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ARTICLE

Efficacy of Otoliths and First Dorsal Spines for Preliminary Age and Growth Determination in Atlantic Tripletails

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Abstract
The Atlantic Tripletail *Lobotes surinamensis* is a popular sport fish for which age and growth data are scarce in general and nonexistent for Georgia (GA), USA, waters. These data are necessary to ensure that management regulations are adequate to protect this species, especially given its popularity as a sport fish. We evaluated whether otoliths and spines were suitable for determining the estimated age (hereafter, "age") and growth rates of Atlantic Tripletails, and we ascertained whether one method was more accurate than the other. Atlantic Tripletails were sampled by angling and trawling during March 30–August 10, 2009, and March 14–August 6, 2010, in nearshore GA waters of the Atlantic Ocean. During the study, 243 Atlantic Tripletails were captured and sampled for aging structures. Sagittal otoliths and the first dorsal spine were removed from each fish and used to estimate the age and growth rate. Mean differences in TL at age for spine and otolith data were evaluated with ANOVA. Estimated ages for males and females ranged from 1 to 5 years based on otoliths and spines. Both otolith and spine mean TLs at ages 1 and 2 were significantly different from each other as well as all other age-classes, whereas mean TLs for ages 3–5 were not significantly different. Differences in Atlantic Tripletail TL among the otolith- and spine-derived age-classes were not significant. Each method used to age Atlantic Tripletails had advantages and disadvantages. Otoliths had higher initial reader agreement than spines, although agreement between the structures was 84.1%. However, otoliths require sacrifice of the fish, whereas a spine can be taken without sacrificing the fish. The lack of concrete life history data and population estimates suggests that when feasible, nonlethal aging methods would be preferred over lethal methods to ensure the survival of Atlantic Tripletail populations.

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The Atlantic Tripletail *Lobotes surinamensis* is a popular sport fish that occurs in the western Atlantic, including the Gulf of Mexico and the Caribbean Sea (Streich et al. 2013; Parr et al. 2016). Atlantic Tripletails occur in a variety of habitats from estuarine waters to the open ocean but are commonly found in association with structure (Streich et al. 2013; Parr et al. 2016). Detailed information about many aspects of the species' life history is lacking, but the available data suggest that Atlantic Tripletails feed opportunistically on shrimp, crabs, and teleost fishes (Merriner and Foster 1974; Cooper 2002; Strelcheck et al. 2004). Information on the large-scale movement of this migratory species is scarce, but Streich et al. (2013) recently provided detailed data on Atlantic Tripletail habitat both nearshore and in estuaries. Parr (2011) and Parr et al. (2016) offered brief syntheses of the available information on Atlantic Tripletail reproduction. Still, much remains to be learned about aspects of the life history of this popular and widely distributed sport fish.

In Georgia (GA), USA, Atlantic Tripletails are found around structure in estuaries and on the ocean side of some barrier islands. From March to July, Atlantic Tripletails are popular targets for anglers in nearshore Atlantic Ocean waters immediately east of Jekyll Island beach (Figure 1). Atypically, these fish are not associated with any structure but rather are free swimming at the sea surface in waters ranging from 2 to 4 m deep; this behavior allows anglers to use sight-fishing techniques to capture Atlantic Tripletails. 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 (S. Woodward, Georgia Department of Natural Resources [GADNR], personal communication). The influx of anglers targeting Atlantic Tripletails has raised questions as to whether the two-fish, 457-mm minimum size limit regulations are sufficient for sustaining the populations in GA and neighboring states.

Age and growth data are scarce for Atlantic Tripletails in general, and such data for Atlantic Tripletails in GA waters are nonexistent. Age and growth data are necessary to ensure that management regulations are adequate to protect Atlantic Tripletails, especially given the rising popularity of this species as a sport fish. Determining the age of a fish has been achieved by counting the annual rings in spines, otoliths, and scales, but these structures do not work equally well for all species (Quist et al. 2012). Franks et al. (1998) reported that spines and fin rays were suitable for determining age in Atlantic Tripletails but that the first dorsal spine was the most acceptable of those two structures for aging the fish in their sample. However, otoliths often provide more accurate aging data than other fish hard parts, such as vertebral bones, scales, fin rays, and spines (VanderKooy 2009). Accordingly, the goal of the present study was to evaluate the efficacy of using sagittal otoliths and first dorsal spines for determining age and calculating growth rates for Atlantic Tripletails.

