

ABSTRACT

BICHY, JOHN BROOKE. A Life History Assessment on the Reproduction and Growth of Striped Mullet, *Mugil cephalus*, in North Carolina. (Under the direction of Dr. Steve W. Ross and Dr. John Miller.)

The striped mullet, *Mugil cephalus*, has supported a commercial fishery in North Carolina since the 1800s and today ranks in the top ten of commercially valuable fin-fisheries in the state worth over a million dollars annually. The species is a direct link between lower and higher trophic levels and thus serves an important role in the food web. Despite striped mullet's biological and economic importance, basic life history data from North Carolina are limited and the stock status is unknown.

Objectives of this study were to describe striped mullet growth, reproductive seasonality, size and age at maturity, and fecundity. Monthly samples of striped mullet were collected using both fishery independent and dependent sampling strategies throughout North Carolina. Sagittae otoliths were removed and sectioned for age and growth analyses. Gonads were fixed and histologically prepared for maturity indices and fecundity estimation. Length was highly variable within age classes. Regional growth differences within North Carolina were found as fish collected from the southern sampling regions were smaller at age and lived longer than fish from the northern regions. Growth models suggest growth rates in North Carolina were greater than other areas in the species' range. Based on the presence of recently post-spawned fish and gonadal development, striped mullet spawn between late September and December. The collection of a hydrated female

less than 1 km from an inlet, coupled with the presence of post-ovulatory follicles from fish sampled within the estuary, provided evidence for near-shore spawning. Males matured at a smaller length (L_{50}) than females, 283 mm and 324 mm fork length, respectively. Fecundity correlated well with fork length ($r^2=0.88$) and body weight ($r^2=0.91$), and ranged from 1193 to 2535 eggs per gram of eviscerated body weight. This study provides the first life history assessment of striped mullet reproduction and growth from North Carolina and shows differences in growth, maturity, spawning location, reproductive seasonality, and fecundity compared to other areas in the species' range.

**A Life History Assessment on the Reproduction and Growth of Striped Mullet,
Mugil cephalus, in North Carolina**

By

John Brooke Bichy

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Zoology

Raleigh

17 March 2004

APPROVED BY:

Co-chair: Dr. Steve W. Ross

Co-chair: Dr. John Miller

Dr. Joseph E. Hightower

Dr. Kenneth H. Pollock

BIOGRAPHY

I was lucky enough to spend my summers fishing and exploring the marshes and waterways of Maryland's coastal bays. It was these summer experiences that helped guide me into the study of Biology as I entered Salisbury State University in 1990. In 1992, I transferred to the University of North Carolina Wilmington (UNCW) to study marine biology and graduated in 1995 with a bachelor's of science degree. After graduation, I lived in Wilmington, NC for several years as a research assistant for both the North Carolina Estuarine Research Reserve and UNCW. These were good times, learning analytical skills and spending time sampling on North Carolina's beautiful rivers, estuaries, and coastal oceans. My graduate career at NCSU provided many necessary lessons for the future. Overall, my life to this point has been full of wonderful experiences that were made possible because of my loving family and good friends.

Acknowledgements

I would like to thank my advisors and committee members for their contributions to my study and development as a fisheries biologist. Specifically, Dr. Steve Ross provided the idea to study striped mullet biology and was responsible for the initial funding. I need to thank Dr. Ross for the opportunities he has provided me as his research assistant in NCNERR lab and as a student at NCSU. I want to thank co-advisor, Dr. John Miller, for providing me with the much needed logistical and laboratory support and for the discussions in fish biology. I would like to thank committee members Dr. Joe Hightower and Dr. Ken Pollock for their analytical help and assistance in overall project development.

Jim Francesconi of the North Carolina Division of Marine Fisheries sectioned, polished, measured annuli, and made first reads on all otoliths I collected. In addition he provided aging and reproductive information from mullet collected as part of the MARFIN striped mullet sampling program. I am indebted to Jim for his solid hard work. Mark Hamrick of the NCDMF collected mullet samples and provided me with insight into the mullet fishery. Dr. Mary Moser provided samples from the UNCW Cape Fear River fish survey. Sandra Horton of the NCSU College of Veterinarian Medicine processed all mullet gonadal tissue. Dr. Craig Sullivan assisted with gonadal maturity determination. Many people assisted me in the field and laboratory and to them I am indebted. They included, Dr. Steve Roberts, Brian Burke, Chris Taylor, Wes Patrick, Mike Williams, Ann Marie Necaise, and Dan

Tenny. Special thanks go to Dr. Roberts who contributed many hours in the field and lab. I would like to thank Jen Sevin for her support throughout the “writing” phase. I am not sure I would be writing this without her.

Last I want to thank Steve Ethridge and Gene Balance. These commercial fishermen, especially Steve Etheridge, not only provided invaluable mullet samples, but also allowed me to join them on their fishing boats in pursuit of mullet and other species. The experiences and conversations I had with Steve and Gene on the water were insightful into understanding the mullet fishery, mullet behavior, and what life as a commercial fisherman is like and also good fun. I want to thank them for everything, as I will never forget the chilled mornings skimming across “the reef” and sloughs of Pamlico Sound or the sight of a mullet wake.

Table of Contents

List of Tables	vi
List of Figures	vii

Sections

I.	Introduction	1
	Objectives.....	4
II.	General Methods.....	5
	Study Area.....	5
III.	General Catch Results	8
IV.	Age and Growth	10
	Methods.....	10
	Results	12
	Discussion	15
V.	Reproduction.....	21
	Methods.....	21
	Results	24
	Discussion	32
VI.	Literature Cited.....	39
	Tables and figures.....	47

List of Tables

<u>Table</u>	<u>Description</u>	<u>Page Number</u>
Table 1	Striped mullet mean fork length (mm) \pm standard error (SE), and sample size (N) by gear type and area	47
Table 2	Striped mullet female and male mean fork length (mm) at age \pm standard error (SE), and sample size (N). Samples collected in October and November between 1996 and 1999. Means were compared by sex using paired t-test. Significant at $p=0.05$	48
Table 3	Description of the macroscopic gonadal maturity staging system used to code the reproductive activity of female and male striped mullet. Key used on fresh gonads soon after the time of capture.	49
Table 4	Criteria used to stage oocyte development as adopted from Wallace and Stenger (1959), Selman (1981), and Sullivan et al. (1997)	50
Table 5	Key used to stage striped mullet ovarian maturity from histologically prepared gonadal tissue. Staging system modified from Stenger (1959), Wallace and Selman (1981), and Sullivan et al. (1997) for the needs of this study. (POF's = post-ovulatory follicles, FOM = final oocyte maturation)	51
Table 6	Criteria used to stage male gonadal development as adopted from Sullivan et al. (1997) and Taylor et al. (1998)	52
Table 7	Ratio of female to male striped mullet by length class (25 mm). Mullet collected from all gears combined. Ratios tested using Chi-square. * significant at $p=0.01$	53
Table 8	Ratio of female to male striped mullet in North Carolina by gear type. Data are sorted in descending order by F:M ratio within each sampling strategy. Ratio's tested using chi-square analysis. * significant at $p=0.001$	54
Table 9	Striped mullet length (FL mm) of maturity in North Carolina. Table includes summary of raw data and model predictions for both females and males collected between October and December. Raw data summaries include, sample size (N), minimum length of maturity, and the length beyond which maturity is obligatory (100%). Logistic model predictions include, the length which 50% of population is mature (L_{50}), and the slope and intercept parameter estimates (\pm standard error). Parameter significance (*). $P=0.0001$	55

List of Figures

<u>Figure</u>	<u>Description</u>	<u>Page Number</u>
Figure 1	Study area, North Carolina coastal plain, USA. Sample sites (circles) show location of mullet collections between 1996 and 2000. Dashed lines distinguish the three sampling zones	56
Figure 2	Striped mullet length (fork length, mm) frequency histogram. Females (shaded) and males (white) are shown. Mullet collected in North Carolina across all sampling areas and methods.....	57
Figure 3	Length-weight relationship of female striped mullet collected from North Carolina	58
Figure 4	Length-weight relationship of male striped mullet collected from North Carolina	58
Figure 5	Striped mullet length (mm FL) frequency histograms for fish collected from the northern (top), mid (middle), and southern (bottom) sampling areas. Females (shaded) and males (white) are shown. All mullet were collected in North Carolina	59
Figure 6	Striped mullet length (fork length, mm) frequency histograms for mullet collected from fishery dependent (top) and independent gears (bottom). Females (shaded) and males (white) are shown. All mullet collected in North Carolina across all sampling areas....	60
Figure 7	Striped mullet length (mm FL) frequency histograms for fish collected from fishery dependent gears: strike nets (top), beach haul seines (middle), and gill nets (bottom). Females (shaded) and males (white) are shown. All mullet collected in North Carolina across all sampling areas	61
Figure 8	Striped mullet length (mm FL) frequency histograms for fish collected from fishery independent gears: survey strike nets (top), cast nets (middle), and electro-fishing (bottom). Females (shaded) and males (white) are shown. All mullet were collected in North Carolina across all sampling areas.....	62
Figure 9	Monthly mean marginal increment width (SE) by age class for striped mullet collected in North Carolina. Age groups 1 to 5 are shown	63
Figure 10	Female (top) and male (bottom) striped mullet length at age. Fishery dependent (FD, open red circles) and independent (FI, closed circles) samples are shown. Data are fit to von Bertalanffy (VB) growth model using FI data only (solid lines).	

	VB parameters are shown. Female and male growth curves were significantly different ($p < 0.05$).	64
Figure 11	Female striped mullet length at age by sampling region. Fishery dependent (FD, open red circles) and independent (FI, closed circles) samples are shown. Data are fit to von Bertalanffy (VB) growth model using FI data only (solid lines). VB parameters are shown. Growth curves from the south were significantly different from the north and mid regions ($p > 0.05$).	65
Figure 12	Male striped mullet length at age by sampling region. Fishery dependent (FD, open red circles) and independent (FI, closed circles) samples are shown. Data are fit to von Bertalanffy (VB) growth model using FI data only (solid lines). VB parameters are shown. Growth curves from the south were significantly different from the north and growth curves from the north were significantly different from the mid regions ($p > 0.05$).	66
Figure 13	Striped mullet pre-vitellogenic stage oocytes. Secondary (SG) and primary (PG) growth oocytes are shown. Cortical alveoli or granules (CA), oocyte nucleus (N), and nucleoli (NC) are shown .	67
Figure 14	Striped mullet early vitellogenic growth oocytes (EV) with some primary growth oocytes (PG). Cortical alveoli (CA), nucleoli (NC), lipid droplets (LD), and yolk globules (YG) are shown	68
Figure 15	Striped mullet late vitellogenic oocyte. Large lipid droplets (LD) and yolk globules (YG) are shown	69
Figure 16	A striped mullet oocyte undergoing final oocyte maturation (FOM). The lipid droplets and yolk globules are coalescing and the germinal vesicle (GV) or nucleus has migrated to the oocyte periphery. This oocyte is one of a few which has not yet ovulated (released from follicle cells)	70
Figure 17	A post-spawned female striped mullet ovarian cross section. Common post-spawned ovarian characteristics such as post-ovulatory follicles (POF) and the presence of a thick gonadal wall (OW) are depicted. Secondary (SG) and primary (PG) growth oocytes are shown.....	71
Figure 18	A cross section of a male striped mullet testis in late maturation. Lobules (LB) and collecting ducts (CD) are filled with spermatozoa.	72
Figure 19	A cross section of a spent male striped mullet testis. Collecting duct (CD) hold residual spermatozoa, but lobules (LB) are mostly empty.....	73
Figure 20	Percentage of female striped mullet ($n=1011$) mature by 20 mm length classes. Fish were collected between October and December. Data fit using logistic regression (solid line)	74

Figure 21	Percentage of male striped mullet (n=556) mature by 20 mm length bins. Fish were collected between October and December. Data fit using logistic regression (solid line)	75
Figure 22	Female striped mullet monthly mean (+/- standard error) gonadosomatic indices (GSI) for the 1997 (top), 1998 (middle) and 1999 (bottom). Means tested using 1-way ANOVA, Duncan's multiple range tests (P=0.05)	76
Figure 23	Relative frequency (%) of striped mullet ovarian maturity by month. Data represents pooled samples from 1997-1999. Sample sizes are given above each monthly frequency bar. POF's = post-ovulatory follicles	77
Figure 24	Striped mullet monthly mean oocyte diameters (+/- standard error). Means tested using 1-way ANOVA, and Duncan's multiple range tests (P=0.05). Data pooled for all years sampled 1997-2000	78
Figure 25	Striped mullet monthly mean oocyte diameters (+/- standard error) by size class (fork length, mm). Means tested within each month using 1-way ANOVA, and Duncan's multiple range tests (P=0.05). Data pooled for all years sampled 1997-2000.....	79
Figure 26	Male striped mullet monthly mean (+/- standard error) gonadosomatic indices (GSI) for the 1997 (top), 1998 (middle) and 1999 (bottom). Means tested using 1-way ANOVA, Duncan's multiple range tests (P=0.05)	80
Figure 27	Relative frequency (%) of striped mullet testicular maturity by month. Data represents pooled samples from 1997-1999. Sample sizes are given above each monthly frequency bar.....	81
Figure 28	Mean monthly water temperature (C) as measured between 1997 and 1999. Water temperatures were taken at the time of mullet collections throughout the North Carolina sampling area....	82
Figure 29	Female striped mullet fecundity to fork length (mm) relationship (n=120)	83
Figure 30	Female striped mullet fecundity to body weight (g) relationship (n=120)	84

I. Introduction

Striped mullet, *Mugil cephalus*, is a euryhaline species distributed worldwide in subtropical and tropical waters (Murdy et al. 1997). In the western Atlantic, striped mullet range from New England to the Gulf of Campeche in northeast Mexico (Gilbert 1993). Along the US east coast, striped mullet occur year-round from North Carolina southward. During summer months, striped mullet range extends to the Mid-Atlantic where juveniles and to a lesser extent adults are common in coastal bays (Hildebrand and Schroeder 1928, Murdy et al. 1997, Able and Fahay 1998). Striped mullet is one of two common species in the family Mugilidae that occur in North Carolina (Taylor 1951). As juveniles, both striped and white mullet, *Mugil curema*, co-exist in estuarine waters and are difficult to distinguish. Adult white mullet (>250mm FL) are rare north of Florida and thus are not associated with the commercial “roe” mullet fishery in North Carolina (Able and Fahay 1998). The mountain mullet, *Agonostomus monticola*, is a rare species in North Carolina known from one collection (Rhode 1976).

