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## NOTE

# Evaluation of Reproductive Status in Atlantic Tripletail by Traditional and Nonlethal Approaches

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## Abstract

Reproductive biology information is an important tool for fishery management actions such as the identification of spawning areas and the development of protective size limits, bag limits, and seasons. Such information for the management of Atlantic Tripletail *Lobotes surinamensis* is currently limited, particularly in the western Atlantic Ocean, as information regarding the reproductive biology of this species is sparse in the published literature. To this end, we determined the reproductive status of tripletail and compared the results of a nonlethal sampling method, plasma vitellogenin (VTG) analysis, with those of two traditional (lethal) methods, gonadosomatic index (GSI) and gonad histology. A total of 223 (122 male and 101 female) tripletail were sampled over 2 years near Jekyll Island, Georgia.

Gonad histology indicated that 107 (94%) of the male tripletail were in the spawning-capable reproductive phase. Female tripletail were found in all reproductive phases, but only nine (8.9%) were in the spawning-capable phase. Plasma VTG was strongly related to GSI in females ( $R^2 = 0.832$ ,  $n = 77$ ), and female GSI differed significantly among reproductive phases ( $p < 0.0001$ ). The estimated length at which 50% (L50) of female tripletail reached maturity was 463 mm; however, the L50 for male tripletail could not be determined because of the lack of immature fish within the study sample. Our study provides valuable information for the management of tripletail and indicates that a nonlethal approach (plasma VTG) may be useful for differentiating developing and spawning-capable females from males and from females in other reproductive phases.

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Atlantic Tripletail *Lobotes surinamensis* (Bloch 1790) 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 (Hardy 1978), and tripletail are known to occur in the western Atlantic Ocean from Massachusetts to Argentina, including the Gulf of Mexico and the Caribbean Sea, with higher abundances south of North Carolina (Hardy 1978). Tripletail are migratory, though detailed information about their movement patterns is scarce (Merriner and Foster 1974; Franks et al. 1998; Streich et al. 2013). Tripletail are frequently found associated with structure in estuaries and near barrier islands but are also routinely found free swimming near the surface of waters, ranging from 2 to 4 m deep in spring and summer (Breder 1949). The conspicuous free-swimming tripletail are increasingly targeted by anglers using sight fishing techniques, and additional information about tripletail life history such as age, growth, and reproductive biology is needed to ensure the adequate protection of this species.

Atlantic Tripletail appear to be multiple-batch spawners, with the ability to spawn once every 3 to 5 d during the putative 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. 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 exclusively in July through September in >100 m of water in plankton surface tows, suggesting that tripletail spawn offshore in summer months.

Gonad maturation and reproductive phase can be assessed with histological analysis; however, this method requires more time and expense than visual staging based on the macroscopic appearance of the gonads or the calculation of gonadosomatic index (GSI) values (West 1990). Gonad histology and GSI analyses require sacrifice of the fish, and nonlethal alternatives for determining reproductive status are desired for rare species or those for which definitive population dynamics data are lacking or scarce, such as Atlantic Tripletail. Nonlethal methods have been used to determine the reproductive status of other fish species and have included the evaluation of blood plasma for the egg yolk precursor, vitellogenin (VTG; Heppell and Sullivan 2000; Ceapa et al. 2002; Wildhaber et al. 2007; Heise et al. 2009). Vitellogenin is found at highest 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 locations (Heppell and Sullivan 2000; Ceapa et al. 2002; Wildhaber et al. 2007; Heise et al. 2009).

The lack of published tripletail data, particularly for the Atlantic coast of the USA, underscores the general scarcity of

information on the life history of the species. The goal of the current study was to inform fishery management actions by adding to the limited existing information regarding Atlantic Tripletail reproductive biology. We determined the reproductive status of tripletail by the traditional (lethal) methods of GSI and gonad histology, and compared the results with a nonlethal sampling method, plasma VTG analysis. Our study represents the first report of tripletail plasma VTG levels as a potential nonlethal method to determine sex and reproductive phase.