METHODS

Fish and water quality sampling.— Personnel from the GADNR's Coastal Resources Division (CRD) sampled Atlantic Tripletails by angling, trawling, and opportunistically from fishing tournaments during March 30–August 10, 2009, and March 14–August 6, 2010, in nearshore GA waters of the Atlantic Ocean near Jekyll Island (Figure 1). A maximum of 10 fish/week were sacrificed for bony structure removal: five individuals that were smaller than 457 mm TL, and five individuals that were 457 mm TL or larger. Criteria for sacrificing the captured fish were based on a concurrent reproductive assessment study (see Parr et al. 2016). The 457-mm demarcation represents the current GADNR minimum size limit for possession of Atlantic Tripletails. All 610-mm and larger Atlantic Tripletails were sacrificed. Fish captured outside of these criteria were measured to the nearest millimeter for TL and SL, tagged with a uniquely numbered Hallprint plastic dart tag inserted behind the base of the third spine of the spiny dorsal fin, and released.

During the sampling conducted via angling, researchers searched the general area for Atlantic Tripletails. When an Atlantic Tripletail was spotted, it was targeted by casting a spinning rod equipped with 14-kg-test braided line that was attached to a popping cork rig with a size-1 Kahle live-bait hook, which was baited with live white shrimp *Litopenaeus setiferus*, brown shrimp *Farfantepenaeus aztecus*, or Striped Mullet *Mugil cephalus*. Duration of the sampling event was recorded to the nearest minute, as was the total number of Atlantic Tripletails observed and captured. A GPS unit was used to determine and record the capture location for each fish. Upon capture, each fish received an individually labeled tag affixed to its gill; the individual was placed on ice and held until arrival at the CRD laboratory located in Brunswick, GA. At each sampling site, surface water temperature (°C), salinity (‰), and dissolved oxygen concentration (mg/L) were measured with a YSI Model 85 dissolved oxygen meter.

Slightly different angling methods were used to capture Atlantic Tripletails around structures (e.g., channel markers, range markers, and buoys) in St. Simons Sound and the adjacent shipping channel. Sampling occurred about 2 h prior to and 2 h after slack tides. Heavier tackle was necessary to target Atlantic Tripletails around structures to prevent breakoffs around the structures. This heavier tackle included 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 shrimp, brown shrimp, or Striped Mullet. The rig was fished at all levels of the water column next to the