Striped mullet serve a vital role in the aquatic food web as a link from the lower to the higher trophic levels. Striped mullet are primary consumers that feed on macroplant matter, and epiphytic and benthic micro-algae such as, diatoms, dinoflagellates, and cyanobacteria (Odum 1970). In turn, striped mullet are preyed upon by numerous piscivorous fishes (Gunter 1945), marine mammals, (Miller 1992), and birds (Collins 1985).

Worldwide, striped mullet are an economically important species. In the Mediterranean and Southeast Asia, striped mullet are cultured and commercially harvested for food (Shireman 1975, Nash and Shehadeh 1980, Liao 1981). In Taiwan, and other Asian countries, mullet roe is considered a delicacy (Chang et al. 1995). In the United States, mullet are primarily harvested and shipped overseas; however, local consumption is common.

In North Carolina, the striped mullet fishery has existed for several centuries (Earll 1887, Taylor 1992). Initially, in the 1700s and early 1800s mullet served as a subsistence fishery for coastal inhabitants. By the mid 1800s, mullet flesh and roe became a tradable commodity with inland towns in exchange for corn and other goods, and thus the commercial mullet fishery was born. Beaufort became the center for mullet fishing, processing, and export, and by the late 1800's exports were higher in North Carolina than the total exports from all other areas on the Atlantic coast (Earll 1887). Mullet were salted, barreled, and shipped to Raleigh, Goldsboro, Norfolk, and places on the Pamlico and Albemarle Sounds (Earll 1887, Taylor 1992). Today, North Carolina's striped mullet fishery is one of the top ten commercially valuable fin-fisheries in the state, worth over 1 million dollars annually. Since 1995, North Carolina has harvested more mullet (6,995.5 MT) from the Atlantic than any other state. Including Gulf of Mexico landings, only Florida harvests more mullet annually than North Carolina (despite the Florida net ban in 1994). The recent economic importance of mullet is a result of the Asian roe market that developed in North Carolina around 1988. This roe fishery occurs along the coastal areas of

North Carolina during the fall when mullet migrate from rivers and estuaries to the coast to spawn. Roe buyers sample the commercial catch at local fish markets and assess roe value based on the ovarian to body weight ratio. Mullet are typically frozen whole and shipped overseas. During the winter, spring, and summer (non-roe season) mullet are harvested in lower numbers for food and bait.

Published research on striped mullet life history along the east coast is limited. Reproductive biology was described from Georgia (Pafford 1983) and Florida (Greeley et al. 1987). In addition, reproductive seasonality was indirectly described from larval collections in the South Atlantic Bight (Anderson 1958, Collins 1985). No study on the reproductive seasonality or maturity is known from North Carolina. Growth was described from Georgia waters using whole otolith aging techniques (Pafford 1983). In North Carolina a single study on mullet growth exists; however, the study was conducted in the 1920's, used scale aging techniques, and sampled primarily juveniles (Jacot 1920). The lack of life history data in North Carolina, specifically information on striped mullet movements, growth and maturity has hindered the completion of a stock assessment and management plan desired by the North Carolina Division of Marine Fisheries (NCDMF).

Striped mullet life history and population dynamics are better documented from the Gulf of Mexico compared to the South Atlantic Bight. Age and growth in the Gulf of Mexico are known from scale (Broadhead 1958, Cech and Wohlschlag 1975) and otolith (Thompson et al. 1990, Mahmoudi 1991) techniques. Reproductive seasonality is described from Florida (Mahmoudi 1991), Alabama (Dindo and

MacGregor 1981), Louisiana (Render et al. 1995) and Texas (Gunter 1945 and Moore 1974). In December of 1995 the Gulf States Marine Fisheries Commission (GSMFC) developed a management plan for striped mullet and declared the Gulf of Mexico stock viable. However, minimum size restrictions and gear restrictions are utilized on a state-by-state basis (Leard et al. 1995).

Objectives

In 1997 and 1999, I was awarded funding from the North Carolina Sea Grant Fishery Resource Grant program to study the reproductive biology of striped mullet in North Carolina. Results from my research are to be integrated into a fishery management plan (FMP) developed by the NCDMF. This thesis provides the results of my work. It is my goal to provide fundamental life history information on striped mullet in order to provide the tools to help conserve striped mullet populations for proper harvest. I will describe striped mullet (1) age and growth, (2) size and age at maturity, (3) reproductive seasonality, and (4) fecundity. I expect to find life history differences between my results compared to other areas of the east coast and Gulf of Mexico, as is commonly found with other species, such as, weakfish (Shepherd and Grimes 1983), Atlantic croaker (Barbieri et al. 1994), tautog (Hostetter and Monroe 1993), and American shad (MacKenzie et al. 1985).

II. General Methods

Study Area

Mullet were sampled throughout North Carolina from riverine, estuarine, and near-shore ocean waters. Three sampling zones, North, Mid, and South, were designated (Figure 1). The northern sampling zone (zone 1) included areas of Albemarle and Pamlico sounds, the Pamlico River, and the Outer Banks. The mid sampling zone (zone 2) included the Neuse River, Bogue Sound, Core Sound, White Oak River, and New River systems. Masonboro Sound and the Cape Fear River yielded the majority of mullet collected from the southern sampling zone (zone 3).

Year-round collections of mullet were made using both fishery independent and dependent gears. Commercial fishermen primarily use gill nets and beach haul seines, with and without the use of stop nets, to catch mullet. Gill nets were primarily used as “strike nets” to actively encircle mullet schools. Mesh sizes varied with season, but 4” and 3-7/8” (10.2 and 9.8 cm) stretch mesh net dominated gears during the fall “roe” mullet season in the northern sampling area of Pamlico Sound. Fishermen typically use flat bottom skiffs of 17 to 22 feet (5.2-6.7 m) powered by jet drive engines, which enable shallow water sampling where mullet are most easily captured. When a school of mullet is observed, a gill net is quickly set in a circle around the aggregation. Often these nets are over 400 yards (365 m) in length. To increase catch efficiency the net is pursed or collapsed to force fish into the webbing. Francesconi (1994) described the beach seine fishery. In general, this fishery is open for short durations in the fall on portions of Bogue Banks. A stop net

is set off the beach to stop and corral mullet as they migrate out of the inlets and down the beach. The first leg of the net is set perpendicular to the beach followed by the second leg that turns and runs parallel to the beach. When mullet are observed within the stop net, a boat is launched from shore that deploys a seine around the mullet school. Tractors are used to haul the seine back onto the beach. Strike nets and the beach seines can result in catches as high as 1000 and 5000 pounds, respectively, per haul.

Fishery-independent samples were collected using primarily strike nets, cast nets, electrofishing, and set gill nets. Strike nets were either set by boat (similar to commercial strike nets) or set from shore as a seine. Cast nets were used in tidal creeks and around large mullet aggregations found often around boat basins, jetties, and docks. Mullet were also provided from a monthly electrofishing survey on the Cape Fear River. In addition, the North Carolina Wildlife Resource Commission (NCWRC) provided samples from both the Neuse and Cape Fear Rivers using electrofishing methods. The NCDMF provided mullet samples from a set gill net survey (passive approach), fish house sampling, and strike nets as part of the MARFIN program.

Regardless of the sampling strategy, mullet were taken from each catch, iced, and processed within 24-48 hours. Fork length (mm) and body weight (g) were measured for each fish. Because mullet samples were obtained from various sources the types of data collected from each fish was not always consistent. Typically, wet gonad weight (g) was taken. For fish that I collected, standard length,

total length, and body weight with and without viscera were taken. In addition, I recorded location, time, gear type, and water temperature. For samples from the NCDMF, gear type and location were not always known.

The number of mullet removed from each catch depended on catch size. If relatively few mullet were collected (<50) all mullet were typically processed. If catches were large a sub-sample was taken. Sub-sampling techniques varied. In many cases random sub-sampling was attempted by quickly selecting mullet from the catch regardless of size; although large fish (>450mm FL) if observed were always removed for processing. In other cases, sub-sampling was stratified by length classes where up to 10 individuals from 50 mm length groupings were removed.

III. General Catch Results

From July 1996 to April 2000, a total of 4,303 mullet was collected, which included 2,709 females (63%), 1,297 males (30%), and 297 undetermined (7%). Mullet ranged from 77 to 660 mm FL (83 to 697 mm TL) for an average of $336 \text{ mm} \pm 1.2$ (SE) (Figure 2). Females were larger than males on average and ranged from 172 to 660 mm (369 ± 1.3) and 109 to 453 mm (298 ± 1.4), respectively. Body weights ranged from 21.5 to 3550.0 g (644 ± 6.4) and females were on average heavier than males, $801 \text{ g} \pm 8.2$ and $422 \text{ g} \pm 5.6$, respectively. Mullet body weights (g) and fork lengths (mm) were fit to the power function, $W = aL^b$. The relationship for females (Figure 3) and males (Figure 4) was strong.

$$\text{Females: } W = 0.000017 (L)^{2.98}, \quad n = 2238, \quad (r^2 = 0.97)$$

$$\text{Males: } W = 0.000014 (L)^{3.01}, \quad n = 1144, \quad (r^2 = 0.97)$$

Striped mullet length distributions varied by gear and area. Lengths were generally greater in the northern sampling zone (mean = 368 mm) compared to mid (319 mm) and southern (300 mm) zones and for mullet collected from commercial strike nets compared to other gears (Table 1). Large fish over 400 mm were common from the northern sampling area ($n=510$) and less common from the mid sampling area ($n=157$) and rare from the southern sample area ($n=28$) (Figure 5). Mean length of fishery independent samples were similar between sampling regions. In comparison, fish collected from fishery dependent gears had higher mean lengths in the northern area compared to the mid and southern areas. Striped mullet length

was on average larger for fishery dependent samples compared to fishery independent samples (Figure 6). Commercial strike nets, the most utilized gear, generally collected mullet > 300 mm FL. However, the commercial beach seine provided samples from a wide size range (Figure 7). Fishery independent samples had similar length distributions across gear types (Figure 8). Of the three dominant fishery independent gears, cast nets sampled the greatest size range, based on a larger variance around the mean. Overall, the largest size range of mullet was observed from commercial beach seine samples.

IV. Age and Growth

Methods

Mullet were aged using sectioned sagittal otoliths. Both sagittae were removed, cleaned in 2-3% bleach, rinsed in water and/or 70% alcohol, and stored dry. To prepare otoliths for reading, a dorso-ventral half-section was made on left otoliths through the core perpendicular to the anterior-posterior plane with a Hillquist sectioning machine (Cowan et al. 1995). The posterior half-section was air dried, mounted to a frosted microscope slide with UV curing glue, and cured for 20 minutes. The mounted otoliths were ground on a wet grinding lap to approximately 1 mm thick and then thickness gauge ground to 0.45 mm. The otolith thin-section was sanded by hand with 600-grit wet-dry sandpaper. Using two progressively smaller grits of aluminum oxide and deagglomerated alumina on felt, each otolith was polished to remove all scratches and improve clarity.

Fish age was determined by the number of annuli or opaque rings found on each left otolith. If the left otolith was not available the right otolith was used. Each fish was aged using a reflected light-dissecting microscope viewed from a high-resolution monitor. A second reader using a dissection microscope without the aid of a high-resolution monitor aged approximately ½ of the otoliths independently. When readers did not agree, both readers made second counts independently. After second counts, otolith reader agreement was 96%. A quality code from 1 to 4 was assigned to each otolith (1= good, 2=average, 3=poor, 4= no read possible) based on otolith clarity. For all analyses, only otoliths with a good (1) to average (2)

quality code were used. For fish captured between January 1 (assumed birth-date) and the date of annuli formation, as determined by marginal increment analysis, the age was increased by one.

Monthly mean marginal increment analysis was used to validate otolith annuli formation for as many age classes as possible (Bagenal and Tesch 1978). A marginal increment is the distance from the outer edge of the otolith to the closest opaque ring. The mean marginal increment width was calculated for each month and age class. Measurements were taken along a straight line from the focus to each annulus and out to the edge using a digitizer. The system was calibrated with an ocular micrometer before each reading session. These measurements were taken on each otolith that had a clear image of the annuli where they meet the ventral edge of the sulcus acusticus.

Striped mullet growth was described using mean length at age at the end of the growing season (October and November fish only) and the von Bertalanffy growth model (VB). The VB model was developed from the observed length at age data for both males and females by region and sampling strategy. The VB model is expressed as (Ricker 1975),

$$L_T = L_{\infty} * (1 - \exp(-k * (t - t_0))),$$

L_{∞} is the asymptotic average maximum size, k is the rate at which L_{∞} is achieved, t_0 is the hypothetical age which the species has zero length, and L_T is the length at age t . Parameters were estimated using a non-linear least squares approach. Model fit

was assessed using the linear relationship (r^2) between predicted versus observed fork length at age. To provide additional data on size at age 0, post-larval mullet collected during the winter recruitment period were incorporated into all VB models. These post-larvae (n=47) were considered age-0 based on their size (20-43 mm TL) and time of collection (January – April). Post-larvae were collected using a 500 μ ichthyoplankton net towed at the surface, small mesh seines near Masonboro Island, and electro-fishing gear on the Cape Fear River.

The analysis of the residual sum of squares (ARSS) was used to test if growth curves differed by sex and sampling region (Chen et al. 1992). The ARSS analysis compares data from two or more curves, but does not compare parameters separately. Because fishery dependent samples were rare from the southern sampling region and these gears generally select for mullet >300 mm FL (see section III), VB models were compared using fishery independent samples only. In addition, growth curves were only compared across representative age classes (Hadden 2001).

Results

Marginal increment analysis was used to validate the time of annuli formation for age groups 1 through 5 (n=2,992). For older age classes (>5 yrs), marginal increment analysis was not possible due to a lack of monthly otolith samples. The minimum mean marginal increment width occurred between June and July for each age class (Figure 9). Following June/July, mean marginal increment width within

each age class increased throughout the year suggesting the formation of one opaque region a year. For subsequent analyses, I assumed all mullet formed one opaque region each year, despite the inability to validate annuli for older age classes (>5 yrs).

Sexual dimorphic growth was observed. By the end of each growing season, mean length at age was larger for females than males (Table 2). Female and male VB growth curves differed ($p < 0.001$). Growth models predicted a size at age 2 of 312 mm FL and 287 mm FL for females and males respectively. Females had a higher L_{∞} (354 mm FL) than males (296 mm FL), but a lower growth rate coefficient (k) of 1.07 and 1.74, respectively (Figure 10). Observed maximum age was older for females (age=12) than males (age=9).