## STUDY SITE

Atlantic Tripletail sampling was conducted in the western Atlantic Ocean near Jekyll Island, Georgia. Sampling occurred inshore, primarily along the northeast to central part of the island and on channel markers, range markers, buoys, and other structures within St. Simons Sound, north and west of Jekyll Island (Figure 1).

## METHODS

**Field sampling.**—Sampling for Atlantic Tripletail and associated water quality variables was performed on multiple dates during April to August 2009 and 2010. On each sampling trip, surface water temperature (°C), salinity

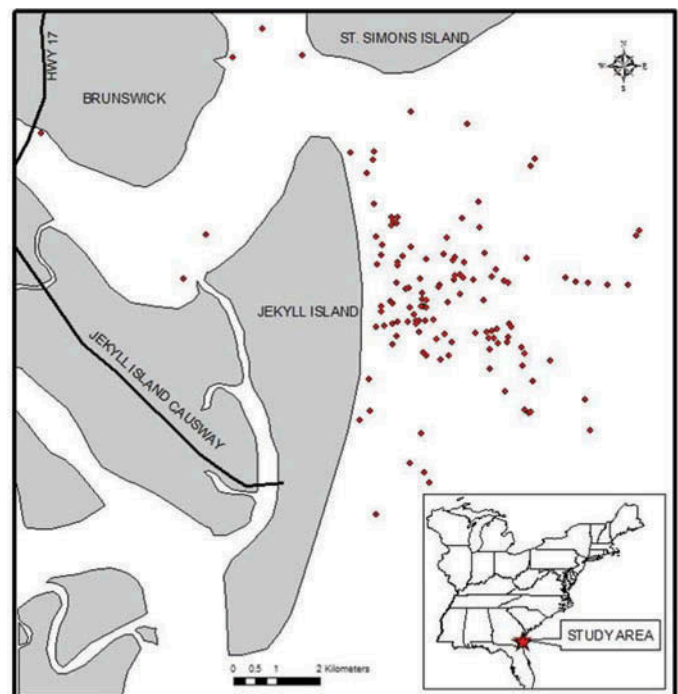


FIGURE 1. Atlantic Tripletail sampling location near Jekyll Island, Georgia. Inset shows a map of the southeast United States, with the Jekyll Island area indicated by the arrow. Each point on the map indicates capture location of one or more tripletail. All fish were captured in April to August 2009 and 2010.

(‰), and dissolved oxygen (mg/L) were measured with a YSI 85 dissolved oxygen meter and recorded upon arrival at the sample site (Figure 1). Nearshore tripletail were sampled with sight fishing methods by casting a baited rig at fish observed near the surface. Tackle consisted of a spinning rod equipped with braided line (14-kg test) attached to a popping cork rig with a Kahle 140 hook (size 1), baited with a live shrimp or Striped Mullet *Mugil cephalus*. Tripletail were captured with a similar approach approximately 2 h before and after slack tide around structures in the St. Simons Sound and adjacent shipping channel.

The sampling target was a maximum of 10 fish/week for histological evaluation: five individuals <457-mm TL and five individuals ≥457-mm TL. The 457-mm (18-in) designation represents the minimum size limit for possession of Atlantic Tripletail in Georgia, established by the Georgia Department of Natural Resources (GADNR). Any captured fish that exceeded the target number were measured, tagged, and released. Upon capture, location was recorded with a GPS and blood samples (2 mL) were immediately obtained from the caudal vein with a heparinized 3-mL syringe equipped with a Luer-Lok 18-gauge hypodermic needle. Blood samples were expelled into 2.0-mL heparinized centrifuge tubes and immediately placed on ice for up to 6 h. To minimize degradation of VTG, blood samples were treated with a protease inhibitor cocktail (P2714; Sigma-Aldrich, St. Louis, Missouri). All fish were measured for TL (nearest mm) and weighed (nearest 1.0 g) with an electronic platform scale (maximum 20 kg). Fish were then marked with a unique tag and placed on ice until processing at the GADNR Coastal Regional Division (CRD) laboratory in Brunswick, Georgia. In the laboratory, whole gonads were dissected from the fish, weighed (nearest 0.1 g), and preserved in a 10% solution of buffered formalin until further processing for histological analysis. Gonadosomatic index values were calculated based on the equation originally described by Nikolsky (1963). Trends in GSI for each sex were examined across the reproductive phases and capture months.

