AGE, GROWTH, AND REPRODUCTIVE STATUS OF TRIPLETAIL (*LOBOTES SURINAMENSIS*) IN THE AGGREGATION NEARSHORE JEKYLL ISLAND, GA, USA

by

RUSSELL TURNER PARR

(Under the Direction of Cecil Jennings and Robert Bringolf)

ABSTRACT

Demographic information is necessary for effective management of fish but was lacking for tripletail (*Lobotes surinamensis*) in Georgia (USA). Therefore, the current study evaluated age, growth, and reproduction of tripletail off the coast of Jekyll Island, GA from March - August 2009 and April - August 2010. Strong agreement (84%) between sagittal otoliths (lethal) and first dorsal spine (non-lethal) documented that both were useful for aging tripletail. Gonad histology revealed that male tripletail were in spawning condition from April – August but most female tripletail were not in spawning condition. Plasma vitellogenin was high in the few spawning-capable females captured, but generally the protein was not useful for distinguishing males from non-spawning females. Non-lethal sampling techniques may be useful for tripletail age and growth analysis as well as identification of spawning females. Future research should focus on validation of non-lethal aging techniques and vitellogenin analysis for determination of reproductive status.
INDEX WORDS: Tripletail, otoliths, spines, growth models, age comparison, demographics, reproduction, non-lethal, histology, vitellogenin
AGE, GROWTH, AND REPRODUCTIVE STATUS OF TRIPLETAIL (*LOBOTES SURINAMENSIS*) IN THE AGGREGATION NEARSHORE JEKYLL ISLAND, GA, USA

by

RUSSELL TURNER PARR

B.S., University of Georgia, 2007

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2011
AGE, GROWTH, AND REPRODUCTIVE STATUS OF TRIPLETAIL (*LOBOTES SURINAMENSIS*) IN THE AGGREGATION NEARSHORE JEKYLL ISLAND, GA, USA

by

RUSSELL TURNER PARR

Major Professor: Cecil Jennings
Robert Bringolf

Committee: Carolyn Belcher
Robert Warren

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2011
DEDICATION

- To my parents, sister, and fiancé: for their moral support, love and understanding.
ACKNOWLEDGEMENTS

I would like to thank the Georgia Department of Natural Resources, Coastal Resources Division (CRD) and the Federal Sportfish Restoration Fund for funding the project.

This project could not have been completed without the help of many people. First, I thank Spud Woodward for his unwavering support and confidence from start to finish. I thank Doug Haymans who gave me my start the GADNR and helped all along the way to the project’s completion. I thank my co-advisors Robert Bringolf and Cecil Jennings who were key to my professional development. Carolyn Belcher, a committee member, provided critical guidance and technical assistance. Bob Warren, a committee member, provided insightful editorial comments. Jim Franks provided invaluable technical advice.

I also thank all of the GADNR CRD staff including Pat Geer, Cason Kinstle, Chris Kalinowsky, Gabe Gaddis, Shawn Jordan, Eric Robillard, Donna McDowell, Kathy Herrin, Linda Willis, Kirby Wolfe, Shane Kicklighter, Geoffrey Meeks, Jeff Mericle, Dominic Guadagnoli, Paul Medders, William Hughes, Jim Page, Billy Readdick, Rusty Flournoy, Ed Butler and Dwight Varnadoe for field and technical assistance.

I appreciate the efforts of all of the anglers including those from the Coastal Conservation Association of Georgia and especially Greg Hildreth who offered assistance with field sampling. Lab mates of the Jennings and Bringolf labs provided valuable advice and assistance.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGEMENTS</th>
<th>LIST OF TABLES</th>
<th>LIST OF FIGURES</th>
<th>CHAPTER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52</td>
</tr>
</tbody>
</table>

---

1 Introduction and Literature Review .................................................................1

   Literature Review ........................................................................................... 3

   Literature Cited .................................................................................................9

2 Evaluating the utility of spines and otoliths for aging tripletail, *Lobotes surinamensis*,
   nearshore Jekyll Island, GA, USA .........................................................................15

   Introduction .......................................................................................................16

   Study Site .........................................................................................................18

   Methods ............................................................................................................19

   Results ..............................................................................................................25

   Discussion .........................................................................................................27

   Conclusion ........................................................................................................31

   Literature Cited.................................................................................................33

3 Evaluation of reproductive status for tripletail, *Lobotes surinamensis*, nearshore
   Jekyll Island, GA, USA .......................................................................................51

   Introduction .......................................................................................................52
Methods ......................................................................................................................55
Results .....................................................................................................................64
Discussion .................................................................................................................67
Literature Cited .........................................................................................................73
Summary ....................................................................................................................87
Conclusion ...............................................................................................................90
APPENDICES

A  Tripletail reproduction capture data ...................................................................91
LIST OF TABLES

Table 2-1: Summary information for male (n = 126 total length; n = 125 total weight) and female (n = 105 total length; n = 101 total weight) tripletail captured near Jekyll Island, Georgia, USA in 2009 and 2010........................................................................................................36

Table 2-2: Otolith-based, back-calculated total length-at-age for tripletail captured in 2009 (A) and 2010 (B) near Jekyll Island, GA (2009 n = 125, 2010 n = 118). ........................................37

Table 2-3: Spine-based, back-calculated total length-at-age for tripletail captured in 2009 (A) and 2010 (B) near Jekyll Island, GA (2009 n = 123, 2010 n = 115). ........................................38

Table 2-4: Mean total length-at-age for otolith- and spine-derived ages at the time of capture compared with otolith and spine back-calculated total length-at-age for tripletail captured in the summer of 2009 and 2010 near Jekyll Island, GA. Otolith back-calculated total length-at-age appear to most closely follow the mean length at time of capture. ........39

Table 3-1: Reproductive phases of individual male tripletail (n = 122) captured in 2009 and 2010 near Jekyll Island, GA, USA. All males in this study in the regressing phase were spawning capable but were no longer undergoing active spermatogenesis. GE = germinal epithelium. ..................................................................................................................................78

Table 3-2: Reproductive phases of individual female tripletail (n = 102) captured in 2009 and 2010 near Jekyll Island, GA, USA..................................................................................................................................79
LIST OF FIGURES

Figure 1-1: Map of the study area off the coast of Jekyll Island, GA. The inset displays the southeastern United States and the arrow represents the location of the study site...........13

Figure 1-2: Picture of a tripletail caught off the Jekyll Island, GA coast. Notice how the second dorsal and anal fin sweep back and give the appearance of three tails with the caudal fin.14

Figure 2-1: An illustration of the tripletail sampling location near Jekyll Island, GA, USA. Inset shows a map of GA with the Jekyll Island area darkened. Each point on the map indicates that one or more tripletail were captured at that location from March to August 2009 and 2010. Not all fish capture locations for the study are located on the map.........................40

Figure 2-2: Examples of otoliths (A) and spines (B) taken from individual tripletail captured in 2009 and 2010 near Jekyll Island, GA, USA. 1A and 1B represent an age-1 fish, the arrow indicates where striations terminate into a translucent band denoted as an annulus. 2A and 2B represent an age-2 tripletail. 3A and 3B represent an age-3 tripletail. 4A and 4B represent an age-4 tripletail, notice the compression of the bands near the edge. 5A and 5B represent an age-5 tripletail, notice the small first annulus that we believe is a result of compression as the tripletail ages, which we suggest should not be skipped. These pictures were not taken at the same scale and are to be used as examples. ............41

Figure 2-3: Number of male and female tripletail (n = 231) captured during sampling off the coast of Jekyll Island, GA during 2009 and 2010. Sex ratio was not different than the expected ratio of 1:1 (X^2 = 9.74, P = 0.0828).........................................43
Figure 2-4: Total length (TL) and total weight regression for tripletail captured (n = 229) from the aggregation near Jekyll Island, GA, USA from March – August 2009 and 2010. .....44

Figure 2-5: Plot of reader 1 versus reader 2 initial otolith based age estimates for tripletail captured in the Jekyll Island, GA, USA, area during the 2009 and 2010 sampling seasons. The equal-age line represents 1:1 agreement of age estimates. Numbers located on the plot represent the number of observations for a given reader 1 and reader 2 age combination..............................................................45

Figure 2-6: Plot of reader 1 versus reader 2 initial spine based age estimates for tripletail captured in the Jekyll Island, GA, USA, area during the 2009 and 2010 sampling seasons. The equal-age line represents 1:1 agreement of age estimates. Numbers located on the plot represent the number of observations for a given reader 1 and reader 2 age combination..............................................................46

Figure 2-7: Plot of agreed otolith and spine derived ages from tripletail captured near Jekyll Island, GA in 2009 and 2010. (n = 239). The equal-age line represents 1:1 agreement of age estimates. Numbers located on the plot represent the number of observations for agreed otolith age and agreed spine age combination. ...........................................47

Figure 2-8: Box plots for tripletail age data for (A) spines and (B) otoliths near Jekyll Island, GA, USA captured from March to August 2009 and 2010. The central line indicates the median, the grey box represents the middle 50% of the data and the whiskers represent the area where 90% of the data fell. Outliers are denoted by the black dots. ......................48

Figure 2-9: Comparison of back-calculated and observed length-at-age estimates from otoliths and spines from tripletail captured near Jekyll Island, GA, USA captured from March to August 2009 and 2010. Error bars represent standard errors of the mean.....................49
Figure 2-10: Spine and otolith von Bertalanffy growth curves for tripletail captured near Jekyll Island, GA, USA during March to August 2009 and 2010. Only ages 1-5 were captured during this study.

Figure 3-1: Tripletail sampling location near Jekyll Island, GA, USA. Inset shows a map of the southeast United States with the Jekyll Island area indicated by the arrow. Each point on the map indicates that one or more tripletail were captured at that location from March to August 2009 and 2010.

Figure 3-2: Non-linear regression of sexually mature female tripletail in 50-mm length bins captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Closed data points represent actual cumulative percent maturity of females, whereas males are represented by the open data points. The dashed line denotes the length (459 mm) at which 50% of female tripletail are mature. The dotted line represents the minimum size limit (457 mm) currently enforced by the Georgia Department of Natural Resources. Non-linear regression models for males were unable to converge and therefore were unable to be modeled; however, only one male captured in this study was classified as immature.

Figure 3-3: Non-linear regression of sexually mature female and male tripletail by age class captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Closed data points represent actual cumulative percent maturity of females; males are represented by the open data points. The dotted line denotes the age at which 50% of males (0.55 years) and females (1.17 years) are mature.
Figure 3-4: Mean gonadosomatic index values for (A) female (n = 101) and (B) male (n = 116) tripletail captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Error bars represent ± 1 standard error...

Figure 3-5: Blood plasma vitellogenin concentrations of tripletail captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Females are represented by the triangles and males are represented by open circles. Reproductive phases are represented by Imm- immature, Dev- developing, SC- spawning capable, Regress- regressing, and Regen- regenerating...

Figure 3-6: Mean plasma vitellogenin concentrations (μg/ml) for (A) female (n = 77) and (B) male (n = 98) tripletail captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Error bars represent ± 1 standard error...

Figure 3-7: Comparison of plasma vitellogenin and gonadosomatic index values across reproductive phases for (A) female (n=77) and (B) male (n=98) tripletail captured near Jekyll Island, GA, USA during March to August 2009 and 2010. Vitellogenin means are represented by triangles and GSI mean values are represented by circles. Error Bars represent 95% confidence intervals. Reproductive phases are represented by Imm- immature, Dev- developing, SC- spawning capable, Regress- regressing, and Regen- regenerating...
In Georgia, tripletail are found in large numbers in the nearshore Atlantic Ocean waters immediately east of Jekyll Island from March to July (Figure 1-1). Atypically, these fish are not associated with any structure, but rather are free swimming at the surface in shallow waters ranging in depth from 2 to 4 meters. This free-floating behavior allows anglers to locate (sight) individual fish, which can be approached stealthily and presented a lure or natural bait. The draw of sight fishing for relatively large fish and their highly sought-after flesh have created an increase in the number of anglers pursuing this species off of the Jekyll Island coast. Juvenile and adult tripletail are commonly observed or captured around floating docks and other fixed structures within estuarine waters but are typically not on the surface at these locations. Targeting fish associated with structure is gaining popularity among anglers, further increasing angling pressure on this species in Georgia. The catch in Georgia varies from small (< 300 g) specimens to very large individuals (> 11 kg). In response to pressure from recreational anglers and charter captains, the Georgia Department of Natural Resources (GA-DNR) implemented harvest regulations to decrease the likelihood of overfishing. In 2006, the State of Georgia enacted a 457-mm (18”) minimum-size limit and a 2 fish per person creel limit for tripletail based on the limited amount of available tripletail life history information.

