the study led to growth in both salinity treatment groups, although growth rates were lower than values recorded in wild fish (Jud and Layman 2012). The losses in mass and minimal increases in length observed between day 16 and 28 (which were similar between the two salinity treatments) were likely caused by the reduced feeding frequency implemented during the second half of the experiment as a means of improving water quality. Since lionfish can survive for three months without food (Fishelson 1997), we were not concerned that our feeding regime would result in mortality for either group of fish (potentially confounding the effects of salinity).

The ability of lionfish to survive at low salinities may play an important role in shaping the eventual spatial extent of the invasion in the Western Hemisphere. While lionfish have spread rapidly throughout the Caribbean, Gulf of Mexico, and Northwest Atlantic, they have yet to colonize the coast of South America, south of the Amazon-Orinoco plume (AOP). The AOP has been proposed as a potential barrier to southward dispersal of lionfish (Côté et al. 2013a); however, our findings support the prediction of

Luiz et al. (2013) that lionfish will eventually cross the AOP and spread along the Atlantic coast of South America. When exposed to reduced salinities in the wild, adult and post-settlement juvenile lionfish (demersal life history stages) have been observed utilizing benthic water layers (Jud et al. 2011), which typically have higher salinities than the overlying water column. The presence of a brackish layer of bottom water under the AOP would potentially allow post-settlement lionfish to traverse areas of low salinity created by the plume. However, the ability of pelagic eggs and larvae of lionfish to cross the low-salinity surface waters of the AOP is not known.

While the future establishment of lionfish south of the AOP is a likely scenario, a more pressing concern is identifying the distribution and impacts of lionfish that are currently utilizing estuarine ecosystems within the presently invaded range. Even as lionfish research has progressed at a rapid pace in other ecosystems, the inherent difficulties associated with detecting and observing lionfish in estuarine systems has hindered our understanding of this aspect of the invasion. There exists a paucity of data on habitat utilization by lionfish in their native range; however, individuals are occasionally captured in or near estuarine systems (Kulbicki et al. 2012). Prakash et al. (2012) have identified native *P. volitans* utilizing estuarine habitats in India; however, all occurrences were <2.3 km from the ocean, where salinities were >12 ‰. In contrast, we have demonstrated that lionfish from the invaded range can survive considerably further from the ocean, at much lower salinities. Because recreational SCUBA diving and snorkeling are not commonly carried out in estuaries (and the fact that visibility is often poor), we feel that the presence of lionfish in these ecosystems is likely being underreported. Two ecologically and economically important estuarine systems on the

east coast of Florida – the Indian River Lagoon and Biscayne Bay – have already been documented to support populations of lionfish (Z. Jud, unpubl. data; E. Dark, unpubl. data). However, without increased efforts to identify lionfish in other invaded estuaries and document their effect on native estuarine organisms, we may fail to fully recognize the potential impacts of the lionfish invasion on these ecosystems.

Acknowledgments

This project was made possible by a close partnership with the Loxahatchee River District. Lauren Arrington (King's Academy, West Palm Beach, FL) conducted preliminary laboratory experiments that helped give rise to our experimental design. We thank Joel Trexler for facilitating our use of Florida International University's aquarium facilities and Diana Churchill for assistance during the laboratory portion of the study. Research protocols were approved by Florida International University's Institutional Animal Care and Use Committee (IACUC-13-030-AM01), and a Florida Fish and Wildlife Conservation Commission Special Activities License (SAL-13-1487-SR).

Table 5.1. Lionfish standard length (SL) and mass on day 0, day 16, and day 28 of the study designed to assess effects of long-term exposure to low salinity (7 ‰). Fish in the high-salinity (35 ‰) control treatment are labeled “H” and fish in the low-salinity (7 ‰) treatment are labeled “L.” Asterisks indicate a fish in the high-salinity control treatment that died on day 20.

Fish label	Day 0		Day 16		Day 28	
	SL (mm)	mass (g)	SL (mm)	mass (g)	SL (mm)	mass (g)
L1	115	47.11	117	46.97	117	44.61
H1	98	28.44	100	27.82	100	27.01
L2	74	8.91	76	9.16	77	9.97
H2	77	10.75	79	10.97	*	*
L3	69	7.58	71	7.76	73	7.27
H3	68	7.48	69	7.55	70	7.54
L4	120	46.7	122	50.38	122	48.58
H4	125	57.85	129	58.34	129	57.6
L5	64	6.14	65	6.35	65	5.87
H5	66	5.97	68	6.02	68	5.82
L6	142	75.39	146	81.53	146	77.7
H6	139	78.38	141	80.6	141	79.92
L7	126	48.4	128	51.35	128	49.71
H7	127	54.66	129	56.14	129	47.33
L8	54	3.53	55	3.56	56	3.42
H8	61	5.06	62	5.02	62	4.81

Fig. 5.1. Effects of fluctuating salinity on the survival of caged lionfish (*Pterois* spp.) at (a) downstream (2.4 km from ocean), (b) midstream (6.2 km from ocean), and (c) upstream (7.0 km from ocean) sites in the Loxahatchee River estuary. Salinity varied over time as a product of freshwater inflow (long term) and tidal incursion of marine water (twice daily). Estimated time of lionfish death is indicated in the bar across the top

of each panel. Note that scale on the x-axis differs among panels. The period of heavy precipitation is indicated by a thick black bar at the top of each panel. Asterisks in (a) represent data gaps due to equipment malfunction.

Fig. 5.2. Kaplan-Meier survival curve for lionfish exposed to salinities of 4 ‰. No mortality was observed at salinities ≥ 5 ‰, the approximate survival salinity minimum ($SS_{\min}$) for lionfish. After 3 hours at 4 ‰, two individuals experienced a complete loss of equilibrium (our predetermined endpoint for $SS_{\min}$ determination), combined with a lack of response to tactile stimulation, and a reduction in the frequency of opercular movements. These two individuals were included in the Kaplan-Meier curve, since their condition was an immediate precursor to death. All fish were dead after 94 hours.

References

- Albins M, Hixon M (2011) Worst case scenario: potential long-term effects of invasive predatory lionfish (*Pterois volitans*) on Atlantic and Caribbean coral-reef communities. *Environmental Biology of Fishes*:1-7
- Albins MA, Hixon MA (2008) Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. *Marine Ecology Progress Series* 367:233-238
- Albins MA, Lyons PJ (2012) Invasive red lionfish *Pterois volitans* blow directed jets of water at prey fish. *Marine Ecology Progress Series* 448:1-5
- Barbour AB, Montgomery ML, Adamson AA, Diaz-Ferguson E, Silliman BR (2010) Mangrove use by the invasive lionfish *Pterois volitans*. *Marine Ecology Progress Series* 401:291-294
- Beck MW, Heck KL, Jr, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* 51:633-641
- Biggs CR, Olden JD (2011) Multi-scale habitat occupancy of invasive lionfish (*Pterois volitans*) in coral reef environments of Roatan, Honduras. *Aquatic Invasions* 6:447-453
- Cheng SY, Chen CS, Chen JC (2013) Salinity and temperature tolerance of brown-marbled grouper *Epinephelus fuscoguttatus*. *Fish Physiology and Biochemistry* 39:277-286. doi:10.1007/s10695-012-9698-x
- Claydon JAB, Calosso MC, Traiger SB (2012) Progression of invasive lionfish in seagrass, mangrove and reef habitats. *Marine Ecology Progress Series* 448:119-129. doi:10.3354/meps09534
- Côté IM, Green SJ, Hixon MA (2013a) Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. *Biological Conservation* 164:50-61
- Côté IM, Green SJ, Morris JA, Jr., Akins JL, Steinke D (2013b) Diet richness of invasive Indo-Pacific lionfish revealed by DNA barcoding. *Marine Ecology Progress Series* 472:249-256. doi:10.3354/meps09992
- Courrat A, Lobry J, Nicolas D, Laffargue P, Amara R, Lepage M, Girardin M, Le Pape O (2009) Anthropogenic disturbance on nursery function of estuarine areas for marine species. *Estuarine Coastal and Shelf Science* 81:179-190. doi:10.1016/j.ecss.2008.10.017

- Fishelson L (1997) Experiments and observations on food consumption, growth and starvation in *Dendrochirus brachypterus* and *Pterois volitans* (Pteroinae, Scorpaenidae). *Environmental Biology of Fishes* 50:391-403
- García LN, Pesq I, Sierra CL, Perez J, Esquivel F, Chapman FA (2013) Osmoregulation of juvenile marine goliath grouper (*Epinephelus itajara*) in low-salinity water. *Revista Colombiana de Ciencias Pecuarias* 26:127-135
- Green S, Akins J, Côté I (2011) Foraging behaviour and prey consumption in the Indo-Pacific lionfish on Bahamian coral reefs. *Marine Ecology Progress Series* 433:159-167
- Green SJ, Akins JL, Maljkovic A, Côté IM (2012) Invasive lionfish drive Atlantic coral reef fish declines. *Plos One* 7:e32596
- Jian CY, Cheng SY, Chen JC (2003) Temperature and salinity tolerances of yellowfin sea bream, *Acanthopagrus latus*, at different salinity and temperature levels. *Aquaculture Research* 34:175-185. doi:10.1046/j.1365-2109.2003.00800.x
- Jud ZR, Layman CA (2012) Site fidelity and movement patterns of invasive lionfish, *Pterois* spp., in a Florida estuary. *Journal of Experimental Marine Biology and Ecology* 414-415:69-74
- Jud ZR, Layman CA, Lee JA, Arrington DA (2011) Recent invasion of a Florida (USA) estuarine system by lionfish *Pterois volitans* / *P. miles*. *Aquatic Biology* 13:21-26. doi:10.3354/ab00351
- Kimball ME, Miller JM, Whitfield PE, Hare JA (2004) Thermal tolerance and potential distribution of invasive lionfish (*Pterois volitans/miles* complex) on the east coast of the United States. *Marine Ecology Progress Series* 283:269-278
- Kulbicki M, Beets J, Chabanet P, Cure K, Darling E, Floeter SR, Galzin R, Green A, Harmelin-Vivien M, Hixon M, Letourneur Y, de Loma TL, McClanahan T, McIlwain J, MouTham G, Myers R, O'Leary JK, Planes S, Vigliola L, Wantiez L (2012) Distributions of Indo-Pacific lionfishes *Pterois* spp. in their native ranges: implications for the Atlantic invasion. *Marine Ecology Progress Series* 446:189-205. doi:10.3354/meps09442
- Lambert Y, Dutil JD, Munro J (1994) Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* 51:1569-1576. doi:10.1139/f94-155
- Layman CA, Allgeier JE (2012) Characterizing trophic ecology of generalist consumers: a case study of the invasive lionfish in The Bahamas. *Marine Ecology Progress Series* 448:131-141