Female growth curves were different between regions across representative age classes 0 to 5 ($p < 0.05$). Females over age 5 were not collected from the northern region using fishery independent gears. By modeling each region separately, model fit was improved from an r^2 of 0.69 for all regions combined to an r^2 of 0.84, 0.92, and 0.93 for south, mid, and north regions, respectively (Figure 11). The southern growth curve was significantly different ($p < 0.0001$) from the mid and northern sampling curves; however, no difference between the north and mid growth curves was found ($p = 0.06$). Females were larger at age in the north (age 1 = 223 mm FL, age 5 = 428 mm FL) compared to the south (age 1 = 190 mm FL, age 5 = 337 mm FL). The oldest females were collected in the south which had the smallest L_{∞} .

estimate. Increases in L_{∞} estimates and decreases k estimates (as expected with increase in L_{∞}) were found from south to north.

Male growth curves were different between regions across representative age classes 0 to 3 ($p < 0.05$). Mullet over age 3 were not collected from the northern region using fishery independent gears. Similar to females, model fit (r^2) was improved when reduced from a full model (all regions combined) to regional models (Figure 12). Unlike females, growth curves between the southern and mid regions were not significantly different ($p > 0.05$) and curves between mid and north regions were significantly different ($p = 0.02$). However, like females, growth curves between the south and north were different ($p = 0.01$). Regardless of the growth curve differences, males reached older ages in the south but appeared to grow slower after age 1. Based on VB growth model predictions of size at age, males were larger at age in the north (age 1 = 251 mm FL, age 3 = 322 FL) compared to the south region (age 1 = 213 mm FL, age 3 = 281 FL) across all ages; however, age 1 estimates were slightly lower from the middle region (203 mm FL) compared to the southern region. By age 3, estimates were higher from the middle region (312) compared to the southern region. Trends in female VB model parameter estimates were not consistent with males. Unlike females, the southern region had a lower estimate of k (1.36) than the northern region (1.49).

Discussion

Observed length at age data and growth model estimates indicate high variability in length at age and a rapid growth rate in the first years of life in North Carolina. Previous studies on striped mullet growth found high variability in length for a given age but the degree of length variability at age was not well described for each age class (Thompson et al. 1989, McDonough and Wenner 2003). Great variability in length at age is common in other estuarine dependent species, such as bluefish (Chiarella and Conover 1990) and Atlantic croaker (Barbieri et al. 1993). I estimated greater growth rates than previously recorded for mullet populations in other areas of the USA. Female von Bertalanffy growth model estimates indicate mullet are much larger at ages 1 and 2 in North Carolina (232 mm FL and 312 mm FL) compared to areas along the Gulf coast of Florida (156 mm FL and 251 mm FL: Mahmoudi 1990) and Louisiana (133 mm FL and 223 mm FL: Thompson 1992). The rapid growth observed in my study is not beyond possibility. For instance, other estuarine dependent species on the Atlantic coast have greater estimates of length at ages 1 and 2 than what I found for striped mullet, such as bluefish (265 mm FL and 435 mm FL: Chiarella and Conover 1990) and weakfish (225 mm FL and 299 mm FL: Shepherd and Grimes 1983).

The apparent greater growth observed in my study compared to previous studies could possibly be explained through environmental and evolutionary mechanisms. Environmental controls include temperature, food availability, and food quality, while evolutionary controls are formed through selection, such as size-

selective mortality (Conover 1990, Schultz et al. 2002). North Carolina contains the second largest estuary and largest lagoonal ecosystem in the USA, the Albemarle-Pamlico Sound. In areas where mullet are residents (North Carolina and south) no estuary comes close to the size or unique structure of the Albemarle-Pamlico Sound. The larger and more open estuarine system of Albemarle-Pamlico Sound compared to other striped mullet environments could provide a surplus in food supply that results in increased consumption rates and thus higher growth. These regions outside of the Albemarle-Pamlico Sounds could have lower growth potential due to density-dependent mechanisms from an inadequate food supply. In addition, striped mullet in North Carolina are at the northern extent of their home range which for other species is suggested to increase growth potential by the existence of a new and superior food niche (Shepherd and Grimes 1983). Therefore, given the differences in environments between North Carolina and regions to the south, I hypothesize that growth potential is greater in North Carolina compared to other regions due to a surplus of energy resources and a superior food supply. No data are yet available on mullet diet in North Carolina compared to these regions to the south, so a study examining mullet diet is needed to address this hypothesis.

A latitudinal gradient in growth can be observed due to a selection for rapid growth with an increase in latitude (Conover and Present 1990). Individuals of a species that reach a larger size at the end of year 1, before the winter limited growth period, exhibit higher survival or are selected over smaller individuals as a result of lower over-winter mortality. This decrease in over-winter mortality with an increase

in body size is thought to be caused by a greater capacity to store energy and thus survive colder temperatures and starvation that most often occur at the northern extent of a species range (Conover 1992, Henderson et al. 1988). Striped mullet populations in North Carolina are likely subjected to colder winter water temperatures than populations to the south. As a result, size-selective mortality could be selecting for larger sized mullet in North Carolina compared to regions to the south, which would increase length at age and growth rate estimates.

Growth curve comparisons indicate a difference in longevity and growth between female and male mullet collected in the southern and northern sampling areas of North Carolina. For both females and males, longevity is greater in the south (age 12 females, age 9 males) compared to the north (age 8 females, age 3 north); however length at age is much smaller in the south compared to the north. Regional growth differences are further supported by improved VB model fit estimates (r^2) when modeled by sampling region versus all regions combined, suggesting that these areas have unique growth patterns. The greater longevity observed in the south could be potentially a result of a lower rate of fishing mortality. Lower fishing mortality is possible considering fewer mullet reach the length targeted by the commercial fishery (typically >300mm FL) in the south compared to the north (see general results). Differences in diet and habitat between the southern and northern sampling areas could also account for the difference in growth patterns. Unlike the mid and northern sampling zones which are associated with the large and unique estuaries of Pamlico and/or Albemarle Sounds, the southern sampling zone

is restricted to small tidal creeks and areas in the Cape Fear River, similar to habitats found in other regions of the species distribution along the southeast USA and Gulf of Mexico, which I have hypothesized might limit growth potential (see above). From my observations at the time of gonad and otolith removal, I have noticed a difference in gut content odor and appearance between the respective sampling regions. In the south, fish are typically filled with thick black sediments that exhibit a strong odor. In the north, I typically did not see black sediments in the digestive track; instead the gut contents are relatively odorless and filled with an off-white almost creamy substance. The apparent regional difference in gut contents indicates distinct food supplies between the Albemarle-Pamlico Sound area to that of the Cape Fear River and tidal flats of Masonboro Island. I therefore hypothesize that prey type and quality are different between sampling regions which could result in a greater capacity for growth in the northern estuaries of North Carolina compared to the southern areas.

An alternative explanation for regional growth differences could be related to gear selectivity and sampling behavior. Fishery independent samples from the south were primarily collected from small schools of mullet in small tidal creeks and tributaries of the Cape Fear River and Masonboro Sound. Fishery independent samples from the North were often taken from large mullet aggregations prior to or during the spawning season. These different sampling behaviors could be selecting for different segments of the population that may be also growing differently and may have biased my observed age length distributions.

I was unable to provide a quantitative estimate of the level of accuracy with the aging method. I was able to validate opaque regions on the otoliths as occurring once a year for the five youngest ages using marginal increment analysis, but this did not provide an estimate of aging accuracy. The 96% reader agreement observed was good, but only implies the two readers agreed on the counts. In the future, the potential for aging error should be addressed in more detail. To achieve a higher level of confidence with striped mullet ages, an age validation study that measures age accuracy should be conducted using a mark-recapture study or other validation methods (Beamish and McFarlane 1983, Francis 1995).

The regional growth difference observed within North Carolina should not be affected by the concern for aging accuracy. Under or over-aging fish, if the errors were consistent across all fish, would have minimal affect on the shape of the growth curves and therefore the growth curve comparisons should not be affected. However, these regional growth differences should be confirmed using an appropriate sampling design. A proper sampling design should involve a standardized gear and sampling behavior over similar time periods. By using the same gear and sampling strategy, comparisons can be made by region without a concern for sampling regional bias.

My study represents the first attempt to age striped mullet in North Carolina using sectioned otoliths. The objectives of my study were to describe striped mullet growth from as many samples and size classes across North Carolina as possible and compare growth estimates to previous studies. Growth patterns observed

include high variability in growth within North Carolina, differences in growth within North Carolina, and greater length at age estimates than previously found in other areas of the USA. Several hypotheses are provided to explain the differences in growth both within and outside North Carolina and suggest more work is needed in order to validate otolith aging accuracy and minimize gear bias.

V. Reproduction

Methods

Fish reproductive status or condition was determined from both a macroscopic and microscopic gonad examination. Prior to gonad fixation, fish were classified as inactive, active, mature, or post-spawned based on macroscopic criteria (Table 3). Gonads were preserved in 12-15% formalin for two weeks, rinsed in water, and stored in 70% ethanol for later histological analysis. Striped mullet ovarian and testicular development is uniform throughout the ovaries and testis (Shehadeh et al. 1973, Greeley et al. 1987, Render et al. 1995); therefore, random samples of gonadal tissues could be removed for microscopic maturity analyses (Hunter et al. 1992). Monthly ovarian samples were provided to the North Carolina State College of Veterinarian Medicine for histological preparation. A sample was prepared by first inserting a cross section of gonadal tissue approximately 5mm thick into a tissue cassette. These sections were embedded in paraffin, sliced (5-6 microns), stained with hematoxylin, counter-stained with eosin, and mounted on glass slides. A compound microscope was used to view and stage each histologically-prepared gonadal section.

Oocyte maturity stage is described using 5 major developmental stages (Table 4) (Stenger 1959, Wallace and Selman 1981, and Sullivan et al. 1997). From each histologically-prepared gonadal section, the leading stage oocyte or most advanced stage out of 100 oocytes was recorded. Ovarian maturity was assigned to each gonad or fish based on the leading oocyte stage, as described in Table 5, and

other ovarian characteristics (Table 5). Male gonadal development was described using criteria from Sullivan et al. (1997) and Taylor et al. (1998). Testicular maturity was assigned to each gonadal section based on the most advanced developmental stage present (Table 6).

Largest oocyte diameters (LOD) were measured (0.01 mm) from both histologically prepared and non-preserved (fresh) ovaries. A dissecting microscope and lens micrometer were used to measure fresh oocytes. Fresh oocytes were carefully teased apart from the ovarian connective tissue and placed in a saline solution for measurement. Only the largest undamaged oocytes were measured. A compound microscope and lens micrometer were used to measure oocyte diameters from histologically prepared gonadal sections. Linear regression was used to predict fresh oocyte diameters from histological measured oocytes ($P < 0.001$) in order to standardize future analyses on development and reproductive seasonality to fresh oocyte diameters. A total of 20 leading staged oocytes (nucleus present) were measured from each ovarian section.

Both microscopic and macroscopic staged fish were used to describe the age and size of maturity. Microscopically, a female was mature if vitellogenic oocyte growth was present (stage 2) or if the female was post-spawned (stage 4 and 5) as indicated in Table 5. A male mullet was mature if spermatogenesis (early maturation) was occurring or if the fish was regressing (stage 2 to 5). Macroscopically all mullet staged 2, as described in Table 3, were considered mature. Fish considered active from a macroscopic stage were never staged

inactive based on a microscopic exam. However, some fish macroscopically staged as inactive were later found to be active based on a microscopic examination.

Size of maturity was modeled using linear logistic regression by the method of maximum likelihood. The binary response model is,

$$\text{logit}(p) = \alpha + x' b$$

where α is the intercept parameter and b is a vector of slope parameters. This value ($\text{logit}(p)$) is the estimated logit of the probability that a fish is mature. To calculate the predicted probability that a fish is mature the following equation was used:

$$p = e^{\text{logit}(p)} / (1 + e^{\text{logit}(p)})$$

The size of sexual maturity was determined for each sex from the model estimate of $p = 0.50$ (L_{50}) (Roa et al. 1998). Logistic regression was used to model age of maturity but these results were insignificant due to the poor length at age relationship. Therefore, the age estimated from the VB growth model at the size of maturity (L_{50}) was used to estimate the age of maturity (A_{50}).

Reproductive seasonality was determined by monthly mean gonadosomatic index (GSI) values, relative frequencies of microscopic maturity stages, and monthly mean oocyte diameters. Only mullet greater than or equal to the minimum size (FL) of maturity were used in these analyses to reduce bias from immature fish. The timing of gonadal development was compared to water temperature data measured at the time of many mullet collections.

The GSI provides an index of the gonad weight relative to body weight and was calculated as follows,

$$\text{GSI} = [\text{GW} / (\text{BW} - \text{GW})] * 100,$$

where GW = gonad weight (g) and BW = body weight (g). Monthly mean GSI's were calculated to examine monthly variation in gonadal size. To test if monthly GSI's differed within each spawning year (May to April) a one-way ANOVA (Duncan's Multiple Range test) was used. Reproductive seasonality from microscopic maturity stages was analyzed by calculating the monthly distribution (% of total examined) of each stage. To evaluate the potential effect of fish length on gonadal recrudescence, monthly mean oocyte diameter comparisons were made across four 50 mm length classes.

Sub-samples of preserved ovaries in the middle to late vitellogenic stage were used to determine fecundity. Whole ovaries were patted dry and weighed (to nearest 0.001g). Six gonadal tissue samples, three from each lobe, were removed and weighed (0.1 mg). An average count of oocytes per sample was calculated and multiplied by the total weight of the gonad. Fecundity to body length and body weight relationships were developed using regression models.

Results

All five stages of oocyte development were verified from a microscopic examination (N=757), including final oocyte maturation that had previously never

been documented from wild mullet. The presence of primary growth (PG) and secondary growth (SG) oocytes (Figure 13) indicated resting or pre-vitellogenic fish. SG oocytes rarely occurred as the leading stage and when present, accounted for minimal change in gonad weight and no change in macroscopic appearance. An inactive fish or pre-vitellogenic fish could refer to both a resting mature fish and an immature fish. A fish with vitellogenic oocytes (VG) was considered active regardless of the stage of vitellogenesis. During early stages of vitellogenesis, PG, SG, and VG oocytes were present (Figure 14). However, as these oocytes developed and prior to maturation or the release of gonadotropin II (GTH II) from the pituitary, all VG oocytes will have recruited into a single clutch of late VG oocytes (Figure 15) with similar oocyte diameters. At this time, only VG and PG oocytes persist in the ovary and only these late VG oocytes will mature for the annual spawn.