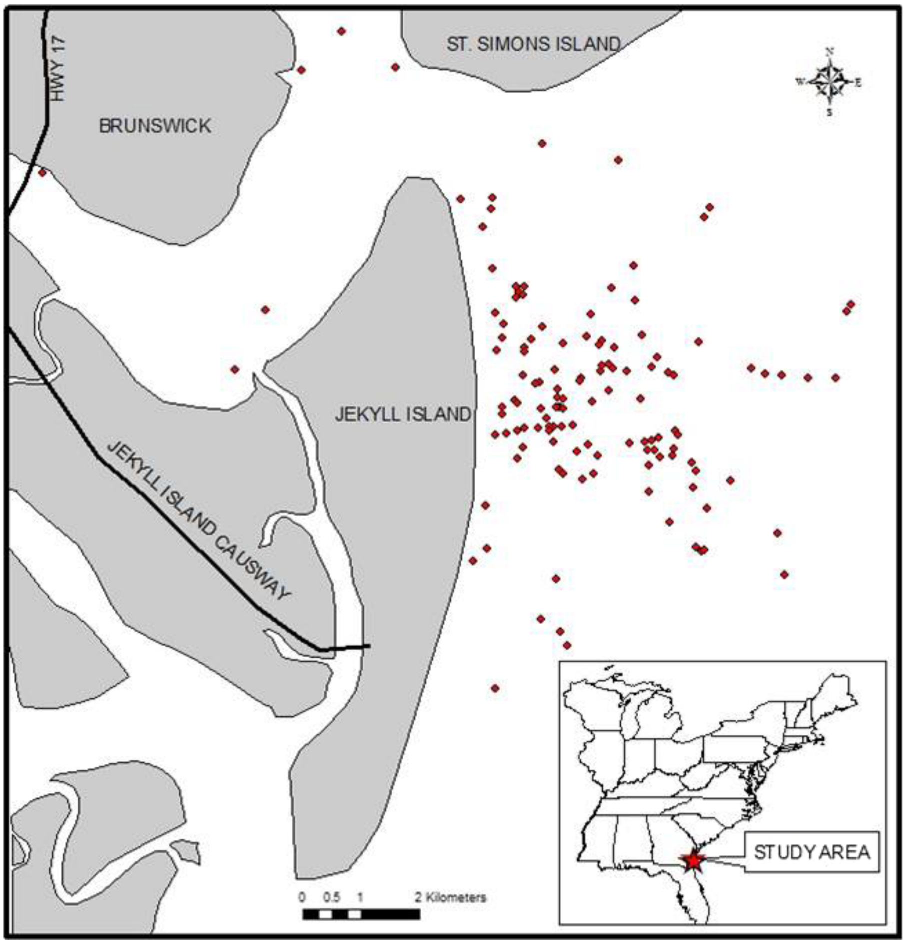

FIGURE 1. Atlantic Tripletail sampling locations near Jekyll Island, Georgia, USA. Inset shows a map of the southeast United States, with the Jekyll Island area indicated by the star. Each point on the map indicates the capture location of one or more Atlantic Tripletails. All fish were captured during March–August 2009 and 2010. Reprinted from Parr et al. (2016).

structure. Upon capture of a fish, procedures were identical to the methodology described previously for sight fishing. Sampling around structure in 2009 was performed opportunistically when weather prevented sight fishing. In 2010, the two fishing methods were split 50:50; catch success determined where effort was best allocated on a weekly basis.

In addition to angling, a 12.2-m fish trawl with 76.2-mm bar mesh was used in 2009 to capture Atlantic Tripletails offshore from Jekyll Island. The trawl was deployed from the GADNR research vessel R/V *Anna* with a maximum tow time of 10 min. Vessel location (determined with a GPS unit) and heading of the R/V *Anna* were recorded at the beginning (trawl net in) and at the end (trawl net out) of each tow. 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 Atlantic Tripletails caught was recorded. Surface water temperature (°C), salinity (‰), dissolved oxygen concentration (mg/L), tide stage, wind direction and speed, and atmospheric condition data were collected and recorded prior to the sampling trip.

Atlantic Tripletails were also sampled opportunistically in cooperation with local tournaments and anglers. These fish often were donated carcasses; therefore, sex and total weight (TW) often could not be determined. Atlantic Tripletails of unknown sex and those lacking weight data were excluded from statistical analyses.

Atlantic Tripletails 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 examined macroscopically, and sex was recorded. Sagittal otoliths were removed. First dorsal spines (henceforth, "spines"), including the condyle base, were removed with a hacksaw and placed into the same labeled envelope as the otoliths.

Age and growth.— Spines were prepared for reading by placing them in bleach for 2 min, and all excess tissue and skin were removed by using a scalpel and forceps (Jim Franks, University of Southern Mississippi, personal communication). One sagittal otolith and the spine from each fish were embedded individually in West System 205 and 206 epoxy (Gougeon, Inc., Bay City, Michigan) within Pelco 10530 numbered trays (Ted Pella, Inc., Redding, California). Aging structures were sectioned with a Buehler Isomet low-speed saw (Buehler, Lake Bluff, Illinois) equipped with two diamond-tipped wafering blades (Buehler Series H-15) and a 0.1-mm spacer between the blades. At least 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 the initial sections were illegible (see Franks et al. 1998). Multiple transverse sections from the dorsoventral plane of each otolith were taken to ensure that a quality core section was obtained. Cytoseal XYL clear bonding agent (Richard-Allan Scientific, Kalamazoo, Michigan) was used to mount the specimen sections onto glass microscope slides. Ages were evaluated via a computer-based image analysis system consisting of a Leica MZ8 stereomicroscope (Leica Microsystems, Wetzlar, Germany) with transmitted light, a Photometrics Coolsnap Camera, and Optimas version 6.51 imaging software (Optimas Corp., Bothell, Washington).

Ages were determined based on the number of opaque bands on the otoliths and the number of 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 Atlantic Tripletails. Otoliths were aged based on standard aging procedures, although the opaque bands were not always easy to distinguish (DeVries and Frie 1996). Examining the periphery of the otolith section to find where the bands had formed often was beneficial. Opaque bands on Atlantic Tripletail otoliths were typically very thin, followed very close behind the translucent bands, and were often difficult to distinguish (Figure 2).

Spine-derived ages were based in part on techniques described by Franks et al. (1998) to create the criteria used to determine spine age. In this approach, “multiples” were considered to be annual marks; a multiple, also known as a “doublet” or a “triplet” (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 were counted, one band was counted when two or three small translucent bands occurred more closely together than the distance to the preceding and subsequent translucent bands (Gonzales-Garces and Farina-Perez 1983; Franks et al. 1998). 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. If band counts differed between readers, they jointly re-examined the counts and attempted to reach a consensus on the estimated age of the individual. If the readers could not reach an agreement, the ages were excluded from further analyses. Precision was evaluated by a single reader who re-aged (1) a random subsample of 50% of both otoliths and spines for age-classes with sample sizes greater than 50 and (2) all otoliths and spines from age-classes with sample sizes less than 50.