**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. Briefly, gonads from the subsampled fish 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 were used including dehydration, embedding in paraffin, thin sectioning (4 µm), and staining with hematoxylin and eosin. Developmental stage was evaluated for each of the three sections per fish and confirmed to be uniform throughout the gonad, so all subsequent gonad samples were collected only from the central portion of the preserved gonad. Reproductive phases for both males and females were evaluated based on criteria described by Brown-Peterson et al. (2011). Two observers independently

evaluated the reproductive status of a randomly selected subsample ( $n = 25$ ) for both males and females for quality assurance and control; agreement between observers was 96%, so a single observer evaluated the remainder of the samples.

**Length at 50% maturity.**—The length at which 50% of individuals reached maturity (L50) for male and female Atlantic Tripletail was estimated by linear regression, with fish length as the independent variable and the proportion of mature adults in each 50-mm length bin (<350 to >500) as the dependent variable. Sample sizes for all bins (male and female) ranged from 14 to 28 fish.

**Plasma vitellogenin.**—Plasma was separated from blood cells by centrifugation ( $1,500 \times g$ ) for 10 min and was then placed into cryogenic vials and frozen in liquid nitrogen. Plasma samples were shipped on dry ice via overnight express courier to the University of Florida for VTG analysis. Atlantic Tripletail plasma VTG was quantified with the methods of Denslow et al. (1999) by a direct enzyme-linked immunosorbent assay with monoclonal antibody 3G2 (HL1393) developed for VTG of Striped Bass *Morone saxatilis*. All samples were assayed in triplicate, and CV ( $100 \cdot \text{SD}/\text{mean}$ ) was <12% for each sample. Inter- and intra-assay variability were measured by analyzing the positive controls on each 96-well plate ( $n = 5$ ) and were <10% and <5%, respectively.

**Statistical analysis.**—Total length and weight data were log transformed to achieve normality. Differences in TL and weight were compared between years with ANOVA. A chi-square analysis was used to determine if monthly sex ratios varied from the expected 1:1 ratio ( $\alpha = 0.05$ ). Plasma VTG data were log transformed to achieve normality, and differences in VTG and GSI among reproductive phases and capture months were evaluated by PROC GLM to allow for differing sample sizes among groups (i.e., phases and months). Linear regression was used to evaluate the relationships between VTG and GSI for males and females. All statistical analyses were conducted with SAS 9.1 (SAS Institute, Cary, North Carolina). Tukey's test was used to evaluate significant differences among means for all one-way ANOVAs ( $\alpha = 0.05$ ).

## RESULTS

### Field Sampling

The project included 177 sampling events, with a total of 385 h of "nearshore" sampling and an additional 95 h of "structure" sampling (during slack tides). A total of 432 Atlantic Tripletail were captured, and 223 (122 males and 101 females) were sampled for reproductive evaluation. The remaining 209 fish were tagged and released. One hundred twelve of 122 (91.8%) males and 81 of 101 (80.2%) females were captured in May–July. Monthly sex ratios did not differ from the expected 1:1 ratio ( $\chi^2 = 2.3937$ ,  $P = 0.0828$ ). Tripletail TL ranged from 241 to 822 mm, and weight ranged from 265 to 14,152 g. Mean TL ( $F_{1, 216} = 0.87$ ,  $P = 0.3517$ )

and weight ( $F_{1, 216} = 0.89$ ,  $P = 0.3476$ ) were not significantly different between years; therefore, the data from 2009 and 2010 were pooled for all subsequent analyses.