- Lee K, Kaneko T, Aida K (2005) Low-salinity tolerance of juvenile fugu *Takifugu rubripes*. Fisheries Science 71:1324-1331
- Lonnstedt OM, McCormick MI (2013) Ultimate predators: Lionfish have evolved to circumvent prey risk assessment abilities. Plos One 8:e75781
- Luiz OJ, Floeter SR, Rocha LA, Ferreira CEL (2013) Perspectives for the lionfish invasion in the South Atlantic: Are Brazilian reefs protected by the currents? Marine Ecology Progress Series 485:1-7. doi:10.3354/meps10383
- Morris JA, Jr, Akins JL (2009) Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago. Environmental Biology of Fishes 86:389-398. doi:10.1007/s10641-009-9538-8
- Morris JA, Jr., Whitfield PE (2009) Biology, Ecology, Control and Management of the Invasive Indo-Pacific Lionfish: An Updated Integrated Assessment. NOAA Technical Memorandum NOS NCCOS 99:1-57
- Prakash S, Balamurugan J, Kumar TTA, Balasubramanian T (2012) Invasion and abundance of reef-inhabiting fishes in the Vellar estuary, southeast coast of India, especially the lionfish *Pterois volitans* Linnaeus. Current Science 103:941-944
- Valdez-Moreno M, Quintal-Lizama C, Gómez-Lozano R, Garcia-Rivas MdC (2012) Monitoring an alien invasion: DNA barcoding and the identification of lionfish and their prey on coral reefs of the Mexican Caribbean. Plos One 7:e36636
- Whitfield PE, Gardner T, Vives SP, Gilligan MR, Courtenay WR, Jr, Ray GC, Hare JA (2002) Biological invasion of the Indo-Pacific lionfish *Pterois volitans* along the Atlantic coast of North America. Marine Ecology Progress Series 235:289-297
- Woo NYS, Chung KC (1995) Tolerance of *Pomacanthus imperator* to hypoosmotic salinities: changes in body composition and hepatic enzyme activities. Journal of Fish Biology 47:70-81. doi:10.1006/jfbi.1995.0112
- Wu RSS, Woo NYS (1983) Tolerance of hypo-osmotic salinities in 13 species of adult marine fish - implications for estuarine fish culture. Aquaculture 32:175-181. doi:10.1016/0044-8486(83)90279-x

CHAPTER VI

CHANGES IN MOTILE BENTHIC FAUNAL COMMUNITY STRUCTURE FOLLOWING LARGE-SCALE OYSTER REEF RESTORATION IN A SUBTROPICAL ESTUARY

Abstract

Assessing the success of oyster restoration efforts is often hampered by a lack of appropriate long-term data from natural reference sites. When reference data are available, many studies rely entirely on oyster-related metrics (e.g., oyster density, abundance, survival, etc.) to quantify restoration success. However, it is also important to examine a variety of other factors (e.g., other reef-associated organisms, sedimentation rates, water quality, etc.) when attempting to identify ecological convergence between natural and restored oyster reef systems. Here, we compare community composition of motile benthic oyster reef-associated organisms (small fishes and motile invertebrates) over time at natural and restored oyster reefs in the Loxahatchee River estuary (Florida, USA) as one means of assessing the success of oyster reef restoration. Motile benthic communities at a 1.93 hectare section of restoration reef gradually began to resemble natural communities in the months following reef construction. Within ~22 months, biomass and community composition were similar between natural and restored habitats. At that point, mean biomass of motile benthic organisms at the restoration site had reached 83.6 g/m² (versus 89.8 g/m² at nearby natural reefs), and the restoration supported >1,600 kg of small, motile, oyster-associated organisms. Biomass values increased more rapidly in high-relief sections of the restored reef (30 cm vs. 15 cm reef height, relative to surrounding benthos), particularly during the first year following restoration. High-relief areas were also characterized by increased oyster growth, greater rugosity, and decreased sedimentation, suggesting that small differences in reef design can have important implications for restoration success.

Introduction

With more than 60% of Earth's population living in the coastal realm, estuarine ecosystems have been extensively altered by human activities (Ray 2006). In many temperate and subtropical estuaries, oyster reefs represent a critical habitat type, providing numerous ecosystem services to humans (Officer et al. 1982; Coen et al. 2007; Grabowski et al. 2012). Oysters are a key foundation species (Bruno et al. 2003), and their presence can facilitate the colonization, survival, and growth of myriad other organisms, including crabs, shrimp, mollusks, and fishes (Tolley and Volety 2005; Stunz et al. 2010). This community of small motile oyster reef-associated organisms serves as a food source for numerous ecologically, commercially, and recreationally important species (Grabowski et al. 2005; Abeels et al. 2012). Furthermore, oyster reefs provide an important nursery habitat for many marine and estuarine organisms (Coen et al. 2007).

Over the past century, oyster reefs throughout North America have experienced significant declines as a result of overharvest, degraded water quality, altered salinity patterns, and disease (Rothschild et al. 1994; Jackson et al. 2001; Kirby 2004; Beck et al. 2011). As the ecological and economic importance of oyster reefs has become more widely recognized, habitat restoration is increasingly being used to slow or reverse these declines (Taylor and Bushek 2008; Brumbaugh and Coen 2009; Schulte et al. 2009). Although some oyster reef restorations are designed primarily to increase oyster production for commercial purposes, a more common goal is to restore multiple ecosystem services associated with an intact natural oyster reef community (Coen and Luckenbach 2000; Palmer et al. 2004; Luckenbach et al. 2005; Grabowski and Peterson 2007; Benayas et al. 2009). For example, the construction of living oyster reefs has the

potential to enhance populations of many organisms that utilize these habitats during all or part of their life history, including a variety of commercially and recreationally valuable species (Peterson et al. 2003; Tolley and Volety 2005). For this reason, the success of an oyster reef restoration should be measured not only by the recovery of a population of living oysters, but also by the reestablishment of ecosystem function and an eventual convergence with natural oyster reef community structure (Coen and Luckenbach 2000). While many studies focus entirely on oyster-related metrics (e.g., oyster density, abundance, size, recruitment rates, survival, etc.) as a means of assessing the success of an oyster restoration project over time (Nestlerode et al. 2007; Schulte et al. 2009), there are a number of other important factors, including community composition of motile benthic oyster-associated organisms, that should be examined when attempting to quantify convergence between natural and restored oyster systems (Rodney and Paynter 2006; Humphries et al. 2011b). However, because long-term data for motile benthic faunal communities at nearby natural oyster reefs are often lacking, selecting ecologically appropriate restoration goals (from the perspective of oyster-associated motile fauna), and determining when those goals have been reached, can be difficult.

Here, we utilize a long-term dataset to characterize the structure of motile benthic faunal communities (e.g., small crustaceans, motile mollusks, and demersal fishes) that utilize natural and restored oyster (*Crassostrea virginica*) reefs in the Loxahatchee River (Jupiter, Florida). Specifically, we identified patterns in biomass and community composition of motile benthic organisms at several natural oyster reef “reference sites” in the system, creating baselines to facilitate comparisons between natural reefs and a large-

scale human-made restoration reef. We then used these baseline values to track the development of motile benthic faunal communities at the restored reef over time as one means of assessing the success of the restoration project. Additionally, we tested the hypothesis that very small increases in habitat complexity (i.e., greater vertical relief) within an oyster restoration reef lead to increased biomass of motile benthic organisms, as well as a more rapid convergence with a natural oyster reef community. This study focuses on the southernmost large-scale oyster restoration reef along the Atlantic coast of the United States, and because of the geographic location of the system, represents an important addition to the existing oyster reef restoration literature.

Methods

Study system

The Loxahatchee River (26°57' N, 80°06' W) is a 27-kilometer-long coastal river that flows into the Atlantic Ocean near Jupiter, Florida, USA (Fig. 6.1) (VanArman et al. 2005). In the Loxahatchee River, oyster reefs have been significantly degraded, largely as a result of anthropogenic alteration of salinity. Widening and stabilization of Jupiter Inlet beginning in the 1920s (as well as extensive dredging in the lower estuary during the 1940s and 1970s) increased the amount of marine water entering the river, while freshwater flow into the system has steadily decreased since the 1930s as a result of dam construction and flood control practices. These disturbances combined to increase overall salinity in the estuary, resulting in an upstream shift in the optimal salinity zone for oysters, i.e., 10-28 ppt (Loosanoff 1965). Oysters reefs presently occur ~4-7.5 km upriver from their historical location, at (and upstream of) an area where a sudden

narrowing and shallowing of the river channel create a geomorphic barrier to marine water intrusion (VanArman et al. 2005; SFWMD 2006). The section of river where salinities presently favor oyster growth is substrate limited, with a benthos composed largely of sand and silt that lacks settlement habitats for larval oysters (e.g., remnants of historical oyster reefs). Present-day oyster reef development in this section of the Loxahatchee River is limited to patchy, subtidal, fringing reefs, often associated with mangrove shorelines (SFWMD 2006). It is likely that fallen mangrove branches and roots represent the only hard substrate available for oyster settlement, facilitating the formation of these fringing reefs. Natural reefs in the system are structurally complex, and are characterized by ridges, depressions, exposed sediment patches, and rapid dropoffs (Loxahatchee River District, unpublished data). These reefs are generally 20-30 cm thick. Relic oyster shell can be found in historical locations close to the ocean; however, benthic salinities in these areas are presently too high (consistently >30 ppt) to support healthy reef development (SFWMD 2006).

Identifying natural oyster reef communities

Between May 2007 and May 2012, we conducted bimonthly sampling of motile benthic organisms at three natural oyster reef reference sites (upstream, midstream, downstream) in the Loxahatchee River (Fig. 6.1), to characterize temporal (wet season vs. dry season) and spatial (upstream vs. downstream) patterns in the communities that were present on naturally occurring oyster reefs in the system. These sites were located between 6.2 and 9.2 km upstream from the ocean, spanning the entire upstream-to-downstream range of present-day oyster reef development in the main branch of the river.

To sample motile benthic macroinvertebrates and small demersal fishes, we deployed benthic sampling trays ($n = 4/\text{site}$) at $\sim 2\text{-}10$ m intervals at each of the three natural reef reference sites. These trays were $64 \times 52 \times 10$ cm plastic bakery trays lined with polyethylene mesh shade cloth (Plunket and La Peyre 2005; Rodney and Paynter 2006). Each tray was filled with 19 l of cleaned, dried oyster shell obtained from local restaurants. The design of these benthic trays allowed us to collect motile organisms that occupied interstitial spaces within the reef, a habitat that is difficult to sample using other methodologies.

At the time of deployment, each sampling tray was placed into a shallow depression that we excavated into the natural oyster reef substrate, such that the top surface of the shell in the tray was flush with the surrounding live oyster matrix. Tray depth ranged from ~ 0.6 to 0.8 m below mean low water. After a two-month soak time, trays were lifted vertically by a pair of divers using snorkeling gear, allowing water to run through the mesh shade cloth on the tray bottom, trapping motile benthic macroinvertebrates and small demersal fishes within the tray. By lifting the trays slowly, we found that demersal fishes would typically take shelter in the bottom of the tray, rather than swimming up and over the tray's edge, negating the need to utilize a cover during retrieval. All fishes, crabs, shrimp, and motile mollusks were collected by hand, kept on ice in the field, and returned to the laboratory for later processing (identification to lowest possible taxonomic level, counting, measuring wet mass). We did not quantify (1) fishes >10 cm, (2) smaller invertebrates such as amphipods and polychaetes, or (3) sessile invertebrates, as our tray methodology was not designed to consistently collect

these organisms. After trays were sampled, they were refilled with shell and returned to their original location in the oyster reef.