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

Initial reader agreement within and between structures was determined as the percentage of ages that were classified as being the same by both readers. Precision of aging was determined with the average percent error (APE) method (see Beamish and Fournier 1981).

An ANOVA was used to determine whether there were significant differences between TLs of the age-classes for both otoliths and spines. Normality of TL data was evaluated with the Shapiro–Wilk test; these data were nonnormal and were $\log_{10}$ transformed. Levene’s test was used to evaluate homogeneity of variances. We also used ANOVA to evaluate mean TL among age cohorts, and all age-groups that were significantly different were evaluated with a means separation test to determine where the differences occurred. Analysis of variance was used to evaluate mean differences between the TLs at age for both spine and otolith data. Back-calculated TL-at-age estimates were derived using the Fraser–Lee method (DeVries and Frie 1996). For both otoliths and spines, growth was calculated with von Bertalanffy (1938) growth models represented by the formula:

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

where L is the predicted TL (mm); L_∞ is the asymptotic maximum TL; K is the growth rate; t is time (age); and t_0 is theoretical age at a length of zero.

Nonlinear regression (NLIN procedure in SAS; Freund and Littell 1991) was used to model the parameters for the von Bertalanffy growth models. An α of 0.05 was used to determine the significance of all statistical tests.

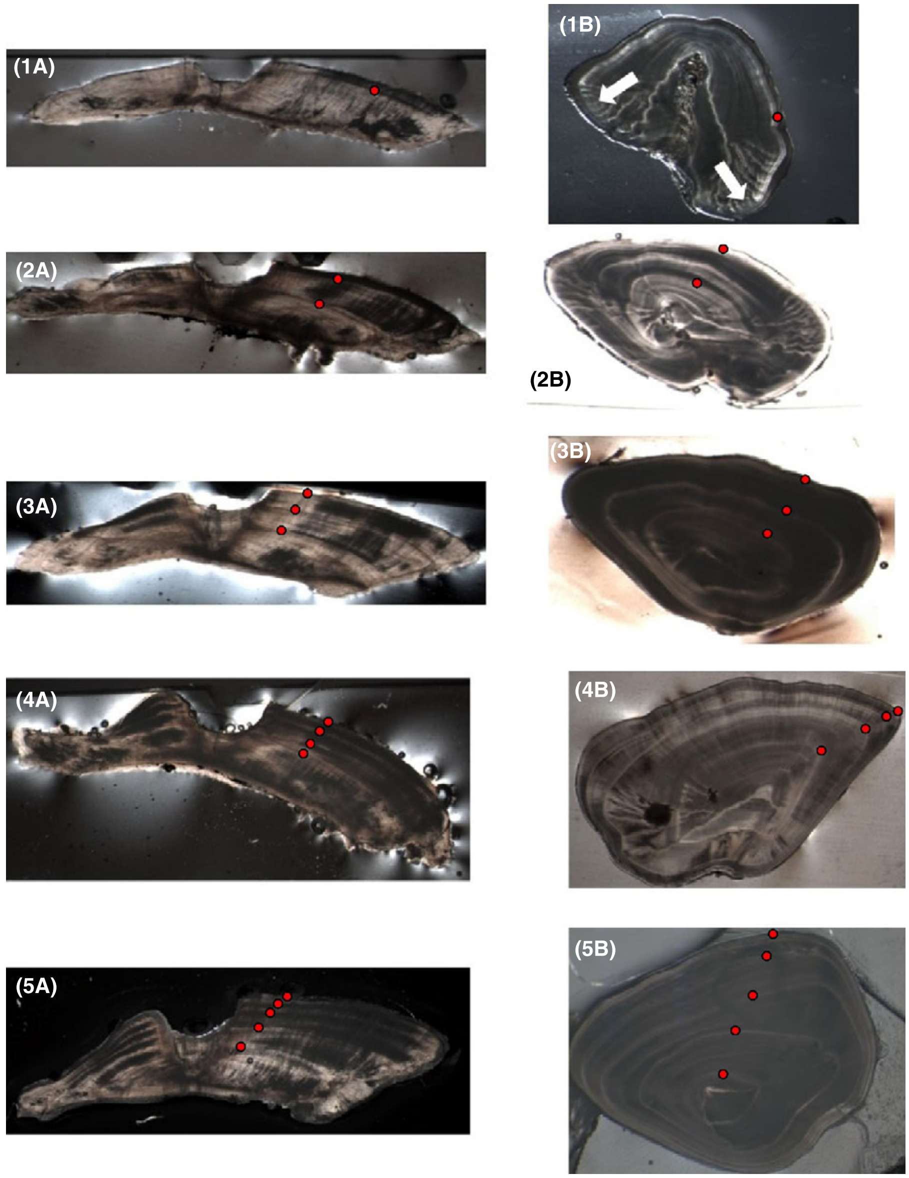