Why tripletail aggregate offshore of Jekyll Island during the spring and summer is unknown. Other such aggregations have not been documented along the Atlantic Coast or in the
Gulf of Mexico; however, anecdotal accounts suggest similar aggregations may exist in other locations in Georgia and off the coast of Cape Canaveral, FL. Macroscopic examination has revealed ovaries in late stages of development in females harvested by anglers fishing this aggregation, although running-ripe males have not been reported. Anglers claim to have observed a single large fish accompanied by two or three small fish in what appears to be courtship behavior. Despite the anecdotal evidence suggesting that tripletail in the aforementioned aggregation are spawning, conclusive evidence is lacking. Whether this aggregation is comprised of pre-spawn, actively spawning, or post-spawn tripletail is unknown.

The lack of data for tripletail found off the Jekyll Island coast and other coastal Georgia areas presents a major gap in scientific data used to manage the species in State waters. The area offshore of Jekyll Island presents a unique opportunity to study these fish because of the large aggregation in close proximity to the GA-DNR Coastal Regional Headquarters (CRH) during a timeframe suggested as the spawning season by prior research (Brown-Peterson and Franks 2001; Cooper 2002; Strelcheck et al. 2004) and anecdotal evidence. This aggregation is targeted by recreational anglers and the area is impacted by commercial shrimp trawlers. Although direct impacts of commercial shrimp trawling are unknown, an interaction between the two sectors occurs. These activities make determining the status of this aggregation a high priority. This determination is a prerequisite for determining if this aggregation should be afforded higher protection and will allow the GA-DNR to evaluate whether current fishery management policy for the conservation of tripletail is appropriate.

The goal of this study was to describe the reproductive status and age structure of the tripletail aggregation near Jekyll Island, GA, USA and to evaluate the efficacy of non-lethal sampling techniques. The use of traditional histological and aging methods requires researchers
to collect and sacrifice large numbers of fish to produce meaningful results. Results of non-lethal sampling techniques were compared to traditional methods for evaluation of age determination and reproductive status. Three primary methods were used to determine the reproductive status of the tripletail offshore of Jekyll Island: gonadosomatic index (GSI), histological examination, and analysis of blood plasma for vitellogenin (VTG). We determined age and growth of tripletail by otoliths and dorsal spines, henceforth spine, and compared the efficacy of the non-lethal (spine) approach to the traditional, lethal (otolith) approach.

**Objectives**

1) use non-lethal measures of reproductive physiology (e.g. blood plasma vitellogenin levels) for assessment of tripletail reproductive status

2) validate non-lethal reproductive assessment methods by comparing results with gonad histology

3) compare sagittal otolith and first dorsal spine for determining age and calculating growth rates of tripletail in the Jekyll Island aggregation

**Literature Review**

Tripletail are medium-sized, deep-bodied fish that occur in tropical and subtropical waters worldwide (Figure 1-2; Baughman 1941). Elongated soft rays of the dorsal and anal fins project backwards around the caudal fin and create the appearance of three tails, hence the common name, tripletail. The tripletail is also called eddyfish, buoy bass or blackfish in the southeastern United States (Gudger 1931).
**Habitat**

Tripletail occur in a variety of habitats from estuarine waters to the open ocean, but are usually found in association with submerged, emergent, or floating structure (Benson 1982; Gudger 1931; Kelly 1923). The species has the unique habit of floating on its side at the surface apparently in an attempt to mimic a floating object, or perhaps to avoid predation while also attracting potential prey seeking cover (Gudger 1931). Examination of the gut contents of specimens from the northern Gulf of Mexico suggests the species feeds opportunistically, with a diet comprised of shrimp, crabs, and teleost fishes, although the primary bait for recreational fishers is live shrimp (Franks et al. 2003; Strelcheck et al. 2004).

**Age and Growth**

Otoliths often provide more accurate aging data when compared to other fish hard parts, such as vertebral bones, scales, fin rays and spines (VanderKooy 2009). This increased accuracy results from the continuous accretion and the limited resorption found in otoliths (VanderKooy 2009). Spine-based aging has the advantage of being non-lethal and a minimally invasive age determination technique (VanderKooy 2009). Disadvantages of using spines include vascularization and resorption that can obscure the first few annuli and lead to underestimation of fish age by the reader (VanderKooy 2009). Advantages and disadvantages of both otolith- and spine-based aging suggest that the evaluation of both structures can elucidate the optimal aging structure and technique.

Scale-based ages of tripletail from North Carolina were: age-0 (n=1; 190 mm TL); age-1 (n=6; 445- 591 mm TL); age-2 (n=5; 562-706 m TL); and age-3 (n=2; 568-706 mm TL) (Merriner and Foster 1974). Franks et al. (1998) and Strelcheck et al. (2004) found otoliths
unsuitable for aging and selected the spine, whereas Armstrong et al. (1996) found otoliths as acceptable aging structures. Although differences occurred among studies as to whether otoliths were acceptable aging structures, length-at-age data were similar for scale, otolith and spine-based ages (Merriner and Foster 1974; Strelcheck et al. 2004; Franks et al. 1998). Scales are generally considered less accurate aging structures and were therefore not evaluated in the current study. The present study represents the largest sample size for tripletail aging in which both otolith and spine-based ages were determined to evaluate the utility of the structures for aging.

Reproduction

Tripletail may attain sexual maturity by age 1+ (Brown-Peterson and Franks 2001; Cooper 2002); however, Strelcheck et al. (2004) suggests that age at maturity could range from one to two years of age based on the reproductive phase used to determine maturity status. The species appears to be a multiple batch spawner, with the ability to spawn once every three to five days during the spawning season (Brown-Peterson and Franks 2001; Cooper 2002). Gudger (1931) and Baughman (1941) both documented females containing roe during the months of July and August in North Carolina, Florida, Mississippi, and Texas. Brown-Peterson and Franks (2001) captured running-ripe males off the coast of Mississippi from May through September and females in late-developing ovarian maturation stages from June through August, with peak spawning condition occurring in July. Strelcheck et al. (2004) found similar results in coastal Alabama waters but did not document running ripe males. These studies indicated tripletail spawn at an early age and over a protracted spawning season throughout their United States range.
The exact temporal and spatial occurrence of tripletail spawning has not been determined, with some of the literature conflicting. Some researchers suggest that spawning occurs offshore because of the occurrence of larval tripletail in sea surface tows in the Gulf of Mexico in waters >100m (Ditty and Shaw 1994). Researchers have found females in early and late stages of gonadal maturation near and inshore waters; however, no hydrated (mature) oocytes have been documented which may suggest offshore spawning (Brown-Peterson and Franks 2001; Cooper 2002; Strelcheck et al. 2004). Alternatively, in North Carolina, the presence of small larvae inshore suggests inshore spawning locations (Merriner and Foster 1974); however, these authors cite a possible role of ocean currents in transporting larvae to capture locations that may be distant from spawning locations. No evidence of anadromy has been suggested in previous literature but this strategy has not been ruled out.

Gonadosomatic Index

The gonadosomatic index (GSI) is a measure that can be used to evaluate spatial and temporal trends in gonadal development (Nikolsky 1963; de Vlaming et al. 1980). The GSI is the ratio of gonadal material to the body mass of the animal (Nikolsky 1963). Gonadosomatic indices can be used to detect hydrated ovaries because the wet weight of hydrated ovaries ranges from two to four or more times the weight of gonads at other maturity phases (Hunter and Macewicz 1985). Brown-Peterson and Franks (2001) found that the mean monthly female GSI values for tripletail elevated throughout the summer and were highest in July in the northern Gulf of Mexico. Cooper (2002) also found that GSI values were elevated in the summer but found peak values in August for tripletail caught off the Atlantic coast of Florida. Male GSI values were elevated from May through September in both studies. The values decreased to resting
(quiescent) levels by September for both sexes in the northern Gulf of Mexico (Brown-Peterson and Franks 2001) and in October in the western Atlantic (Cooper 2002). Although Brown-Peterson and Franks (2001) and Cooper (2002) did find elevated GSI values in summer months, the values were relatively low compared to other species, most likely because of the lack of gravid females with hydrated oocytes.

Histology

Histological analysis yields very accurate information on oocyte development; however, this analysis is more time consuming compared to GSI or visual staging based on macroscopic appearance of the gonads (West 1990). Many studies have reviewed oocyte development in teleosts, including tripletail (Hunter and Macewicz 1985; Brown-Peterson et al. 1988; West 1990; Brown-Peterson and Franks 2001; Cooper 2002; Strelcheck et al. 2004). Brown-Peterson et al. (2011) attempted to standardize classification of gonadal reproductive phase to prevent confusion across species and these methods were used to determine reproductive phase in tripletail. Brown-Peterson et al. (2011) classified females in one of five categories: immature, developing (sub-phase: early developing), spawning capable (sub-phase: actively spawning), regressing, or regenerating. Males were also classified in one of five categories: immature, developing (sub-phase: early developing), spawning capable (sub-phase: early germinal epithelium (GE), mid-GE, and late-GE ), regressing or regenerating. Occurrence and relative abundance of the five developmental stages of the oocytes (primary growth, cortical alveoli, partially yolked, advanced yolk, and hydrated) and occurrence and intensity of atresia were used as the criteria for histological classification (Wallace and Selman 1981).
Blood Sex Steroid Hormones and Vitellogenin

Previously mentioned methods of determining reproductive status require that the fish be sacrificed, which is an undesirable approach for an aggregation lacking population dynamics information. Sex steroid hormones and the yolk precursor, VTG, have been linked with sturgeon gender and maturity stages in aquaculture facilities (Fox 2001). Gender and gonadal maturation stage have been determined by quantifying levels of VTG and gonadal steroids in other teleosts by enzyme-linked immunosorbent assays (ELISA) and radioimmunoassay (RIA) (Heppell and Sullivan 2000). Vitellogenin is found at high concentrations during the final oocyte maturation phase in most fishes. Vitellogenin is relatively simple to purify, specific to maturing females, and cost effective to measure using an ELISA (Heppell and Sullivan 2000). Gonadosomatic index values have also been positively correlated with an increase in VTG in rainbow trout and gag grouper (van Bohemen and Lambert 1981; Heppell and Sullivan 2000). Researchers have successfully used ELISA to quantify VTG in fish plasma in place of standard histological techniques to accurately identify maturing females (Heppell and Sullivan 1999). No published data exists for the VTG levels in tripletail.

Format

This thesis was written in manuscript format. Chapter 1 is an introductory chapter that includes a literature of all tripletail age, growth, and reproduction studies. In Chapter 2, the utility of spines and otoliths for tripletail aging were evaluated. In Chapter 3, the reproductive status of tripletail nearshore Jekyll Island, GA and the utility of blood plasma VTG as indicator of reproductive status were evaluated. Chapter 4 summarizes the findings of this project and describes management implications.
Literature Cited


(Mycteroperca microlepis). Canadian Journal of Fisheries and Aquatic Sciences 57:148-159.


Figure 1-1. Map of the study area off the coast of Jekyll Island, GA. The inset displays the southeastern United States and the arrow represents the location of the study site.
Figure 1-2. Picture of a tripletail caught off the Jekyll Island, GA coast. Notice how the second dorsal and anal fin sweep back and give the appearance of three tails with the caudal fin.
CHAPTER 2

Evaluating the utility of spines and otoliths for aging tripletail, *Lobotes surinamensis*, nearshore Jekyll Island, GA, USA

Introduction

Tripletail (*Lobotes surinamensis*, Lobotidae) are medium-sized, deep-bodied fish that occur in tropical and subtropical waters worldwide (Baughman 1941; Hardy 1978). Peer-reviewed literature with data about tripletail is scarce, and the majority of the most recent studies remain in unpublished agency reports. Tripletail is the only member from the monophyletic family Lobotidae; the species is found in the western Atlantic Ocean (Hardy 1978). They are known to occur from Massachusetts, USA in the Northern Hemisphere to Argentina in the Southern Hemisphere; their westward range includes the Gulf of Mexico and the Caribbean Sea, with higher abundances south of North Carolina, USA (Hardy 1978). Tripletail are migratory; however, detailed information about their exact movement patterns is scarce or lacking (Merriner and Foster 1974; Franks et al. 1998).