### Gonadosomatic Index

Female GSI ranged from 0.07 to 7.67 ( $n = 101$ ); generally, the lowest GSI values were measured in immature or regenerating reproductive phases and the highest values were in the spawning-capable phase (Table 1). Seven of the nine (77.8%) spawning-capable females were captured in July and August. Male ( $n = 122$ ) GSI values ranged from 0.01 to 0.22 and followed a pattern similar to that of females, with the lowest GSI in the immature and developing phases and the highest GSI in spawning-capable males (Table 2).

### Gonad Histology

Female Atlantic Tripletail were captured in all reproductive phases (Table 3), but 42.6% were immature, only 8.9% were spawning capable, and 31.7% were regenerating. Nearly all (107 of 122, or 87.7%) of the male tripletail captured throughout the study were in the spawning-capable phase; only one (0.8%) was immature and six (4.9%) were developing (Table 4). Spawning-capable males were captured in all months, but 92.5% (99 of 107) in the spawning-capable phase were captured from May to July.

### Length at Maturity

The proportion of mature females in the 50-mm length bins ranged from 0 to 1.0 and fit ( $R^2 = 0.92$ ) a linear model (Figure 2). The L50 for female Atlantic Tripletail was esti-

TABLE 1. Mean ( $n$ ) GSI by reproductive phase for female Atlantic Tripletail captured near Jekyll Island, Georgia.

Month	Immature	Developing	Spawning capable	Regressing	Regenerating
Apr	0.61 (7)	0.96 (6)	3.91 (1)	(0)	0.64 (2)
May	0.59 (14)	0.83 (3)	(0)	1.17 (2)	0.71 (5)
Jun	0.49 (18)	(0)	7.67 (1)	1.50 (2)	0.72 (15)
Jul	0.54 (4)	0.74 (3)	4.39 (5)	(0)	0.68 (9)
Aug	(0)	(0)	4.83 (2)	2.09 (1)	0.65 (1)
Total	0.54 (43)	0.88 (12)	5.13 (9)	1.31 (5)	0.70 (32)

TABLE 2. Mean ( $n$ ) GSI by reproductive phase for male Atlantic Tripletail captured near Jekyll Island, Georgia.

Month	Immature	Developing	Spawning capable	Regressing	Regenerating
Apr	(0)	0.08 (1)	0.10 (4)	(0)	(0)
May	(0)	0.10 (5)	0.13 (27)	0.05 (2)	(0)
Jun	0.01 (1)	(0)	0.10 (46)	0.06 (3)	(0)
Jul	(0)	(0)	0.11 (26)	0.08 (2)	(0)
Aug	(0)	(0)	0.12 (4)	0.07 (1)	(0)
Total	0.01 (1)	0.09 (6)	0.11 (107)	0.06 (8)	(0)

TABLE 3. Reproductive phases of individual female Atlantic Tripletail captured near Jekyll Island, Georgia. Values for phases are a percentage of the monthly total, with the number of fish in parentheses.

Month	Immature	Developing	Spawning capable	Regressing	Regenerating	Total
Apr	43.8 (7)	37.5 (6)	6.3 (1)	0.0 (0)	12.5 (2)	16
May	58.3 (14)	12.5 (3)	0.0 (0)	8.3 (2)	20.8 (5)	24
Jun	50.0 (18)	0.0 (0)	2.8 (1)	5.6 (2)	41.7 (1)	36
Jul	19.0 (4)	14.3 (3)	23.8 (5)	0.0 (0)	42.9 (9)	21
Aug	0.0 (0)	0.0 (0)	50.0 (2)	25.0 (1)	25.0 (1)	4



TABLE 4. Reproductive phases of individual male Atlantic Tripletail captured near Jekyll Island, Georgia. Values for phases are a percentage of the monthly total, with the number of fish in parentheses.