FIGURE 2. Images of **(A)** otoliths and **(B)** first dorsal spines taken from individual Atlantic Tripletails captured in 2009 and 2010 near Jekyll Island, Georgia. Images 1A and 1B represent an age-1 fish; the arrow indicates where striations terminate into a translucent band denoted as an annulus. Images 2A and 2B represent an age-2 fish; images 3A and 3B represent an age-3 individual. Images 4A and 4B represent an age-4 fish; notice the compression of the bands near the edge. Images 5A and 5B represent an age-5 individual; notice the small first annulus, which we believe resulted from compression as the individual aged and should not be skipped. These pictures were not taken at the same scale and are meant to be used as examples only.

RESULTS

Sampling and Water Quality

During the project, 177 sampling events resulted in 385 h of sight fishing, 95 h of structure sampling, and 9 h of trawl sampling. In total, 243 Atlantic Tripletails were captured and sampled for aging structures. An additional 208 Atlantic Tripletails were 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‰ (range = 15–35‰), and dissolved oxygen averaged 6.18 mg/L (range = 3.34–9.02 mg/L).

Morphometrics

Of the 243 Atlantic Tripletails that were collected and sampled for age structures, 105 were females, 126 were males, and the remaining 12 were of undetermined sex. Significant differences were not detected between the expected monthly 1:1 sex ratio of males to females

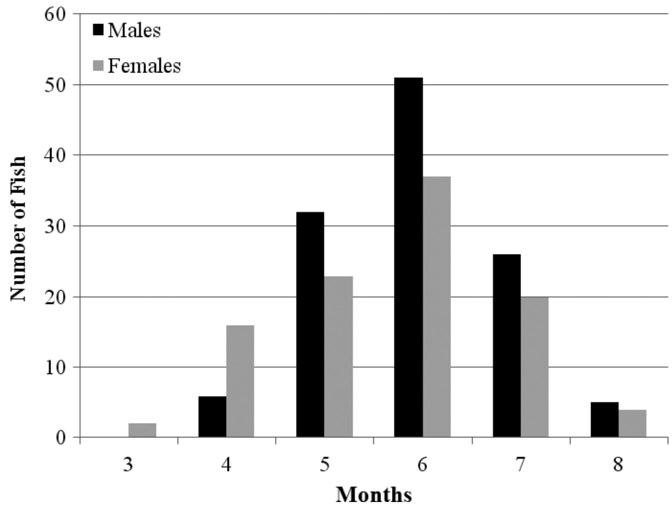

FIGURE 3. Numbers of male and female Atlantic Tripletails (n = 231) captured during sampling off the coast of Jekyll Island, Georgia, during 2009 and 2010. Sex ratio did not differ from the expected ratio of 1:1 (χ^2 = 9.74, P = 0.0828).

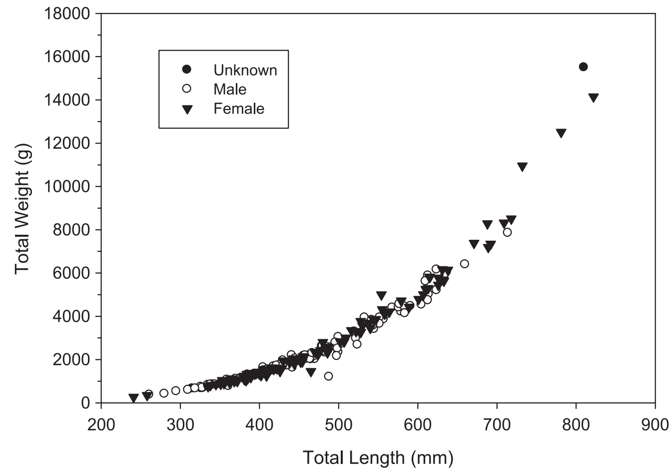

FIGURE 4. Scatter plot of total weight (g) versus total length (mm) of Atlantic Tripletails (n = 229) captured during March–August 2009 and 2010 from the aggregation near Jekyll Island, Georgia.

TABLE 1. Summary information for male (TL: n = 126; total weight: n = 125) and female (TL: n = 105; total weight: n = 101) Atlantic Tripletails captured near Jekyll Island, Georgia, in 2009 and 2010.