To characterize natural oyster reef-associated communities, we used a one-way analysis of variance (ANOVA) to compare overall mean biomass and density values for motile benthic fauna among the three natural reef sites across all five years of sampling. Post-hoc comparisons were made using the Tukey HSD test (SPSS v.16). We then used a series of nonparametric multivariate analyses to compare patterns of community composition among sites and across sampling dates. A Bray-Curtis similarity matrix was created using the mean biomass (g/m^2) of each taxonomic group (averaged at the site level for each sampling date). Biomass values were fourth-root transformed to down-weight abundant taxa and allow less common taxa to influence similarity values (Clarke and Warwick 2001). A non-metric multidimensional scaling (NMDS) ordination was created to provide a visual representation of community similarity or dissimilarity among the three natural reference sites. Each data point in the NMDS ordination represents the community that was present during a single sampling date at a single site (mean of four trays per data point). The relative proximity of two points to one another on the NMDS ordination reflects the relative similarity of the communities represented by those points. A 1-way analysis of similarities (ANOSIM) was used to test for significant differences in community composition among the three reference sites. Finally, we used similarity percentages (SIMPER) to identify which taxa were most responsible for differences in community structure among sites. All community-level analyses were carried out using PRIMER v.6.1.6 software.

Restoration reef construction

In July 2010, 2.36 hectares of oyster restoration reef were constructed in the Loxahatchee River (Fig. 6.1) as part of a project funded by the National Oceanic and Atmospheric Administration (NOAA) through the American Recovery and Reinvestment Act (AARA). Since the Loxahatchee River does not support an oyster fishery, the goal of the restoration was to create a self-sustaining living oyster reef with similar structure and function to natural oyster reefs in the same system. The intent of the project was to construct a carbonate-based reef in a substrate-limited section of the estuary to provide suitable settlement habitat for larval oysters, while simultaneously creating essential habitat for numerous other oyster reef-associated organisms. Prior to reef construction, the benthos at the restoration site was largely 2-dimensional, composed primarily of sand and coarse silt substrates. The reef was constructed by spreading a continuous and relatively homogeneous 15 cm thick layer of limestone/sandstone rocks and mollusk shells (~5 to 20 cm in diameter) across the river bottom, such that the entire restoration site was uniformly covered by a thin layer of calcareous material. The resulting reef was thinner and less structurally complex than natural reefs in the system. Rock and shell that were used to build the reef were obtained as a byproduct of a nearby beach nourishment project. Because of the large scale of the restoration project, heavy equipment was used to deploy the rock and shell aggregate. All areas of the completed reef remained submerged at low tide. Our sampling was conducted on a continuous 1.93 hectare section of the restoration reef (“restoration reef site” for the remainder of the paper), which was located ~6.75 km from the ocean, in the part of river that supports natural oyster reef growth (~2.5 km from the upstream reference site, ~100 m from the

midstream reference site, and ~500 m from the downstream reference site of our long-term natural oyster reef community structure study). This section of the restoration reef had a roughly rectangular footprint, measuring ~165 m x 120 m.

Comparing restored and natural oyster reef communities

To identify motile benthic organisms utilizing the restoration site prior to the construction of the reef, we began sampling this area six months before the reef was built. At that time (January 2010), we deployed four benthic sampling trays (see above) within the future footprint of the restoration reef. Since our goal was to document community composition on the 2-dimensional soft-bottomed habitat prior to the addition of a 3-dimensional calcareous restoration reef, we filled each sampling tray with 19 l of ambient sand/coarse silt substrate excavated directly from the site (rather than oyster shell, as described above). Trays were then placed into the resulting holes, flush with the surrounding river bottom. We sampled these trays two times (March and May 2010) before reef construction began. After sampling in March 2010, trays were refilled with sand/coarse silt, and redeployed at their original location for two additional months. Trays were temporarily removed from the river after the May 2010 sampling event in preparation for the construction phase of the project. Data collected from these trays allowed us to compare motile benthic communities at the site before and after reef construction.

Following reef construction in July 2010, the four sampling trays were redeployed at the restoration reef site. Each tray was filled with 19 l of the loose limestone/sandstone rock and mollusk shell aggregate that was used to build the reef. For the next 22 months,

these trays were sampled bimonthly, using the same methodology outlined above (two-month soak time, replacement of substrate after each sampling).

To assess convergence between motile benthic communities on natural and restored oyster reefs, we began by comparing biomass and organismal density between the three natural reef reference sites and the restoration site. We then used nonparametric multivariate analyses to compare community structure at the restoration reef site to all three natural reef sites over time. Organismal biomass data from each restoration reef sampling date were incorporated into the natural-reef NMDS ordination (see above) to visualize changes in community composition following reef construction (to avoid redundancy, a single ordination plot is shown, containing natural and restoration reef data). Each restoration reef data point in the NMDS ordination represents the community composition found at the restoration site on a single sampling date (mean of four trays per data point). Hierarchical agglomerative cluster analysis was used to identify groupings of similar restored and natural communities. We conducted a 1-way ANOSIM to test for differences among natural reef communities (i.e., the reference sites), pre-restoration communities, and post-restoration communities. For this analysis, we divided the post-restoration period into four to six month time blocks (two to three sampling dates) to look for community convergence over time. We then used SIMPER to identify the primary taxa that contributed to the dissimilarity between natural reefs and the restored reef during each time block in the 22 months following reef construction. Community-level analyses were carried out using PRIMER v.6.1.6 software.

Habitat complexity in a restored oyster reef

To test the effects of habitat complexity on motile benthic community structure at a restored oyster reef, we created three parallel 10 m x 7 m experimental blocks within the continuous restoration reef matrix, each containing two levels of bottom relief. The three experimental blocks were located near the center of the restoration reef, and were spaced at ~25 m intervals. We created a 1 m border around each block by clearing away the rock and shell aggregate down to the natural sand/silt substrate. Within each of the three experimental blocks, we built a high-relief plot and a paired low-relief plot, where “high relief” refers to rapid (i.e., sub-meter scale) changes in reef height relative to the surrounding benthos. The three high-relief plots (constructed using an excavator and hand tools) were 10 m x 2 m, and 30 cm thick, the greatest elevation allowed by the construction permit (the approximate height of most natural reefs in the system). Each paired low-relief plot was 10 m x 4 m, and 15 cm thick, the same thickness as the surrounding restoration reef matrix. Plot sizes were selected so that both treatments utilized the same volume of aggregate (6 m^3). The paired high- and low-relief plots within each experimental block were immediately adjacent to each other (with parallel long axes), and were separated by a 1 m strip of exposed sand substrate. Based on pre-restoration bathymetric surveys (conducted by Continental Shelf Associates, Inc., Stuart, FL, USA), all three blocks were placed at the same initial base elevation. Since each pair of high- and low-relief treatments within an experimental block were parallel, and were only separated by a 1 m border, they were likely subject to the same environmental and physical conditions (e.g., current velocity and direction, distance to mangroves, salinity, etc.).

In August 2010, one week after reef construction was completed, we deployed 42 benthic sampling trays across the three experimental blocks (14 paired trays per block). Within each experimental block, we created two parallel rows of sampling trays, with seven trays running down the long axis of the high-relief plot, paired with seven trays running down the long axis of the low-relief plot. Trays were spaced ~1 m apart within rows. Each tray was filled with 19 l of rock and shell substrate that was excavated directly from the reef surface. Trays were then placed into the resulting depressions, such that the surface of the material in the tray was even with the surrounding substrate. The initial 19 l of material that was collected from the reef and placed into each tray was treated as the day 0 sample (i.e., all motile benthic organisms were removed from the substrate and retained prior to the initial filling of each tray in order to characterize the community that was present at the start of the study). Rather than sampling this set of trays at a fixed bimonthly time interval, we chose *a priori* to sample at approximately day 0 (date of deployment), 14, 28, 60, 120, 240, 365, and 480. On each sampling date, one randomly selected pair of trays (high/low) was removed from each experimental block and processed (six trays per sampling date). Unlike the sampling protocol described in the above sections, these trays were left undisturbed from the time of deployment to the time of sampling, at which point they were permanently removed from the river. By utilizing a range of different soak times (rather than re-sampling every two months), we feel that we were able to more accurately identify cumulative changes in community structure that occurred in the 16 months following reef construction (i.e., motile faunal communities developed over time without being disturbed every two months). To compare biomass between high- and low-relief treatments over time, we ran a General

Linear Model using relief level and days since construction as fixed factors (SPSS v.16). We initially included location of each experimental block within the reef as a random factor, but location was not a significant predictor of biomass, so we removed it from the model. Data were fourth-root transformed to meet assumptions of homogeneity of variance. To visualize changes in community structure between the two vertical relief treatments over time, we created a NMDS ordination from a Bray-Curtis similarity matrix using fourth-root transformed biomass values (g/m^2) from each tray (Primer v.6.1.6). We then used a 2-way crossed ANOSIM to test for differences in community composition between relief treatments and across sampling dates.

Although our study focused primarily on motile benthic organisms, we observed changes in oyster density and surface rugosity in high- and low-relief sampling trays that had been allowed to soak undisturbed for extended periods of time. At the time of our final sampling (day 485), we quantified the number of live oysters in the three remaining pairs of high- and low-relief sampling trays. Additionally, we measured surface rugosity in these trays by pressing a piece of copper wire into the contours, recesses, and surface irregularities along lines running across the center of each tray's long and short axes (two bent-wire measurements per tray). The bent piece of wire was then straightened and measured. Rugosity measurements were reported as bent-wire distance/straight-line distance. A paired t-test was used to compare final rugosity between treatments (SPSS v.16).

Based on preliminary observations of differences between high- and low-relief communities during the above study, we added four benthic sampling trays to one of the former high-relief experimental plots upon completion of the habitat complexity

experiment (December 2011). The purpose of these new trays was to allow us to continue to make biomass and community structure comparisons between high- and low-relief sections of the restored reef even though the initial habitat complexity study had ended. These trays were sampled on a bimonthly basis along with the original four trays at the restoration site, using the same methodology outlined in earlier sections (two-month soak time, replacement of substrate after each sampling).

Results

Temporal and spatial variability in natural oyster reef communities

Between May 2007 and May 2012, we collected and identified nearly 27,000 individual organisms representing 11 fish and 19 invertebrate taxa from natural oyster reefs in the Loxahatchee River (Table 6.1, 6.2). We were able to identify many taxa at the species level. In cases where we were not able to make positive identifications (typically as a result of difficulties in differentiating juveniles of closely related species), organisms were grouped at the genus or family level (e.g., mud crabs <9 mm carapace width were combined as *Panopeidae* spp. for our analyses). Dominant organisms (by biomass) in these motile natural oyster reef communities were black-fingered mud crabs – *Panopeus herbstii*, followed by depressed mud crabs – *Eurypanopeus depressus*, crested gobies – *Lophogobius cyprinoides*, unidentified mud crabs <9 mm – *Panopeidae* spp., snapping shrimp – *Alpheus* spp., green porcelain crabs – *Petrolisthes armatus*, and frillfin gobies – *Bathygobius soporator* (Table 6.1). Each of the remaining 24 taxa accounted for $\leq 2\%$ of total natural reef biomass. Unidentified *Panopeidae* spp. <9 mm were the most numerically abundant benthic organisms at natural oyster reefs, followed

by *Alpheus* spp., *Petrolisthes armatus*, *Eurypanopeus depressus*, *Lophogobius cyprinoides*, and *Panopeus herbstii*, with each of the remaining taxa representing $\leq 2\%$ of the total sample (Table 6.2).