Month	Immature	Developing	Spawning capable	Regressing	Regenerating	Total
Apr	0.0 (0)	20.0 (1)	80.0 (4)	0.0 (0)	0.0 (0)	5
May	0.0 (0)	14.7 (5)	79.4 (27)	5.9 (2)	0.0 (0)	34
Jun	2.0 (1)	0.0 (0)	92.0 (46)	6.0 (3)	0.0 (0)	50
Jul	0.0 (0)	0.0 (0)	92.9 (26)	7.1 (2)	0.0 (0)	28
Aug	0.0 (0)	0.0 (0)	80.0 (4)	20.0 (1)	0.0 (0)	5

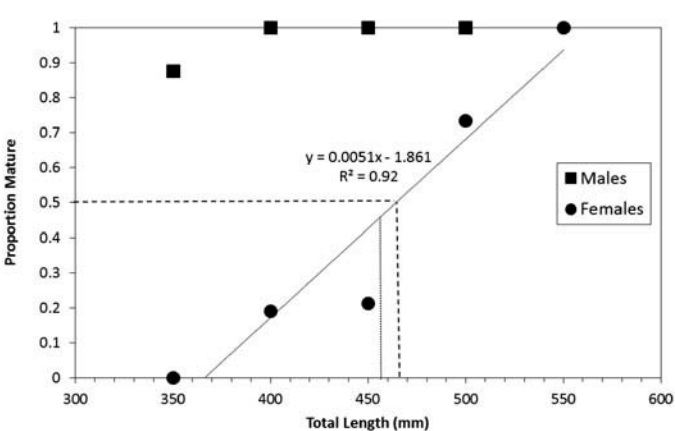


FIGURE 2. Proportion of sexually mature Atlantic Tripletail (by 50-mm length bin;  $n = 14\text{--}28$ ) captured near Jekyll Island, Georgia, from April to August 2009 and 2010. The dashed line is the estimated length (463 mm) at which 50% of female tripletail were mature. The dotted line represents the minimum size limit (457 mm) currently enforced by GADNR.

mated at 463 mm, with a slope of 0.0051 and  $y$ -intercept of  $-1.861$ . A lack of immature male fish in the sample precluded estimation of an L50 for males; the single immature male was 300–350 mm (Figure 2), suggesting that the L50 for males is  $<350$  mm.

Vitellogenin

Plasma VTG concentrations for female Atlantic Tripletail ( $n = 77$ ) ranged from below detection ( $1.0\text{ }\mu\text{g/mL}$ ) to  $4,000\text{ }\mu\text{g/mL}$  (Figure 3A). The highest individual VTG values in females were measured in June, July, and August; however, VTG means did not differ ( $F_{4, 76} = 3.33$ ,  $P = 0.164$ ) among months. Male tripletail ( $n = 98$ ) VTG levels ranged from below detection to a maximum of  $170\text{ }\mu\text{g/mL}$  (Figure 3B). The highest individual male VTG values were measured in June and July, but mean VTG did not differ ( $F_{4, 97} = 3.10$ ,  $P = 0.249$ ) among months. Plasma VTG concentration was significantly higher in spawning-capable females than in all other phases (ANOVA:  $F_{4, 76} = 9.09$ ,  $P < 0.0001$ ). Differences were not detected among female plasma VTG in the developing and regressing phases. Plasma VTG was a significant and strong