	TL (mm)		Total weight (g)	
Sex	Mean	SD	Mean	SD
Male	455	93	2,314	1,524
Female	489	117	3,116	2,595

(χ^2 = 9.74, P = 0.0828; Figure 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 years; therefore, the data for 2009 and 2010 were pooled. Mean female TL (mean ± SD = 489 ± 117 mm) was not significantly different from male TL (455 ± 93 mm; $F_{1,216}$ = 3.85, P = 0.0512; Table 1). Mean female TW (mean ± SD = 3,116 ± 2,595 g) was significantly higher than mean male TW (2,314 ± 1,524 g; $F_{1,216}$ = 4.96, P = 0.0270; Table 1). Models based on sex were poor predictors for both TL (r^2 = 0.02) and TW (r^2 = 0.03); therefore, the data for both sexes were combined for all further analyses. The length–weight regression indicated that there was a strong relationship between TL and TW ($\log_{10}$[TW] = −5.223 + 3.210·$\log_{10}$[TL]; r^2 = 0.985, n = 229), and TW was nearly a cubic function of TL (Figure 4).

Age and Growth

Otoliths and spines had alternating opaque and translucent bands (Figure 2), and the quality of bands varied among individual fish as well as between aging structures. Legible otolith sections were obtained from all Atlantic Tripletails. Spines were not retrieved from all fish, and some of the spine cores (n = 5) displayed vascular erosion and were not suitable for aging; those spines were removed from further analysis. Initial reader agreement was 90.1% for otoliths (219 of 243) and 82.8% for spines (198 of 238). All spine and otolith band counts were mutually agreed upon for each structure during the concert reading. Otolith-based ages were more precise (APE = 6%) than the spine-based ages (APE = 21%). 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. Within-reader agreement was high for both spines (90.8%) and otoliths (96.2%), as was the precision between readers for a given age. The two readers produced age estimates that were within 1 year of each other in most cases (99% each for both structures). Agreement between structures based on agreed-upon ages was 84.1% (201 of 238), and the APE between structures was 6.7%.

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

Otolith-based, back-calculated TLs at age showed similar results to mean TLs at age from our capture data (Figure 6). Spine-based, back-calculated TLs at age were lower than the mean TLs at age from capture data (Figure 6). Parameter estimates derived from the von Bertalanffy growth model for otolith- and spine-estimated ages paralleled each other over the range of ages observed within this study (Figure 7). The spine-based von Bertalanffy

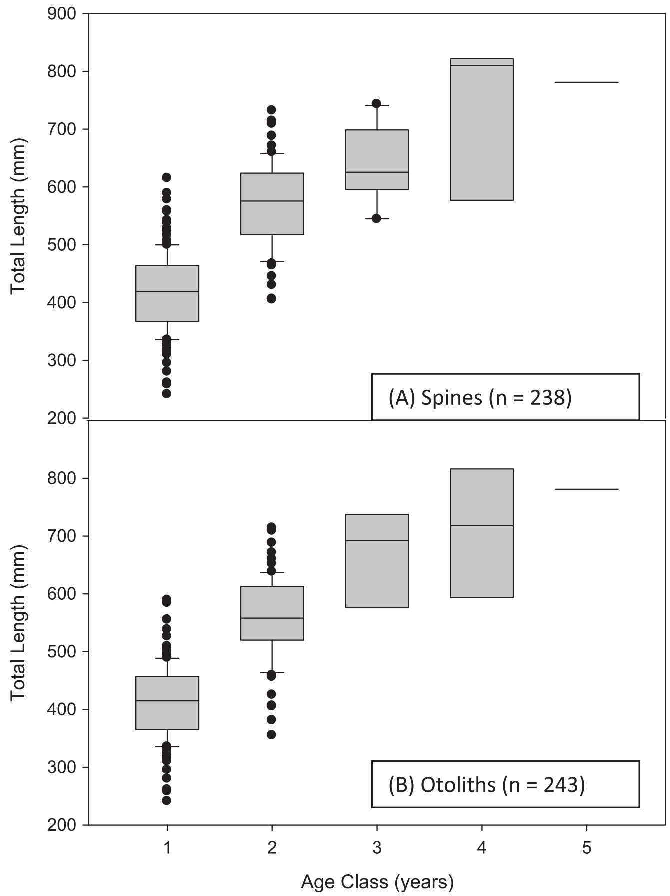

FIGURE 5. Box plots of **(A)** spine-based ages (i.e., first dorsal spine) and **(B)** otolith-based ages for Atlantic Tripletails captured during March–August 2009 and 2010 near Jekyll Island, Georgia. The central line indicates the median, the gray box represents the middle 50% of the data, the whiskers represent the area where 90% of the data fell, and the black dots denote outliers.