We observed a distinct seasonality in overall biomass of motile reef-associated organisms. Although there was year-to-year and site-to-site variability, we found that biomass at natural oyster reefs in the Loxahatchee River was typically greatest during May or July. The timing of annual biomass minima was less consistent among years, but usually occurred between November and March. When averaged across all natural reef reference sites and all months, mean biomass of motile oyster reef-associated organisms at natural reefs was $93.8 \pm 34.6 \text{ g/m}^2$ (mean $\pm$ SD) and mean organismal density was $266.6 \pm 158.4 \text{ individuals/m}^2$.

In addition to seasonal variability, long-term mean biomass of motile oyster-reef associated organisms at natural reef reference sites showed considerable spatial variability. There were significant differences in average biomass among sites ($F_{2, 84} = 8.79, p < 0.001$), with values increasing along an upstream-to-downstream gradient (Table 6.3). Post-hoc testing revealed that the downstream natural site had significantly greater biomass than the midstream site and the upstream site (Table 6.3). Differences in biomass between the midstream and upstream sites were not significant. We observed similar spatial differences in mean organismal density (organisms/ m^2) among sites ($F_{2, 84} = 9.42, p < 0.001$), where densities at the downstream site ($372.4 \pm 181.5 \text{ individuals/m}^2$) were significantly greater than the midstream site ($218.2 \pm 94.9 \text{ individuals/m}^2$) and the upstream site ($229.5 \pm 153.1 \text{ individuals/m}^2$). Densities at the upstream and midstream sites were not significantly different.

Seasonal shifts between maximum and minimum annual biomass values occurred more rapidly at the upstream natural reef site than at the downstream natural reef site. At the upstream reference site, biomass typically peaked in July and reached annual minimum values in November, a four-month transition between maximum and minimum annual biomass. The transition between maximum and minimum biomass took twice as long at the downstream reference site, with average peak biomass occurring two months earlier (May) and average minimum values occurring two months later (January). At all three reference sites, mean annual maximum biomass values (spring/summer) were approximately two times greater than mean annual minimum biomass values (fall/winter) (Table 6.3).

Community composition of motile benthic organisms differed among the three natural reef reference sites across 31 sampling dates (Fig. 6.2; ANOSIM Global $R = 0.54$, $p = 0.001$). Pairwise comparisons suggested that the upstream and downstream sites had the most dissimilar communities ($R = 0.80$, $p = 0.001$). *Petrolisthes armatus*, *Panopeus herbstii*, *Eurypanopeus depressus*, *Lophogobius cyprinoides*, *Nassarius vibex*, and *Lupinoblennius nichols* were the primary taxa driving community-level differences between the upstream site and the downstream site (based on biomass, Table 6.1). *Petrolisthes armatus*, *P. herbstii*, and *N. vibex* were more abundant downstream, while *E. depressus*, *L. cyprinoides*, and *L. nichols* were more abundant upstream (Table 6.1). In most cases, biomass values for these taxa at the midstream reference site were intermediates of upstream and downstream values. Species richness was greater at the downstream reference site (25 species) than at the midstream or upstream sites (20 species each).

Convergence between natural and restored reef communities

In 26 months of bimonthly sampling at the restoration site (March 2010 to May 2012), we collected ~4,000 motile benthic organisms representing 20 invertebrate taxa and 10 fish taxa (Table 6.1, 6.2). Ten of these taxa, including the economically important Florida stone crab (*Menippe mercenaria*), were not found at natural reef sites during the study. During the four months prior to restoration, biomass values measured from the sandy and silty substrate at the future restoration site were substantially lower than values from natural reefs, representing 10% of the mean biomass present at the three natural oyster reef reference sites (Fig. 6.3). Motile benthic organisms began to colonize the restoration reef site shortly after construction ended. Two months after reef construction (the time of our first post-restoration sampling), biomass values at the restored reef site were just 22% of the mean biomass of the three natural reef reference sites; however, abundance values had already reached 72% of the mean organismal density on natural reference reefs. Biomass increased slowly during the first 6 months following the completion of the restoration project. Between months 6 and 8, mean biomass at the restoration site doubled (Fig. 6.3). From month 8 to 18, biomass values at the restored reef began to exhibit seasonal fluctuations that were similar to those observed at nearby natural reference reefs. By the end of the study (months 20 and 22), biomass values at the restored reef were very similar to mean biomass values at the natural reference reefs (Fig. 6.3). The simultaneous increase in biomass between months 20 (March) and 22 (May) at both natural and restored sites is indicative of the seasonal variation we detected in our long-term dataset.

When viewed across the duration of the study, there were significant differences among natural, pre-restoration, and post-restoration communities (Fig. 6.2; ANOSIM Global $R = 0.70$, $p = 0.001$). Pre-restoration communities, which were characterized by low species richness (15 species) and low biomass ($8.8 \pm 4.2 \text{ g/m}^2$), differed from natural reef communities ($R = 1.00$, $p = 0.001$). At the time of our first post-restoration sampling (two months after reef construction), several taxa that were common at natural reference reefs were already present at the restoration site (e.g., *Eurypanopeus depressus*, *Alpheus* spp., Panopeidae spp. <9 mm, *Petrolisthes armatus*, *Gobiosoma bosc*), primarily as small, newly recruited, juveniles. Several larger benthic species (e.g., *Panopeus herbstii*, *Lophogobius cyprinoides*, *Bathygobius saporator*, *Lupinoblennius nichols*) that were abundant at nearby natural reference reefs were initially absent from the restoration reef community.

In the 22 months following the construction of the restoration reef, motile benthic communities at the restoration site slowly began to resemble natural reference reef communities (Fig. 6.2 – over time, restoration reef data points get closer to the cluster of natural reef data points in ordination space). Convergence of community structure occurred gradually, with post-restoration communities differing from natural reef communities during the first six months after restoration ($R = 0.96$, $p = 0.001$), the second six months after restoration ($R = 0.85$, $p = 0.001$), and the third six months after restoration ($R = 0.42$, $p = 0.008$). In the first six months following restoration reef construction, *L. cyprinoides*, *P. herbstii*, and *B. saporator* were the primary taxa affecting community differences between natural and restored reefs. Juvenile *P. herbstii* were first found at the restoration reef six months after construction was completed; however, it

took eight months for biomass and abundance values to approach those found at natural reference reefs. Following the appearance of *P. herbstii* in month six, differences between restored and natural reef communities during the second six month period following restoration were driven primarily by *L. cyprinoides*, *Portunus* spp. (swimming crabs), and *B. saporator*. While *Gobiosoma bosc* recruits were present within two months of reef construction, colonization of the restoration reef by other demersal fish species occurred more slowly. *Lophogobius cyprinoides* did not appear at the restored reef until the third six-month period following restoration (month 14), during which time community differences between natural and restored reefs were largely affected by *Bathygobius saporator*, *Petrolisthes armatus*, and *Portunus* spp.

Communities present towards the end of the study (months 16, 20, and 22) were more similar to natural reference reef communities (at a 60% similarity level) than they were to earlier post-restoration communities. In the last four-month period of the study (the final two sampling dates, 20 and 22 months post-construction), motile benthic community composition at the restoration reef closely resembled that found at natural reference reefs ($R = 0.17$, $p = 0.22$), particularly the downstream reference site (Fig. 6.2 – note that data points representing three of the final four sampling dates lie within the cluster of natural reef data points in ordination space). With the appearance of *B. saporator* 20 months post restoration, community differences during months 18-22 of the study were primarily driven by *Stramonita haemastoma* (Florida rock shell), *P. armatus*, and *Palaemonetes* spp. (grass shrimp).

Effects of habitat complexity at a restored oyster reef

To assess effects of vertical relief on post-restoration oyster reef communities, we sampled paired high-relief and low-relief experimental plots within the restoration site eight times during the 16 months immediately following reef construction. During this period, we collected >3,000 motile benthic organisms from the experimental treatments. Throughout the study, mean biomass at high-relief plots was significantly greater than at low-relief plots ($F_{1,26} = 68.1, p < 0.001$), and there was a significant effect of time since construction on biomass values for both levels of vertical relief, with a general trend of increasing biomass over time ($F_{1,26} = 24.7, p < 0.001$; Fig. 6.4). Additionally, we observed a significant interaction between effects of relief and time since construction on the biomass of benthic organisms ($F_{6,26} = 3.20, p = 0.017$). For the first eight months of the study, biomass increased at both high- and low-relief plots; however, the overall rate of increase at high-relief plots during this time period was 10 times greater than at adjacent low-relief plots. After peaking in month eight (April), biomass values at the high-relief plots slowly began to decrease. The timing of this decrease corresponded to seasonal biomass declines that were simultaneously occurring at nearby natural reference reefs. Low-relief plots experienced a similar decline in biomass, but the decrease began three months later (July). When high-relief biomass peaked on day 240, we recorded a single-tray biomass of 388 g/m^2 , higher than any natural reef biomass value measured during the course of the study. At that time, mean high-relief biomass was >900% greater than mean low-relief biomass. Community composition at high- and low-relief treatments changed over time, but for any single sampling date, communities at both treatments levels exhibited overlap. We observed significant differences in community

structure between the two treatment levels across all sampling dates ($R = 0.47$, $p = 0.001$), as well as among dates for both treatments levels ($R = 0.60$, $p = 0.001$).

At the culmination of the habitat complexity experiment (day 485), high- and low-relief treatments exhibited differences in live oyster densities and surface rugosity. On average, high-relief treatments had more than twice as many live oysters per m^2 as low-relief treatments (420 ± 100 vs. 206 ± 114 oysters per m^2 ; mean $\pm$ SD). As a result, surface rugosity was significantly greater for the high-relief treatments than for the low-relief treatments (1.64 ± 0.15 vs. 1.20 ± 0.13 ; $t_5 = 4.66$, $p = 0.006$). Additionally, the interstitial spaces in two of the three low-relief trays that were sampled on the final day of the experiment were densely packed with sediment. Sediment accumulations were minimal in high-relief trays.

By the end of the habitat complexity experiment (December 2011), at which point trays had been left undisturbed for 485 days, high-relief biomass (147 g/m^2) was ~700% greater than low-relief biomass (18 g/m^2). After the completion of this phase of the study, we continued to sample high- and low-relief sections of the restoration reef for six additional months, using a different protocol (the bimonthly sampling protocol – see above), where trays were emptied and refilled every two months. At the end of this sampling period, which extended through May 2012, high-relief biomass (130 g/m^2) was only 55% greater than low-relief biomass (84 g/m^2). Sediment buildup appeared to be reduced in low-relief trays that were emptied and refilled regularly, as compared to trays that were left undisturbed for long periods of time.

Discussion

Natural oyster reefs in the Loxahatchee River provide critical habitat for a variety of ecologically and economically important motile benthic organisms. The most abundant taxa on oyster reefs – small mud crabs (Panopeidae), porcelain crabs (Porcellanidae), snapping shrimp (Alpheidae), and gobies (Gobiidae) – represent key links in the estuarine food web (Yeager and Layman 2011). These small detritivores, primary consumers, and mesopredators are an important food source for larger estuarine predators, linking estuarine primary production to higher trophic levels (Abeels et al. 2012). While less abundant, a number of economically important species utilize natural oyster reefs in the estuary as nursery habitat. Although benthic sampling trays are not designed to efficiently capture larger, more motile organisms, our long-term sampling of natural reefs revealed juvenile snapper, grouper, blue crabs, and commercial shrimp sheltering in the reef matrix.