Tripletail occur in a variety of habitats, from estuarine waters to the open ocean, but are commonly found in association with structure (Gudger 1931; Kelly 1923; Benson 1982; Ditty and Shaw 1994). Examination of the gut contents of northern Gulf of Mexico tripletail suggests the species feeds opportunistically. Diets are comprised of shrimp, crabs, and teleost fishes; live shrimp is the primary bait used by recreational anglers targeting tripletail (Merriner and Foster 1974; Cooper 2002; Strelcheck et al. 2004).

Scale-based ages of tripletail from North Carolina were: age-0 (n=1; 190 mm total length (TL)); age-1 (n=6; 445-591 mm TL); age-2 (n=5; 562-706 mm TL); and age-3 (n=2; 568-706 mm TL) (Merriner and Foster 1974) and spine-derived ages of tripletail show similar results (Franks et al. 1998; Strelcheck et al. 2004). Otoliths have been cited as poor aging structures for tripletail (Franks et al. 1998; Strelcheck et al. 2004); however, Armstrong et al. (1996) used
otoliths to produce similar lengths-at-age as spine-derived ages (Franks et al. 1998; Strelcheck et al. 2004).

Nearly all data on the reproductive biology of tripletail are unpublished, with some of the data conflicting. Estimates for age- and length-at-sexual maturity range from one to two years and from 350 – 500 mm TL. The species is thought to be a multiple-batch spawner, with the ability to spawn once every three to five days throughout the spawning period (Brown-Peterson and Franks 2001; Cooper 2002). Baughman (1941) documented ovaries containing roe during the months of June, July and August in the Gulf of Mexico. Spawning in the Gulf of Mexico occurs from June through August with a peak in July (Brown-Peterson and Franks 2001), although larval data indicate spawning may occur through September (Ditty and Shaw 1994). Female tripletail in the Western Atlantic Ocean are in spawning condition from April through September (Cooper 2002) and running-ripe males have been captured from May through September in the Gulf of Mexico (Brown-Peterson and Franks 2001; Strelcheck et al. 2004). Offshore spawning has been suggested for tripletail because of the lack of hydrated female tripletail in previous studies and larvae have been captured in >100 m of water in plankton surface tows (Ditty and Shaw 1994; Brown-Peterson and Franks 2001; Cooper 2002; Strelcheck et al. 2004).

In Georgia (GA, USA), tripletail are found in estuaries around structure and nearshore off some of the barrier islands. Free-floating tripletail are found in large numbers in the nearshore Atlantic Ocean waters immediately east of Jekyll Island beach from March to July (Figure 2-1). Atypically, these fish are not associated with any structure, but rather are free swimming at the sea surface of shallow waters ranging from 2 to 4 m deep. This behavior allows anglers to use sight-fishing techniques to capture tripletail. The draw of sight fishing for relatively large fish
and their highly sought-after flesh have created an increase in the number of anglers pursuing this species off the Jekyll Island coast. This influx of anglers targeting tripletail has raised questions as to whether the 2-fish, 457-mm minimum size limit regulations are sufficient for sustaining the population.

Most modern tripletail research has been conducted in the north-central Gulf of Mexico (Armstrong et al. 1996; Franks et al. 1998; Brown-Peterson and Franks 2001; Strelcheck et al. 2004); there has been one study from the western Atlantic Ocean (Cooper 2002). The lack of published reports underscores the general scarcity of information on the life history of the species, as well as a specific lack of data for tripletail in GA. As angling pressure increases in GA, there is a need to understand the life history of the tripletail, specifically age and growth data, to ensure management regulations are adequate to protect this species.

The goal of this study was to determine the age structure of the tripletail aggregation off the coast of Jekyll Island, GA. The objectives were to evaluate the efficacy of using sagittal otoliths and first dorsal spines for determining age and calculating growth rates for tripletail in the Jekyll Island aggregation.

**Study Site**

Field sampling was conducted March 30 - August 10, 2009 and March 14 - August 6, 2010 in the Atlantic Ocean nearshore Jekyll Island, GA, USA. The aggregation was targeted primarily around the northeastern to central part of the island (Figure 2-1).
Methods

Sampling

Upon arriving at the nearshore sampling area (Figure 2-1) by boat, surface water temperature (°C), salinity (ppt), and dissolved oxygen (mg/L) were measured using an YSI® 85 dissolved oxygen meter and recorded. Hook-and-line methods were used to sample the fish. Researchers visually searched the general area in an center-consoled, fiberglass boat and scanned the ocean for tripletail. When a tripletail was spotted, the researchers targeted the fish by casting a spinning rod equipped with 14 kg test braided line attached to a popping cork rig with a size 1 Kahle® live-bait hook, baited with live white or brown shrimp (*Litopenaeus setiferus* and *Farfantepenaeus aztecus*, Penaeidae) or striped mullet (*Mugil cephalus*, Mugilidae). Duration of the sampling event was recorded to the nearest minute. Total numbers of tripletail observed and captured were recorded. The capture location for each fish was recorded by a global positioning system (GPS). Upon capture, fish were given an individually-labeled tag affixed to their gill, placed on ice, and held until researchers returned to the Georgia Department of Natural Resources’ (GA-DNR) Coastal Regional Headquarters (CRD) located in Brunswick, GA.

Tripletail were also captured around structure (e.g., channel markers, range markers, and buoys) in the St. Simons Sound and adjacent shipping channel. Hook-and-line sampling methods were used to sample structures; however, the sampling tactics were different. Researchers would sample approximately 2 hours prior to and post slack tides. Heavier tackle was necessary to prevent break offs around the structure. A heavy spinning rod equipped with 36.2-kg test braided line with a slip-float rig, a 36.2-kg monofilament leader, and a 7-g, jig-head hook baited with live white or brown shrimp (*Litopenaeus setiferus* and *Farfantepenaeus aztecus*, Penaeidae) or
striped mullet (*Mugil cephalus*, Mugilidae). During this sampling method, researchers would approach a piece of structure and cast the rig next to the structure and fish all levels of the water column. Procedures upon capture of a fish were identical to the beach sampling methodology. Sampling around structure in 2009 was performed opportunistically when weather did not permit sampling off the beach. In 2010, the beach and structure were sampled at a 50:50 ratio; catch success determined where our effort was best allocated on a weekly basis.

In addition to the hook-and-line gear, a 12.2-m fish trawl with 76.2-mm mesh (bar) deployed from the GA-DNR Research Vessel *Anna* with a maximum tow time of 10 minutes was used experimentally in 2009 to capture tripletail from the aggregation offshore Jekyll Island. Vessel location (latitude and longitude determined with global positioning system-GPS) and heading of the R/V *Anna* was recorded at the beginning of each tow (trawl-net-in) and at the end of each tow (trawl-net-out). Speed was determined based on the amount of time required to get from the trawl-net-in GPS point to the trawl-net-out GPS point. The number of tripletail caught was recorded. Surface water temperature (°C), salinity (ppt), dissolved oxygen (mg/l), tide stage, wind direction and speed, and atmospheric condition data were collected and recorded prior to the sampling trip.

Tripletail were also sampled opportunistically in co-operation local tournaments and anglers. These fish often were donated carcasses; therefore, sex and total weight often were not able to be determined. Tripletail of unknown sex and those lacking weight data were excluded from the length-weight regression analysis and factorial analysis of variance (ANOVA).

Criteria for sacrificing the captured fish were based on a concurrent reproductive study. A maximum of 10 fish per week were sacrificed for bony structure removal: five individuals <457 mm total length (TL) and five individuals ≥ 457 mm (TL). The 457 mm cut-off represents the
current GA-DNR minimum size limit for possession of tripletail. All tripletail ≥ 610 mm were sacrificed. Fish captured outside of these criteria were measured to the nearest millimeter for TL and standard length (SL). The fish were then tagged with a uniquely numbered Hallprint™ plastic dart tag inserted behind the base of the third dorsal spine of the spiny dorsal fin and released.

Sacrificed tripletail were measured for TL and SL (nearest mm) and then weighed (nearest 1.0 g) with a Northern Industrial™ R-2553 20 kg electronic platform scale. Gonads were macroscopically examined and sex was recorded. Sagittal otoliths were removed and placed into uniquely-labeled coin envelopes. First dorsal spines, including the condyle base, were removed with a hacksaw and placed into the same labeled envelope as the otoliths.

**Age and Growth**

First dorsal spines, henceforth spines, were placed in bleach for a maximum of two minutes, and all excess tissue and skin were removed by a scalpel and forceps (Jim Franks, University of Southern Mississippi, personal communication). One sagittal otolith and spine from each fish were embedded individually in West System™ 205 and 206 (Gougeon, Inc.-Bay City, MI) epoxy in Pelco™-10530 (Ted Pella- Inc; Redding, CA) numbered trays. Aging structures were thin-sectioned with a low-speed, Buehler™ Isomet saw (Buehler-Lake Bluff, IL) equipped with two diamond-tipped wafering blades (Series H-15; Buehler-Lake Bluff, IL) with a 0.1 mm spacer between the blades. A minimum of three sections were cut serially beginning at approximately 25% of the total length of the spine starting at the condyle base, additional sections were cut if sections were illegible (see Franks et al. 1998). Multiple otolith transverse sections from the dorsoventral plane were taken from the otoliths to ensure a quality core section.
was obtained. Cytoseal™ XYL (Richard-Allan Scientific-Kalamazoo, MI) clear bonding agent was used to mount the specimen sections on glass microscope slides. Ages were evaluated via a computer-based image analysis system comprised of a Leica MZ8 stereomicroscope (Leica Microsystems-Wetzlar, Germany) with transmitted light, a Photometrics Coolsnap Camera, and Optimas 6.51 (Optimas Corporation- Bothell, Washington) imaging software.

Ages were determined based on the number of opaque bands on the otoliths and translucent bands on the sectioned dorsal spines. Opaque and translucent bands were assumed to be annuli on otoliths and spines respectively; however, this assumption has not been validated by independent studies of known-age tripletail.

Otoliths were aged based on standard aging procedures, although the opaque bands were not always easy to distinguish (Devries and Frie 1996). Researchers often benefited from looking at the periphery of the otolith section to find where bands had formed. Opaque bands on tripletail otoliths were often very thin and followed very close behind the translucent bands and were often difficult to distinguish (Figure 2-2).

Spine-derived ages were based in part on techniques described by Franks et al. (1998) to create the criteria to determine spine age. In this approach, “multiples” were considered to be annual marks. A “multiple”, also known as “doublets” and “triplets” (Cayre and Diouf 1983), was defined as two and occasionally three small, conspicuous, adjacent translucent rings that were separated by a small opaque zone (Franks et al. 1998). When “multiples” are counted, one band is counted when two or three small translucent bands occurred more closely together than the distance to the preceding and following translucent bands (Gonzalez-Garces and Farina-Perez 1983; Franks et al. 1998). Franks et al. (1998) methodology defines the first annulus as the second multiple; however, after evaluation of spine sections with corresponding otoliths, we
believe that the need to skip the first multiples may be necessary for tripletail that have not reached age-2. As tripletail grow and age, the multiple bands appear to become compressed into a single band. Tripletail >2 years of age often had the compressed distinct translucent bands near the edge of the spine sections. If these translucent bands appeared to be distinct bands around the majority of the spine they were considered individual annuli (Figure 2-2). Striations often appear on the spine sections and were used as part of the criteria in determining whether an annulus was present. Striations that are not interrupted by a translucent band are not counted as an annulus, whereas a translucent band that interrupts the striations would be counted as an annulus (Figure 2-2). Spines with significant core erosion were removed from further analysis.

Band counts and measurements were performed on otoliths and spines separately by two independent readers using the Optimas 6.51 imaging software (Optimas Corporation, Bothell, Washington) to evaluate between-reader agreement. If band counts differed, the readers jointly re-examined the counts and came to a consensus as to the estimated age of the individual. If the readers could not reach an agreement, the ages were excluded from further analyses. Within-reader agreement was evaluated by a single reader re-aging a random subsample from both otoliths and spines. A 50% random subsamples selected by a random number generator of otoliths and spines were evaluated for all age classes with n > 50, all samples were evaluated for age classes with n<50.

**Data Analysis**

Total length and total weight (TW) data were examined for normality with the Shapiro-Wilk test. These data were not normally distributed; therefore, they were log$_{10}$ transformed. Levene’s test was used to evaluate homogeneity of variances. A 2 X 2 factorial ANOVA was
used to determine if there were significant differences among TL and TW of samples collected in 2009 and 2010, sexes, or the interaction between capture year and sex. Simple linear regression was used to evaluate the relationship between TL and TW. Data were pooled if the relationships were not significantly different. A chi-square analysis was used to determine if monthly sex ratios varied from the expected 1:1 ratio.