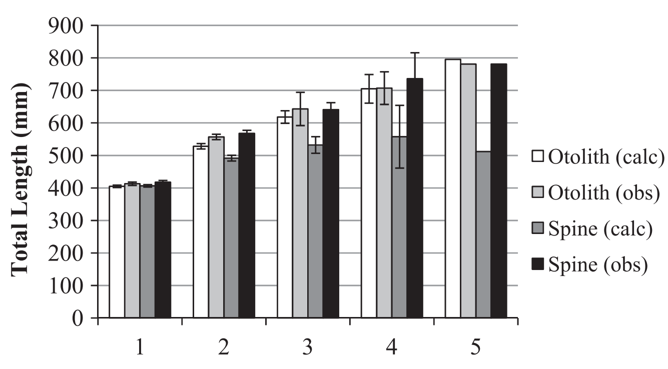

FIGURE 6. Comparison of mean (±SE) back-calculated (calc) and observed (obs) total length-at-age estimates based on otoliths and first dorsal spines from Atlantic Tripletails captured during March–August 2009 and 2010 near Jekyll Island, Georgia.

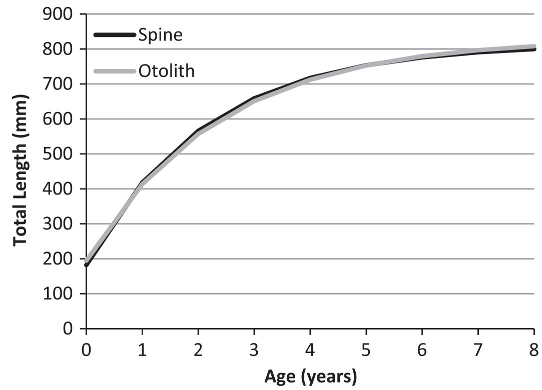

FIGURE 7. von Bertalanffy growth curves based on first dorsal spines and otoliths from Atlantic Tripletails captured during March–August 2009 and 2010 near Jekyll Island, Georgia. Only ages 1–5 were captured during this study.

growth model estimates (L_∞ = 815 mm, K = 0.46, t_0 = −0.5414) were similar to the otolith-based von Bertalanffy growth model estimates (L_∞ = 830 mm, K = 0.42, t_0 = −0.63289).

DISCUSSION

We detected slight but significant differences between sexes in mean TL and TW; however, these differences probably resulted from the lack of males captured in the upper size range (>700 mm TL) and a relatively large sample size. This finding is consistent with the results of studies on Atlantic Tripletails in the Gulf of Mexico (Armstrong et al. 1996; Franks et al. 1998; Strelcheck et al. 2004) and off Cape Canaveral, Florida (Cooper 2002). Despite these findings, we deem these modest differences to be biologically irrelevant to fisheries managers and base this conclusion on the minor differences in the length–weight relationship between males and females (Figure 4). Our results suggest that treating males and females as separate groups would not yield any better inferences about Atlantic Tripletail growth rates than pooling those data for analysis.

The average size at age and general age demographics of Atlantic Tripletails in our study were similar to the data that have been presented in the limited published literature focused on this species. Our findings of very rapid growth during the first few years of life for Atlantic Tripletails agree with the findings of Merriner and Foster (1974), Armstrong et al. (1996), Franks et al. (1998), and Strelcheck et al. (2004). Atlantic Tripletails are thought to live about 7–10 years (Merriner and Foster 1974). However, in our study, we observed no Atlantic Tripletails belonging to those age-classes. The age range (1–5 years) we observed is closer to the maximum ages reported by Franks et al. (1998) and Strelcheck et al.

(2004). This finding suggests that hook-and-line methods mostly target 1–3-year-old Atlantic Tripletails. However, the GA state record of 17.63 kg and the International Game Fish Association record of 19.19 kg for Atlantic Tripletails suggest that some individuals can reach an age of 7 years.

Both otoliths and spines produced legible bands for age determination, and we successfully aged Atlantic Tripletails from the lethal technique (otoliths) and nonlethal technique (spines). Furthermore, ages derived from both structures agreed consistently. This result is noteworthy because Franks et al. (1998) and Strelcheck et al. (2004) did not find agreement between the structures and deemed the otoliths illegible. Their results may have differed from ours because of their small sample sizes (<50 samples) as well as geographic and climatic differences. The agreement between otolith- and spine-derived ages observed in the present study establishes the spine-based method as a viable, nonlethal alternative for producing Atlantic Tripletail age estimates, especially in situations when lethal sampling is undesirable.