A single fish was collected undergoing final oocyte maturation (FOM). This fish was collected on 18 November 1997 by a commercial strike net in the ocean about 1 km north of Hatteras Inlet and 100-200 m from the beach. When the fish was landed, it was the only fish actively releasing “semi-clear” eggs. Under microscopic examination this fish had an ovary filled with ovulated oocytes and oocytes in FOM (Figure 16), indicating a complete hydration of nearly all oocytes at the time of spawn.

Post-spawned fish were distinguished microscopically by a relatively thick gonad wall and an abundance of interstitial cells with or without residual ovulated oocytes and/or post-ovulatory follicles (Figure 17). Well after the spawning season,

a post-spawned or regressed fish and a resting mature fish were difficult to distinguish because both could have dark purple ovaries, thick gonadal walls, and similar microscopic structures. It is unknown how long females will exhibit this post-spawn macroscopic appearance, but by May all fish had small, dark purple or red ovaries, indicating the complete return to the resting stage.

Fresh oocyte diameters were compared to oocyte diameters from histologically prepared ovarian samples taken from the same fish (n=19). Oocyte diameters taken from fresh gonadal material were on average larger than oocytes taken from histological samples. To convert histologically prepared oocyte diameters to fresh oocyte diameters the following equation was developed using linear regression,

$$\text{Fresh Diameter} = 1.0695 (\text{Histological Diameter}) + 0.0444, \quad (r^2 = 0.87)$$

Male gonadal development followed the general staging criteria exhibited by most teleosts; therefore, a separate maturity key, as needed with females was unnecessary. Inactive males exhibited small, white, ribbon-like gonads (2-3 mm in diameter) regardless of maturity or body size. All males that exhibited early, mid, and late maturation development (Figure 18) were considered active and easily distinguished with a macroscopic exam. Post-spawn males exhibited a more vascularized and flaccid gonad with an overall reduction in gonad size from a late maturation stage (Figure 19). Residual free flowing sperm was present in many post-spawned males.

Sex Differentiation and Sex Ratios

The smallest male and female distinguished from a microscopic exam was 203 mm FL and 221 mm FL, respectively. Only eight mullet < 250 mm FL could be sexed from a microscopic exam; therefore, sexual differentiation is thought to occur between 200 and 250 mm FL. Only fish \geq 200 mm FL were used to determine sex ratios by gear. No hermaphroditic mullet were collected, although one male did contain a limited number of primary oocytes.

The female to male ratio was never equal across length classes. As fish length increased the proportion of females to males increased (Table 7). Most mullet < 300 mm FL were male and mullet > 300 mm FL were usually female. Males were rare above 375 mm FL. By gear type, females dominated catches except for cast nets (Table 8). However, despite a higher male to female ratio for cast nets the sex ratio was not significantly different from 1:1. Overall, fishery dependent gears had a higher female to male ratio (2.9:1) than fishery independent gears (1.5:1). The commercial beach seine had a similar female to male ratio (1.6:1) as the overall fishery independent ratio.

Maturity

Length was a good predictor of maturity. For females, maturity was rare below 300 mm. The smallest mature female was 250 mm FL as confirmed from an elevated gonadosomatic index and macroscopic gonadal maturity exam. By 320

mm, 50% maturity was reached and females >367 mm were all mature (Figure 20). Males matured at a smaller size than females with 50% mature by 280 mm FL (Figure 21). The smallest mature male was 241 mm FL and by 333 mm FL males were all mature. Logistic regression models predicted maturity (L_{50}) for females and males at 324 mm and 283 mm FL, respectively. Intercept and slope parameter estimates (Table 9) were significantly different from 0 ($p < 0.0001$).

Age was not a good predictor of maturity. The old, apparently slow growing, fish from the southern sampling region were often immature up to age 7. Samples from the north, especially fishery dependent samples, typically contained mature fish over 300 mm FL that were collected from large mullet aggregations prior to or during the spawning season. Age of maturity was based on the length of maturity (L_{50}) and growth model estimates of age. Females at 324 mm FL (L_{50}) were estimated at 2.3 years and males at 283 mm FL (L_{50}) were estimated at 1.8 years. In general, striped mullet appear to reach maturity at approximately age 2.

Reproductive Seasonality

Gonadal recrudescence was initiated in September for all sampling years. Female monthly mean gonadosomatic indices peaked in November of 1997 (14.2 ± 1.05) and 1998 (16.8 ± 0.82) and in October (13.7 ± 0.57) of 1999 (Figure 22). The month following each peak had significantly lower GSI's ($p < 0.05$). The most drastic changes in GSI's were observed from September to October as vitellogenic

growth caused a great increase in gonad size. By January, mean GSI's had returned to pre-spawning levels. Overall, GSI patterns were similar between years.

The spawning duration can be best described by oocyte development. Vitellogenic oocyte growth first occurred in early September in direct relation to an increase in GSI's. By late September, most females were in the latter stages of vitellogenesis (Figure 23). Evidence of spawning, presence of post-spawned fish, was observed as early as the end of September (1999). Such post-spawned fish were collected throughout October and November. Most of the post-spawned fish were collected within the estuary and were commonly sampled together with ripe or maturing fish. To catch only post-spawned fish in a sample was rare and usually restricted to the end of the spawning season when most mature fish had spawned. Post-spawned fish with post-ovulatory follicles were collected from October through December and generally had the macroscopic appearance of recent spawning, such as residual ovulated oocytes loose in the lumen and a highly vascularized ovary. A small percentage of mullet had atretic oocytes in September. By December atresia was common as the spawning season was ending. A few fish were collected in the winter months of February and March with large ovaries; however, from a microscopic exam these fish were atretic. After January, no fish were microscopically staged vitellogenic or viably active.

Monthly largest oocyte diameter (LOD) frequencies supported results obtained from GSI, macroscopic, and microscopic analyses. Oocyte size increased from a pre-vitellogenic range of 0.08 to 0.22 mm, observed from February through

August, to a late vitellogenic size near 0.60 mm in September (Figure 24). By October and November, LOD distributions were primarily 0.5-0.6 mm with a maximum diameter of 0.69 mm. Oocytes undergoing final oocyte maturation had a LOD of 0.72 mm. Ovulated oocytes taken on residual oocytes loose in the lumen of recently post-spawned fish had diameters of 0.78 to 0.91 mm.

Female length had an effect on the timing of gonadal development. Larger females developed earlier and may spawn earlier than smaller female mullet. In September, females ≥ 400 mm FL had mean LOD's above 0.40 mm, significantly larger than smaller mullet (Figure 25). Mean LOD's increased for each length class in October. Larger females above 350 mm FL remained more developed than smaller mullet. By November, mullet > 330 mm FL (L_{50}) had similar LOD's to one another.

Male seasonality patterns were similar to females. Each year gonadal growth was initiated in September. Peak GSI's varied from year to year, but by January testis returned to pre-spawning levels (Figure 26). The 1997 and 1998 GSI patterns were most similar to each other. GSI's increased rapidly in October ($>6.0\%$) and peaked in November (12.5%) or early December (15.6%). In contrast, the 1999 GSI pattern had rapid development in September (7.3%) with a peak in October (9.9%). Like females, the mean GSI peak in 1999 was lower than the peaks in 1997 or 1998. A monthly analysis of the microscopic exam of testicular maturation also indicates initial gonadal growth in September (Figure 27). By October males were primarily in

late maturation stage and ready to spawn. No obvious signs of post spawning were observed until November and by February all males were resting.

Gonadal growth was initiated in September when monthly mean estuarine water temperature for all years combined (1997-1999) was 26°C (Figure 28). The greatest drop in mean temperature occurred between September and October when evidence of spawning was first observed. The majority of spawning is thought to occur between October and November when mean monthly water temperatures were 15-20°C. The warmest temperature a reproductively active female (vitellogenic) or male (spermatogenic) was collected was 28°C. The earliest post-spawned female, was collected when the water temperature at the time and site of collection was 24.5°C, however, 98% of all post-spawned fish were collected in water temperatures below 17°C.

Fecundity

Potential annual fecundity estimates were determined from a wide size range of mullet (302 to 597 mm) in the mid to late vitellogenic stages (N=120). Fecundity estimates ranged from 4.8×10^5 to 4.2×10^6 . Strong relationships between fecundity and body length (Figure 29) and weight (Figure 30) were observed, with correlation coefficients (r^2) of 0.88 and 0.92, respectively. Relative fecundity ranged from 1,193 to 2,535 eggs per gram of eviscerated body weight. The equations to estimate fecundity based on body weight (BW, g) and fork length (FL, mm) were:

$$\text{Fecundity} = 0.2407(\text{FL}^{2.98}) \quad (P < 0.001)$$

$$\text{Fecundity} = 0.0015(\text{BW}) - 0.0654 \quad (P < 0.001)$$

Discussion

Before sex differentiation, mullet juveniles have two thin triangularly shaped gonadal lobes. At the time of sexual differentiation, females develop oocytes causing the gonad to slightly enlarge into a cylindrical shape. It is at this time when macroscopic sexual determination becomes possible. Stenger (1959) reported sex differentiation at 175 to 225 mm SL (205-260 mm FL), but found females as small as 150 mm SL (175 mm FL). In my study, sexual differentiation occurred between 200 and 250 mm FL, consistent with Stenger.

Hermaphroditic mullet are reported in the literature (Kesteven 1942, Stenger 1959, Moe 1966, Thompson et al. 1990, and Franks et al. 1998). Usually in these cases, male and female gonadal tissue develop together and are described as ovotestes. However, in one case development was synchronous and each gonadal lobe had a separate testis and ovary (Franks et al. 1998). Hermaphrodites are rare and striped mullet are not thought to undergo sex reversal (protogyny or protandary) during their life cycle (Stenger 1959, Franks et al. 1998). However, the occurrence of oocyte-like tissue in adult males (intersexuality) is not uncommon (Stenger 1959). In my study, one male possessed a limited number of what appeared to be primary oocytes, indicating intersexuality does exist in North Carolina. These structures were only detectable from a microscopic exam. No true hermaphrodites were found in my study.

Striped mullet maturity is determined by fish size regardless of age and thus length should be used to describe mullet maturity. A threshold is observed with size and maturity so that after a certain size maturity is 100%. No such threshold is observed with age and maturity. Fishery dependent strike nets effectively harvest mature females (324 mm FL, ♀ L_{50}) with approximately 98% of these samples over 324 mm FL. Because nearly all fish sampled by the commercial gears are mature, as expected from a fishery that targets adults, length at maturity determinations made solely from these gears are biased. Likewise a bias is associated with fishery independent gears that are most effective at sampling smaller mullet, although fishery independent gears did collect both mature and immature fish over a more even ratio than fishery dependent gears. Together these sampling strategies provided samples from a broad size and age range needed for proper maturity determinations.

Mullet in North Carolina, in general, reach maturity at a greater length compared to other regions. In Louisiana, females and males are mature (L_{50}) at 230 and 220 mm FL, respectively (Thompson et al. 1991). Compared to my results (324 and 283 mm FL), this is a difference of 94 mm for females and 63 mm for males. No other studies evaluated maturity based on L_{50} criteria. Previous studies often used minimum size of maturity and/or a size beyond which maturity was 100% (obligatory maturity). On the Florida west coast, maturity was observed between 290 to 380 mm FL; however, maturity determination criteria were not provided (Leard et al. 1995). In my study, the smallest mature female and male was 250 mm and 241

mm, respectively. In eastern Florida, female maturity was observed at a minimum size of 230 mm SL (267 mm FL) (Greeley 1985). In Georgia, the minimum size of maturity was 247 and 231 mm FL for females and males, respectively (Pafford 1983). Obligatory or mandatory maturity occurred at a larger size for females (367 mm FL) in North Carolina compared to other regions. Female obligatory maturity is observed at 290 mm in Louisiana (Thompson et al. 1990) and 310 mm in eastern Florida (Greeley 1985). Male obligatory maturity is 280 mm FL in Louisiana (Thompson et al. 1991), no different from my study. The age and maturity relationship was poor for both females and males, which resulted in the inability to model age and maturity with confidence. The age of maturity results I presented are based on the age at the size of maturity (L_{50}). Based on these age of maturity estimates (A_{50}) females and males are mature by age 2. In all other studies outside of North Carolina, mullet were found to mature (A_{50}) at ages 2-3 (Broadhead 1958, Thompson et al. 1991, Pafford 1983).

Striped mullet in North Carolina spawn earlier in the year compared to other regions of the USA. I found mullet began spawning in September from the earliest collection of a post-spawned fish. Using this criteria, spawning begins in late November in both Florida (Greeley et al. 1987) and Georgia (Pafford 1983) and in late December in Louisiana (Thompson 1991). Using the occurrence of late vitellogenic oocytes in the ovary, most mullet in North Carolina are ready to spawn by late September or early October compared to October for Georgia (Pafford 1983) and October/November for the east coast of Florida (Greeley et al. 1987).

Previous inferences on striped mullet spawning locations in the USA are based largely on indirect evidence from larval surveys and behavioral observations. Larval studies suggested mullet spawn in offshore oceanic waters (Anderson 1958, Fahay 1975, Finucane et al. 1978, Powells 1981, Collins 1989). However, one study reported offshore spawning in Gulf of Mexico waters greater than 900 m deep from observations of spawning mullet (Arnold and Thompson 1958). Powells (1981) and Collins (1989) found an inverse larval length to depth relationship across the SAB shelf water, suggesting spawning was in outer shelf waters. However, larval size is not always indicative of spawning location. For example, from these same larval studies white mullet larvae were found in a similar latitudinal distribution as striped mullet and the same larval size to depth relationship existed (Anderson 1957, Collins 1989). Adult white mullet are restricted to offshore waters as far north as Florida (Anderson 1957). The occurrence of small white mullet (< 6 mm) offshore of Cape Fear River, NC, can only be a result of larval drift and does not support a spawning location off North Carolina because adult white mullet have never been reported from North Carolina. Larval drift implications do not disprove the theory that striped mullet spawn offshore in the SAB, but do indicate the limited value of using larval size to predict spawning location. To more accurately determine striped mullet spawning location from larval data the larvae should be aged.

My study focused on striped mullet reproductive condition in the estuary, although some ocean samples were collected usually near inlets. No mullet in spawning condition was collected from the estuary and thus estuarine spawning is

unlikely. However, I found direct evidence of near-shore ocean spawning from one actively spawning female. The ovary was full of ovulated oocytes with a limited number of primary oocytes, which contradicts the theory that mullet spawn "batches" of eggs over successive hours or nights due to limited body cavity space (Render et al. 1995). This fish represents the first naturally spawning female mullet ever collected from the wild that was verified using ovarian histology.