An ANOVA was used to determine if there were significant differences between TL of the age classes for both otoliths and spines. Normality of TL data was evaluated with the Shapiro-Wilk test, these data were found to be non-normal and were log10 transformed. Levene’s test was used to evaluate homogeneity of variances. An ANOVA was used to evaluate mean TL among age cohorts and all age groups that were significantly different were evaluated with a means separation test to determine the significance of the differences. An ANOVA was used to evaluate mean differences between the TL at age for both spine and otolith data. Back-calculated estimates for length-at-age were derived using the Fraser-Lee method (Devries and Frie 1996). For both otoliths and spines, growth was calculated with von Bertalanffy (von Bertalanffy 1938) growth models, represented by the formula:

$$L = L_\infty \left[ 1 - e^{-K(t-t_0)} \right].$$

where: $L =$ Predicted TL (mm), $L_\infty =$ Maximum (asymptotic) TL (mm), $K =$ Growth rate, $t =$ time (age), $t_0 =$ Time at length = zero.

Non-linear regression (PROC NLIN) was used to model the parameters for the Von Bertalanffy growth models (SAS; Freund and Littell 1991). An $\alpha = 0.05$ was used to determine the significance of all statistical tests.
Results

**Sampling and water quality**

Over the course of the 2-year project, 385 hours of beach sampling, 95 hours of structure sampling and 9 hours of trawl sampling took place over 177 sampling events. A total of 243 tripletail was captured and sampled for aging structures. An additional 208 tripletail was captured that did not meet the retention criteria and were subsequently tagged and released. Average water temperature was 25.6 °C (range = 13.7 – 33.0 °C). Average salinity was 30 ppt (range = 15 - 35 ppt). Dissolved oxygen averaged of 6.18 mg/L (range = 3.34 – 9.02 mg/L).

**Morphometrics**

Of the 243 tripletail collected and sampled for age structures, 105 were females, 126 were males, and gender was not determined for the remaining 12. Significant differences were not detected between the expected monthly 1:1 sex ratio of males to females ($X^2 = 9.74, P = 0.0828$; Figure 2-3). Differences in mean TL ($F_{1,216} = 0.87, P = 0.3517$) and TW ($F_{1,216} = 0.89, P = 0.3476$) were not significant between the years; therefore, the data for both years were pooled. Mean female TL (489 ± 117mm) was not significantly different from male TL (455 ± 93mm) ($F_{1,216} = 3.85, P = 0.0512$; Table 2-1). Mean female TW (3,116 ± 2,595g) was significantly higher than mean male TW (2,314 ± 1,524g) ($F_{1,216} = 4.96, P = 0.0270$; Table 2-1). Models based on sex were poor predictors for both TL ($r^2 = 0.024$) and TW ($r^2 = 0.0296$); therefore, the sexes were combined for all further analyses. The length-weight regression indicated that the there was a strong relationship between TL and TW ($\log_{10}TW = -5.223 + (3.210 \times \log_{10}TL)$, $r^2 = 0.985, n = 229$) (Figure 2-4). Total weight was nearly a cubic function of TL (Figure 2-4).
Age and Growth

Otoliths and spines had alternating opaque and translucent bands (Figure 2-2). Quality of bands varied among individual fish as well as between aging structures. Legible otolith sections were obtained from all tripletail. Spines were not retrieved from all fish, and some of the spine cores (n = 5) displayed vascular erosion and were not suitable for aging and were removed from further analysis. Initial reader agreement was 90.1% for otoliths (219 of 243; Figure 2-5) and 82.8% for spines (198 of 238; Figure 2-6). All spine and otolith band counts were mutually agreed upon for each structure during the concert reading. Estimated ages for males and females ranged from 1 to 5 years based on the opaque and translucent band counts on the otoliths and spines respectively (Figure 2-4). Within reader agreement was 90.8% and 96.2% for spines and otoliths, respectively. Agreement between structures based on agreed-upon ages was 84.1% (201 of 238; Figure 2-7).

Total lengths varied greatly within age classes, and lengths overlapped among the otolith age classes (Figure 2-8). Spine-based aging yielded similar results for length at age (Figure 2-8). Both otolith and spine mean TL at age classes 1 and 2 were significantly different from each other, as well as all other age classes, whereas mean TL for age classes 3 – 5 were not significantly different. Differences in tripletail TL among the otolith- and spine-derived age classes were not significantly different (ANOVA, F_{1,478} = 0.01, P = 0.9289).

Back-calculated length at age from otoliths showed similar results to mean TL at age at size from capture data for our two-year study (Figure 9). Back-calculated lengths-at-age derived from spines produced TL estimates that were lower than the mean length-at-age from capture data (Figure 9). Parameter estimates derived from the von Bertalanffy growth model for otolith- and spine-estimated ages parallel each other over the range of ages observed within this study.
(Figure 2-10). The spine based von Bertalanffy growth model length at age estimates are similar to those of the otolith based von Bertalanffy growth model estimates.

Discussion

This study demonstrated agreement between ages derived from structures collected by lethal (otolith) and non-lethal (spine) means for tripletail. Otoliths and spines produced legible bands for age determination. This result is noteworthy, because Franks et al. (1998) and Strelcheck et al. (2004) did not find agreement between the structures and deemed the otolith illegible, although their results may have been hampered by small sample sizes (< 50 samples), whereas the current study evaluated 243 tripletail. The agreement between these structures observed in the present study will allow future researchers to produce non-lethal, viable age estimates for tripletail.

The current study found good agreement (84.1%) between ages derived from otoliths and spines. Initial reader agreement was higher for otoliths (90.1%) than spines (82.8%). Back-calculated otolith ages closely resemble age and length at time of capture which likely resulted from the more uniform and consistent shape of the otoliths as compared to spines (Table 2-4). Spines represent a non-lethal aging technique for tripletail, when accurate back-calculated age estimates are not required. The use of both structures when lethal sampling occurs would likely produce the most accurate ages.

The current study detected significant differences between sexes in mean TL and TW; however, these differences are likely a result of the lack of males captured in the upper size range (> 700 mm). Previous researchers have also concluded that male tripletail are slightly smaller
than females at similar ages in the Gulf of Mexico (Armstrong et al. 1996; Franks et al. 1998; Strelcheck et al. 2004). Researchers also found females sampled from Cape Canaveral, FL were slightly heavier than males (Cooper 2002). However, we deem these modest differences to be biologically irrelevant to fisheries managers. Length-weight regression also suggested that treating males and females as separate groups would be unnecessary (Figure 2-4).

Otolith-based age disagreements between readers often stemmed from the lack of knowledge of when annulus formation occurred (i.e. when to add a year based on imminent formation of an annulus). Band formation on the edge appears to occur around June 1 in tripletail captured near Jekyll Island; however, bands occurred before and after this date and had to be evaluated by the individual reader.

Translucent band appearance and overall shape of the spine vary greatly among individual tripletail. As tripletail age the bands appear to compress and become clearer; however, this compression increases the difficulty in identifying the first annulus. Using the criteria described by Franks et al. (1998) tends to be effective for fish up to age-2. Skipping of the first multiple does not appear to be necessary in older fish, which is likely a result of the compression of the spine. Age-1 fish tend to have many multiples that occur prior to the formation of a definitive annulus which we believe can lend itself to over aging. Back-calculated spine-based ages appear to underestimate length at age, again likely caused by the compression of bands within the spine (Table 2-4). We measured the distance of each annulus from the core on the plane with the greatest distance from the core, which did not always occur in the same location on each spine section. Multiple planes for measurement or measuring area of annuli could prove to be beneficial in future studies.
The average size-at-age and general age demographics of fish in our study were similar to the data presented in limited published literature available, which suggests that most tripletail that are targeted by hook and line methods are between 1 and 3 years of age. Previous studies have suggested tripletail could reach > 7 years old (Armstrong et al. 1996; Franks et al. 1998; Strelcheck et al. 2004). In our study, tripletail ages ranged from 1 – 5 years of age for both otolith and spine estimated ages. The Georgia state record 17.63 kg and the International Game Fish Association (IGFA) record 19.19 kg tripletails suggest that it is likely tripletail could tripletail reach an age ~ 7 years.

Although scales (Merriner and Foster 1974), otoliths (Armstrong et al. 1996), and spines (Franks et al. 1998; Strelcheck et al. 2004) have been used to age tripletaill, estimates of length-at-age have been similar. Our findings agree with the previous literature, which suggest that tripletail growth is very rapid in the first few years of life (Merriner and Foster 1974; Armstrong et al. 1996; Franks et al. 1998; Strelcheck et al. 2004). Tripletail both captive and wild tripletail are capable of lengths >500 mm and weights >4 kg in their first year of life.

The ages obtained in our study were very similar to the results from studies conducted in the northern Gulf of Mexico; however, our study did not contain age-0 fish (Franks et al. 1998; Strelcheck et al. 2004). All specimens from the current study appeared to be from the previous year class based on band formation and therefore were assumed to be at least one year of age. This assumption resulted in the lack of age-0 fish. Strelcheck et al. (2004) assigned fractional ages; but for our study, we deemed the use fractional ages without definitive evidence of time of annulus deposition and accurate spawning data to be inappropriate. Offshore spawning has been suggested based on the capture of larval and juvenile tripletail in offshore waters in the Gulf of Mexico (Ditty and Shaw 1994; Franks et al. 1998; Cooper 2002; Strelcheck et al. 2004), which
could also account for the lack of age-0 tripletail in the study. Gear selectivity may play an important role in the lack of age-0 fish as well. An increased understanding of the reproductive life history of tripletail and the timing and cause of annulus formation are needed to determine if this assumption is valid.

Previous age and growth research for tripletail found wide ranges of TL at a given age, with significant overlap in TL between ages (Armstrong et al. 1996; Franks et al. 1998; Strelcheck et al. 2004). Our study demonstrated that age-1 and age-2 fish were significantly different from one and other, as well as from all other age classes. Significant overlap occurred for ages 3-5. The protracted batch spawning characteristics of the tripletail could explain the wide range of TL within an age class and overlap among the age classes. The open ocean environment can be patchy for distribution of food and habitat which may also contribute to overlap within TL at age. Perhaps a larger sample size than the one used in this study would help determine if significant size differences do occur among ages and sexes and are only masked by the current small sample size.

Initially fish captured free floating off the Jekyll Island beach were evaluated separately from fish captured off structure within the estuary; however, tag returns from the current study and another ongoing tripletail research project (Chris Kalinowsky, Georgia Department of Natural Resources and Matt Streich, University of Georgia, Personal Communication) have indicated movements as far as southeastern Florida. This movement information indicates that fish exhibiting different behaviors (i.e., free floating or associating with structure) within close proximity of one and other should be treated as one aggregation of fish.

Future research will require a greater number of larger and older fish to be sampled to evaluate both effectiveness of the aging technique and size at age data for tripletail greater than 3
years old. Although this study represents a “multiple lines of evidence” approach to aging tripletail, the need for age validation is necessary. Determination of the ring deposition timing and cause is also a necessary step towards the validation of aging techniques. Studies have shown and have suggested that the use of first dorsal spines could be used as a non-lethal aging technique that could be validated through mark-recapture (Beamish and McFarlane 1987; Strelcheck 2004). Strelcheck et al. (2004) suggests removing the first dorsal spine at the time of tagging and evaluating the first dorsal spine against other spines for age comparison upon recapture. We suggest that upon recapture, the fish would be sacrificed and otoliths could be compared to spine ages based on the original capture date. Strelcheck et al. (2004) also suggests that an area of high concentration of tripletail would be needed to complete a study with this methodology. The tripletail aggregation near Jekyll Island may lend itself to this type of study based on preliminary tagging data with relatively high recapture rates within a season and throughout the year.

Conclusions

Aging techniques with both otoliths and spines have their advantages and disadvantages. Otoliths had higher initial reader agreement than spines, although agreement between the structures was relatively high at 84.1%. Compression within the spine as the tripletail age does not lend itself to logical back-calculated ages such as the otolith back-calculated ages. Otoliths require that the fish be sacrificed, whereas a spine can be taken and the fish can be released. We believe the greatest accuracy would be achieved by reading the structures concurrently and analyzing growth measurements only with the otoliths. However, the lack of concrete life history data and population estimates suggest non-lethal aging methods would be preferred over lethal
methods to ensure the survival of tripletail populations. In summary, we believe spines are sufficient aging structures for tripletail if back-calculated ages are not required, which would allow researchers to practice non-lethal sampling.
Literature Cited


Table 2-1. Summary information for male (n = 126 total length; n = 125 total weight) and female (n = 105 total length; n = 101 total weight) tripletail captured near Jekyll Island, Georgia, USA in 2009 and 2010.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total Length (mm)</th>
<th>Total Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Males</td>
<td>455</td>
<td>93</td>
</tr>
<tr>
<td>Females</td>
<td>489</td>
<td>117</td>
</tr>
</tbody>
</table>
Table 2-2. Otolith-based, back-calculated total length-at-age for tripletail captured in 2009 (A) and 2010 (B) near Jekyll Island, GA (2009 n = 125, 2010 n = 118).

