

PROTANDRIC SEX CHANGE IN *CENTROPOMUS UNDECIMALIS*, THE COMMON  
SNOOK

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. John Baldwin, Department of Biological Sciences, and has been approved by all members of the supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

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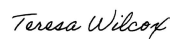


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## ABSTRACT

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Otolith increment morphology of the protandric hermaphrodite *Centropomus undecimalis* (snook) was analyzed in relation to age and length for sex specific growth differences to retroactively determine the time of sex change. Growth spurts in the otoliths of female snook, the terminal sex, were identified between ages 4 and 8, corroborating the current understanding of when sex change occurs (between 3 and 8). No such growth increases were identified on the otoliths of male snook, the primary sex. Otolith growth, however, was found to decouple from length, so these growth spurts are not reflected in the length of the fish. This study marks the first time that a distinct growth pattern differentiating the primary and terminal sexes, similar to that seen in the protogynous transitional growth spurt hypothesis, has been identified on the otoliths of a protandric species.

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*COMMON SNOOK*

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## 1. INTRODUCTION

A sequentially hermaphroditic sexual pattern is one in which a fish initially matures as a primary sex and later changes to a terminal sex. When the primary sex is female and the terminal sex is male, sequential hermaphrodites are protogynous, while the inverse sequence of the sexes is protandry (de Mitcheson & Liu, 2008). The change between the primary sex and terminal sex follows the progression of age and size (de Mitcheson & Liu, 2008) and may result in a clear sexual size dimorphism where terminal sex animals fill different size classes than primary sex animals (Iwata et al., 2008; Walker & McCormick, 2004). The interactions among size, age, and sex are central to understanding the occurrence and timing of sequential hermaphroditism in fish.

Multiple “growth” hypotheses have been suggested to explain the observed linkage between size and sex change in protogynous fishes. Although yet unsubstantiated, the “larval growth hypothesis” characterizes hermaphroditic animals that experience early divergence in larval growth patterns where greater larval growth increases the likelihood of sex change (Walker & McCormick, 2004). Similarly, the “juvenile growth hypothesis” suggests that juvenile growth patterns, instead of larval growth patterns, may affect the probability of becoming the terminal sex. In these cases, early divergence in juvenile growth dictates eventual terminal maturation (e.g. *Plectropomus maculatus*) (Adams & Williams, 2001; R. C. Francis & Barlow, 1993). Lastly, the “transitional growth spurt hypothesis” describes those hermaphroditic animals that experience a growth spurt at the time of sex change so that the terminal sex is larger

than the primary sex (e.g. *Parapercis cylindrica* and *Scarus frenatus* among others) (Munday et al., 2004; Walker & McCormick, 2004).

Although the “larval growth hypothesis,” “juvenile growth hypothesis,” and “transitional growth spurt hypothesis” have yet to describe growth in protandric species (Walker & McCormick, 2004), preliminary evidence has suggested their applicability (Munday et al., 2006). In a number of protandric species (e.g. *Centropomus undecimalis*, *Lates cacarifer*), young primary males are nearly all the same size, but terminal females average greater sizes when compared to same age males (Andrade et al., 2013; McDougall, 2004; Taylor et al., 2000). Whether this difference in growth is better described by a protogynous growth hypothesis or a difference in lifetime growth rate remains to be shown (McDougall pers. Comm.).

A growth acceleration when changing from male to female could benefit females with higher fecundity and genetically favor larger individuals that arise from a growth spurt (Hixon et al., 2014; Munday et al., 2006). Larger females of some species, including snook, are assumed to have increased fecundity because they produce more and often larger eggs than their smaller female counterparts (Hixon et al., 2014; Rhody et al., 2014; Young et al., 2014). Consequently, the young of large females have an increased probability of survival because they are more resistant to starvation at spawn and grow larger, faster (Hixon et al., 2014; Kamler, 2005). Furthermore, because larger females have higher fecundity, their young likely compose a larger proportion of the population (Hixon et al., 2014; Rhody et al., 2014).

In order to determine if protandric fishes experience growth patterns described by the three protogynous growth hypotheses, the chronological pattern of individual growth

must be understood. Otoliths are ideal for this purpose because they are non-resorbable structures that record the metabolic growth rate of a fish (Campana, 1999; Payan et al., 2004). As metabolic rate alternates between faster and slower rates with environmental temperature fluctuations, the otolith grows alternating translucent and opaque increments (Rodríguez Mendoza, 2006). The periodicity of these increments may be interpreted as a record of growth and age (Campana, 2001). Furthermore, somatic growth may be indirectly connected to otolith growth because both otolith and somatic growth are dictated by metabolic rate (Campana, 1999; Molony & Choat, 1990). The linkage between otolith growth and age as well as otolith growth and somatic growth, however, should be verified on a species-specific basis (Campana, 2001). Otoliths may also record extreme physiological fluctuations as abrupt interruptions in an increment. These interruptions, known as check marks, have been associated with settlement from larva (Wilson & McCormick, 1997), changes in feeding behavior (Shen & Tzeng, 2002), and, in a few species, sex change (Munday et al., 2009; Walker & McCormick, 2004).

The aim of this study was to determine if one of the three protogynous growth hypotheses could be identified in the protandric hermaphrodite, *Centropomus undecimalis* the common snook, hereafter snook. The snook is an ideal candidate for this investigation because female snook attain larger sizes than their male cohort, suggesting a sex-specific growth difference between males and females (Andrade et al., 2013; Muller et al., 2015). They spawn with random mates in group aggregations, so male size should not influence female mating preference (Taylor et al., 1998; Young et al., 2014). Furthermore, the increment periodicity of snook otoliths has been validated in previous studies (Andrade et al., 2013; Taylor et al., 2000). The objectives of this study are (1) to

determine if snook otolith radius and total length are linearly linked and can be used to back calculate length-at-age, (2) to identify sex specific growth differences, and (3) to determine if a protogynous growth spurt hypothesis may describe growth differences in the protandric snook, and (4) to determine if check marks were specific to one sex.