In the Loxahatchee River, the timing of biomass maxima and minima for motile benthic communities appears to be related to seasonal patterns of precipitation and freshwater inflow. The annual peaks in biomass that we observed in late spring and early summer corresponded to the end of the dry season or early stages of the wet season. Annual minimum biomass values occurred in late fall and winter, at the start of the dry season. While the timing of biomass peaks was relatively similar from year-to-year, timing of annual minima was more variable. At all three natural reef reference sites, long-term mean biomass values doubled between the end of the wet season and the end of the dry season. A similar temporal pattern was observed in the Caloosahatchee Estuary in southwest Florida, where oyster reef communities exhibited greater biomass

during the dry season than during the wet season (Tolley et al. 2005). While intra-annual fluctuations in biomass may represent a direct response to water conditions, such as changes in salinity related to precipitation, or changes in water temperature (Lehnert and Allen 2002; Shervette and Gelwick 2008), it is also possible that the fluctuations were a result of an ingrained behavioral response associated with seasonality (e.g., change in day length).

The spatial variability in biomass of motile benthic organisms that we observed may also be attributed to salinity differences within the estuary. The upstream natural site, which had the lowest mean biomass, was closest to the freshwater source of the river and experienced more rapid fluctuations in salinity, as well as longer periods of reduced salinity (Loxahatchee River District, unpublished data). The downstream reference site, where biomass values were typically highest, may have experienced smaller fluctuations in environmental parameters (e.g., salinity, temperature) as a result of its proximity to the ocean. A similar change in oyster reef community structure along an upstream-to-downstream salinity gradient has been observed in other systems (Tolley et al. 2005; Shervette and Gelwick 2008; Quan et al. 2012).

Patterns of motile benthic community composition that we identified at natural oyster reef reference sites in the Loxahatchee River allowed us to quantify the amount of time required for restored reef communities to begin to resemble natural reef communities. In this case, the restored reef motile benthic community was similar to natural reef communities (in terms of biomass and species composition) after ~20-22 months. This timeframe was comparable to the convergence times identified by Meyer and Townsend (2000). However, other studies have documented changes in oyster reef

communities continuing over longer time frames, up to 3 to 5 years following restoration (Quan et al. 2009; Quan et al. 2012), so it is possible that further community-level convergence will occur, with additional rare taxa appearing at the restoration reef over time. At the end of the study (22 months), the restoration reef community most closely resembled the communities found at the downstream natural reef reference site.

Although the downstream reference site was not closest to the restoration reef spatially, both were located in the same shallow, open embayment (as opposed to the other two reference sites that were located in narrow, mangrove-lined channels). This similarity in landscape context between the restoration and the downstream reference site may account for the close resemblance in community composition.

Gradual development of the motile benthic community at the restoration reef was likely driven by a complex interaction between habitat quality, specific settlement cues, and the presence of previous plant and animal colonists. Initial colonists may have been generalist species that possessed broader habitat or dietary requirements than later arrivals. It is also possible that some of the later colonizers (e.g., certain blenny and goby species) were more reliant on living oysters or articulated oyster shells as habitat, and as such, may have required a certain level of live oyster growth before successfully settling on the new reef. The continued accumulation of live oyster biomass at the restoration reef will be particularly important over time, since positive interactions between living oysters and other oyster reef-associated species have been shown to help to shape a natural post-restoration community (Meyer and Townsend 2000; Halpern et al. 2007). Further convergence between motile benthic communities at natural and restored reefs

(i.e., the appearance of additional rare taxa at the restored reef) will likely be facilitated by the continued presence of living oysters at the restoration site into the future.

Motile benthic organisms that colonized the restoration reef likely represented new secondary production in the system. The low biomass and high abundance values we observed shortly after the reef was constructed imply that the restoration reef was initially colonized by large numbers of tiny organisms. Most taxa first appeared at the restoration reef as small juveniles, suggesting that they had recently recruited from the plankton. While biomass of motile benthic fauna steadily increased at the restoration reef site for the first 10 months following reef construction, we did not observe a simultaneous reduction in biomass at nearby natural reefs that would have been indicative of a redistribution of existing production to the new reef. Since habitat was likely limited for benthic oyster reef-dependent species in the Loxahatchee River, the addition of new structurally complex restoration reef habitat provided more places for larval organisms to settle (Bohnsack 1989; Pickering and Whitmarsh 1997). Based on our final biomass estimate from the restored reef site (83.6 g/m^2), the 1.93 hectare section of restoration reef that we studied supported $>1,600 \text{ kg}$ of new biomass of motile benthic organisms in May 2012, 22 months after the reef was constructed. Since restored oyster reefs are utilized by a variety of larger transient fish species (Harding and Mann 2001), this new benthic production may also serve to increase production at higher trophic levels, potentially linking oyster reef production to other ecosystems.

Habitat complexity plays an important role in the outcome of oyster reef restoration. We have shown that even very small differences (i.e., 15 cm) in vertical relief can have large effects on restored oyster reef communities, particularly during the

first year after restoration. Increased vertical relief in restoration reefs appears to facilitate convergence with natural reef communities as a result of a variety of factors. Similar to Schulte et al. (2009), we observed greater live oyster densities in treatments with slightly higher vertical relief. High-relief reefs have been found to experience increased current flow velocities, decreased sedimentation rates, and reduced occurrence of hypoxia (Lenihan and Peterson 1998; Lenihan 1999), all of which favor survival and growth of oysters (Schulte et al. 2009). Increased oyster growth can gradually lead to greater surface rugosity (another form of habitat complexity), which was apparent in the high-relief treatment at the end of our study. Increased rugosity, in turn, leads to hydrological conditions that favor larval oyster recruitment (Soniati et al. 2004; Whitman and Reidenbach 2012), creating a positive feedback that results in increased oyster recruitment on high-relief reefs (Gregalis et al. 2008). Reduced sedimentation and compaction rates can also lead to greater rugosity by maintaining open interstitial space in high-relief reefs, creating refuge for reef-dwelling organisms. Additionally, habitat complexity can affect community composition on oyster restoration reefs as a result of altered predator-prey interactions (Grabowski 2004; Grabowski and Powers 2004; Hughes and Grabowski 2006; Grabowski et al. 2008; Humphries et al. 2011a).

While many possible mechanisms could explain the differences in biomass we detected between high- and low-relief sites, our observations suggest that increased sedimentation in low-relief areas, and its related impact on live oyster growth and rugosity, may be the primary driver of reduced motile benthic biomass in low-relief sections of reef. Initial surface rugosity did not differ between treatments, since both were constructed from the same substrate. Over time, low-relief areas appeared to lose

surface rugosity as a result of sedimentation and compaction, while rugosity at high-relief areas remained constant or increased as a result of oyster growth. Early in the post-restoration phase, before live oysters had started to grow, sedimentation in the low-relief treatments likely reduced the amount of interstitial space available for colonization by motile benthic organisms. This pattern is apparent in our data, as high-relief biomass was more than five times greater than low-relief biomass within the first month following reef construction, despite just a 15 cm difference in vertical relief. Over time, as some oysters began to grow in low-relief areas, the negative impacts of sedimentation appeared to decrease slightly, resulting in the gradual convergence in biomass values that we observed. These findings may also explain why biomass differences between high- and low-relief treatments were smaller when sampling trays were emptied every other month (i.e., bimonthly sampling protocol) than when sampling trays were left undisturbed for many months (i.e., experimental habitat complexity protocol). By emptying and refilling bimonthly sampling trays on a regular basis, we may have reduced the effects of sediment accumulation, resulting in increased low-relief biomass compared to values that were observed at the end of the habitat complexity experiment (where trays had been left undisturbed for 16 months prior to sampling).

The results of our study emphasize the importance of incorporating even small increases in vertical relief into the design of future oyster restoration projects. While flat, 2-dimensional restorations have been shown to increase the abundance of macroinvertebrates and small fishes when compared to unstructured (i.e., non-reef) habitats (Plunket and La Peyre 2005), studies (like ours) that directly compare high- and low-relief habitats typically show an increased response with greater vertical relief

(Harding and Mann 2001; Gratwicke and Speight 2005). Whereas high-relief restoration reefs may become permanent, self-sustaining habitats in the years following construction (the ultimate goal of most restoration efforts), low-relief reefs are less likely to persist over time due to burial by sediments, and insufficient oyster accretion rates (Taylor and Bushek 2008; Schulte et al. 2009)

Overall, our findings illustrate a relatively rapid convergence in motile benthic community structure between restored and natural oyster reefs. From the perspective of motile oyster-associated organisms, the restoration project in the Loxahatchee River appears to have successfully achieved the pre-construction goal of creating a self-sustaining oyster reef with similar structure and function to a natural reef, through the addition of carbonate-based material to a substrate-limited section of estuary. While healthy motile benthic communities only represent one component of the ecological success of a large-scale oyster restoration project, these findings are of broad importance, as they illustrate just how quickly a critical ecosystem service provided by a natural system can be restored as a result of restoration efforts. This recovery of ecosystem services represents a rapid ecological (as well as economic) return on the initial investment made to create the restoration, and hopefully serves to further promote future restoration efforts in other systems.

Acknowledgments

Our study represents one component of a larger project funded by the government of Martin County (Florida) as part of an oyster restoration grant awarded by the National Oceanic and Atmospheric Administration (NOAA), through the American Recovery and

Reinvestment Act (ARRA) of 2009. While we have focused exclusively on the motile benthic organisms that utilize oyster reef habitats as one metric to assess the success of a large-scale oyster restoration project, other groups were tasked with tracking additional restoration success criteria (e.g., sedimentation rates, substrate compaction, water quality, sessile epifaunal community structure, oyster settlement and growth, etc.) Our research on the Loxahatchee River was made possible by a long-term partnership and collaboration with the Loxahatchee River District. We would like to thank Jerry Metz for extensive support of our field operations, Lauren Yeager for map preparation, and Continental Shelf Associates Inc. (Stuart, FL) for assisting with the construction of the high-relief experimental plots.

Table 6.1. Relative gravimetric abundance of motile benthic organisms collected in sampling trays at natural (upstream, midstream, downstream) and restored oyster reefs in the Loxahatchee River (Florida, USA). Taxa are arranged by total overall gravimetric abundance (natural and restored sites combined). Restored Reef column includes all organisms collected during bimonthly sampling following reef construction, as well as the high/low-relief time series. Asterisks indicate taxa that were identified only at the restored oyster reef. NP = not present.