A)

<table>
<thead>
<tr>
<th>Capture Year</th>
<th>Age Group</th>
<th>Year Class</th>
<th>Length at Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>1</td>
<td>2009</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2008</td>
<td>379</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2007</td>
<td>365</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2006</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2005</td>
<td>476</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>418</td>
</tr>
</tbody>
</table>

B)

<table>
<thead>
<tr>
<th>Capture Year</th>
<th>Age Group</th>
<th>Year Class</th>
<th>Length at Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>1</td>
<td>2010</td>
<td>390</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2009</td>
<td>426</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2008</td>
<td>392</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>403</td>
</tr>
</tbody>
</table>
Table 2-3. Spine-based, back-calculated total length-at-age for tripletail captured in 2009 (A) and 2010 (B) near Jekyll Island, GA (2009 n = 123, 2010 n = 115).

A)

<table>
<thead>
<tr>
<th>Capture Year</th>
<th>Age Group</th>
<th>Year Class</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>1</td>
<td>2009</td>
<td>428</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2008</td>
<td>394</td>
<td>483</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2007</td>
<td>404</td>
<td>515</td>
<td>568</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2006</td>
<td>414</td>
<td>695</td>
<td>760</td>
<td>777</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2005</td>
<td>258</td>
<td>330</td>
<td>412</td>
<td>478</td>
<td>512</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>380</td>
<td>506</td>
<td>580</td>
<td>628</td>
<td>512</td>
</tr>
</tbody>
</table>

B)

<table>
<thead>
<tr>
<th>Capture Year</th>
<th>Age Group</th>
<th>Year Class</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>1</td>
<td>2010</td>
<td>398</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2009</td>
<td>395</td>
<td>510</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2008</td>
<td>359</td>
<td>442</td>
<td>512</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2007</td>
<td>443</td>
<td>462</td>
<td>484</td>
<td>497</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>399</td>
<td>472</td>
<td>498</td>
<td>497</td>
<td></td>
</tr>
</tbody>
</table>
Table 2-4. Mean total length-at-age for otolith- and spine-derived ages at the time of capture compared with otolith (Calc-Otolith) and spine back-calculated (Calc-Spine) total length-at-age for tripletail captured in the summer of 2009 and 2010 near Jekyll Island, GA. Otolith back-calculated total length-at-age appear to most closely follow the mean length at time of capture.

<table>
<thead>
<tr>
<th>Age Class</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otolith</td>
<td>413</td>
<td>556</td>
<td>664</td>
<td>707</td>
<td>781</td>
</tr>
<tr>
<td>Calc-Otolith</td>
<td>410</td>
<td>538</td>
<td>628</td>
<td>712</td>
<td>795</td>
</tr>
<tr>
<td>Spine</td>
<td>418</td>
<td>567</td>
<td>640</td>
<td>736</td>
<td>781</td>
</tr>
<tr>
<td>Calc-Spine</td>
<td>389</td>
<td>489</td>
<td>539</td>
<td>562</td>
<td>512</td>
</tr>
</tbody>
</table>
Figure 2-1. An illustration of the tripletail sampling location near Jekyll Island, GA, USA. Inset shows a map of GA with the Jekyll Island area darkened. Each point on the map indicates that one or more tripletail were captured at that location from March to August 2009 and 2010. Not all fish capture locations for the study are located on the map.
Figure 2-2. Examples of otoliths (A) and spines (B) taken from individual tripletail captured in 2009 and 2010 near Jekyll Island, GA, USA. 1A and 1B represent an age-1 fish, the arrow indicates where striations terminate into a translucent band denoted as an annulus. 2A and 2B represent an age-2 tripletail. 3A and 3B represent an age-3 tripletail. 4A and 4B represent an age-
4 tripletail, notice the compression of the bands near the edge. 5A and 5B represent an age-5 tripletail, notice the small first annulus that we believe is a result of compression as the tripletail ages, which we suggest should not be skipped. These pictures were not taken at the same scale and are to be used as examples.
Figure 2-3. Number of male and female tripletail (n = 231) captured during sampling off the coast of Jekyll Island, GA during 2009 and 2010. Sex ratio was not different than the expected ratio of 1:1 ($X^2 = 9.74, P = 0.0828$).
Figure 2-4. Total length (TL) and total weight regression for tripletail captured (n = 229) from the aggregation near Jekyll Island, GA, USA from March to August 2009 and 2010.
Figure 2-5. Plot of reader 1 versus reader 2 initial otolith based age estimates for tripletail captured in the Jekyll Island, GA, USA, area during the 2009 and 2010 sampling seasons. The equal-age line represents 1:1 agreement of age estimates. Numbers located on the plot represent the number of observations for a given reader 1 and reader 2 age combination.
Figure 2-6. Plot of reader 1 versus reader 2 initial spine based age estimates for tripletail captured in the Jekyll Island, GA, USA, area during the 2009 and 2010 sampling seasons. The equal-age line represents 1:1 agreement of age estimates. Numbers located on the plot represent the number of observations for a given reader 1 and reader 2 age combination.
Figure 2-7. Plot of agreed otolith and spine derived ages from tripletail captured near Jekyll Island, GA in 2009 and 2010. (n = 239). The equal-age line represents 1:1 agreement of age estimates. Numbers located on the plot represent the number of observations for agreed otolith age and agreed spine age combination.
Figure 2-8. Box plots for tripletail age data for (A) spines and (B) otoliths near Jekyll Island, GA, USA captured from March to August 2009 and 2010. The central line indicates the median, the grey box represents the middle 50% of the data and the whiskers represent the area where 90% of the data fell. Outliers are denoted by the black dots.
Figure 2-9. Comparison of back-calculated (calc) and observed (obs) length-at-age estimates from otoliths and spines from tripletail captured near Jekyll Island, GA, USA captured from March to August 2009 and 2010. Error bars represent standard errors of the mean.
Figure 2-10. von Bertalanffy growth curves based on spines and otoliths from tripletail captured near Jekyll Island, GA, USA during March – August 2009 and 2010. Only ages 1-5 were captured during this study.
CHAPTER 3

Evaluation of reproductive status for tripletail, *Lobotes surinamensis*, nearshore Jekyll Island, GA, USA

Introduction

Tripletail (*Lobotes surinamensis*, Lobotidae) are medium-sized, deep-bodied fish that occur in tropical and subtropical waters worldwide (Baughman 1941; Hardy 1978). The species is the only member from the monophyletic family Lobotidae, which is found in the western Atlantic Ocean (Hardy 1978). Tripletail are known to occur from Massachusetts, USA to Argentina, including the Gulf of Mexico and the Caribbean Sea, with higher abundances south of North Carolina, USA (Hardy 1978). Tripletail are migratory; however, detailed information about their exact movement patterns is scarce or lacking (Merriner and Foster 1974; Franks et al. 1998).

Tripletail occur in a variety of habitats from estuarine waters to the open ocean, but are usually found in association with structure such as pilings, buoys, and channel markers (Gudger 1931; Kelly 1923; Benson 1982; Ditty and Shaw 1994). Tripletail will often use eddies created by structure to ambush prey flushed out of the estuary by tidal movement. The species has a unique behavior of floating on its side at the surface in open waters; this behavior is hypothesized to mimic a floating object to avoid predation while also attracting potential prey that may be seeking cover (Gudger 1931; Breder 1949).

Tripletail growth appears to be rapid during early life stages; wild-caught and captive fish have demonstrated the ability to grow greater than 500 mm total length at age-1 (Armstrong et al. 1996; Franks et al. 1998; Strelcheck et al. 2004; Chapter 2). Scale-based total length (TL) at age of tripletail from North Carolina was: age-0 (n=1) 190 mm, age-1 (n=6) 445-591 mm, age-2 (n=5) 562-706 mm, and age-3 (n=2) 568-706 mm (Merriner and Foster 1974). Spine-derived ages of tripletail also show similar results (Franks et al. 1998; Strelcheck et al. 2004).
Peer-reviewed literature with tripletail reproductive data is scarce, and the majority of information remains in unpublished agency reports. The size at which 50% of males reach maturity has not been determined because of a lack of data for age-0 and immature males; however, Brown-Peterson and Franks (2001) estimate that 50% of males are mature by at least 290 mm. Estimates for female age-at-sexual maturity range from one to two years and from 350 – 500 mm TL depending upon criteria used for determination of maturity status (Brown-Peterson and Franks 2001; Strelcheck et al. 2004). Brown-Peterson and Franks (2001) and Strelcheck et al. (2004) estimate that 50% of females reach maturity at approximately one year and approximately 490 mm TL. Strelcheck et al. (2004) note that size at 50% maturity for females could change significantly if the presence of vitellogenic oocytes is used as the gauge for maturity rather than presence of oocytes in the cortical alveolar stage.

Tripletail are thought to be a protracted multiple-batch spawners, with the ability to spawn once every three to five days during the spawning season (Brown-Peterson and Franks 2001; Cooper 2002). Baughman (1941) documented gravid females during the months of July and August on the Atlantic Coast, but other anecdotal reports of gravid females have not been confirmed. In the Gulf of Mexico, running-ripe males have been captured from May through September, and females in late ovarian maturation phases have been found from June through August (Brown Peterson and Franks 2001; Strelcheck et al. 2004). Ditty and Shaw (1994) captured larval tripletail in >100 m of water in plankton surface tows and suggested that tripletail may use offshore spawning. Juvenile tripletail have also been captured in offshore waters in the Gulf of Mexico in association with sargassum patches (Franks et al. 2001).

Histological analysis can yield accurate information on oocyte development and reproductive phase; however, this analysis requires more time and expense compared to visual
staging based on macroscopic appearance of the gonads or calculation of gonadal somatic index (GSI) values (West 1990). However, all of the aforementioned techniques require sacrifice of the fish, and non-lethal alternatives for determining reproductive status are desired for species such as tripletail, for which definitive population dynamics data are scarce or lacking. Non-lethal methods have been used to determine reproductive status of other fish species and often include evaluation of vitellogenin (VTG), the egg yolk precursor, concentrations in the blood plasma (Heppell and Sullivan 2000; Ceapa et al. 2002; Wildhaber et al. 2007; Heise et al. 2009). Vitellogenin is found at high concentrations during the final oocyte maturation phase in most fishes. Therefore, VTG can be an effective tool for determining sex, and in some cases, reproductive phase -- although increased VTG levels are not always indicative of spawning location (Heppell and Sullivan 2000; Ceapa et al. 2002; Wildhaber et al. 2007; Heise et al. 2009).

In Georgia (GA, USA), tripletail are found associated with structure in estuaries and nearshore of some of the barrier islands. Tripletail are found free-floating in the nearshore Atlantic Ocean waters immediately east of Jekyll Island from March to July (Figure 3-1) and anglers commonly report sighting 40-50 fish per day. Some investigators (Brown-Peterson and Franks 2001; Cooper 2002; Strelcheck et al. 2004) have suggested that this period coincides with the spawning season for tripletail. Atypically, the fish near Jekyll Island are not associated with any structure, but rather are free swimming at the surface of waters ranging from 2 to 4 meters deep. This unique swimming behavior makes the tripletail vulnerable to anglers using sight-fishing techniques, and in recent years numbers of anglers pursuing this species off the Jekyll Island coast have increased substantially (R. Parr, personal observation). Tripletail associated with structure are also increasingly being targeted by recreational anglers in Georgia estuaries (S. Woodward, Georgia Department of Natural Resources (GA-DNR) personal communication).
The increase in recreational fishing effort for tripletail has raised questions about the effectiveness of current regulations (2-fish daily creel limit, 457-mm minimum size limit) for sustaining the population.

As tripletail angling pressure increases in GA and elsewhere, additional information about their life history information such as age, growth, and reproduction data, is needed to inform fisheries managers to adequately protect this species. The lack of published tripletail data, particularly for the western Atlantic Ocean, underscores the general scarcity of information on the life history of the species. The goal of the current study was to describe reproductive characteristics of a nearshore tripletail aggregation in the western Atlantic Ocean, specifically fish found near Jekyll Island, GA, USA. Specific objectives were to evaluate non-lethal techniques to determine the gender and reproductive status of tripletail and to determine size at maturity to inform fishery management actions. The current study represents the first attempt to evaluate tripletail VTG levels as a non-lethal method to determine gender and reproductive phase.