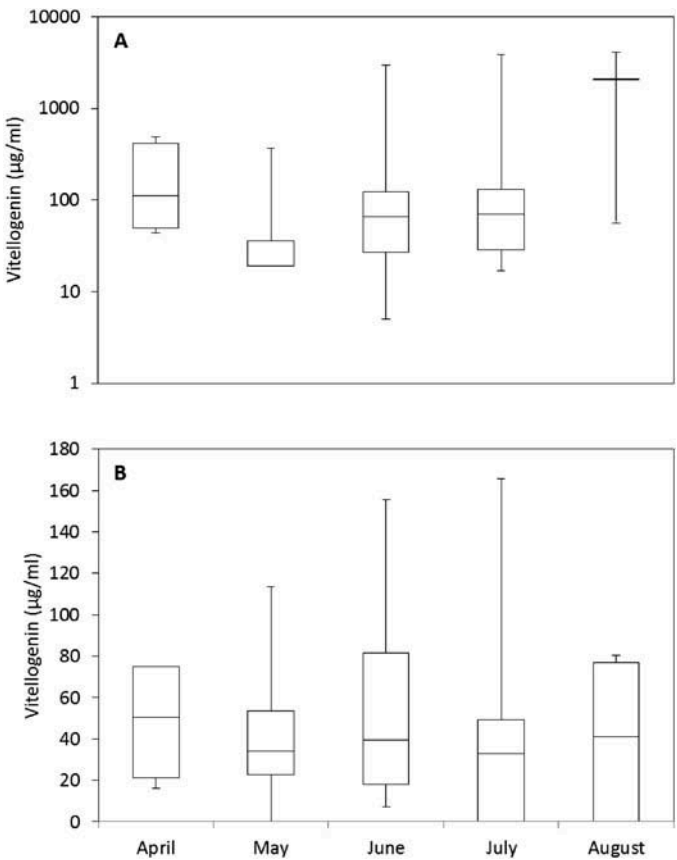


FIGURE 3. Plasma vitellogenin concentration of (A) female ( $n = 77$ ) and (B) male ( $n = 98$ ) Atlantic Tripletail captured near Jekyll Island, Georgia, from April to August 2009 and 2010. Box and whiskers represent minimum, 25th percentile, median, 75th percentile, and maximum values. Values did not differ significantly among months (Tukey's:  $\alpha = 0.05$ ) for males or females.

predictor ( $R^2 = 0.832$ ,  $n = 84$ ,  $p < 0.0001$ ) of GSI in females (Figure 4). In males, plasma VTG was not related to GSI ( $R^2 = 0.027$ ,  $n = 101$ ,  $p = 0.102$ ).

DISCUSSION

The observed relationship between female plasma VTG and GSI suggests that plasma VTG may be useful as a nonlethal method for differentiating spawning-capable females from

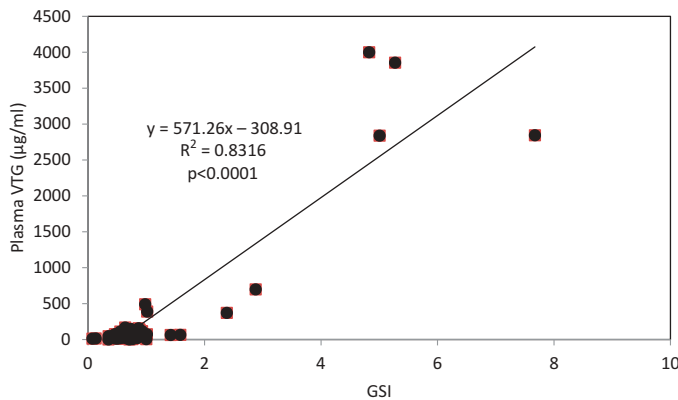


FIGURE 4. Relationship between plasma VTG and GSI in female Atlantic Tripletail collected from April to August 2009 and 2010 near Jekyll Island, Georgia.