Indirect evidence for near-shore ocean spawning was observed from the common occurrence of post-ovulatory follicles found in post-spawned mullet. Post-ovulatory follicles (POF's) persist in the ovary for short time periods (24-48 hours) after spawning (Hunter and Goldberg 1980, Hunter and Macewicz 1985, Cuellar et al. 1996) and are often used to indicate location and time of spawn. No other study on striped mullet reproduction has ever found POF's. Because no spawning females or eggs have ever been collected from estuarine waters the presence of POF's in fish collected within North Carolina estuaries indicates spawning in the near-shore ocean environment and leaves open the possibility of inlet or lower estuarine spawning. Together, the limited direct evidence of a spawning female and the abundance of indirect evidence from the common occurrence of POF's in mullet ovaries collected within the estuary, strong evidence for near-shore spawning is provided.

Monthly mean GSI's provide a reasonable indicator of reproductive seasonality for both males and females. The initial increase in GSI's in September is directly related to the production of vitellogenic oocytes and spermatozoa. By

October, GSI's are near peak and nearly all females are late vitellogenic with large oocyte diameters. In December, GSI's begin to decline with the completion of spawning. GSI analyses fail to provide information on the initiation of spawning and gonad reabsorption. Without a macroscopic or microscopic gonadal examination, detection of post-spawned fish, hydrated females, and atresia are not possible. Therefore, GSI analyses alone should not be used to determine the complete reproductive seasonality of a species.

The oocyte diameter to stage of oocyte maturity relationship was similar to results from studies in Florida (Greeley et al. 1987) and Louisiana (Render et al. 1995). In this study, the transition from a pre-vitellogenic oocyte (immature) to a vitellogenic oocyte occurred at 0.22 mm (0.16 mm from histological oocyte diameters). In Florida and Louisiana this transition occurred at 0.18 mm and 0.21 mm, respectively. The maximum vitellogenic oocyte diameter was 0.69 mm in this study, compared to 0.72 mm and 0.61 from Florida and Louisiana, respectively. Residual ovulated oocytes were measured from recently post spawned fish to be 0.78 to 0.91, which closely matches reports from Pien and Liao (1975) (0.90-0.95 mm) who spawned mullet in laboratory tanks. Larger mullet appear to mature earlier in the season than smaller mullet, a result consistent with Greeley et al. (1987).

A decrease in water temperature is the primary factor that determines the onset of vitellogenesis or gonadal development in female striped mullet (Kelly 1990). Water temperatures decline earlier in the year in North Carolina compared to Florida and the Gulf of Mexico, as observed from data collected by the National Estuarine

Research Reserve water quality monitoring program. Thus, water temperature is likely responsible for the earlier spawning season in North Carolina compared to areas in lower latitudes. From a aquaculture study, 21°C was the most efficient water temperature to complete oogenesis (Kuo et al. 1974). Results from my study support these findings as I found most mullet were not ready to spawn until October and November when mean monthly water temperatures were 15-20°C.

Latitudinal variation in fecundity occurs for species along the east coast (MacKenzie et al. 1985, Mercer 1989). Estimates of fecundity are higher in my study compared to the east coast of Florida (Greeley et al. 1987) and Louisiana (Render et al. 1995, Thompson 1990) where fecundity estimate methodologies were similar to the present study. On the west coast of Florida fecundity estimates are higher than in my study (Leard et al. 1995).

My study is the first comprehensive study on the reproductive life history of striped mullet in North Carolina. Results indicate differences in maturity, spawning location, reproductive seasonality, and fecundity compared to other areas in the species' range. These results provide the most accurate information on striped mullet reproductive biology in North Carolina, much of which is needed for the current North Carolina striped mullet fishery management plan.

VI. Literature Cited

- Able, K. W. and M. P. Fahay. 1998. The first year in the life of estuarine fishes in the middle Atlantic bight. Rutgers University Press, New Brunswick, New Jersey, 333pp.
- Anderson, W.W. 1958. Larval development, growth, and spawning of striped mullet (*Mugil cephalus*) along the south Atlantic coast of the United States. U.S. Fish and Wildlife Service, Fishery Bulletin 58:501-519.
- Anderson, W.W. 1957. Early development, spawning, growth, and occurrence of the silver mullet (*Mugil curema*) along the south Atlantic coast of the United States. U.S. Fish and Wildlife Service, Fishery Bulletin 57:397-414.
- Arnold, E. L. and J. R. Thompson. 1958. Offshore spawning of the striped mullet (*Mugil cephalus*) in the Gulf of Mexico. Copeia. 2:130-132
- Bagenal, T.B. and F.W. Tesch. 1978. Age and Growth. In T.B. Bagenal ed. Methods for assessment of fish production in fresh waters, 3rd ed. P.101-136. Blackwell Scientific Publications, Oxford.
- Barbieri, L. R., M. Chittenden Jr., and S. K. Lowerre-Barbieri. 1994. Maturity, spawning, and ovarian cycle of Atlantic croaker, *Micropogonias undulates*, in the Chesapeake Bay and adjacent waters. Fish. Bull. 92:671-685.
- Barbieri, L. R., M. Chittenden Jr., and C. M. Jones. 1994. Age, growth, and maturity of Atlantic croaker, *Micropogonias undulates*, in the Chesapeake Bay region, with a discussion of apparent geographic changes in population dynamics. Fish. Bull. 92:1-12.
- Beamish, R. J. and G.A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Transaction of the American Fisheries Society 112:735-743.
- Breder, C.M. 1940. The spawning of *Mugil cephalus* on the Florida west coast. Copeia. 2:138-139.
- Broadhead, G.C. 1958. Growth of the black mullet (*Mugil cephalus*) in west and northwest Florida. State of Florida, Board of Conservation, Marine Laboratory, Technical Series. 25. 29pp.

- Chang, C. F., S.-C. Lan, and H.-Y. Chou. 1995. Gonadal histology and plasma sex steroids during sex differentiation in grey mullet, *Mugil cephalus*. The Journal of Experimental Zoology. 272:395-406.
- Chen, Y., D. A. Jackson, and H. H. Harvey. 1992. A comparison of von Bertalanffy and polynomial functions in modeling fish growth data. Canadian Journal of Fisheries and Aquatic Sciences. 49: 1228-1235.
- Cech, J.J. and D.E. Wohlschlag. 1975. Summer growth depression in the striped mullet, *Mugil cephalus*, Linnaeus. Contributions in Marine Science. 19:91-100.
- Collins, M. R. 1985. Species profiles : Life histories and environmental requirements of coastal fishes and invertebrates (South Florida)- Striped mullet. U.S. Fish and Wildlife Service, Biological Report 82 (11.34). U.S. Army Corps of Engineers, TR EL-82-4, 11 pp.
- Collins, M. R. 1989. Larval striped mullet, *Mugil cephalus*, and white mullet, *Mugil curema*, off the southeastern United States. Bull. Of Marine Science, 43:580-589.
- Conover, D. O. 1992. Seasonality and the scheduling of life history at different latitudes. Journal of fish Biology 41:161-178.
- Conover, D. O. 1990. The relation between capacity for growth and length of growing season: evidence for and implications of countergradient variation. Trans. Of the Am. Fish. Society, 119:416-430.
- Conover, D. O. and W. M. C. Present. 1990. Countergradient variation in growth rate: compensation for lengths of the growing season among Atlantic silversides from different latitudes. Oecologia, 83:316-324.
- Cowan, J. H. Jr, R. L. Shipp, H. K. Bailey IV and D. W. Haywick. 1995. Procedure for rapid processing of large otoliths. Transactions of the American Fisheries Society. 124:280-282.
- Cuellar, N., G.R. Sedberry, and D.M. Wyanski. 1996. Reproductive seasonality, maturation, fecundity, and spawning frequency of the vermilion snapper, *Rhomboplites aurorubens*, off the southeastern United States. Fishery Bulletin, U.S. 94:635-653.
- Dindo, J. J. and R. MacGregor III. 1981. Annual cycle of serum gonadal steroids and serum lipids in striped mullet. Transactions of the American Fisheries Society. 110:403-409.

- Earll, E.R. 1887. The mullet fishery. In The Fisheries and Fishery Industries of the United States, ed. George Brown Goode, 5 sections, Washington D.C.: Commission of the Fish and Fisheries, 1884-1887, 1:564.
- Fahay, M.P. 1975. An annotated list of larval and juvenile fishes captured with surface-towed meter net in the South Atlantic Bight during four R/V DOLPHIN cruises between May 1967 and February 1968. NOAA Tech. Rep. NMFS SSRF-685, 39pp.
- Finucane, J.H., L. A. Collins and L.E. Barger. 1978. Spawning of the striped mullet, *Mugil cephalus*, in the northwestern Gulf of Mexico. Northeast Gulf Science 2(2):148-151.
- Francesconi, J. J. 1994. Effect of stop nets on pier and beach angler catch rates and general fish movement along Bogue Banks, NC. North Carolina Department of Environment, Health and Natural Resources, North Carolina Division of Marine Fisheries, Morehead City, North Carolina. 19pp.
- Francis, R. I. C. C. 1995. The analysis of otolith data – A mathematician's perspective (What, precisely, is your model?). Recent developments in fish otolith research. Belle Branch Institute for Marine Biology and Coastal research by the Univ. of South Carolina Press, 19:81-96.
- Franks, J.S., N.J. Brown-Peterson, D.P. Wilson, R.J. Russell, and J.K. Welker. 1998. Occurrence of a synchronous hermaphroditic striped mullet, *Mugil cephalus*, from the northern Gulf of Mexico. Gulf Research Reports. 10:33-39.
- Goodyear, C.P. 1995. Mean size at age: An evaluation of the sampling strategies with simulated red grouper data. Transactions of the American Fisheries Society 124:746-755.
- Gilbert, C.R. 1993. Geographic distribution of the striped mullet (*Mugil cephalus* linnaeus) in the Atlantic and Eastern Pacific oceans. Florida Scientist. 56:204-210.
- Greeley, M. S., D. A. Calder and R. A. Wallace. 1987. Oocyte growth and development in the striped mullet, *Mugil cephalus*, during seasonal ovarian recrudescence : Relationship to fecundity and size at maturity. Fisheries Bulletin 85:187-200
- Gunter, G. 1945. Studies on the Marine Fishes of Texas. Publications of the Institute of Marine Science, University of Texas, Austin, Texas. 1(1):1-90.

- Hadden, M. 2001. Modeling and quantitative methods in fisheries. Chapman and Hall, CRC Press LLC, Boca Raton, Florida. 406pp.
- Henderson P. A., R. H. A. Holmes, and R. N. Bamber. 1988. Size-selective overwintering mortality in the sand melt, *Atherina boyeri*, and its role in population regulation. J. Fish. Biol 33:221-233.
- Hilborn, R. and C. J. Walters. 1992. Quantitative fisheries stock assessment: Chioce, dynamics, and uncertainty. Chapman and Hall, New York, NY.
- Hildebrand, S.F. and W.C. Schroeder. 1928. Fishes of Chesapeake Bay. Bulletin of the U.S. Bureau of Fisheries 48 (1):1-366.
- Hostetter, E. Brian and T.A. Monroe. 1993. Age, growth, and reproduction of *Tautog onitis* (Labridae: Perciformes) from coastal waters of Chesapeake Bay. Fisheries Bulletin, U.S. 91:45-64.
- Hunter, J.R. and B.J. Macewicz. 1985. Measurement of spawning frequency in multiple spawning fishes. In R. Lasker (ed.), An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax*, p67-77. U.S. Dep. Commerce., NOAA Technical Report NMFS 36.
- Hunter, J.R., B.J. Macewicz, N.C. Lo, and C.A. Kimbrell. 1992. Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. Fishery Bulletin, U.S. 90:101-128.
- Hunter, J.R. and S.R. Goldberg. 1980. Spawning incidence and batch fecundity in northern anchovy, *Engraulis mordax*. Fishery Bulletin, U.S. 77:641-652.
- Jacot, A.P. 1920. Age, growth, and scale characters of the mullets, *Mugil cephalus* and *Mugil curema*. Transactions of the American Fisheries Society 39(3):199-229.
- Kelly, Christopher D. 1990. Effects of photoperiod and temperature on ovarian maturation in the striped mullet, *Mugil cephalus*. Pacific Science. 44(2):187.
- Kesteven, G. L. 1942. Studies in the biology of Australian mullet. 1- Account of the fishery and preliminary statement of the biology of *Mugil dobula*. Commonw. Australia Council Sci. and Ind. Res. Bull. 157. 149 pp.
- Kimura, D. K. 1977. Statistical assessment of the age-length key. J. Fish. Res. Board Can. 34:317-324.

- Kuo, C.-M., C. E. Nash, and Z. H. Shehaden. 1974. The effects of temperature and photoperiod on ovarian development in captive grey mullet (*Mugil cephalus*). *Aquaculture*, 3(1):25-43.
- Leard, R., B. Mahmoudi, H. Blanchet, H. Lazauski, K. Spiller, M. Buchanan, C. Dyer and W. Keithly. 1995. The striped mullet fishery of the Gulf of Mexico, United States : A regional management plan. Gulf States Marine Fisheries Commission, No.33.
- Leaman, B. M. and R. J. Beamish. 1984. Ecological and management implications of longevity in some northeast Pacific groundfishes. *Int. North Pac. Fish. Comm.* 42:85-97.
- Liao, I.C. 1981. Cultivation methods: In: *Aquaculture of Grey Mullet*. O.H. Oren, ed. Cambridge University Press, Cambridge, pp361-389.
- MacKenzie, C., L.S. Weiss-Glanz, and J.R. Moring. 1985. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic) – American shad. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.37). U.S. Army Corps of Engineers, TR EL-82-4. 18pp.
- Mahmoudi, B. 1990. Population assessment of the black mullet (*Mugil cephalus*) in the eastern Gulf of Mexico. Final report of Cooperative Agreement (MARFIN) NA89-WC-H-MF003, Florida Department of Environmental Protection, St. Petersburg, Florida, 78 pp.
- Mahmoudi, B. 1991. Population assessment of the black mullet (*Mugil cephalus*) in the eastern Gul of Mexico. Final report of Cooperative Agreement (MARFIN) NA90-WC-H-MF003, Florida Department of Environmental Protection, St. Petersburg, Florida, 69 pp.
- McDonough, C. J. and C. W. Wenner. 2003. Growth, recruitment, and abundance of juvenile striped mullet (*Mugil cephalus*) in South Carolina estuaries. *Fish. Bull.* 101:343-357.
- McEvoy, L. A. and J. McEvoy. 1992. Multiple spawning in several commercial fish species and its consequences for fisheries management, cultivation, and experimentation. *J. of Fish Biology*. 41:125-136.
- Mercer, L. P. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic) – weakfish. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.109). U.S. Army Corps of Engineers, TR EL-82-4. 17pp.