## 2. METHODS

### 2.1 OTOLITH COLLECTION AND PREPARATION

All snook in this project were harvested in the Florida Fish and Wildlife Conservation Commission (FWC) Fishery Independent Monitoring (FIM) program, logbook program, and fish biology program. Both the FIM program and fish biology program collected snook independent of recreational harvest regulations and seasons by seine, hook-and-line, spear, and electrofishing. FWC's logbook program receives donated carcasses from fish caught in the recreational fishery throughout Florida. All fish used in this study came from the Atlantic coast of Florida between 2008 and 2014, were sexed and measured for total length, and were not undergoing sex change when harvested. Total length was ignored when selecting samples to ensure a random distribution of fish growth rates.

Both left and right sagittal otoliths were removed and processed by FWC. The left sagittal otolith was dried and stored. The right sagittal otolith was cut into three 0.5mm transverse sections (centered on the core and posterior and anterior the core) with a microtome. All sections from a unique otolith were mounted under liquid cover slip on a single glass microscope slide. Otoliths were aged by at least 2 independent readers at the Age and Growth Lab at the Florida Wildlife Research Institute (FWRI) where standard protocol requires that readers agree. Otoliths ages were determined again when

reader interpretation differed, and otoliths were not aged if the readers could not come to a consensus on the otolith age.

## 2.2 SAMPLING PROTOCOL

Three hundred and ninety-nine slide-mounted transverse otolith sections were obtained. Each slide was inspected at 10x magnification with a Leica stereo microscope. A single transverse section of the three slide-mounted sections was selected for imaging using two criteria: (1) close proximity to the otolith longitudinal center and (2) distinctive increment junctions ([Figure 1](#)). After selection, transverse sections were individually photographed at 10x magnification using the Zen Blue software (ver. 2) to manipulate an AxioCam Zeiss camera affixed to the Leica stereo microscope. All imaging was conducted using transmitted light. Otolith morphology of the imaged transverse section was analyzed using the ObjectJ (v1.04r) plugin for ImageJ (v1.52n) and a macro for ObjectJ written for otolith processing.

Otolith measurement locations were standardized. The core was identified on a selected transverse section ([Figure 1 D](#)). A line tangential to the proximal edge of the core was drawn parallel to the distal edge of the otolith ([Figure 1 E](#)). A line connecting the tangential line to the proximal edge of the otolith was drawn parallel to the dorsal edge of the sulcus ([Figure 1 F](#)).

All 3 readers measured the otolith transverse section images in microns along the standardized locations in the same way. First, the diameter of the core was measured across its center. Second, the radius of the otolith along the line parallel to the sulcus was measured. Third, the alternating translucent and opaque bands were measured along the

same line used to measure the radius of the otolith. Fourth, each reader assigned the transverse section a quality ranging from 1 to 3. An otolith section of quality 1 came from the exact center of the otolith, had clearly defined translucent and opaque increments, and was unbroken. Quality 2 otolith sections had clearly defined increments, came from near the center of the otolith, and may have had breaks that did not inhibit measuring the otolith. Otoliths were assigned quality 3 if the otolith section had poorly defined increments, breaks that inhibited measurement, or were far from the center of the otolith. Any otolith section assigned a quality of 3 was removed from analysis. All but 28 otoliths were analyzed by three readers. These 28 samples were later obtained and analyzed by a single reader following the same criteria as the rest of the samples.

It was important to retain only centered transverse otolith sections for measurement and analysis to prevent inaccurate comparisons of otolith growth rates from different longitudinal locations on the otolith. The otolith forms as a non-spherical accreting 3-D structure with location dependent rates of accretion, so the quantity of material accreted varies with the observed location. Consequently, transverse sections may lack or combine increments if they are longitudinally removed from the center axis of the structure. By analyzing sections that come from the same longitudinal location on the otolith the increments hidden or missed would be controlled in each of the transverse sections.

### 2.3 OTOLITH GROWTH

Beginning with the first translucent increment after the core, each translucent increment was summed with the subsequent opaque increment to obtain yearly otolith



growth for each snook. As such, age 0 growth incorporates only core growth and represents larval growth, age 1 growth is growth from the first year of life, age 2 growth is growth from the second year of life and so on until the otolith edge. Juvenile growth is treated as the growth occurring after larval growth and before the growth decline expected with maturity.

#### 2.4 SIZE-AT-AGE BACK CALCULATION

To determine if otolith growth can be used as a proxy for somatic growth, the relationship between otolith growth and length as well as age and length were modelled with linear regressions. The residuals from linear models between otolith growth and length and length and age were compared with Pearson's correlation test. Insignificant correlation between the standard residuals indicates that otolith growth may be used as a proxy of somatic growth. All variables were log transformed to normalize the data and reduce heteroscedasticity.

Size-at-age of snook was determined via the Fraser-Lee back calculation model (Rodríguez Mendoza, 2006) which takes the form

$$L_x = \frac{R_x}{R_h} * (L - c) + c$$

Where  $L_x$  is the total length of the animal at time  $x$ ,  $R_x$  is the radius of the otolith at time  $x$ ,  $R_h$  is the radius of the otolith at harvest,  $L$  is the length of the animal at measurement, and  $c$  is the average length of the animal at the time of otolith formation found by taking the intercept of the relationship between total length at measurement and concurrent otolith radius (R. I. C. C. Francis, 1990).