males as well as females in other reproductive phases. These results should be interpreted with caution because of the relatively small sample of females with a GSI >3.0, and the capture of a limited number of spawning-capable females in the present study (nearshore) is consistent with previous findings that suggest Atlantic Tripletail spawn offshore (Ditty and Shaw 1994). To the best of our knowledge, no other studies have measured VTG in tripletail; however, the plasma VTG in spawning-capable females was similar to that reported in other fish species, including spawning Stellate Sturgeon *Acipenser stellatus* (Ceapa et al. 2002), Gag *Mycteroperca microlepis* (~3,000 µg/mL; Heppell and Sullivan 2000), and presumed spawning female Gulf Sturgeon *Acipenser oxyrinchus desotoi* (>1,000 µg/mL; Heise et al. 2009). Female tripletail VTG levels were generally greater than concentrations reported in spawning female Shovelnose Sturgeon *Scaphirhynchus platyrhynchus* (~400 µg/mL; Wildhaber et al. 2007). Increased VTG levels are not always clear indicators of the temporal and spatial distribution of spawning fish, but they can be used as a line of evidence for elucidating reproductive activities (Ceapa et al. 2002).

Cooper (2002) reported that Atlantic Tripletail spawning seems to occur primarily between April and September off the eastern coast of Florida, and the high proportion of spawning-capable male tripletail in our study, particularly in June–August, corroborates this assertion. Male GSI data were relatively constant throughout the sampling period, as would be expected from a spawning-capable male population. Preliminary tagging data (Streich et al. 2013) 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.

The L50 estimate for female Atlantic Tripletail in our study is generally consistent with previous studies that have reported an L50 of approximately 490 mm for female tripletail in the Gulf of Mexico (Brown-Peterson and Franks 2001; Strelcheck

et al. 2004). Brown-Peterson and Franks (2001) also reported that 100% of females >525 mm were sexually mature. The current minimum size limit (457 mm) for tripletail caught in Georgia waters is lower than the L50 we estimated, indicating that female tripletail off the Georgia coast may be harvested prior to spawning. Male tripletail in Georgia are likely able to mature prior to becoming vulnerable to harvest because our data suggest that the L50 for males is <350 mm, which is consistent with Brown-Peterson and Franks (2001) and Strelcheck et al. (2004) who suggested that the L50 for males in the Gulf of Mexico would likely be ≤290 mm. 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 male and female tripletail to reach maturity prior to susceptibility to exploitation by anglers.

The highest plasma VTG concentrations measured in males (approximately 100–170 µg/mL) in the present study are comparable to the VTG levels measured in male fish exposed to exogenous estrogens, which are commonly associated with municipal wastewater and other types of pollution (Denslow et al. 1999; Jones et al. 2000; Marin and Matozzo 2004). Jekyll Island and St. Simons Island each have municipal wastewater treatment facilities, and nearby Brunswick, Georgia, has three municipal wastewater treatment facilities that discharge into St. Simons Sound. Additionally, four Superfund sites are located <10 km from all Atlantic Tripletail collection sites in Brunswick, Georgia (<http://www.epa.gov/region4/superfund/sites/sites.html>), and contaminants from these sites may also contribute to endocrine disruption in fish in the area. Several persistent organic compounds confirmed as endocrine disruptors, such as polychlorinated biphenyls and toxaphene, have been documented at very high concentrations in aquatic organisms in and around the St. Simons Sound area (Balmer et al. 2011), where the present study occurred. Additional research is needed to better understand VTG patterns in male tripletail and the effects that contaminants may be having on fish and wildlife populations in this area.

The inability to use entanglement gear to capture Atlantic Tripletail in their primary habitat presented us with a challenge when attempting to obtain representative samples across all size ranges. The use of hook-and-line sampling is likely 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 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. 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; however, a large offshore sampling effort may be cost prohibitive because of the random distribution of tripletail and their association with nonstationary flotsam.

The current study provides evidence that plasma VTG concentrations could provide an effective nonlethal sampling technique for determining the reproductive status of female Atlantic Tripletail. The primary need for future research is an increased sample size of sexually mature female tripletail (greater than ~500 mm) throughout the year to further elucidate temporal trends in GSI and plasma VTG concentrations. Future laboratory studies of plasma VTG and sex steroid hormone (e.g., testosterone, estradiol, progesterone) concentrations in maturing tripletail may be a cost-effective means for better understanding tripletail reproductive biology.

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