Methods

Study Site

Tripletail sampling was conducted from March 30 to August 10, 2009 and March 14 - August 6, 2010 in the Atlantic Ocean nearshore Jekyll Island, GA, USA. Tripletail were targeted primarily around the northeast to central part of the island (Figure 3-1). Tripletail were also
targeted on channel markers, range markers, buoys and other structures within St. Simons Sound, north and west of Jekyll Island.

**Field Sampling**

On each sampling trip we measured surface water temperature (°C), salinity (ppt), and dissolved oxygen (mg/L) were measured with an YSI 85 dissolved oxygen meter and recorded upon arrival at the sample site (Figure 3-1). Hook-and-line methods from an open cockpit fiberglass boat were used to sample tripletail. For near shore sampling, the study area was searched visually for tripletail near the surface and when a tripletail was spotted they were directly targeted fish by casting a live white or brown shrimp (*Litopenaeus setiferus* and *Farfantepenaeus aztecus*, Penaeidae) or striped mullet (*Mugil cephalus*, Mugilidae) with a spinning rod equipped with braided line (14 kg test) attached to a popping cork rig with a Kahle™ live bait hook (size 1). Total numbers of tripletail observed and captured were recorded and duration of the sampling event was recorded to the nearest minute. The capture location for each fish was recorded with a global positioning system (GPS).

Tripletail sampling also occurred around structures in the St. Simons sound and adjacent shipping channel. We used hook-and-line sampling methods to sample structures but with different tactics than described for near shore (open water) sampling. We sampled approximately two hours prior to and post slack tide. Heavier tackle was necessary to prevent break offs around the structure; we used a heavy-action spinning rod equipped with braided line (36.2 kg test) with a slip float rig, a monofilament leader (36.2 kg), and a 7-g jig head hook baited with live penaeid shrimp or striped mullet. We approached the structure by boat and fished the bait throughout the water column next to the structure. Upon capture of a fish, procedures were identical to the
aforementioned sampling methodology. In 2009, we sampled opportunistically on inshore structures when weather did not permit sampling nearshore. In 2010, we began sampling the nearshore area and structures (in St. Simons sound) at a 50:50 ratio by sampling day and allowed weekly catch success to determine where our effort was best allocated the following week.

In addition to sampling with hook-and-line gear nearshore Jekyll Island, tripletail from the aggregation were also sampled with a 12.2-meter fish trawl with 76.2-mm mesh (bar) deployed from the GA-DNR Research Vessel (R/V) Anna. Vessel location (latitude and longitude was determined with global positioning system-GPS), and heading of the R/V Anna was recorded at the beginning of each tow, with trawl net--in, and at the end of each tow, with trawl net--out. Speed was determined based on the amount of time required to get from the trawl net in GPS point to the trawl net out GPS point. A maximum tow time of 10 minutes was used to minimize the risk of lethal interactions with tripletail and other non-target species. The number of tripletail caught was recorded and procedures for handling the fish were identical to nearshore sampling methodology by hook and line. Tripletail were also sampled opportunistically from local tournaments and recreational anglers. As a result of the differing condition of the donations (i.e., carcass, fresh dead fish, live fish), not all fish sampled were included in all statistical analyses.

The sampling target was a maximum of 10 fish ≤ 610 mm per week for histological evaluation: five individuals <457 mm total length (TL) and five individuals ≥ 457 mm (TL). The 457-mm (18 inch) designation represents the minimum size limit for possession of tripletail in Georgia, established by the GADNR. Based on the literature, all tripletail ≥ 610 mm were assumed to be mature fish and therefore were sacrificed. Any captured fish that exceeded the target number were measured to the nearest millimeter for TL and standard length (SL), tagged
with a uniquely numbered Hallprint™ PDS plastic dart tag inserted behind the base of the third dorsal spine, and released.

Upon capture of tripletail within the sampling target size range, blood samples were immediately obtained from the caudal vein with a 3-mL syringe equipped with a Luer-lok 18-gauge hypodermic needle. Immediately prior to blood collection, syringes and needles were flushed with a heparin sodium solution to prevent clotting. The blood samples were placed in numbered 1.0-mL centrifuge tubes and immediately placed on ice. Blood samples collected in 2009 contained the protease inhibitor aprotinin and 2010 samples were treated with a protease inhibitor cocktail (P2714, Sigma-Aldrich, St. Louis, MO, USA). Specimens were then placed on ice and returned to the lab within six hours of collection. Once blood samples were collected, fish were marked with a unique tag affixed to the fish through their mouth and out of their operculum via a zip tie and placed on ice until processing when researchers returned to the lab at the GADNR Coastal Regional Division (CRD) headquarters in Brunswick, GA.

At the CRD lab, plasma was separated from blood cells by centrifugation (1500 x g) for 10 min and was then extracted with a micropipette, placed into a cryogenic vial and frozen in liquid nitrogen. Samples were later transferred from liquid nitrogen to an ultracold (-80ºC) freezer until VTG analysis. Sacrificed tripletail were measured for TL and SL (nearest mm) and weighed (nearest 1.0 g) with an electronic platform scale (max 20 kg; Northern Industrial R-2553). Gonads were dissected from the fish, weighed (nearest 0.1g), and preserved in 10% buffered formalin until further processing for histological analysis.

An analysis of variance (ANOVA) was used to evaluate differences between male and female tripletail mean TL and TW. A chi-square analysis was used to determine if monthly sex
ratios varied from the expected 50:50 ratio ($\alpha = 0.05$). March samples were excluded from the chi-square analysis based on insufficient data (< 5 samples) during this month.

Gonad Histology

A subsample of 15 males and 15 females was used to determine if development within the gonads was uniform throughout the length of the gonad. Gonads were removed from 10% buffered formalin and three sections (i.e., one each from the posterior, central and anterior portions of the gonads) were placed in tissue cassettes. Standard histological procedures including dehydration, embedding in paraffin, thin sectioning (4 um), staining with hematoxylin, and counterstaining with eosin, were performed at the University of Georgia Veterinary Medicine Diagnostic Lab. Development of gametes was uniform throughout the gonad and all subsequent gonad samples were collected only from the central portion of the gonad.

Reproductive phases for both males and females were evaluated based on criteria described by Brown-Peterson et al. (2011) by examination of the mounted and stained gonad sections with a compound microscope. Females were classified as immature, developing (sub-phase: early developing), spawning capable (sub-phase: actively spawning), regressing or regenerating. Immature females contained only oogonia and primary growth (PG) oocytes. Females entered the developing phase with the appearance of cortical alveoli (CA) oocytes. Primary vitellogenic and secondary vitellogenic oocytes are also present during the developing reproductive phase. Primary, secondary, and tertiary vitellogenic oocytes are determined based on the relative increase in yolk deposition (Lowerre-Barbieri et al. 2011). Females that contained CA oocytes as the most advanced oocyte type were classified as the sub-phase of early development. Females in the spawning--capable reproductive phase contained tertiary
vitellogenic oocytes. The actively spawning sub-phase of spawning capable females was characterized by oocytes undergoing germinal vesicle migration, germinal vesicle breakdown, hydration, or ovulation. Regressing females were characterized by increased atresia, post-ovulatory follicles (POFs), and few if any healthy vitellogenic oocytes. Regenerating females contained PG oocytes, late-stage atresia, and a thicker ovarian wall than seen in immature fish.

Males were classified as immature, developing (sub-phase: early developing), spawning capable (sub-phase: early germinal epithelium (GE), mid-GE, and late-GE), regressing or regenerating (Brown-Peterson et al. 2011). Immature males contained only primary spermatogonia (Sg1) and without lumen within the lobules. Developing males were characterized as having all phases of spermatogenic development: secondary spermatogonia (Sg2), primary and secondary spermatocytes (Sc1 and Sc2), spermatids (St), and spermatozoa (Sz). Spermatozoa were not present in the lumen of the lobules or in sperm ducts. Spawning--capable males were characterized by having spermatozoa in the lumen of the lobules. The sub-phases of the spawning--capable phase were evaluated based on the level of continuous or discontinuous GE in spermatocysts. Early GE was characterized by continuous GE throughout the testes, mid-GE contained continuous GE in spermatocysts at testis periphery and discontinuous GE in spermatocysts near the ducts, and late-GE was characterized by discontinuous GE in all spermatocysts throughout the testes. The active spawning sub-phase was only distinguishable macroscopically, when milt was released with gentle pressure to the abdomen. Regressing males contained residual Sz in the lumen of the lobules and sperm ducts but contained little, if any, active spermatogenesis. Spermatagonal proliferation and regeneration of GE was common in the periphery of testes. Males in the regressing phase in the current study are still capable of spawning but not actively undergoing spermatogenesis.
Regenerating males were characterized by the absence of spermatocysts, the absence of lumen of the lobule, and proliferation of spermatogonia throughout the testes. A second reader evaluated the reproductive status of a randomly selected subsample (n ~25) for both males and females for quality assurance and control.

Length at 50% Maturity

Length at which 50% of individuals reached maturity for male and female tripletail was evaluated for TL and age, based on the equation described by Goncalves and Erzini (2000):

\[ P_i = \frac{1}{1 + e^{-b(L_i-L_{50})}} \]

where \( P_i \) represents the proportion of mature adults in each 50-mm length bin, \( b \) represents the slope of the maturity curve, \( L_{50} \) is the length at which 50% of the fish are mature, and length or age class at 50% maturity (\( L_t \)). Ages for fish in this study were based on a concurrent tripletail aging project (Chapter 2). All male and female tripletail that were not in the immature reproductive phase were classified as mature for determination of length at 50% maturity. Non-linear regression (PROC NLIN) was used to model the parameters for the length at 50% maturity (SAS 9.1; Freund and Littell 1991).

Gonadosomatic Index

Gonadosomatic Index (GSI) values were calculated based on the equation originally described by Nikolsky (1963):

\[ GSI = \left( \frac{\text{Gonad Weight}}{\text{Total Weight}} \right) \times 100 \]
Gonadosomatic Index values were not normally distributed and could not be transformed to fit a normal distribution; therefore, the GSI values were ranked for further (nonparametric) statistical analyses. As a result of the relatively small annual sample sizes, data were combined from the two years of the study. Sex, reproductive phase, and capture month variables were analyzed individually with one-way ANOVA to evaluate their relationships with the GSI values. Small sample sizes in some months and reproductive phases precluded the use of a factorial ANOVA design to evaluate the interaction and effects of month and reproductive phase on GSI (Appendix A). Trends were examined for GSI values across the reproductive phases and capture months.

**Vitellogenin**

Tripletail plasma samples were shipped on ice overnight via express courier to the University of Florida for VTG analysis. Vitellogenin was purified by anion exchange chromatography with the BIOCAD Sprint Perfusion system (Perseptive Biosystems) and estrogenized male plasma as described by Denslow et al. (1999). Plasma was diluted in running buffer (10mM bis-tris propane, 50 mM NaCl, pH 9.0) and loaded onto a strong anion exchange resin (POROS 20 HQ). Vitellogenin was eluted from the column with a linear gradient of NaCl (50→1000 mM). The VTG fractions were pooled, pH adjusted to 7.0, and were then concentrated with a 30,000 MWCO Centricon (Amicon) filter. Protease inhibitor, (Aprotinin, 10 KIU/ml), bacteriocide (azide, 0.02%), and cryoprotectant (glycerol, 1:2) were added to the purified VTG. The standard stored in this form is stable for 1-2 years (-20°C) and circumvents freeze/thaw protein fracture (K. Kroll and N. Denslow, University of Florida, personal communication).
Concentrations of plasma VTG were determined by direct Enzyme-Linked Immunosorbent Assay (ELISA) by using the monoclonal antibody, 3G2 (HL1393), that was developed for striped bass VTG. Plasma samples were diluted 1:100, 1:10,000, and 1:100,000 with 10 mM phosphate, 150 mM NaCl, 0.02% azide, 10 KIU/mL Aprotinin, pH 7.6 (PBSZ-AP). Tripletail VTG standards (0, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 µg/mL) containing 1:100, 1:10,000 and 1:100,000 male plasma (in PBSZ-AP) were used to make a species-specific standard curve. Male plasma was added to the standards to account for matrix effect found in direct ELISAs. Samples and standards were loaded onto a 96-well ELISA plate in triplicate and stored overnight at 4 ºC in a humidified Tupperware® container. The following day the plates were washed four times with PBSZ and were then blocked with 1% BSA in 10 mM Tris, 150 mM NaCl, 0.05% tween, 0.02% azide, 10 KIU/mL Aprotinin, pH 7.6 (1% BSA/TBSTZ-AP) for 2 hr at room temperature. The plates were rewashed with PBSZ (4 times) and the 1° monoclonal antibody, 3G2, was loaded into wells on each plate. The lowest dilution (1:100) was probed with 1µg/mL of the monoclonal antibody and dilution ≥ 1:10,000 with 0.1 µg/mL. After the addition of the monoclonal antibody, the plates were stored at 4 ºC overnight in the humidified container. The following day the plates were washed and the biotinylated secondary antibody, goat anti-mouse IgG-biotin (Pierce) was added to each well at 1:1,000 dilution in 1% BSA/TBSTZ-AP and incubated at room temperature for 2 hr. The plates were washed and a strepavidin-alkaline phosphatase conjugate (Pierce) was added at 1:1,000 dilution in 1% BSA/TBSTZ-AP and incubated for 2 hr at room temperature. After a final wash of the plates, the color was developed by adding 1 mg/ml p-nitro-phenyl phosphate in carbonate buffer (30 mM carbonate, 2 mM MgCl₂, pH 9.6) and the color was measured with an ELISA plate reader (SpectraMax Plus384, Applied Biosystems) at 405 nm. Concentrations of the unknowns
were determined from the standard curves by using SoftMax® Pro analysis program. The limit of
detection for tripletail VTG was 1µg/mL. All assays were performed in triplicate and reported as
the mean of the three measurements. The coefficient of variation was <10% for all samples
analyzed. Inter- and intra-assay variability were measured by analyzing positive controls on
several plates and were <10%, and <5%, respectively.