## 2.5 GROWTH DIVERGENCE AND SEX CHANGE

To investigate the possibility of a sex-change related growth divergence in snook, yearly otolith growth from the core until age 8 was compared between male and female snook using a multivariate analysis of variance (MANOVA) that incorporated birth year as a mixed effect ( $\alpha = 0.05$ ) to explore the effects of environment on growth. Snook born in the same year were assumed to experience the same environmental effects during the same period of growth. Sphericity of the data was checked using Mauchly's test. Additional analysis to identify the years in which growth diverged were conducted using analysis of variance ( $\alpha = 0.05$ ) and Tukey's test ( $\alpha = 0.05$ ).

## 2.6 SEX SPECIFIC CHECK MARKS

Male and female otoliths were inspected for check marks regardless of assigned quality (1, 2, or 3) because the identification of check marks did not require otolith measurements. Each of the three transverse otolith sections mounted on a sample slide were inspected at 400x magnification for check marks.

### 3. RESULTS

A near equivalent number of male and female otoliths were obtained from fish age 8 (67 males, 59 females, total 126), age 9 (54 males, 47 females, total 101), age 10 (52 males, 47 females, total 99), and age 11 (33 males, 33 females, total 66). Fish older than age 11 were obtained when available, but an unequal number of male and female fish that were age 12 (1 male, 5 females, total 6) and age 13 (0 males, 1 female, total 1) were obtained due to the rarity of males at these ages (Table 1). Of the 399 otoliths, 226 were ranked quality 1 or 2 and retained for analysis. These otoliths were from fish aged 8 (46 males, 29 females, total 75), 9 (36 males, 27 females, 63 total), 10 (34 males, 23 females, 57 total), and 11 (20 males, 11 females, 31 total) years of age (Table 1).

Female snook ranged between 620mm and 1136mm in total length, and male snook spanned 357 and 993mm. Female otolith core diameters ranged between 65 and 271 microns (Figure 2) while otolith radii ranged between 1874 and 3892 microns (Figure 3). Male snook otolith core diameters ranged between 62 and 297 microns (Figure 2) while otolith radii ranged between 2249 and 4431 microns (Figure 3). Male snook otolith cores (mean=145.15 microns) averaged greater diameters than female snook otolith cores (mean=142.25 microns), but this was not statistically significant (MANOVA,  $p$ -value = 0.07). Female otoliths averaged larger radii (2977.33 microns) than male otoliths (2918.62 microns).

### 3.1 GROWTH DIVERGENCE AND SEX CHANGE

Snook exhibit sex specific growth patterns (MANOVA, sphericity adjusted  $p$ -value  $< 0.01$ ,  $Df = 9$ ) that were significantly different even when environmental conditions were taken into account through birth year (Mixed-effect ANOVA unadjusted  $p$ -value for the effect of sex =  $< 0.01$  and for the effect of birth year =  $0.6$ ). During the 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> ages (Figure 4), male and female growth was significantly different (ANOVA  $p$ -value =  $< 0.01$ ,  $< 0.01$ ,  $< 0.01$ ,  $< 0.01$ , and  $< 0.01$  respectively). A Tukey's test run on the ages 4, 5, 6, 7, and 8 verified that females averaged higher growth during each of these years. Otolith growth during age 0 and the subsequent 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> ages were not statistically different between those fish that remained male and those that changed sex (MANOVA unadjusted  $p$ -value =  $0.07$ ,  $0.31$ ,  $0.65$ , and  $0.83$  respectively).

### 3.2 SIZE-AT-AGE BACK CALCULATION

Both otolith size ( $p$ -value  $< 0.01$ ) and age ( $p$ -value  $< 0.01$ ) were significantly positively correlated with total length. Otolith size explained more variation seen in the total length ( $R^2 = 0.2579$ ) than did age ( $R^2 = 0.05243$ ). A significant positive correlation ( $p$ -value  $< 0.01$ ) was found between the standardized residuals from the regression between otolith radius and total length and the standardized residuals from the regression between total length and age (Figure 5) indicating that other variables not in the model better explain length of snook than otolith growth and age. Consequently, back-calculated total lengths reported here are not expected to reflect snook total lengths at each age (Table 2).

### 3.3 VISUAL INSPECTION FOR OTOLITH CHECK MARKS

Check marks (Figure 6) appeared inconsistently and were ambiguous on both male and female otoliths.

#### 4. DISCUSSION

Many of the events that induce sex change in protandric sequential hermaphrodites remain poorly explored or unidentified. Some categories, such as social interactions (Iwata et al., 2008), relative size, sex ratio, and population density (Munday et al., 2006) encompass interactions throughout the lifetimes of hermaphroditic animals. Indeed, research on protogynous fishes suggests that control of sex change is not limited to interactions immediately preceding the transition from the primary to terminal sex and that events leading to sex change occur at any point prior to sex change; a detail no better exemplified than by the juvenile growth hypothesis of the 3 protogynous growth hypotheses (Adams & Williams, 2001; R. C. Francis & Barlow, 1993). Moreover, many protandric species lack external distinctions between the sexes, so identifying the occurrence of sex change and distinguishing between the sexes is invasive and possibly lethal.

Previous studies have begun to link growth and sex change in protogynous fishes by using otoliths (McCormick et al., 2010; Munday et al., 2009; Walker et al., 2007; Walker & McCormick, 2004, 2009). Growth, measured on otoliths, that occurs during 3 age categories (larval, juvenile, and mature) appears to increase the probability of or coincide with sex change in hermaphrodites. The recognition of the importance of these three growth patterns to hermaphrodites has generated 3 respective growth hypotheses – the larval growth hypothesis, the juvenile growth hypothesis, and the transitional growth spurt hypothesis (Walker & McCormick, 2004)– that are yet to be explored in protandric

fishes. In this study, forays into understanding the linkage between otolith growth and fish metabolic and somatic growth are conducted before the transitional growth spurt hypothesis is identified in the growth of the protandric snook.