- Miller, W.G. 1992. An investigation of bottlenose dolphin, *Tursiops truncatus*, deaths in east Matagorda Bay, Texas, January 1990. Fishery Bulletin, U.S. 90:791-797.
- Moe, M. A. 1966. Hermaphroditism in mullet, *Mugil cephalus*, Linneaus. Quarterly Journal of the Florida Academy of Sciences. 29:111-116.
- Moore, R.H. 1974. General ecology, distribution, and relative abundance of *Mugil cephalus* and *Mugil curema* on the south Texas coast. Contributions Marine Science. 18:241-255.
- Murdy, E. O., R. S. Birdsong and J. A. Musick. 1997. Fishes of Chesapeake Bay. Smithsonian Institution Press, Washington D.C.
- Nash, C.E. and Z.H. Shehadeh. 1980. Review of the breeding and propagation techniques for grey mullet, *Mugil cephalus*, L. ICLARM Studies and Reviews, 387pp. International Center for Living Aquatic Resources Management, Manila.
- Odum, W.E. 1970. Utilization of the direct grazing and plant detritus food chains by the striped mullet, *Mugil cephalus*. P222-240. In J.J. Steele (editor), Marine Food Chains, Oliver and Boyd, Ltd., Edinburgh, Scotland.
- Pafford, J.M. 1983. Life history aspects of the striped mullet, *Mugil cephalus*, in Georgia's St. Simons estuarine system. Master's Thesis, Georgia Southern College, Statesboro, Georgia. 80pp.
- Pien, P. C. and I. C. Liao. 1975. Preliminary report of histological studies on the grey mullet gonad related to hormone treatment. Aquaculture. 5:31-39.
- Powells, H. 1981. Distribution and movement of neustonic young of estuarine dependent (*Mugil spp.*, *Pomatomus saltatrix*) and estuarine independent (*Coryphaena spp.*) fishes off the southeastern United States. Rapp. P.-v. Reun. Cons./ Int. Explor. Mer. 178:207-209.
- Render, J. R., B. A. Thompson and R. L. Allen. 1995. Reproductive development of striped mullet in Louisiana estuarine waters with notes on the applicability of reproductive assessment methods for Isochronal species. Transactions of the American Fisheries Society 124:26-36.
- Ricker, W.E. 1969. Effects of the size-selective mortality and sampling bias on estimates of growth, mortality, production, and yield. J. of the Fisheries Research Board of Canada. 26(3).

- Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Fisheries Research Board of Canada, Bulletin 191, 382 p.
- Rhode, F. C. 1976. First record of the mountain mullet, *Agonostomus monticola* (Bancroft), from North Carolina. Fla. Sci., 39(2), 126, (1976)
- Roa, Ruben, B. Ernst, and F. Tapia. 1998. Estimates of size at sexual maturity: an evaluation of analytical and resampling procedures. U.S. Fish. Bulletin. 97:570-580.
- Ross, J.L., T.M. Stevens, and D.S. Vaughan. 1995. Age, growth, mortality, and reproductive biology of red drums in North Carolina waters. Transactions of the American Fisheries Society. 124:37-54.
- Shultz, E. T., T. E. Lankford, and D. O. Conover. 2002. The covariance of routine and compensatory growth rates over a seasonality gradient in coastal fish. *Oecologia*
- Shehadeh, Z.H., C.-M. Kuo, and K.K. Milisen. 1973. Induced spawning of grey mullet *Mugil cephalus*, Linnaeus, with fractionated salmon pituitary extract. Journal of Fish Biology. 5(4):471-478.
- Shepherd, G. and C.B. Grimes. 1983. Geographic and historic variations in growth of weakfish, *Cynoscion regalis*, in the Middle Atlantic Bight. U.S. National Marine Fisheries Service Fishery Bulletin. 18:803-813.
- Shireman, J. V. 1975. Gonadal development of striped mullet (*Mugil cephalus*) in freshwater. The Progressive Fish Culturist. 37(4):205-208.
- Smith, H. 1907. Fishes of North Carolina. pp.180-183.
- Stenger, A. H. 1959. A study of the structure and development of certain reproductive tissues of *Mugil cephalus* linnaeus. Zoologica: New York Zoological Society. 44(3):53-69.
- Sullivan, C.V., D. L. Berlinsky and R.G. Hodson. 1997. Striped bass and other *Morone* culture. R.M. Harrell (ed). In Developments in Aquaculture and Fisheries Science. University of Maryland System, Horn Point Environmental Laboratory, Center for Environmental and Estuarine Studies, Cambridge, Maryland. pp.11-73.
- Taylor, H.F et al. 1951. Survey of marine fisheries of North Carolina. University of North Carolina Press. Chapel Hill, North Carolina. pp.114-116.

- Taylor, M. T. 1992. Seiners and tongers : North Carolina fisheries in the Old and New South. The North Carolina Historical Review. Vol LXIX, no.1, pp.1-36.
- Taylor, R.G., H.J. Grier, J.A. Whittington. 1998. Spawning rhythms of common snook in Florida. Journal of Fish Biology. 53:502-520.
- Thompson, B. A., J. H Render, and R. L. Allen. 1989. Life history and population dynamics of commercially harvested striped mullet, *Mugil cephalus*, in coastal Louisiana. Coastal Fisheries Institute, Louisiana State University, Baton Rouge, Louisiana. 79 pp.
- Thompson, B. A., J. H Render, R. L. Allen and D. L. Nieland. 1990. Fishery independent characterization of population dynamics and life history of striped mullet in Louisiana. A report of Cooperative agreement NA88WC-H-MF-197. Coastal Fisheries Institute, Louisiana State University, Baton Rouge, Louisiana. 73pp.
- Thompson, B. A., J. H Render, R. L. Allen and D. L. Nieland. 1991. Fishery independent characterization of population dynamics and life history of striped mullet in Louisiana. Final report of Cooperative agreement NA90AA-H-MF-113. Coastal Fisheries Institute, Louisiana State University, Baton Rouge, Louisiana. 92 pp.
- Yates, F. 1934. Contingency tables involving small numbers and the χ^2 test. J. Royal Statist. Soc. Suppl. 1:217-235.
- Wallace, R. A. and K. Selman. 1981. Cellular and dynamic aspects of oocyte growth in Teleosts. Amer. Zool. 21:325-343.

Table 1: Striped mullet mean fork length (mm) \pm standard error (SE), and sample size (N) by gear type and area.

	<u>South</u>	<u>Mid</u>	<u>North</u>	<u>Total</u>
	Mean (SE) N	Mean (SE) N	Mean (SE) N	Mean (SE) N
Fishery Dependent Gears				
Beach Seine		350 (5.2) 239		350 (5.2) 239
Gill Nets	360 (8.8) 30	344 (3.3) 205	343 (6.4) 67	345 (2.8) 302
Strike Nets	363 (4.2) 15	375 (3.9) 61	410 (1.9) 804	406 (1.8) 880
Other	346 (10.5) 2	578 (0.0) 1	446 (35.6) 6	438 (32.3) 9
<i>Total</i>	360 (5.8) 47	351 (2.9) 506	405 (1.9) 877	384 (1.7) 1430
Fishery Independent Gears				
Cast Nets	291 (5.3) 188	260 (6.3) 51	301 (6.1) 228	293 (3.8) 467
Electro-fishing	283 (3.2) 402	318 (7.0) 140		292 (3.0) 542
Other	361 (10.3) 5	262 (9.7) 85	322 (8.4) 101	296 (6.6) 191
Strike Nets	324 (2.1) 232	287 (3.5) 266	285 (4.1) 154	299 (2.0) 652
<i>Total</i>	297 (2.1) 827	288 (3.1) 542	300 (3.7) 483	295 (1.6) 1852
Total	300 (2.1) 874	319 (2.3) 1048	368 (2.3) 1360	334 (1.4) 3282

Table 2: Striped mullet female and male mean fork length (mm) at age \pm standard error (SE), and sample size (N). Samples collected in October and November between 1996 and 1999.

<u>AGE</u>	<u>Female</u>	<u>Males</u>
	Mean (SE) N	Mean (SE) N
1	341 (4.7) 116	307 (2.8) 190
2	390 (2.7) 349	337 (2.7) 130
3	429 (3.3) 188	327 (15.7) 11
4	446 (9.6) 55	312 (20.7) 6
5	427 (16.8) 30	428 (.) 1

Table 3: Description of the macroscopic gonadal maturity staging system used to code the reproductive activity of female and male striped mullet. Key used on fresh gonads soon after the time of capture.

Stage	Females	Males
1 Inactive Resting	Small rounded ovaries (<10% of body cavity) with no visible oocytes; ovary color varies from dark purple and red to a clear and light pink (immature).	White, small, thin or ribbon like testis (<2-4% of body cavity).
2 Active Developing	Ovary size ranges from small to large (~25 to 100% of body cavity); ovary color orange (early stage) to yellow (late stages); oocytes often visible in later stages	Gonad bright white and enlarged; early maturation characterized by slight increase in gonad size; late maturation characterized by a gonad that fills body cavity, spermiation occurring.
3 Maturation Spawning	Semi-clear, large oocytes free in lumen (ovulated); non-induced release of eggs from genital papilla.	Free-flowing milt or sperm from distal end of testis.
4 Post Spawn Regressed	Ovary contracted (<25% of body cavity) and highly vascularized with thick elastic gonad wall; residual ovulated oocytes maybe visible in early stages.	Testis collapsed and flaccid; residual spermiation maybe present.

Table 4: Criteria used to stage oocyte development as adopted from Wallace and Stenger (1959), Selman (1981), and Sullivan et al. (1997).

Stage		Stage Criteria
<u>1</u>	Primary Growth	a uniform basophilic (stains purple) cytoplasm and large centrally located nucleus (germinal vesicle). A single nucleolus and/or multiple nucleoli on periphery of nucleus.
<u>2</u>	Secondary Growth	Unstained lipid droplets (yolk vesicles) appear around the nucleus and will migrate to the oocyte periphery as they enlarge. Cortical granules stain light pink and form on the periphery of the oocyte. These granules will discharge into the perivitelline space at the time of fertilization.
<u>3</u>	Vitellogenic Growth	a tremendous amount of growth in weight and size occurs due to the production of yolk globules (stain red). In early stages of vitellogenic growth, the yolk globules are small and form on the oocyte periphery. By later stages of vitellogenesis the entire cytoplasm is filled with large yolk globules and lipid droplets.
<u>4</u>	Final Oocyte Maturation	The germinal vesicle (nucleus) migrates to the oocyte periphery followed by the coalescence of yolk globules and lipid droplets. Ovulation (hydration) occurs just prior to spawning and results in the release of the oocyte from the surrounding theca and granulosa cells (somatic cells) into the ovarian lumen. These granulosa and theca cells are distinguishable for 24-48 hours after ovulation and are referred to as post-ovulatory follicles (POFs).
<u>5</u>	Atretic	oocytes in the process of reabsorption. These oocytes usually lack a germinal vesicle, have a highly vacuolated cytoplasm, and are irregularly shaped. Usually occurs when a fish has missed the spawn (total spawners) and/or when the spawning season is over. Atresia can also occur when poor environmental conditions or other factors cause excessive stress.

Table 5: Key used to stage striped mullet ovarian maturity from histologically prepared gonadal tissue. Staging system modified from Stenger (1959), Wallace and Selman (1981), and Sullivan et al. (1997) for the needs of this study. (POF's = post-ovulatory follicles, FOM = final oocyte maturation).

Stage	Microscopic Criteria
<u>1</u> Pre-vitellogenic	Primary and secondary oocytes present; no atresia; thin gonad wall.
<u>2</u> Vitellogenic	Early to late vitellogenic oocytes present; no major atresia of vitellogenic oocytes; gonad wall thin.
<u>3</u> Maturation	Oocytes in FOM and/or ovulation.
<u>4</u> Recent post-spawn	POF's present, residual ovulated oocytes maybe present; gonad wall thick and convoluted from recent gonadal contraction; primary and secondary oocytes present; atresia often present.
<u>5</u> Post-spawn	Gonad wall thick; follicular tissue present; Primary and secondary oocytes present; atresia often present.
<u>6</u> Atretic	Large scale atresia in primary, secondary, and/or vitellogenic oocytes.

Table 6: Criteria used to stage male gonadal development as adopted from Sullivan et al. (1997) and Taylor et al. (1998).

Stage	Stage Criteria
<u>1</u> Regressed	this stage is dominated by the germ cells spermatogonia, but some spermatocytes may also be present. The testis lobules or channels are not well defined and no spermatogenesis is occurring.
<u>2</u> Early Maturation	spermatogenesis begins and the lobules elongate. Spermatocytes, spermatogonia, and spermatocytes are present signaling the completion of the first meiotic division.
<u>3</u> Mid Maturation	spermatogenesis continues and the completion of the second meiotic division occurs producing spermatids. These spermatids differentiate into flagellated spermatozoa (sperm), which begin to fill into the collecting ducts.
<u>4</u> Late Maturation	spermatozoa fill the collecting ducts and the lobules to the distal ends. The individual is ready for spawning.
<u>5</u> Regressing	spermatogenesis ceases and the testis decrease in size. Stem cells begin to restock the spent tubules.

Table 7: Ratio of female to male striped mullet by length class (25 mm). Mullet collected from all gears combined.

Fork Length (mm)	Females	Males	Total	% Female	Ratio (F:M)
200-224	22	46	68	32.4	0.48
225-249	49	110	159	30.8	0.45
250-274	96	185	281	34.2	0.52
275-299	198	276	474	41.8	0.72
300-324	327	241	568	57.6	1.36
325-349	351	190	541	64.9	1.85
350-374	433	146	579	74.8	2.97
375-399	377	42	419	90.0	8.98
400-424	324	12	336	96.4	27.00
425-449	219	5	224	97.8	43.80
450-474	137	1	138	99.3	137.00
>474	169	0	169	100.0	

Tables 8: Ratio of female to male striped mullet in North Carolina by gear type. Data are sorted in descending order by F:M ratio within each sampling strategy.