Taxon	Common Name	Natural Reef (Total) % by biomass	Natural Reef (Up) % by biomass	Natural Reef (Mid) % by biomass	Natural Reef (Down) % by biomass	Restored Reef (Total) % by biomass
<i>Panopeus herbstii</i>	black-fingered mud crab	24.49	8.53	23.97	39.94	20.26
<i>Eurypanopeus depressus</i>	depressed mud crab	16.42	25.32	19.27	5.15	18.65
<i>Lophogobius cyprinoides</i>	crested goby	15.86	24.54	18.43	5.08	2.76
Panopeidae spp.	mud crab (<9 mm)	13.24	15.34	14.46	10.11	11.22
<i>Alpheus</i> spp.	snapping shrimp	8.91	8.36	6.15	12.30	14.86
<i>Petrolisthes armatus</i>	green porcelain crab	7.53	1.21	6.28	14.73	8.63
<i>Bathygobius soporator</i>	frillfin goby	5.40	6.29	7.01	2.88	1.58
<i>Nassarius vibex</i>	bruised nassa snail	2.35	NP	NP	6.99	0.71
<i>Lupinoblennius nicholsi</i>	highfin blenny	1.38	3.55	0.57	0.19	NP
<i>Portunus</i> spp.	swimming crab	0.24	0.52	0.01	0.22	6.44
<i>Gobiosoma bosc</i>	naked goby	0.59	0.60	0.32	0.88	3.85
<i>Menippe mercenaria</i>	Florida stone crab	NP	NP	NP	NP	*6.79
<i>Lutjanus griseus</i>	gray snapper	0.77	1.44	0.43	0.51	0.39
<i>Neritina clenchi</i>	Clench's nerite snail	0.64	2.00	0.01	0.04	0.06
<i>Palaemonetes</i> spp.	grass shrimp	0.16	0.01	0.06	0.40	0.97
<i>Pachygrapsus transversus</i>	mottled shore crab	0.24	0.40	0.32	NP	0.18
<i>Archosargus probatocephalus</i>	sheepshead	0.20	0.63	NP	NP	NP
<i>Erotelis smaragdus</i>	emerald sleeper	0.17	NP	0.48	0.01	NP

Table 6.1. Continued

Taxon	Common Name	Natural Reef (Total) % by biomass	Natural Reef (Up) % by biomass	Natural Reef (Mid) % by biomass	Natural Reef (Down) % by biomass	Restored Reef (Total) % by biomass
<i>Hypleurochilus aequipinnis</i>	oyster blenny	0.01	NP	0.03	0.02	0.71
<i>Stramonita haemastoma</i>	Florida rock shell	NP	NP	NP	NP	*0.68
<i>Epinephelus itajara</i>	goliath grouper	0.10	NP	0.29	NP	NP
<i>Mithrax</i> spp.	clinging crab	NP	NP	NP	NP	*0.45
<i>Farfantepenaeus aztecus</i>	brown shrimp	0.04	0.04	0.06	0.03	0.09
<i>Libinia</i> spp.	spider crab	0.02	NP	0.05	0.01	0.12
<i>Lutjanus synagris</i>	lane snapper	NP	NP	NP	NP	*0.23
<i>Upogebia</i> spp.	mud shrimp	0.02	NP	NP	0.05	0.04
<i>Haemulon</i> spp.	grunt	0.02	NP	NP	0.06	NP
<i>Mercenaria</i> spp.	hard clam	0.02	0.06	NP	NP	NP
<i>Callinectes sapidus</i>	blue crab	0.01	0.01	0.01	NP	0.01
<i>Tagelus</i> spp.	razor clam	0.01	0.01	NP	0.01	NP
<i>Clibanarius vittatus</i>	striped hermit crab	0.01	NP	NP	0.01	0.01
<i>Lysmata wurdemanni</i>	peppermint shrimp	0.01	0.01	NP	0.01	NP
<i>Gobiesox strumosus</i>	skilletfish	0.01	NP	NP	0.01	NP
<i>Eucinostomus</i> sp.	mojarra	NP	NP	NP	NP	*0.01
<i>Malacoctenus macropus</i>	rosy blenny	NP	NP	NP	NP	*0.02
<i>Alpheus formosus</i>	striped snapping shrimp	NP	NP	NP	NP	*0.01
<i>Hypsoblennius ionthas</i>	freckled blenny	NP	NP	NP	NP	*0.01
<i>Syngnathus</i> spp.	pipefish	NP	NP	NP	NP	*0.01
Isopoda spp.	isopod	0.01	NP	NP	0.01	NP
<i>Pinnixa</i> spp.	pea crab	NP	NP	NP	NP	*0.01

Table 6.2. Relative numerical abundance of motile benthic organisms collected in sampling trays at natural (upstream, midstream, downstream) and restored oyster reefs in the Loxahatchee River (Florida, USA). Taxa are arranged by total overall numerical abundance (natural and restored sites combined). Restored Reef column includes all organisms collected during bimonthly sampling following reef construction, as well as the high/low-relief time series. Asterisks indicate taxa that were identified only at the restored oyster reef. NP = not present.

Taxon	Common Name	Natural Reef (Total) % abundance	Natural Reef (Up) % abundance	Natural Reef (Mid) % abundance	Natural Reef (Down) % abundance	Restored Reef (Total) % abundance
Panopeidae spp.	mud crab (<9 mm)	41.21	50.78	44.96	30.03	36.12
<i>Alpheus</i> spp.	snapping shrimp	14.32	8.31	12.09	21.23	24.13
<i>Petrolisthes armatus</i>	green porcelain crab	13.58	3.88	10.41	24.41	7.37
<i>Eurypanopeus depressus</i>	depressed mud crab	11.09	15.84	15.35	3.65	9.68
<i>Lophogobius cyprinoides</i>	crested goby	6.71	11.58	6.75	2.49	0.62
<i>Panopeus herbstii</i>	black-fingered mud crab	3.53	1.81	4.35	4.35	4.00
<i>Gobiosoma bosc</i>	naked goby	1.53	1.30	0.65	2.43	7.90
<i>Palaemonetes</i> spp.	grass shrimp	1.68	0.02	0.69	3.88	7.07
<i>Nassarius vibex</i>	bruised nassa snail	2.06	NP	NP	5.44	0.37
<i>Bathygobius soporator</i>	frillfin goby	1.44	1.41	1.82	1.16	0.40
<i>Lupinoblennius nicholsi</i>	highfin blenny	0.94	2.44	0.27	0.18	NP
<i>Neritina clenchi</i>	Clench's nerite snail	0.41	1.18	0.01	0.07	0.04
<i>Pachygrapsus transversus</i>	mottled shore crab	0.29	0.42	0.51	NP	0.09
<i>Portunus</i> spp.	swimming crab	0.04	0.06	0.01	0.04	0.74
<i>Farfantepenaeus aztecus</i>	brown shrimp	0.10	0.02	0.17	0.11	0.04
<i>Mithrax</i> spp.	clinging crab	NP	NP	NP	NP	*0.40
<i>Menippe mercenaria</i>	Florida stone crab	NP	NP	NP	NP	*0.36
<i>Libinia</i> spp.	spider crab	0.04	NP	0.05	0.08	0.13
<i>Lutjanus griseus</i>	gray snapper	0.07	0.05	0.06	0.09	0.04

Table 6.2. Continued

Taxon	Common Name	Natural Reef (Total) % abundance	Natural Reef (Up) % abundance	Natural Reef (Mid) % abundance	Natural Reef (Down) % abundance	Restored Reef (Total) % abundance
<i>Hypleurochilus aequipinnis</i>	oyster blenny	0.01	NP	0.02	0.02	0.21
<i>Upogebia</i> spp.	mud shrimp	0.03	NP	NP	0.07	0.07
<i>Erotelis smaragdus</i>	emerald sleeper	0.04	NP	0.11	0.01	NP
<i>Mercenaria</i> spp.	hard clam	0.02	0.06	NP	NP	NP
<i>Tagelus</i> spp.	razor clam	0.01	0.02	NP	0.02	NP
<i>Callinectes sapidus</i>	blue crab	0.01	0.01	0.02	NP	0.01
<i>Lysmata wurdemanni</i>	peppermint shrimp	0.01	0.01	NP	0.02	NP
Isopoda spp.	isopod	0.01	NP	NP	0.03	NP
<i>Stramonita haemastoma</i>	Florida rock shell	NP	NP	NP	NP	*0.04
<i>Archosargus probatocephalus</i>	sheepshead	0.01	0.02	NP	NP	NP
<i>Alpheus formosus</i>	striped snapping shrimp	NP	NP	NP	NP	*0.03
<i>Pinnixa</i> spp.	pea crab	NP	NP	NP	NP	*0.03
<i>Clibanarius vittatus</i>	striped hermit crab	0.01	NP	NP	0.01	0.01
<i>Epinephelus itajara</i>	goliath grouper	0.01	NP	0.01	NP	NP
<i>Gobiesox strumosus</i>	skilletfish	0.01	NP	NP	0.01	NP
<i>Haemulon</i> spp.	grunt	0.01	NP	NP	0.01	NP
<i>Eucinostomus</i> sp.	mojarra	NP	NP	NP	NP	*0.01
<i>Hypsoblennius ionthas</i>	freckled blenny	NP	NP	NP	NP	*0.01
<i>Lutjanus synagris</i>	lane snapper	NP	NP	NP	NP	*0.01
<i>Malacoctenus macropus</i>	rosy blenny	NP	NP	NP	NP	*0.01
<i>Syngnathus</i> spp.	pipefish	NP	NP	NP	NP	*0.01

Table 6.3. Spatial variation in mean biomass of motile benthic oyster reef-associated fauna at three natural reef sites along an upstream-to-downstream gradient (mean $\pm$ standard deviation). Overall mean biomass includes all sampling dates. Annual maximum biomass is the mean of each year's maximum biomass value, which typically occurred at the end of the dry season or the beginning of the wet season. Annual minimum biomass is the mean of each year's minimum biomass value, which usually occurred near the beginning of the dry season. Capital letters in parenthesis represent the results of Tukey HSD post-hoc testing, where different letters indicate significantly different overall mean biomass values at $p < 0.05$.

Site	Overall Mean Biomass (g/m ²)	Annual Maximum Biomass (g/m ²)	Annual Minimum Biomass (g/m ²)
Upstream Site	79 $\pm$ 26 ^(A)	108 $\pm$ 22	50 $\pm$ 20
Midstream Site	92 $\pm$ 27 ^(A)	129 $\pm$ 31	62 $\pm$ 14
Downstream Site	114 $\pm$ 42 ^(B)	171 $\pm$ 75	82 $\pm$ 11

Fig. 6.1. Map of the Loxahatchee River estuary (Jupiter, Florida, USA), showing the location of the upstream (Up), midstream (Mid), and downstream (Down) natural reef reference sites, as well as the oyster restoration reef (Rest).

Fig. 6.2. Non-metric multidimensional scaling (NMDS) ordination showing relative similarity/dissimilarity between natural (Up = upstream reference site, Mid = midstream reference site, and Down = downstream reference site) and restored (Pre = pre restoration, Rest = post restoration) motile oyster reef communities. Each data point represents a single sampling date at a single site (mean of four trays). The relative proximity of two points to one another on the NMDS ordination reflects the relative similarity of the communities represented by those points (i.e., closer points indicate more similar communities). Natural reference reef data were collected between May 2007 and May 2012. Pre-restoration data were collected in March and May 2010, and post-restoration data were collected from September 2010 to May 2012.

Fig. 6.3. Changes in biomass of motile oyster reef-associated organisms following oyster reef restoration. Dashed black lines represent biomass at the restoration reef site, before reef construction (first two data points, March and May 2010), and after reef construction (all points after July 2010). Biomass at three natural reef reference sites is represented by black (upstream site) dark gray (midstream site) and light gray (downstream site) solid lines. Asterisk = date of restoration reef construction. Error bars have been omitted for clarity.

Fig. 6.4. Biomass of oyster reef-associated organisms at high- and low-relief experimental plots during the first 16 months following restoration. Throughout the study, mean biomass at high-relief plots was significantly greater than at low-relief plots ($F_{1,26} = 68.1, p < 0.001$), and there was a significant effect of time since construction on biomass values for both levels of vertical relief ($F_{1,26} = 24.7, p < 0.001$). Additionally, we observed a significant interaction between effects of relief and time since construction on the biomass of benthic organisms ($F_{6,26} = 3.20, p = 0.017$). Error bars = standard deviation.