#### 4.1 OTOLITH GROWTH AND FISH LENGTH

In order to use otolith growth as a proxy for somatic growth, both the periodicity of increment formation on the otolith and a linear relationship between otolith and somatic growth need to be verified. Previous studies have verified the timely formation of both translucent and opaque increments on snook otoliths (Andrade et al., 2013; Taylor et al., 2000). No previous studies, however, have explored the linkage between otolith and somatic growth in snook. This study found that otolith growth and total length decouple ([Figure 5](#)), indicating that otoliths should not be used to proxy length or back calculate length at age with methods sensitive to otolith decoupling.

#### 4.2 SEX-SPECIFIC OTOLITH GROWTH

Snook growth and sex change are not described by the larval growth hypothesis. Otolith core growth, representative of snook larval growth, was greater in males than females on average. Although approaching statistical significance (MANOVA,  $p$ -value = 0.07), this growth was statistically indistinguishable between those fish that remained male throughout life and those that changed to female. The slight difference in mean otolith core size likely resulted from the subset of snook that were analyzed in this study, but a larger subset of snook could push the result either towards or away from

significance. Therefore, it is likely that the larval growth hypothesis does not describe the sex change pattern in snook but further analysis should be conducted to determine if larval growth may affect the occurrence of sex change in snook.

During the period identified as juvenile by a fast growth rate (until age 1), those fish that remained male and those that changed to female were statistically indistinguishable. If snook were described by the juvenile growth hypothesis, the lack of statistical distinction in growth rate during the juvenile phase suggests that no snook used in this study should have changed sex (Adams & Williams, 2001; R. C. Francis & Barlow, 1993), which is not the case. Consequently, snook growth and sex change are not described by the juvenile growth hypothesis.

On average, female otoliths grew more than male otoliths during ages 4, 5, 6, 7, and 8. Individual female growth (unpublished data) did not show this multi-year period of high growth, showing instead growth spurts following the growth slowdown of ages 2 and 3. Although additional analysis is needed to determine which growth spurts coincide with sex change, otolith morphometrics depict a growth spurt like that described by the transitional growth spurt hypothesis ([Figure 7](#)).

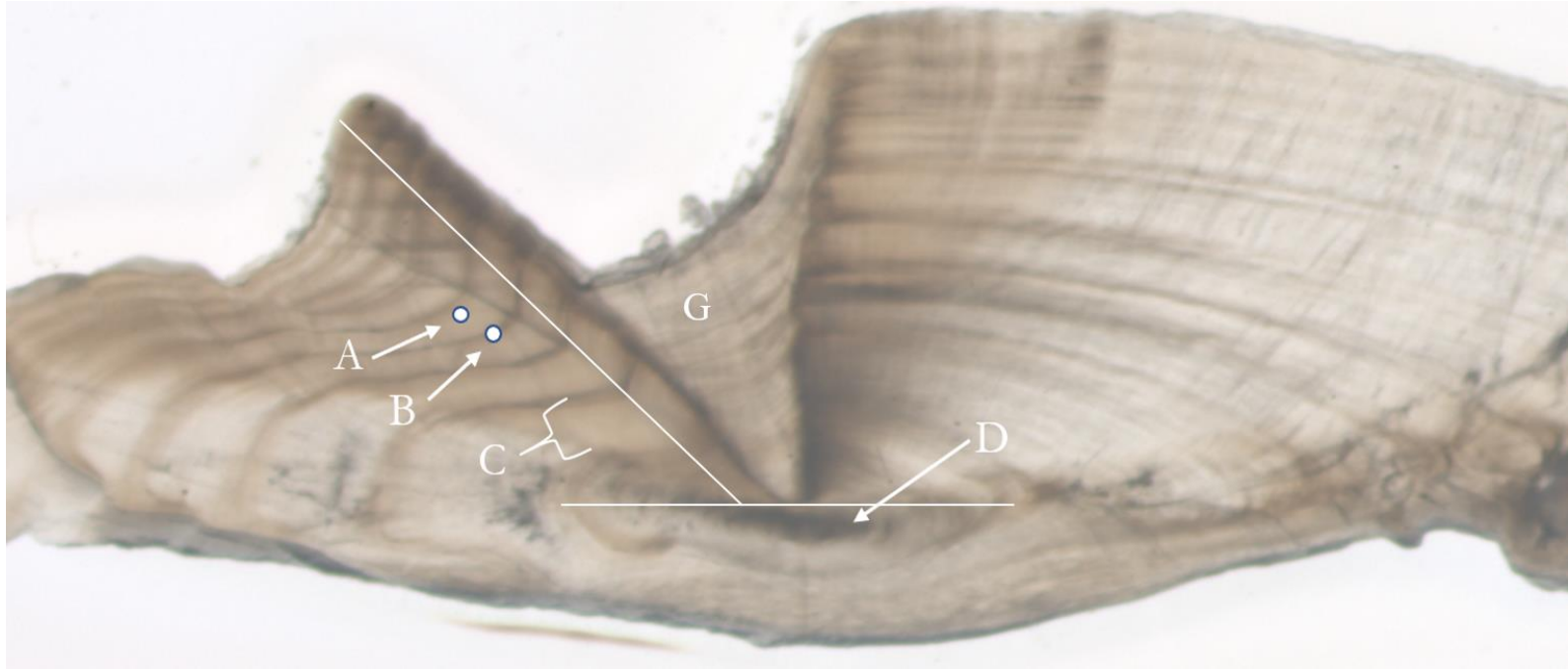
Differences observed between male and female otolith growth in this study are likely related to sex change. This study found that growth in the 3<sup>rd</sup> year through the 7<sup>th</sup> year (ages 4 through 8) was significantly different between males and females, supporting the timing of sex change (between ages 3 and 8) reported by (Young et al., 2020). Furthermore, these findings suggest that sex change may be occurring later in Atlantic populations than initially thought, between ages 1 and 7 (Taylor et al., 2000). A thorough exploration of why snook sex change may be starting at later ages, however, is



outside the scope of this study and requires greater exploration of the life history changes that snook may be undergoing.