	Females	Males	Total	Ratio (F:M)
Fishery Dependent Gears	1028	354	1382	2.9
Strike Net	707	171	878	4.13
Gill Net (unclassified)	179	95	274	1.88
Beach Seine	142	88	230	1.61
Fishery Independent Gears	916	607	1523	1.51
Electro-fishing	379	97	476	3.91
Strike Net	371	283	654	1.31
Cast nets	166	227	393	0.73

Table 9: Striped mullet length (FL mm) of maturity in North Carolina. Table includes summary of raw data and model predictions for both females and males collected between October and December. Raw data summaries include, sample size (N), minimum length of maturity, and the length beyond which maturity is obligatory (100%). Logistic model predictions include, the length which 50% of population is mature (L_{50}), and the slope and intercept parameter estimates (+/- standard error). Parameter significant from 0 (*) tested using chi-square. $P = 0.0001$.

Raw Data	Females	Males
N	1011	566
Minimum Length at Maturity	250 mm	241 mm
100% Mature	367 mm	333 mm
Model Predictions		
L_{50}	324 mm	283 mm
Slope (SE)	0.08 (0.006) *	0.07 (0.006) *
Intercept (SE)	-25.07 (2.18) *	-19.54 (1.74) *

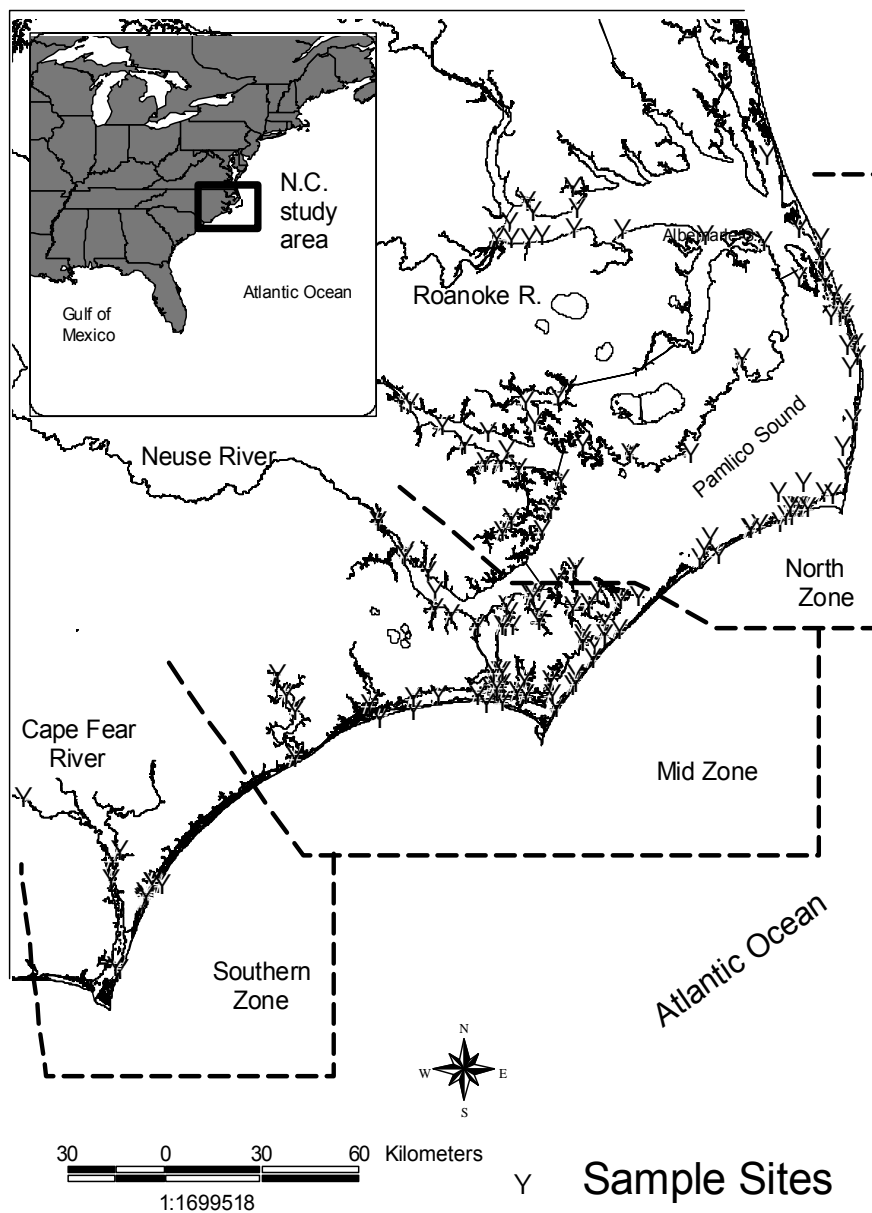


Figure 1: Study area, North Carolina coastal plain, USA. Sample sites (circles) show location of mullet collections between 1996 and 2000. Dashed lines distinguish the three sampling zones.

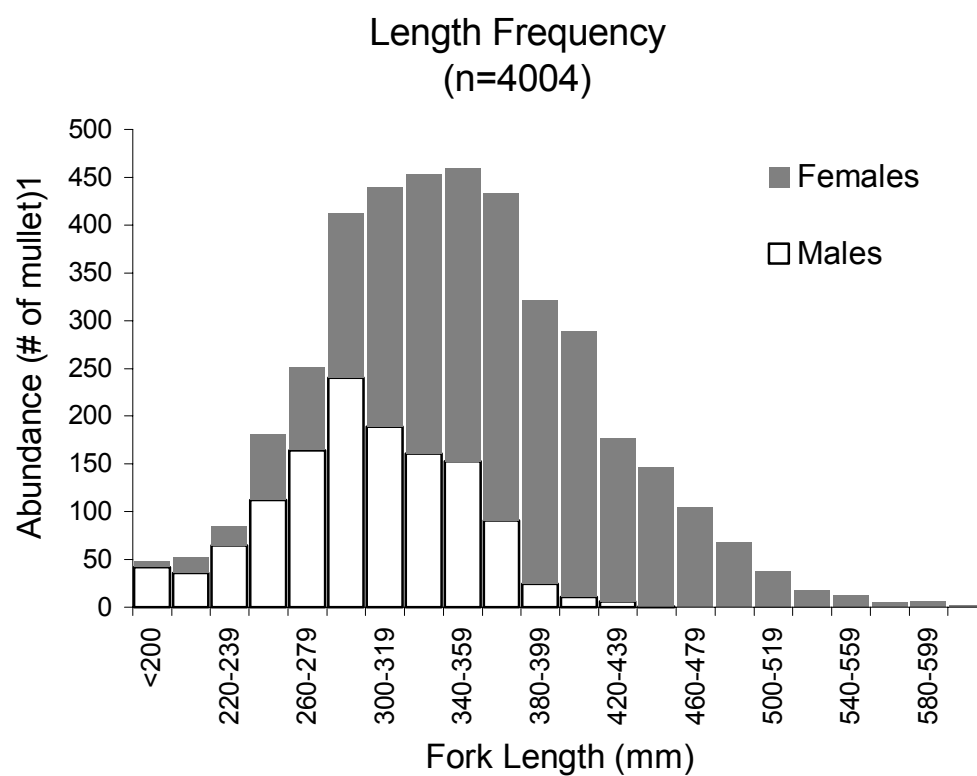


Figure 2: Striped mullet length (fork length, mm) frequency histogram. Females (shaded) and males (white) are shown. Mullet collected in North Carolina across all sampling areas and methods.

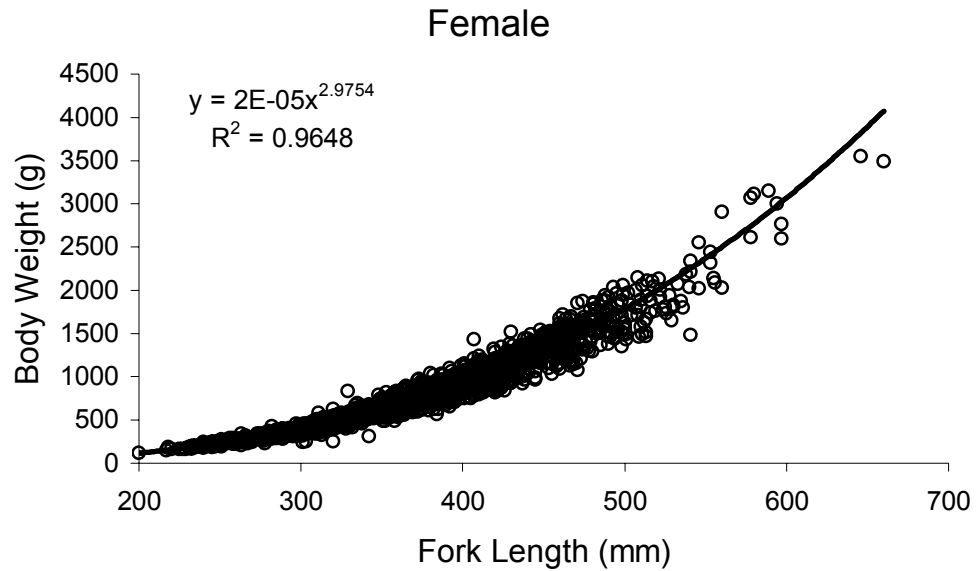


Figure 3: Length-weight relationship of female striped mullet collected from North Carolina.

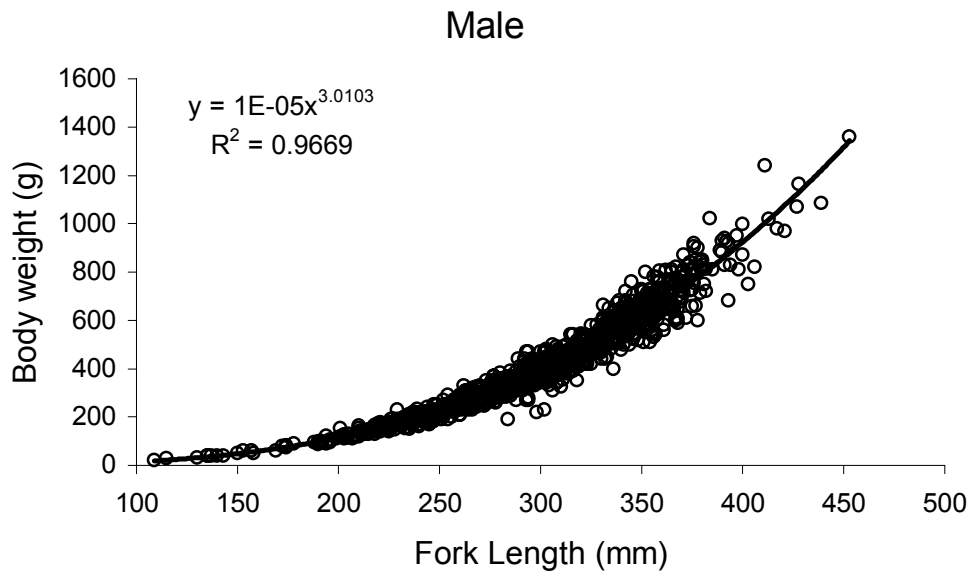


Figure 4: Length-weight relationship of male striped mullet collected from North Carolina.

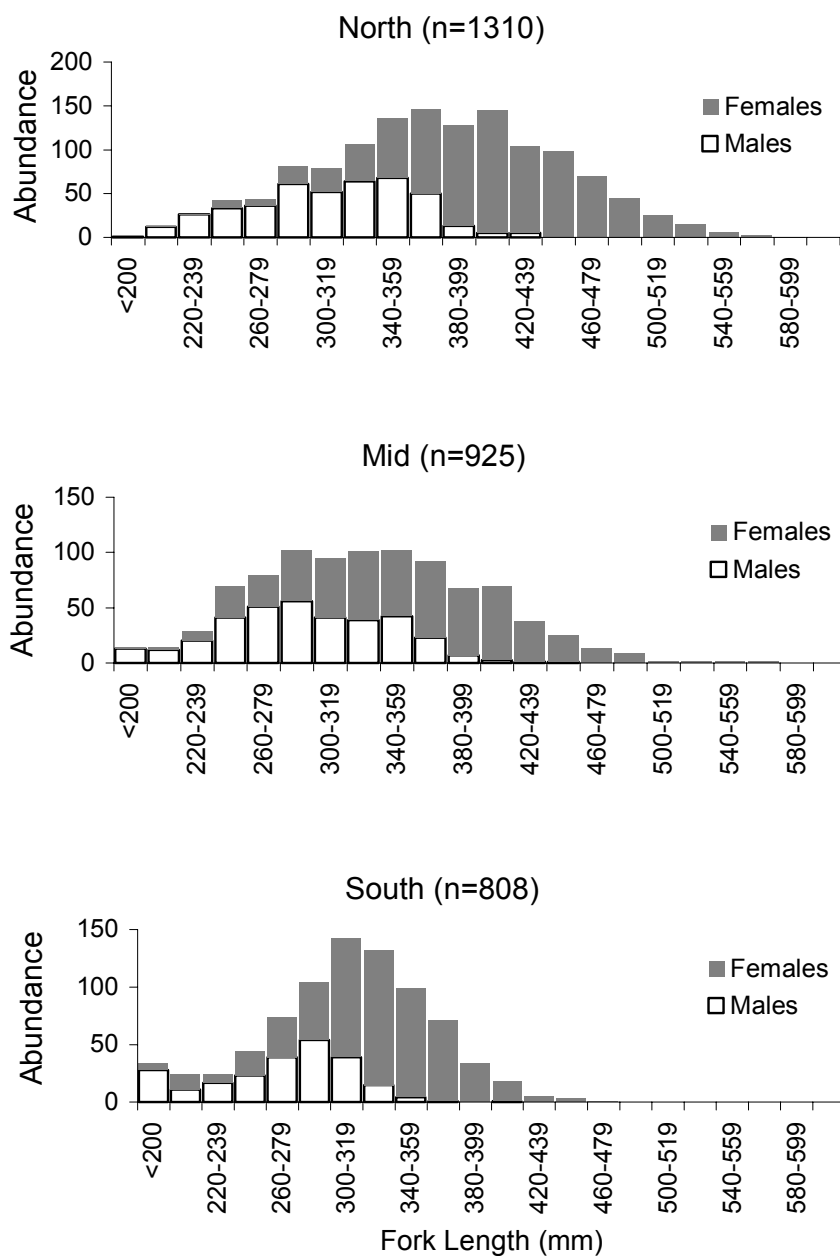


Figure 5: Striped mullet length (mm FL) frequency histograms for fish collected from the northern (top), mid (middle), and southern (bottom) sampling areas. Females (shaded) and males (white) are shown. All mullet were collected in North Carolina.