References

- Abeels HA, Loh AN, Volety AK (2012) Trophic transfer and habitat use of oyster *Crassostrea virginica* reefs in southwest Florida, identified by stable isotope analysis. Marine Ecology Progress Series 462:125-142. doi:10.3354/meps09824
- Beck MW, Brumbaugh RD, Airoidi L, Carranza A, Coen LD, Crawford C, Defeo O, Edgar GJ, Hancock B, Kay MC, Lenihan HS, Luckenbach MW, Toropova CL, Zhang GF, Guo XM (2011) Oyster reefs at risk and recommendations for conservation, restoration, and management. BioScience 61:107-116. doi:10.1525/bio.2011.61.2.5
- Benayas JMR, Newton AC, Diaz A, Bullock JM (2009) Enhancement of biodiversity and ecosystem services by ecological restoration: A meta-analysis. Science 325:1121-1124. doi:10.1126/science.1172460
- Bohnsack JA (1989) Are high densities of fishes at artificial reefs the result of habitat limitation or behavioral preference? Bulletin of Marine Science 44:631-645
- Brumbaugh RD, Coen LD (2009) Contemporary approaches for small-scale oyster reef restoration to address substrate versus recruitment limitation: A review and comments relevant for the Olympia Oyster, *Ostrea lurida* Carpenter 1864. Journal of Shellfish Research 28:147-161
- Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. Trends in Ecology & Evolution 18:119-125
- Clarke KR, Warwick RM (2001) Changes in marine communities: an approach to statistical analysis and interpretation. 2nd Edition edn. PRIMER-E, Plymouth, UK
- Coen LD, Brumbaugh RD, Bushek D, Grizzle R, Luckenbach MW, Posey MH, Powers SP, Tolley SG (2007) Ecosystem services related to oyster restoration. Marine Ecology-Progress Series 341:303-307
- Coen LD, Luckenbach MW (2000) Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? Ecological Engineering 15:323-343
- Grabowski JH (2004) Habitat complexity disrupts predator-prey interactions but not the trophic cascade on oyster reefs. Ecology 85:995-1004
- Grabowski JH, Brumbaugh RD, Conrad RF, Keeler AG, Opaluch JJ, Peterson CH, Piehler MF, Powers SP, Smyth AR (2012) Economic valuation of ecosystem services provided by oyster reefs. BioScience 62:900-909. doi:10.1525/bio.2012.62.10.10

- Grabowski JH, Hughes AR, Kimbro DL (2008) Habitat complexity influences cascading effects of multiple predators. *Ecology* 89:3413-3422. doi:10.1890/07-1057.1
- Grabowski JH, Hughes AR, Kimbro DL, Dolan MA (2005) How habitat setting influences restored oyster reef communities. *Ecology* 86:1926-1935. doi:10.1890/04-0690
- Grabowski JH, Peterson CH (2007) Restoring oyster reefs to recover ecosystem services In: Cuddington K, Byers JE, Wilson WG, Hastings A (eds) *Ecosystem Engineers: Plants to Protists (Theoretical Ecology Series)*. Academic Press, Burlington, MA, pp 281-298
- Grabowski JH, Powers SP (2004) Habitat complexity mitigates trophic transfer on oyster reefs. *Marine Ecology Progress Series* 277:291-295
- Gratwicke B, Speight MR (2005) The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. *Journal of Fish Biology* 66:650-667. doi:10.1111/j.1095-8649.2005.00629.x
- Gregalis KC, Powers SP, Heck KL (2008) Restoration of oyster reefs along a biophysical gradient in Mobile Bay, Alabama. *Journal of Shellfish Research* 27:1163-1169
- Halpern BS, Silliman BR, Olden JD, Bruno JP, Bertness MD (2007) Incorporating positive interactions in aquatic restoration and conservation. *Frontiers in Ecology and the Environment* 5:153-160
- Harding JM, Mann R (2001) Oyster reefs as fish habitat: Opportunistic use of restored reefs by transient fishes. *Journal of Shellfish Research* 20:951-959
- Hughes A, Grabowski J (2006) Habitat context influences predator interference interactions and the strength of resource partitioning. *Oecologia* 149:256-264
- Humphries AT, La Peyre MK, Decossas GA (2011a) The effect of structural complexity, prey density, and "predator-free space" on prey survivorship at created oyster reef mesocosms. *Plos One* 6:7. doi:10.1371/journal.pone.0028339
- Humphries AT, La Peyre MK, Kimball ME, Rozas LP (2011b) Testing the effect of habitat structure and complexity on nekton assemblages using experimental oyster reefs. *Journal of Experimental Marine Biology and Ecology* 409:172-179. doi:10.1016/j.jembe.2011.08.017
- Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293:629-637. doi:10.1126/science.1059199

- Kirby MX (2004) Fishing down the coast: Historical expansion and collapse of oyster fisheries along continental margins. *Proceedings of the National Academy of Sciences of the United States of America* 101:13096-13099.
doi:10.1073/pnas.0405150101
- Lehnert RL, Allen DM (2002) Nekton use of subtidal oyster shell habitat in a southeastern US estuary. *Estuaries* 25:1015-1024. doi:10.1007/bf02691348
- Lenihan HS (1999) Physical-biological coupling on oyster reefs: how habitat structure influences individual performance. *Ecological Monographs* 69:251-275.
doi:10.1890/0012-9615(1999)069[0251:PBCOOR]2.0.CO;2
- Lenihan HS, Peterson CH (1998) How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. *Ecological Applications* 8:128-140
- Loosanoff VL (1965) The American or eastern oyster. U.S. Fish and Wildlife Service, Circular #205, Technical Report, 36pp
- Luckenbach MW, Coen LD, Ross PG, Stephen JA (2005) Oyster reef habitat restoration: Relationships between oyster abundance and community development based on two studies in Virginia and South Carolina. *Journal of Coastal Research Special Issue*: 40:64-78
- Meyer DL, Townsend EC (2000) Faunal utilization of created intertidal eastern oyster (*Crassostrea virginica*) reefs in the southeastern United States. *Estuaries* 23:34-45
- Nestlerode JA, Luckenbach MW, O'Beirn FX (2007) Settlement and survival of the oyster *Crassostrea virginica* on created oyster reef habitats in Chesapeake Bay. *Restoration Ecology* 15:273-283
- Officer CB, Smayda TJ, Mann R (1982) Benthic filter feeding - a natural eutrophication control. *Marine Ecology-Progress Series* 9:203-210
- Palmer M, Bernhardt E, Chornesky E, Collins S, Dobson A, Duke C, Gold B, Jacobson R, Kingsland S, Kranz R, Mappin M, Martinez ML, Micheli F, Morse J, Pace M, Pascual M, Palumbi S, Reichman OJ, Simons A, Townsend A, Turner M (2004) Ecology for a crowded planet. *Science* 304:1251-1252
- Peterson CH, Grabowski JH, Powers SP (2003) Estimated enhancement of fish production resulting from restoring oyster reef habitat: quantitative valuation. *Marine Ecology-Progress Series* 264:249-264
- Pickering H, Whitmarsh D (1997) Artificial reefs and fisheries exploitation: a review of the 'attraction versus production' debate, the influence of design and its significance for policy. *Fisheries Research* 31:39-59

- Plunket J, La Peyre MK (2005) Oyster beds as fish and macroinvertebrate habitat in Barataria Bay, Louisiana. *Bulletin of Marine Science* 77:155-164
- Quan W-m, Zhu J-x, Ni Y, Shi L-y, Chen Y-q (2009) Faunal utilization of constructed intertidal oyster (*Crassostrea rivularis*) reef in the Yangtze River estuary, China. *Ecological Engineering* 35:1466-1475
- Quan WM, Humphries AT, Shen XQ, Chen YQ (2012) Oyster and associated benthic macrofaunal development on a created intertidal oyster (*Crassostrea ariakensis*) reef in the Yangtze River Estuary, China. *Journal of Shellfish Research* 31:599-610. doi:10.2983/035.031.0302
- Ray GC (2006) The coastal realm's environmental debt. *Aquatic Conservation: Marine and Freshwater Ecosystems* 16:1-4
- Rodney WS, Paynter KT (2006) Comparisons of macrofaunal assemblages on restored and non-restored oyster reefs in mesohaline regions of Chesapeake Bay in Maryland. *Journal of Experimental Marine Biology and Ecology* 335:39-51
- Rothschild BJ, Ault JS, Gouilletquer P, Heral M (1994) Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. *Marine Ecology Progress Series* 111:29-39
- Schulte DM, Burke RP, Lipcius RN (2009) Unprecedented restoration of a native oyster metapopulation. *Science* 325:1124-1128. doi:10.1126/science.1176516
- SFWMD (2006) Restoration plan for the Northwest Fork of the Loxahatchee River South Florida Water Management District, Watershed Management Department, Coastal Ecosystems Division, Technical Report, 465 pp.,
- Shervette VR, Gelwick F (2008) Seasonal and spatial variations in fish and macroinvertebrate communities of oyster and adjacent habitats in a Mississippi estuary. *Estuaries and Coasts* 31:584-596. doi:10.1007/s12237-008-9049-4
- Soniat TM, Finelli CM, Ruiz JT (2004) Vertical structure and predator refuge mediate oyster reef development and community dynamics. *Journal of Experimental Marine Biology and Ecology* 310:163-182. doi:10.1016/j.jembe.2004.04.007
- Stunz GW, Minello TJ, Rozas LP (2010) Relative value of oyster reef as habitat for estuarine nekton in Galveston Bay, Texas. *Marine Ecology-Progress Series* 406:147-159. doi:10.3354/meps08556
- Taylor J, Bushek D (2008) Intertidal oyster reefs can persist and function in a temperate North American Atlantic estuary. *Marine Ecology Progress Series* 361:301-306. doi:10.3354/meps07429

- Tolley SG, Volety AK (2005) The role of oysters in habitat use of oyster reefs by resident fishes and decapod crustaceans. *Journal of Shellfish Research* 24:1007-1012
- Tolley SG, Volety AK, Savarese M (2005) Influence of salinity on the habitat use of oyster reefs in three southwest Florida estuaries. *Journal of Shellfish Research* 24:127-137
- VanArman J, Graves GA, Fike D (2005) Loxahatchee watershed conceptual ecological model. *Wetlands* 25:926-942
- Whitman ER, Reidenbach MA (2012) Benthic flow environments affect recruitment of *Crassostrea virginica* larvae to an intertidal oyster reef. *Marine Ecology-Progress Series* 463:177-191. doi:10.3354/meps09882
- Yeager LA, Layman CA (2011) Energy flow to two abundant consumers in a subtropical oyster reef food web. *Aquatic Ecology* 45:267-277

CHAPTER VII
CONCLUSIONS AND FUTURE DIRECTIONS

Although this dissertation spans a diverse range of topics, all of the underlying research questions have interrelated implications associated with the conservation and management of biotic and abiotic resources in estuarine systems. Each chapter provides a different perspective on a particular set of anthropogenic interactions in estuaries, and collectively, my findings may help establish future frameworks for adaptive management in the Loxahatchee River and other similar systems. My work underscores the importance of restoring natural freshwater inflow patterns in estuaries and coastal rivers. To help establish target flow goals, managers have identified Valued Ecosystem Components (VECs) for many coastal rivers in Florida (Alber 2002; Sime 2005; VanArman et al. 2005; SFWMD 2006). Minimum freshwater inflow targets are selected with the goal of protecting VECs (which include specific ecologically and economically plant and animal species or communities). In theory, managing freshwater inflow to protect VECs should also benefit other estuarine organisms. However, I feel that the list of VECs should be expanded to include organisms that have different responses to flow and salinity, and that utilize estuaries at different times of year. Additionally, while efforts have been made to establish ecologically relevant minimum freshwater inflow thresholds to protect VECs in many estuaries in Florida, these thresholds focus on dry season inflow, and largely ignore flow patterns that occur during the wet season. While maintaining critical minimum flow levels during the dry season to prevent ecological harm to salt-sensitive estuarine organisms is one important component of contemporary inflow management plans (Barnes 2005), it should not be the only factor considered by water managers. For the benefit of organisms that utilize estuaries outside of the dry

season, future management objectives should also include duplicating historical temporal flow patterns, and stabilizing flows to reduce unnaturally rapid fluctuations.