This study highlights the importance of otolith morphometry, especially in hermaphroditic species. Snook total lengths overlap extensively between same age male and female fish (Muller et al., 2015) while other external characteristics, such as color and pattern, are indistinguishable between the sexes (Rivas, 1986). Otolith increment measurements, however, demonstrated a distinction between the growth patterns of each sex, providing a way to not only distinguish between the sexes but also identify the timing of sex change (Walker & McCormick, 2004). Although additional research is needed to understand if this growth spurt pre- or proceeds sex change itself in snook, the identification of the transitional growth spurt hypothesis in the snook is the first time that a protogynous growth hypothesis has been identified in a protandric species and may indicate that protandric species undergo profound physiological changes during sex change not evidenced by standard fishery data such as length, age, and weight.

APPENDIX A: FIGURES



*Figure 1 Image of a transverse otolith section. Point A marks a translucent increment. Point B marks an opaque increment. Point C spans a distinct translucent and opaque increment intersection. The otolith core is marked D. E labels the proximally parallel line, and F labels the parallel line. G is the sulcus.*

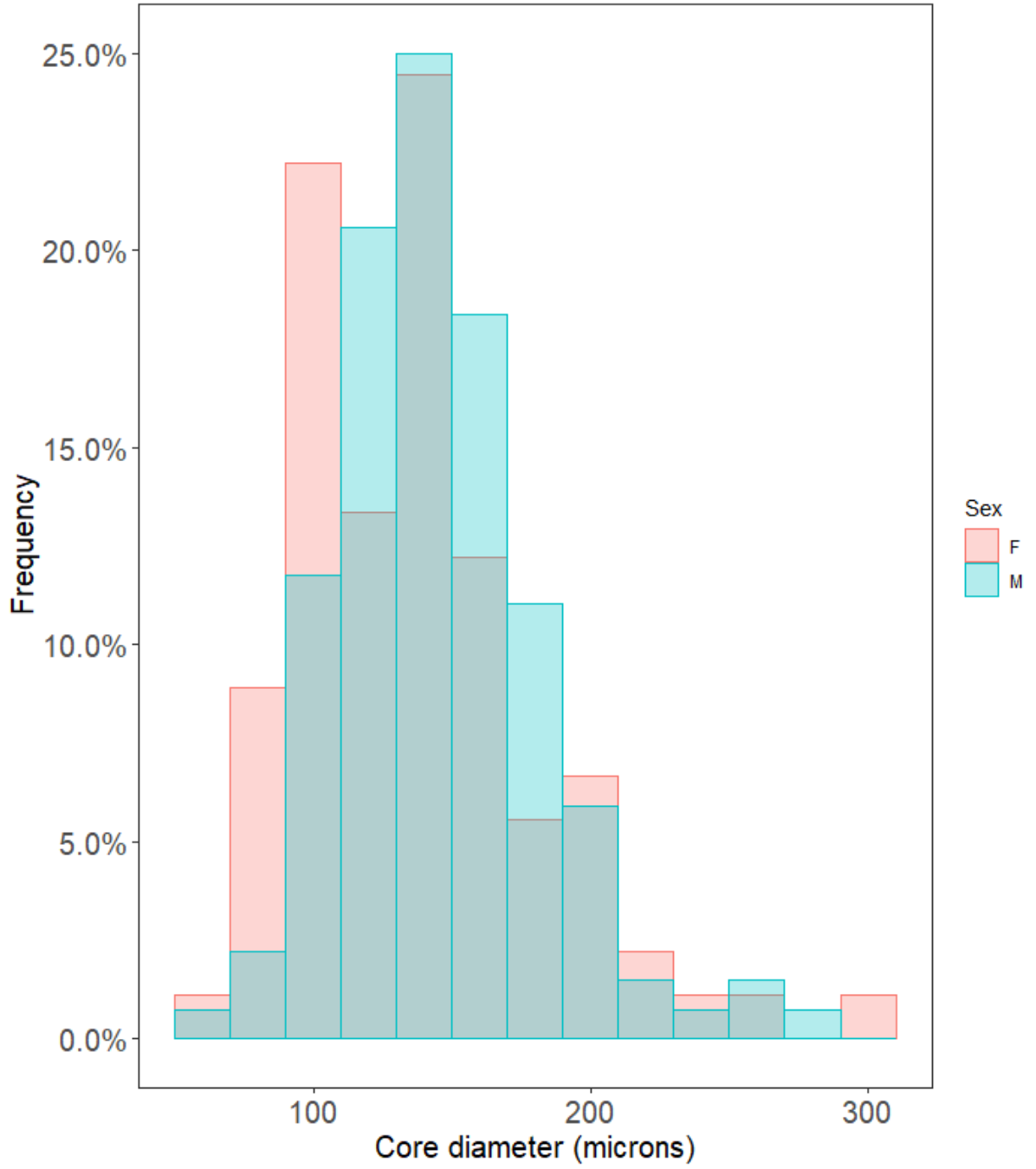


Figure 2 Histogram of male and female core diameter distribution.