Agreement between ages derived from otoliths and spines was 84.1%, but initial reader agreement was slightly higher for otoliths (90.1%) than for spines (82.8%). Back-calculated otolith ages closely resembled age and length at the time of capture (Table 2), which probably resulted from the more uniform and consistent shape of the otoliths compared to spines. When lethal sampling is feasible, the use of both structures would increase the likelihood of more accurate ages if one of the two structures is unreadable for any reason.

Disagreements about otolith-based estimated age 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 Atlantic Tripletails that are captured near Jekyll Island; however, bands occurred before and after this date and had to be evaluated by the individual reader.

TABLE 2. Mean TLs (mm) at age for otolith- and first dorsal spine-derived ages at the time of capture compared with otolith back-calculated (Calc-Otolith) and spine back-calculated (Calc-Spine) TLs at age for Atlantic Tripletails captured in summer 2009 and 2010 near Jekyll Island, Georgia.

	Age-class				
Variable	1	2	3	4	5
Otolith	413	556	664	707	781
Calc-Otolith	410	538	628	712	795
Spine	418	567	640	736	781
Calc-Spine	389	489	539	562	512

Translucent band appearance and the overall shape of the spine varied greatly among individual Atlantic Tripletails. As Atlantic Tripletails age, the bands appear to compress and become clearer; however; this compression increases the difficulty in identifying the first annulus. Franks et al. (1998) defined the first annulus as the second multiple; however, after evaluation of spine sections with corresponding otoliths, we believe that skipping the first multiples may be necessary for age-2 or younger Atlantic Tripletails. As Atlantic Tripletails grow and age, the multiple bands appear to become compressed into a single band. Age-3 and older Atlantic Tripletails often had distinct compressed translucent bands near the edge of the spine sections. If those translucent bands appeared to be distinct bands around most of the spine, they were considered individual annuli (Figure 2). Striations often appeared on the spine sections and were used as part of the criteria in determining whether an annulus was present. Striations that were not interrupted by a translucent band were not counted as an annulus, whereas a translucent band that interrupted the striations was counted as an annulus (Figure 2). Accordingly, Franks et al.'s (1998) criteria seemed effective for fish up to age 2. Skipping the first multiple appeared to be unnecessary in age-3 or older fish, probably as a result of compression of the spine. Age-1 fish tended to have many multiples occurring prior to the formation of a definitive annulus; we believe that this pattern can lead to overestimation of fish age. Back-calculated, spine-based ages seemed to underestimate TL at age; this was probably caused by the compression of bands within the spine (Table 2). 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 be beneficial when using spines to age Atlantic Tripletails.

Future research on Atlantic Tripletail age and growth would benefit from sampling a greater number of age-3 and older fish to evaluate both the effectiveness of the aging technique and size-at-age data for Atlantic Tripletails. Although this study represents a "multiple lines of evidence" approach to aging Atlantic Tripletails, the need for age validation with known-age fish is basic. Determination of the band deposition timing and cause is also a necessary step toward the validation of aging techniques. Beamish and McFarlane (1987) demonstrated that the first dorsal spine was a nonlethal aging structure that could be used in concert with other dorsal spines from the same recaptured individual to validate the frequency of band deposition. This idea has been revived recently by Strelcheck et al. (2004), who suggested removing the first dorsal spine at the time of tagging and then evaluating the first dorsal spine against other spines for age comparison upon recapture. Strelcheck et al. (2004) also recommended that an area with a high concentration of Atlantic Tripletails would be needed to complete a study based on this methodology. Atlantic Tripletails aggregate in large numbers near Jekyll Island and support relatively high recapture rates within season and throughout the year; this aggregation may lend itself to validation studies for annulus formation in wild Atlantic Tripletails.

In conclusion, we found that otolith- versus spine-based methods of Atlantic Tripletail age estimation each had advantages and disadvantages. Otoliths had higher initial reader agreement and lower APE (i.e., higher precision) than spines, although agreement between the structures (84.1%) and aging precision (i.e., low APE = 6.7%) were high. Compression within the spine as the Atlantic Tripletail ages hinders the ability to generate logical back-calculated ages in comparison with otolith back-calculated ages. However, otoliths require that the fish be sacrificed, whereas the fish can be released after a dorsal spine is sampled. We believe that the greatest accuracy would be achieved by reading the structures concurrently and analyzing growth measurements only with the otoliths, but the lack of concrete life history data and population estimates suggests that nonlethal aging methods would be preferred over lethal methods to ensure the survival of Atlantic Tripletail populations.

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