In Chapter II, I examined the effects of freshwater inflow on the abundance and movement patterns of common snook *Centropomus undecimalis*, an ecologically and economically important, estuarine-dependent, fish that can move freely between freshwater and saltwater. I found that common snook were more abundant in the Loxahatchee River during the wet season than during the dry season. Additionally, upstream migrations from the inlet (where spawning occurs) to riverine areas of the estuary occurred more frequently during the wet season. However, acute fluctuations in freshwater inflow did not appear to be the proximate cause for these behaviors. Instead, it seems likely that common snook evolved to spawn during the wet season, and that spawning and recruitment success may somehow be tied to flow. If common snook did evolve to reproduce during higher flows that naturally occur in the wet season, anthropogenic alteration of freshwater inflow into estuarine systems may affect spawning or recruitment success. In particular, human-controlled changes that affect the timing of the wet season or flow levels within the wet season could result in a temporal mismatch between snook spawning time and the ideal flow for spawning success (Drinkwater and Frank 1994). Additionally, anthropogenic flow alteration may affect estuarine current patterns that are responsible for transporting snook larvae to appropriate nursery habitats (Drinkwater and Frank 1994).

Moving forward, it is critical to identify the role that freshwater inflow plays on snook spawning success. This relationship has important management implications, both from the perspective of managing snook stocks, as well as managing freshwater inflow.

Future research should focus on identifying how freshwater inflow affects snook spawning behavior. Additional studies should compare snook recruitment success during periods of high and low freshwater inflow. Finally, efforts should be made to identify why snook make upstream migrations during the breeding season. From a conservation perspective, it is important to identify and protect spawning and overwintering habitats, as these areas are critical to maintaining stable common snook populations. In addition to preserving the physical habitats that common snook utilize, it is equally important to restore natural flow patterns in estuaries that snook use for spawning.

In Chapters III, IV, and V, I examined various aspects of an invasion of a predatory marine fish (Indo-Pacific lionfish *Pterois* spp.) into an estuarine ecosystem. The invasion of lionfish throughout the Western Atlantic and Caribbean was recently characterized as one of the top 15 emerging environmental issues at a global scale (Sutherland et al. 2010). This invasion represents multiple human disturbances occurring simultaneously. While the initial release of non-native lionfish from the pet trade represents a direct human-mediated disturbance, my findings suggest that the estuarine aspect of the invasion may have been facilitated by anthropogenic alteration of freshwater inflow patterns and shoreline habitat modification. Because lionfish are typically associated with marine habitats, I feel that anthropogenic reductions in freshwater inflow, combined with increased saltwater intrusion resulting from estuarine dredging, may have allowed lionfish to colonize estuaries. Additionally, I have shown that lionfish have a strong preference for anthropogenically modified habitats within estuaries. In highly disturbed estuaries like the lower Loxahatchee River, the construction of thousands of residential docks may have facilitated the upstream spread of lionfish.

Future studies should examine effects of lionfish on other estuarine organisms in order to determine whether this facet of the invasion has the potential to impact estuarine health. In response to my findings, additional efforts should be made to assess lionfish populations in other estuarine systems throughout the invaded range. The high site fidelity that I identified in Chapter IV suggests that localized lionfish populations may be kept under control through regular removal efforts. Since lionfish do not seem to move great distances once they settle as juveniles, areas where lionfish removal efforts have been carried out will most likely be repopulated by newly recruited juveniles (rather than larger adult fish moving in from other areas). These smaller individuals will likely have a smaller ecological impact. Recent studies have shown that regular removal efforts can result in a considerable reduction in lionfish predation on native fish species (Green et al. 2014). Since invasive species population control is costly and time consuming, future removal efforts should focus on ecologically important habitats, like estuaries, coral reefs, and marine protected areas. Based on the minimum salinity tolerance values I identified in Chapter V, the restoration of historical freshwater inflow regimes in coastal rivers may help restrict the extent of estuarine colonization by lionfish without the need for physical removal.

In Chapter VI, I examined restored oyster reefs that were constructed to replace natural oyster reefs that had been lost due to anthropogenic reductions in freshwater inflow and increased saltwater intrusion. Oysters represent a critical foundation species (Bruno et al. 2003), providing food, shelter, and nursery habitat for a wide variety of estuarine organisms, including many ecologically and economically important fishes (Grabowski et al. 2005; Abeels et al. 2012). Because of the multitude of ecosystem

services provided by intact oyster reefs (Officer et al. 1982; Coen et al. 2007; Grabowski et al. 2012), oyster reef restoration may play an important role in improving overall estuarine health. I demonstrated that communities of motile benthic organism that colonize restored oyster reefs quickly began to resemble natural oyster reef communities, suggesting that restoration efforts successfully reestablish one critical set of ecosystem services provided by natural oyster reefs (i.e., providing habitat for oyster reef-dependent organisms). Oyster reef restoration creates new benthic production in estuarine systems, which is likely propagated up through the estuarine food web. My findings illustrate the importance of incorporating vertical relief into the design of future oyster reef restoration projects, since higher relief reefs supported much greater biomass of reef-associated organisms in the months following restoration. Although my research demonstrated that restored oyster reefs rapidly support communities that are similar to those found on natural oyster reefs, the reestablishment of historical flow and salinity patterns would allow natural oyster reefs to flourish, reducing our dependence on human-created restoration habitats. Future work should focus on modeling the effects of flow restoration on oyster reef development, and assessing optimal reef design to provide the greatest ecological benefit at the lowest economic cost.

References

- Abeels HA, Loh AN, Volety AK (2012) Trophic transfer and habitat use of oyster *Crassostrea virginica* reefs in southwest Florida, identified by stable isotope analysis. *Marine Ecology Progress Series* 462:125-142. doi:10.3354/meps09824
- Alber M (2002) A conceptual model of estuarine freshwater inflow management. *Estuaries and Coasts* 25:1246-1261
- Barnes T (2005) Caloosahatchee estuary conceptual ecological model. *Wetlands* 25:884-897
- Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution* 18:119-125
- Coen LD, Brumbaugh RD, Bushek D, Grizzle R, Luckenbach MW, Posey MH, Powers SP, Tolley SG (2007) Ecosystem services related to oyster restoration. *Marine Ecology-Progress Series* 341:303-307
- Drinkwater KF, Frank KT (1994) Effects of river regulation and diversion on marine fish and invertebrates. *Aquatic Conservation: Marine and Freshwater Ecosystems* 4:135-151
- Grabowski JH, Brumbaugh RD, Conrad RF, Keeler AG, Opaluch JJ, Peterson CH, Piehler MF, Powers SP, Smyth AR (2012) Economic valuation of ecosystem services provided by oyster reefs. *BioScience* 62:900-909. doi:10.1525/bio.2012.62.10.10
- Grabowski JH, Hughes AR, Kimbro DL, Dolan MA (2005) How habitat setting influences restored oyster reef communities. *Ecology* 86:1926-1935. doi:10.1890/04-0690
- Green SJ, Dulvy NK, Brooks ALM, Akins JL, Cooper AB, Miller S, Côté IM (2014) Linking removal targets to the ecological effects of invaders: a predictive model and field test. *Ecological Applications*. doi:10.1890/13-0979.1
- Officer CB, Smayda TJ, Mann R (1982) Benthic filter feeding - a natural eutrophication control. *Marine Ecology-Progress Series* 9:203-210
- SFWMD (2006) Restoration plan for the Northwest Fork of the Loxahatchee River South Florida Water Management District, Watershed Management Department, Coastal Ecosystems Division, Technical Report, 465 pp.
- Sime P (2005) St. Lucie Estuary and Indian River Lagoon conceptual ecological model. *Wetlands* 25:898-907

- Sutherland WJ, Clout M, Côté IM, Daszak P, Depledge MH, Fellman L, Fleishman E, Garthwaite R, Gibbons DW, De Lurio J, Impey AJ, Lickorish F, Lindenmayer D, Madgwick J, Margerison C, Maynard T, Peck LS, Pretty J, Prior S, Redford KH, Scharlemann JPW, Spalding M, Watkinson AR (2010) A horizon scan of global conservation issues for 2010. *Trends in Ecology & Evolution* 25:1-7
- VanArman J, Graves GA, Fike D (2005) Loxahatchee watershed conceptual ecological model. *Wetlands* 25:926-942

VITA

ZACHARY ROBERT JUD

Born, Baltimore, Maryland

- 2002 B.S., Biology
 University of Scranton
 Scranton, Pennsylvania
- 2008 M.S., Marine Biology
 Florida Institute of Technology
 Melbourne, Florida
- 2008-2014 Doctoral Candidate
 Florida International University
 Miami, Florida

PUBLICATIONS

- Jud ZR, Nichols PK, Layman CA (in press) Broad salinity tolerance in the invasive lionfish *Pterois* spp. may facilitate estuarine colonization. *Environmental Biology of Fishes*
- Layman CA, Jud ZR, Arrington DA, Sabin D (in press) Fish behavior as a means to assess oyster reef restoration. *Ecological Restoration*
- Layman CA, Jud ZR, Archer SK, Riera D (in press) Provision of ecosystem services by human-made structures in a highly impacted estuary. *Environmental Research Letters*
- Jud ZR, Layman CA (2012) Site fidelity and movement patterns of invasive lionfish, *Pterois* spp., in a Florida estuary. *Journal of Experimental Marine Biology and Ecology* 414-415:69-74
- Jud ZR, Layman CA, Lee JA, Arrington DA (2011) Recent invasion of a Florida (USA) estuarine system by lionfish *Pterois volitans* / *P. miles*. *Aquatic Biology* 13:21-26
- Jud ZR, Layman CA, Shenker JM (2011) Diet of age-0 tarpon (*Megalops atlanticus*) in anthropogenically-modified and natural nursery habitats along the Indian River Lagoon, Florida. *Environmental Biology of Fishes* 90:223-233
- Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P, Rosenblatt AE, Vaudo JJ, Yeager LA, Post DM, Bearhop S (2011)

Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews*

Jud ZR, Layman CA (in review) Changes in benthic faunal community structure following large-scale oyster reef restoration in a subtropical estuary. *Aquatic Sciences*

Layman, CA, Jud ZR, Nichols PK (in review) Lionfish alter benthic invertebrate assemblages. *Marine Biology*