Vitellogenin concentration data were evaluated for normality with the Shapiro-wilk test
and evaluated for equality of variances with Levene’s test. Results of these tests indicated the
data were not normally distributed and their variances were unequal; therefore, VTG
concentration levels were ranked to facilitate further (nonparametric) statistical evaluation. A t-
test was used to evaluate differences between male and female VTG ranks. Similar to GSI data,
VTG data from the two years were combined because of the small sample size. A one-way
ANOVA was used to evaluate differences between the VTG ranks for sex, reproductive phase,
and capture month. As with GSI evaluations, some capture months and reproductive phases did
not contain a sufficient minimum sample size and restricted the use of a factorial design. Linear
regression was used to evaluate relationships between VTG and GSI for males and females.

All statistical analyses were conducted with program SAS 9.1(SAS Institute, Inc; Cary,
NC). Duncan’s multiple range test was used to evaluate significant differences among means for
all one-way ANOVAs (α = 0.05).
Results

Field Sampling

The project included 177 sampling events with a total of 385 hours of ‘near shore’ sampling, 95 hours of ‘structure’ sampling, and 9 hours of ‘trawl’ sampling. Average water temperature was 25.6 °C, with a range of 13.7 – 33.0 °C. Average salinity was 30.5 ppt, with a range of 14.5 to 38.7 ppt. Dissolved oxygen averaged of 6.18 mg/L and ranged from 3.34 – 9.02 mg/L. A total of 432 tripletail were captured during the study, and 224 (122 males and 102 females) were sampled for reproductive evaluation; the remaining 208 were tagged and released. Ratio of males and females was not significantly different than the expected 1:1 ratio (X² = 2.3937, P = 0.0828). Tripletail TL ranged from 241 to 822 mm and TW ranged from 265 to 14,152 g. Mean female TL (489 mm) was not significantly different from mean male TL (455 mm) (F₁,216 = 3.85, P = 0.0512). Mean female TW (3,116 g) was significantly heavier than mean male TW (2,314 g) (F₁,216 = 4.96, P = 0.00270).

Gonad Histology

Nearly all (115 of 122, 94%) of the male tripletail captured were in the spawning-capable phase (Table 3-1). Only one male (0.8 %) was in an immature reproductive phase. Female tripletail were captured in all reproductive phases (Table 3-2), but only two (2.0 %) of the 102 females were classified in the ‘actively spawning’ sub-phase of the spawning-capable category. One actively spawning female captured in June 2009 was 615 mm and contained oocytes that were undergoing lipid coalescence, indicative of final oocyte maturation. The other actively
spawning female was 355 mm and was captured in April 2010; the ovaries contained hydrated oocytes and post-ovulatory follicles, which indicated ovulation had occurred within 48 hours.

Length at Maturity
The estimated length at which 50% of females reached maturity (L\textsubscript{50}) was 459 mm with a slope (b) of 0.02, and the age at which 50% of females reached maturity was estimated at 1.17 years with a slope of 4.21 (Figure 3-2 and 3-3). Length at which 50% of males reached maturity could not be estimated by regression techniques because of a lack of convergence caused by the lack of immature fish in the sample (Figure 3-2); however, the age at which 50% of males became mature was 0.55 years with a slope of 9.74 (Figure 3-3).

Gonadosomatic Index
Gonadosomatic index values for female tripletail (n = 101) ranged from 0.07 to 10.9% with a grand mean of 1.1% (Figure 3-4). Male (n = 116) GSI values ranged from 0.04 to 0.22% with a grand mean of 0.11% (Figure 3-4). Females had significantly higher GSI ranks than males (F\textsubscript{1,210} = 535.18, P <0.0001) and female GSI values differed significantly among sample months (F\textsubscript{5,95} = 2.98, P = 0.0153). When the multiple comparison test was applied, differences were not detected among months with the exception of the March values, which were higher than May and June. Significant differences were not detected for male GSI ranks among sample months (F\textsubscript{4,111} = 1.51, P = 0.2040)

Female GSI values were significantly different among reproductive phases (F\textsubscript{4,96} = 25.21, P = <0.0001). Multiple comparison tests indicated GSI values for females in the immature reproductive phase were lower than all other phases, and values for the regenerating phase were higher than the immature phase and lower than all other reproductive phases. Differences were
not detected among the developing, spawning--capable, and regressing female reproductive phases but were higher than all other reproductive phases. Significant differences were not detected among male reproductive phases for GSI values ($F_{3,111} = 1.89, P = 0.1357$).

**Vitellogenin**

Vitellogenin levels for female tripletail ($n = 77$) ranged from below detection (1.0 μg/ml) to 4000 μg/ml with a mean of 209 μg/ml and a standard deviation of 696 μg/ml (Figures 3-5 and 3-6). Male tripletail ($n = 98$) VTG levels ranged from below detection to a maximum of 170 μg/ml with a mean of 36 μg/ml and a standard deviation of 28 μg/ml (Figures 3-5 and 3-6). A t-test indicated that females had significantly higher VTG concentrations than males (DF = 173, t-value = -3.49, $P = 0.0006$). Vitellogenin did not differ among months for males ($F_{4,92} = 0.64, P = 0.6351$). Significant differences were detected among months for female VTG concentrations ($F_{4,73} = 3.33, P = 0.0146$). May female VTG concentration was lower than April and August but was not different from June and July. Vitellogenin differed significantly among reproductive phases in females ($F_{4,73} = 9.09, P < 0.0001$). Immature females had lower VTG concentrations than all other phases except the regenerating phase. Differences were not detected among females in developing, regressing, and regenerating phases. Vitellogenin concentrations in spawning capable females were higher than all phases except the developing phase. Vitellogenin concentrations did not differ among male reproductive phases ($F_{3,93} = 0.24, P = 0.8684$).

Vitellogenin and GSI values showed similar trends across the reproductive phases for males and females (Figure 3-7). Male tripletail did not exhibit any peaks in GSI or VTG during our sampling period and linear regression analysis of VTG and GSI for males showed a very
weak relationship ($R^2=0.186\ n=94$). Female tripletail in the spawning capable phase increased in VTG and GSI values but VTG and GSI across all other reproductive phases were similar. Linear regression demonstrated a strong relationship ($R^2=0.873,\ n=77$) between VTG and GSI in females.

**Discussion**

Tripletail nearshore Jekyll Island, GA appear to have similar reproductive characteristics to those previously reported for tripletail in the Gulf of Mexico (Franks and Brown-Peterson 2001; Strelcheck et al. 2004). Our data are generally consistent with previous tripletail reproductive studies that suggest primary spawning does not occur in nearshore waters (Ditty and Shaw 1994; Franks and Brown-Peterson 2001; Cooper 2002; Strelcheck et al. 2004). Male tripletail captured near Jekyll Island, GA from March to August were in a spawning-capable reproductive phase but, most female tripletail in this aggregation did not appear to be in spawning condition. Vitellogenin analysis was unable to decisively distinguish males and females, although spawning-capable females may be distinguishable from all other reproductive phases. A larger sample size than was available in the present study, particularly of females >525 mm, is needed to provide more conclusive evidence for the utility of VTG to differentiate sexes. Increased sampling of females >500 mm is needed to determine if the variability in the GSI and VTG data occurring during the year is natural or is an artifact of the small data set. The limitations of this data set required us to evaluate relationships between GSI and VTG with one-way ANOVA based on ranks instead of actual data, which increased difficulties in interpreting our results.
Our findings are consistent with other investigators who have reported that male tripletail mature within the first year of life (Brown-Peterson and Franks 2001; Strelcheck et al. 2004; Chapter 2). In the present study 50% of male tripletail reach maturity at approximately 0.5 years old, although our approximation is limited by the lack of age-0 fish (Figure 3-3). The lack of immature males precluded calculation of $L_{50}$ for males; however, the smallest male tripletail found in a spawning capable phase was 261 mm. The only immature male was 336 mm. Data suggests that the $L_{50}$ for males is $< 261$ mm (Figure 3-2). Brown-Peterson and Franks (2001) and Strelcheck et al. (2004) reported that all males in their studies were sexually mature and suggested that length at which 50% of the males were mature would likely be $\leq 290$ mm. Overall, male tripletail captured in the present study near Jekyll Island, GA were spawning capable through the size range (261-714 mm) and the season sampled.

Previous studies have reported that 50% of female tripletail in the Gulf of Mexico reached maturity at approximately 490 mm (Brown-Peterson and Franks 2001; Strelcheck et al. 2004). Similarly, Brown-Peterson and Franks (2001) reported that all females $>525$ were mm sexually mature. We determined that 50% of female tripletail in GA reached maturity at 449 mm or 1.17 years (Figure 3-2 and 3-3). Our study indicates that the $L_{50}$ for females (449 mm) was below the current minimum size limit (457 mm) for tripletail caught in Georgia waters.

Previous estimates of length and age at which 50% of the population is mature and those of the present study are based on the premise that females in a developing stage during the predicted spawning season are mature fish. Other investigators (Strelcheck et al. 2004) have warned that if fish in the developing phase do not spawn in that season, length- and age-at-sexual maturity may be underestimated. Strelcheck et al. (2004) further suggested that if early vitellogenic oocytes were used as the minimum qualification for maturity status, length at which
50% of the females are mature would increase from 494 to 594 mm, which corresponds to an age of 1 or 2 years. In the present study, classification of immature and developing females as immature and all other female reproductive phases as mature resulted in an increase in length at 50% maturity from 449 to 491 mm, which corresponds to an age of 1.17 to 1.47 years of age. This change in maturity classification indicates that a more conservative approach of classifying maturity status yields only slightly higher length and age estimates; however, the increase to 491 mm does raise the length at 50% maturity above Georgia’s current minimum size restriction, of 457 mm, which means less than 50% of female fish are mature when they become vulnerable to harvest.

Gonadosomatic index values for tripletail near Jekyll Island, GA were similar to those found in other studies conducted both in the Gulf of Mexico (Brown-Peterson and Franks 2001) and in Cape Canaveral, FL (Cooper 2002). Male tripletail GSI in the present study ranged from 0.04 to 0.22% with a mean value of 0.11% and remained relatively constant throughout the sampling period. Female tripletail had a wider range of GSI values, 0.07 - 10.9%, with a mean of 1.1%; female GSI generally increased from April to August. The high GSI value in March is a result of a two females captured in 2009 and could be indicative of a protracted spawning period of tripletail or could be an artifact of small sample size. The asynchronous oocyte maturation and relatively low mean GSI values throughout the sampling period is consistent with previous tripletail studies (Brown-Peterson and Franks 2001; Cooper 2002), as well as other multiple-batch spawning species such as wahoo (Acanthocybium solandri, Brown-Peterson et al. 2000).