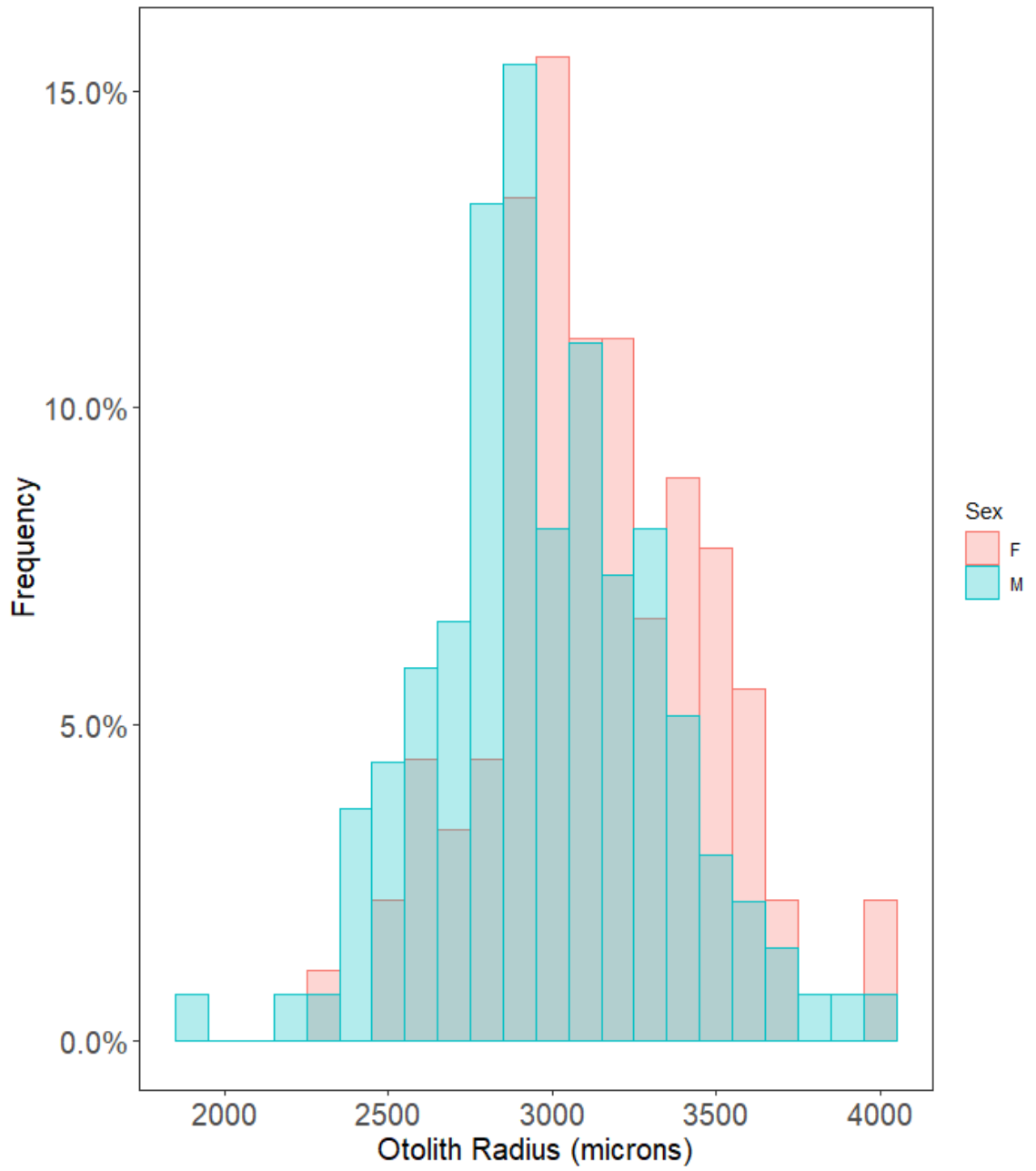


Figure 3 Histogram of the distribution of male and female otolith radii.

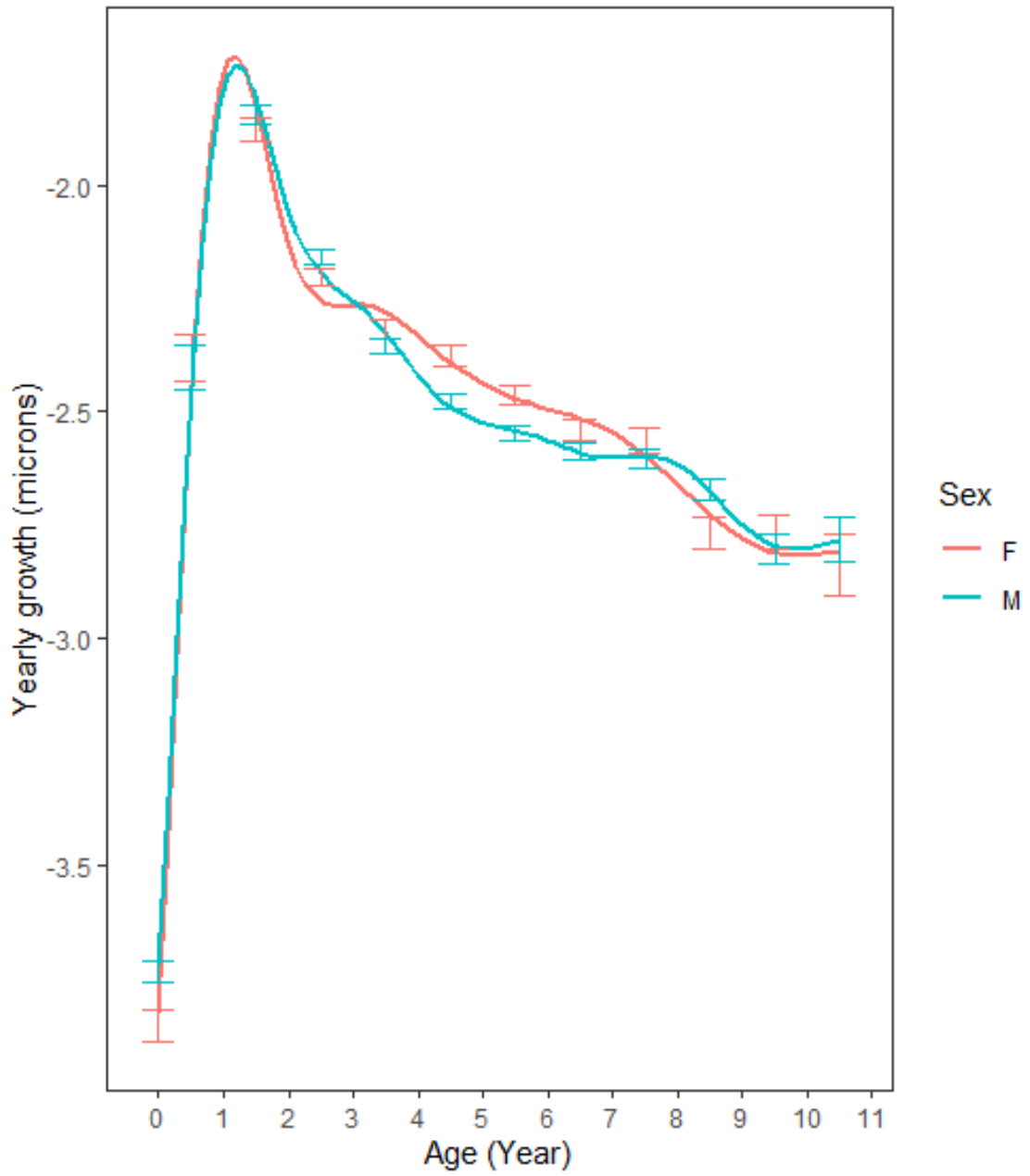
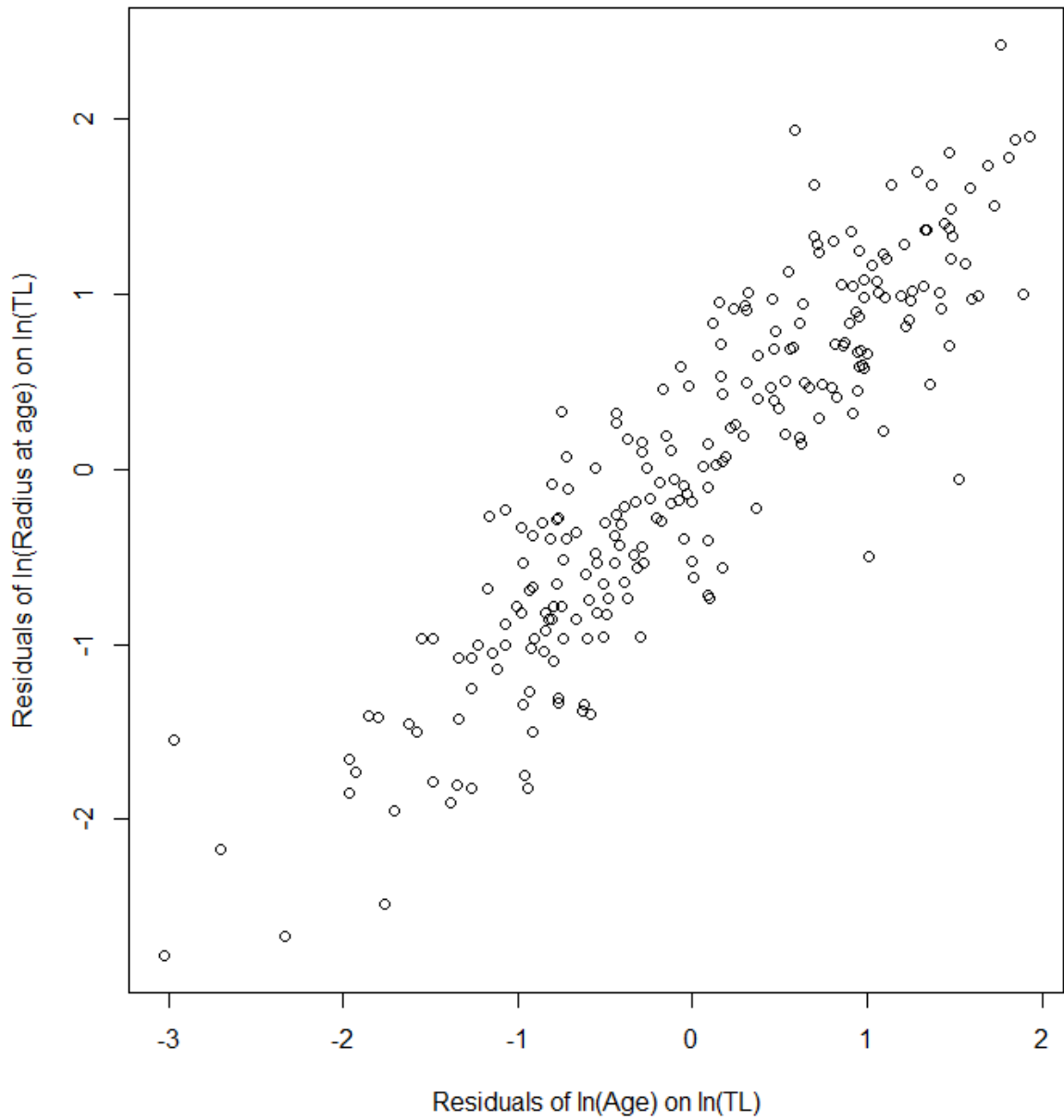


Figure 4 Average yearly otolith growth (natural log transformed) of male and female snook with standard error bars.