Mean and range of VTG concentrations for male tripletail were similar to previously reported values for other male fish, specifically sturgeon (Ceapa et al. 2002; Wildhaber et al. 2007; Heise et al. 2009). As expected, VTG levels were significantly greater in female tripletail
than in males; however, the difference appears to be heavily influenced by high VTG concentrations in a few spawning capable females. Statistical interpretations of the data are limited by the small sample size but the trend of higher GSI and VTG for females in the spawning capable phase suggests that a more robust dataset could provide the ability to differentiate spawning females from other female reproductive phases and from males (Figures 5 and 6). The relationship between VTG and GSI was strong ($R^2 = 0.873$) but should be interpreted with caution because of the small sample size of high VTG and high GSI, which may have disproportionately affected the coefficient of determination. Levels of VTG in spawning capable females were similar to those reported in other fish species including spawning stellate sturgeon *Acipenser stellatus* (Ceapa et al. 2002), gag grouper, *Mycteroperca microlepis*, (~3000 µg/ml) (Heppell and Sullivan 2000) and presumed spawning female Gulf sturgeon *Acipenser oxyrinchus desotoi* (> 1000 µg/ml) (Heise et al. 2009). Tripletail VTG levels were greater (~ 400 µg/ml) than concentrations found in spawning shovelnose sturgeon *Scaphirhynchus platorhynchus* (Wildhaber et al. 2007). The VTG concentration in females also appears to increase into August, which suggests that peak spawning may occur in August or later into the year (Figure 3-6). Increased VTG levels are not always clear indicators of temporal and spatial distribution of spawning fish but can be used as a line of evidence for elucidating reproductive activities (Ceapa et al. 2002).

Preliminary tagging data indicates that tripletail remain near the Georgia coast as late as October; therefore, spawning in the Atlantic could occur later in the year than previously reported for fish captured in the Gulf of Mexico and represents a possible bias in the study design (Chris Kalinowsky, GA-DNR; personal communication). Cooper (2002) suggested that
spawning could occur year-round in tropical waters although spawning seems to occur primarily between April and September off the eastern coast of Florida.

Currently the state of Georgia’s recreational fishing harvest regulations require a minimum size limit of 457 mm and a creel limit of two fish per person per day. This level of harvest seems to allow 100% of the males and approximately 50% of the females to reach sexual maturity prior to vulnerability of harvest. An increase in the minimum size would allow a greater percentage of females to reach sexual maturity. In the present study, only one female tripletail greater than 500 mm (1 of 39) was in an immature reproductive phase; therefore, an increase in the Georgia minimum size limit to 500-525 mm may allow nearly all females to reach maturity prior to susceptibility to exploitation by anglers. Currently, fisheries managers do not have sufficient data to understand the effects of fishing mortality on tripletail populations.

The inability to use entanglement gear to capture tripletail in their primary habitat presents researchers with a challenge when attempting to obtain representative samples across all size ranges. The use of hook-and-line sampling presents the most cost effective method for sampling tripletail; however, the use of these sampling methodologies can bias sampling, often leading to the disproportionate capture of fish in the lower end of the size range, a trend evident in this and other tripletail studies (Brown-Peterson and Franks 2001; Cooper 2002; Strelcheck et al. 2004). The lack of imminently spawning females in the present study could be attributed to the possibility that imminently spawning females may not actively feed; therefore, other methods to capture tripletail must continue to be explored.

The role of nearshore aggregations in the life history of tripletail in the western Atlantic remains unanswered. The primary need for future research is an increased sample size of sexually mature female tripletail (> ~525 mm) throughout the year. Increasing the sample size of
large females throughout the year could elucidate trends in GSI and plasma VTG concentrations. Future research should expand the sampling to a larger temporal and spatial scale and use tagging information to better understand migratory patterns for more cost-effective sampling. A larger spatial scale should include more inshore and nearshore locations on the Atlantic coast but should also include an offshore component. A large offshore effort may be cost prohibitive because of the random distribution of tripletail and association with non-stationary floating debris. Laboratory studies of VTG and sex steroid hormone concentrations in maturing tripletail can be a cost effective means for better understanding tripletail reproductive biology.
Literature Cited


Hardy, J. D. 1978. Development of fishes of the Mid-Atlantic Bight: an atlas of egg,
larval and juvenile stages. U.S. Fish and Wildlife Service, Biological Service Program FSW/BSP78/12.


Table 3-1. Reproductive phases of individual male tripletail (n = 122) captured in 2009 and 2010 near Jekyll Island, GA, USA. All males in this study in the regressing phase were spawning capable but were no longer undergoing active spermatogenesis. GE = germinal epithelium.

<table>
<thead>
<tr>
<th>Month</th>
<th>Immature</th>
<th>Early Developing</th>
<th>Late GE</th>
<th>Mid GE</th>
<th>Early GE</th>
<th>Regressing</th>
<th>Regenerating</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>May</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>June</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>20</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>July</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>August</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 3-2. Reproductive phases of individual female tripletail (n = 102) captured in 2009 and 2010 near Jekyll Island, GA, USA.

<table>
<thead>
<tr>
<th>Month</th>
<th>Immature</th>
<th>Early</th>
<th>Developing</th>
<th>Spawning Capable</th>
<th>Actively Spawning</th>
<th>Regressing</th>
<th>Regenerating</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>May</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>June</td>
<td>18</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>July</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>August</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 3-1. Tripletail sampling location near Jekyll Island, GA, USA. Inset shows a map of the southeast United States with the Jekyll Island area indicated by the arrow. Each point on the map indicates that one or more tripletail were captured at that location from March to August 2009 and 2010.
Figure 3-2. Non-linear regression of sexually mature female tripletail in 50-mm length bins captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Closed data points represent actual cumulative percent maturity of females, whereas males are represented by the open data points. The dashed line denotes the length (459 mm) at which 50% of female tripletail are mature. The dotted line represents the minimum size limit (457 mm) currently enforced by the Georgia Department of Natural Resources. Non-linear regression models for males were unable to converge and therefore were unable to be modeled; however, only one male captured in this study was classified as immature.
Figure 3-3. Non-linear regression of sexually mature female and male tripletail by age class captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Closed data points represent actual cumulative percent maturity of females; males are represented by the open data points. The dotted line denotes the age at which 50% of males (0.55 years) and females (1.17 years) are mature.
Figure 3-4. Mean gonadosomatic index values for (A) female (n = 101) and (B) male (n = 116) tripletail captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Error bars represent ± 1 standard error.
Figure 3-5. Blood plasma vitellogenin concentrations of tripletail captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Females are represented by the triangles and males are represented by open circles. Reproductive phases are represented by Imm- immature, Dev- developing, SC- spawning capable, Regress- regressing, and Regen- regenerating.
Figure 3-6. Mean plasma vitellogenin concentrations (μg/ml) for (A) female (n = 77) and (B) male (n = 98) tripletail captured near Jekyll Island, GA, USA from March to August 2009 and 2010. Error bars represent ± 1 standard error.
Figure 3-7. Comparison of plasma vitellogenin and gonadosomatic index values across reproductive phases for (A) female (n=77) and (B) male (n=98) tripletail captured near Jekyll Island, GA, USA during March to August 2009 and 2010. Vitellogenin means are represented by triangles and GSI mean values are represented by circles. Error Bars represent 95% confidence intervals. Reproductive phases are represented by Imm- immature, Dev- developing, SC- spawning capable, Regress- regressing, and Regen- regenerating.
CHAPTER 4

Summary

Proper management of fish populations requires basic understanding of life history characteristics, including age, growth, and reproduction. This basic life history information for tripletail is lacking in published reports, particularly for the western Atlantic Ocean. The results presented in this thesis increase knowledge about the age, growth, and reproduction for tripletail in the western Atlantic Ocean, specifically off southeastern Georgia. The lack of tripletail life history information led to a collaborative research effort between the University of Georgia and the Coastal Resource Division of the Georgia Department of Natural Resources to evaluate non-lethal sampling techniques for determining age and reproductive status for the species.

In Chapter 2, tripletail age and growth were evaluated, and the results provide the first published account of tripletail ages in the western Atlantic Ocean. Otoliths and first dorsal spines, henceforth spines, were evaluated and compared as aging structures. Otolith removal requires the tripletail to be sacrificed; whereas, the spine represents a non-lethal aging technique. Both structures produced legible, translucent (spine) and opaque (otolith) bands, which were interpreted as annuli. Results from the present study were similar to those of previous studies conducted in the Gulf of Mexico; both studies indicated that tripletail growth is very rapid (>500 mm and >4 kg) during the first few years of life. There were slight differences in growth between males and females, but we deem these to be biologically irrelevant to fisheries managers. Ages
for male and female tripletails ranged from 1 to 5 years of age for both otolith- and spine-estimated ages.

The use of otoliths and spines as aging structures had advantages and disadvantages. Otoliths had higher initial reader agreement than spines, although agreement between the structures was relatively high (84.1%). As a result of compression within the spine as tripletail age, back-calculated length-at-age data are not feasible for spines; however, they were feasible for otoliths. We believe the greatest accuracy for ages would be achieved by reading the structures concurrently and only analyzing measurements from otoliths. However, the lack of existing life history data and population estimates suggest non-lethal aging methods would be preferred over lethal methods. In summary, spines may provide sufficient aging structures for tripletail if back-calculated lengths-at-age are not required, which would allow researchers to practice non-lethal sampling.

The evaluation of the reproductive status of tripletail in the nearshore waters off the Jekyll Island, GA is reported in Chapter 3. Standard histological techniques, which required specimens to be sacrificed, were used to evaluate the efficacy of non-lethal blood plasma vitellogenin (VTG) concentrations as a surrogate method for determining sex and reproductive status. The data were generally consistent with tripletail reproductive studies conducted in the Gulf of Mexico. Our data and data from other studies suggest that primary spawning does not occur in nearshore waters. Most male tripletail captured near Jekyll Island, GA from March to August were in a spawning capable reproductive phase. Results of histological analysis indicate that most female tripletail near Jekyll Island, GA were not in spawning condition. Vitellogenin analysis was unable to distinguish between all males and females; however, spawning capable females may be distinguishable from all other reproductive phases. A larger sample size,
particularly of females >525 mm, is needed to provide conclusive evidence about spawning status.

The estimated length and age at which 50% of females reached maturity (L_{50}) were 459 mm and 1.17 years. Predicted size at which 50% of males reach maturity could not be derived because of the lack of immature males; however, the age at which 50% of males became mature was 0.55 years. The derived size at which 50% of females are mature (449 mm) is below the current minimum size limit (457 mm) for tripletail caught in Georgia waters. Based on our data, we believe all males above the current minimum size (457 mm) would be mature. Georgia’s current minimum size limit of 457 mm and a creel limit of two fish per person per day allow 100% of the males and approximately 50% of the females to reach sexual maturity prior to becoming vulnerable to harvest. These regulations appear to adequately allow the tripletail to mature; however, further population estimates and recruitment rates are needed to determine if regulations are sufficient to maintain a self-sustaining population.

The reproductive status of tripletail throughout the year, their seasonal and annual range, as well as information about their movement patterns all are fruitful areas for future research on tripletail in the western Atlantic. Concurrently developing non-lethal sampling techniques represents an attempt to responsibly manage a species with very little population demographic information. In a time where many of the world’s marine fish stocks are currently undergoing overfishing, the decrease in overall take by both fishers and researchers alike demonstrates the overall goal of responsible stewardship of natural resources. These non-lethal techniques may represent a template for minimizing researcher effects while evaluating and improving knowledge of marine fish species.
**Conclusions**

Our study found both otoliths and spines have their advantages and disadvantages. Otoliths had higher initial reader agreement than spines, although agreement between the structures was relatively high at 84.1%. We believe spines are sufficient aging structures for tripletail if back-calculated length-at-age data are not required, which would allow researchers to practice non-lethal sampling. The current sample size was insufficient to demonstrate VTG blood plasma concentrations can be used as a surrogate for standard histological procedures for determining reproductive status of tripletail. Male tripletail are capable of spawning in the nearshore waters of Jekyll Island, GA; whereas female tripletail are primarily not in spawning condition. The present study has provided valuable demographic information, but the role of nearshore GA aggregations in the life history of tripletail in the western Atlantic remains unknown. Additional research should focus on the validation of aging methods and vitellogenin analysis for determination of reproductive status. An increase in sample size of tripletail, particularly in the upper size range, will increase the robustness of analysis.
Appendix A. Tripletail captured for reproductive analysis near Jekyll Island, GA, USA during 2009 and 2010.

<table>
<thead>
<tr>
<th>Capture Month</th>
<th>2009 Males</th>
<th>2009 Females</th>
<th>2010 Males</th>
<th>2010 Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>May</td>
<td>17</td>
<td>15</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>June</td>
<td>28</td>
<td>17</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>July</td>
<td>7</td>
<td>10</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>August</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>