*Figure 5 Graph showing the relationship between the standardized residuals from the  $\ln(\text{Radius at age})$  on  $\ln(\text{total length})$  and the  $\ln(\text{Age})$  on  $\ln(\text{total length})$  regressions.*



*Figure 6 Close up of a transverse otolith section displaying a check mark indicated by a white arrow.*

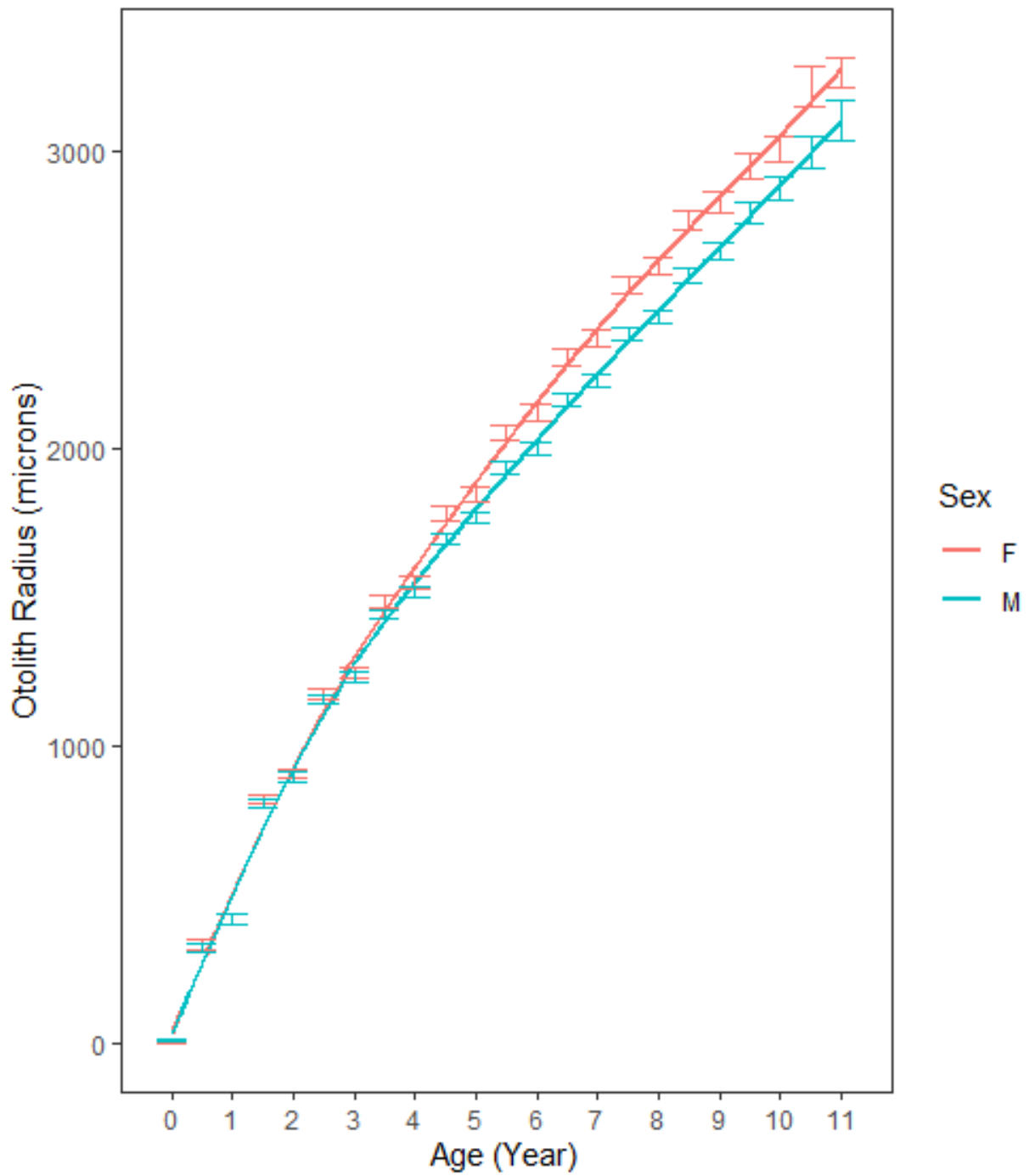


Figure 7 Cumulative average radii of male and female otoliths with standard error bars.



APPENDIX B: TABLES

*Table 1 Numbers of male and female fish of each range in this study from before and after ranking.*

	All Fish			Quality 1 and 2		
	n			n		
Age (Years)	M	F	Total	M	F	Total
8	67	59	126	46	29	75
9	54	47	101	36	27	63
10	52	47	99	34	23	57
11	33	33	66	20	11	31
12	1	5	6			0
13	0	1	1			0
Total			399			226

*Table 2 Total lengths back-calculated from otolith growth using the Fraser-Lee model. Average total length is reported under the range of total lengths for that age group.*

Age	Male Total Length (mm)	Female Total Length (mm)
	37.11-63.30	38.20-77.96
0	47.61	48.52
	74.62-325.82	83.44-320.13
1	149.70	162.58
	148.8-421.7	160.1-417.2
2	269.1	297.2
	247.6-501.2	271.0-498.0
3	352.3	391.1
	305.6-583.5	345.1-598.6
4	422.3	475.9
	355.2-660.1	401.1-698.5
5	485.0	558.9
	398.5-710.1	447.9-804.8
6	543.1	636.6
	431.8-779.8	494.3-898.1
7	600.0	707.2
	464.6-822.7	551.2-989.4
8	654.5	775.3

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