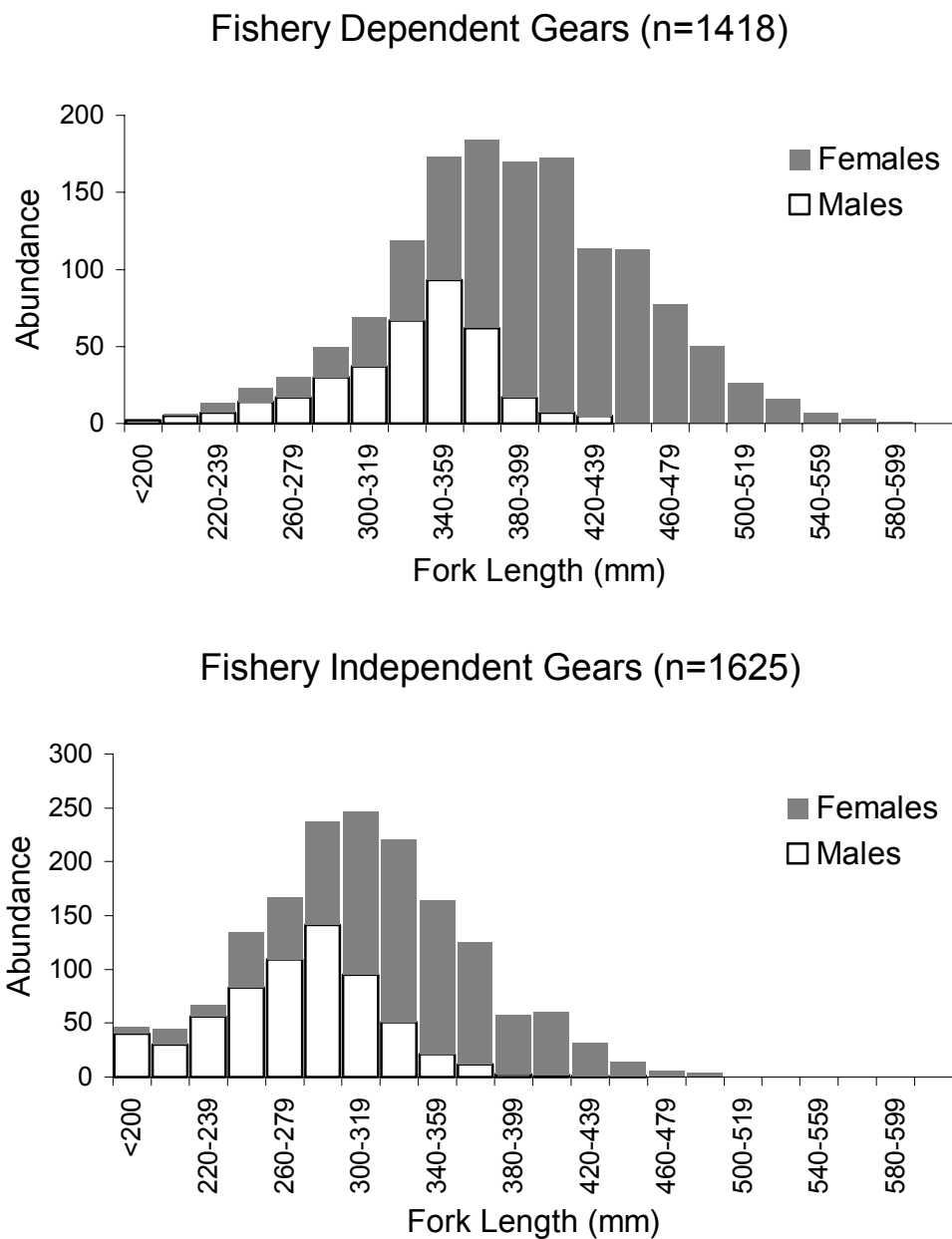


Figure 6: Striped mullet length (fork length, mm) frequency histograms for mullet collected from fishery dependent (top) and independent gears (bottom). Females (shaded) and males (white) are shown. All mullet collected in North Carolina across all sampling areas.

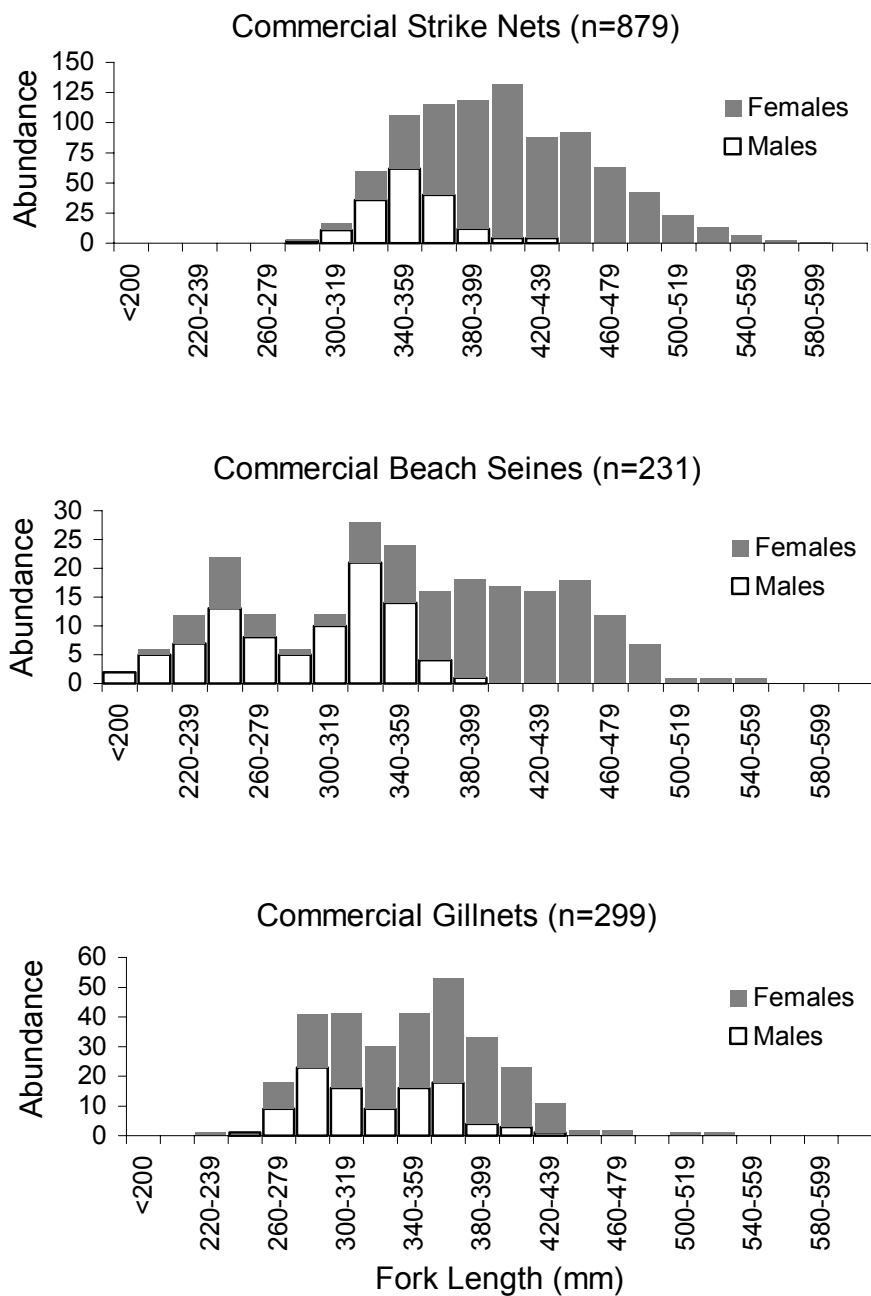


Figure 7: Striped mullet length (mm FL) frequency histograms for fish collected from fishery dependent gears: strike nets (top), beach haul seines (middle), and gill nets (bottom). Females (shaded) and males (white) are shown. All mullet collected in North Carolina across all sampling areas.

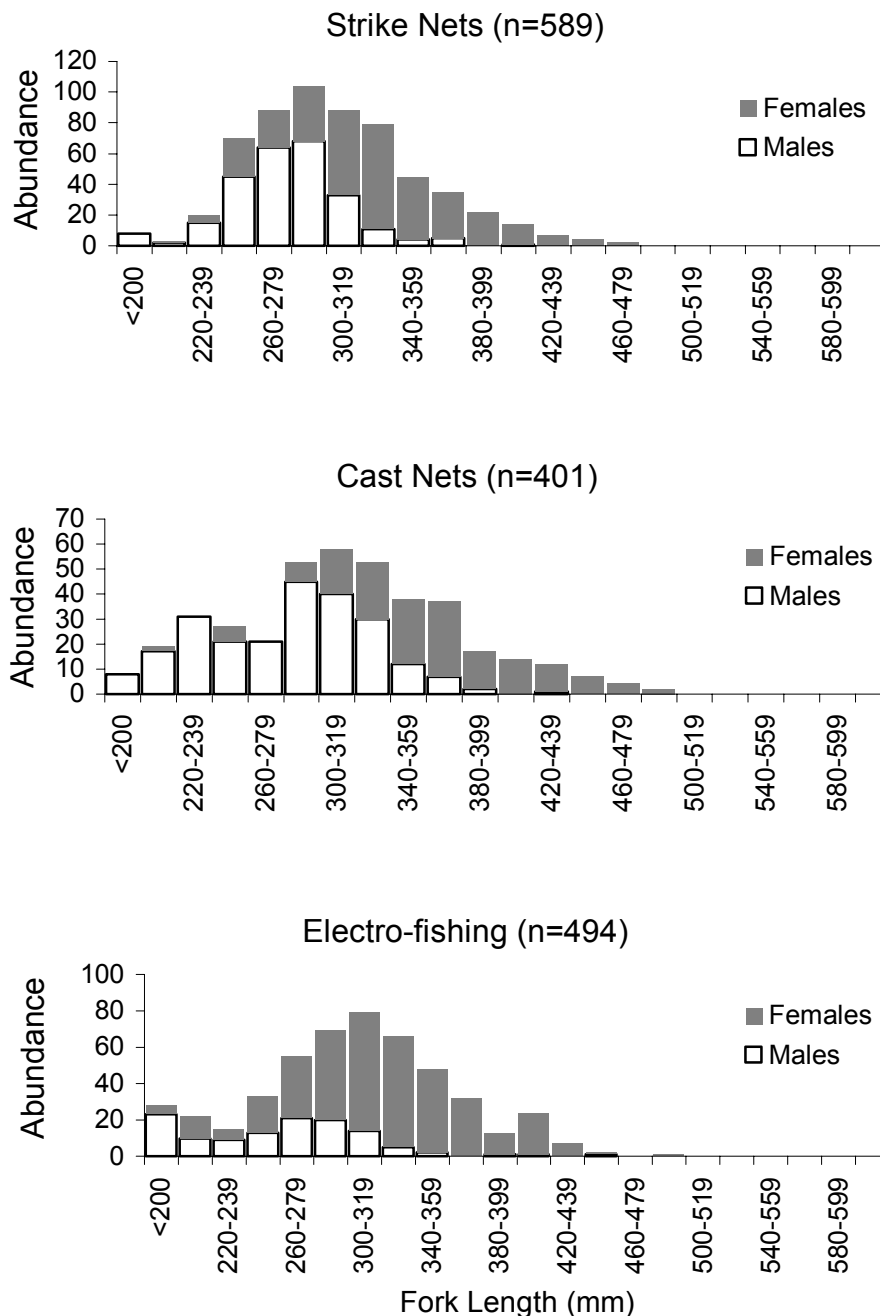


Figure 8: Striped mullet length (mm FL) frequency histograms for fish collected from fishery independent gears: survey strike nets (top), cast nets (middle), and electro-fishing (bottom). Females (shaded) and males (white) are shown. All mullet were collected in North Carolina across all sampling areas.

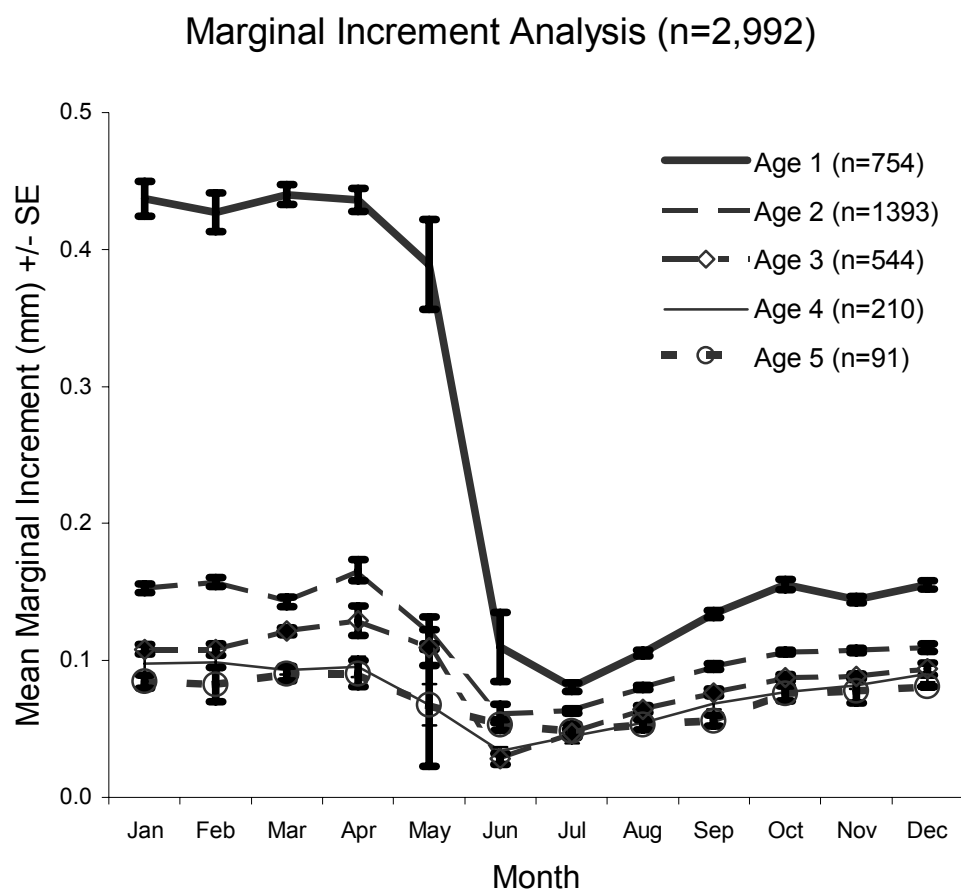


Figure 9: Monthly mean marginal increment width (SE) by age class for striped mullet collected in North Carolina. Age groups 1 to 5 are shown.

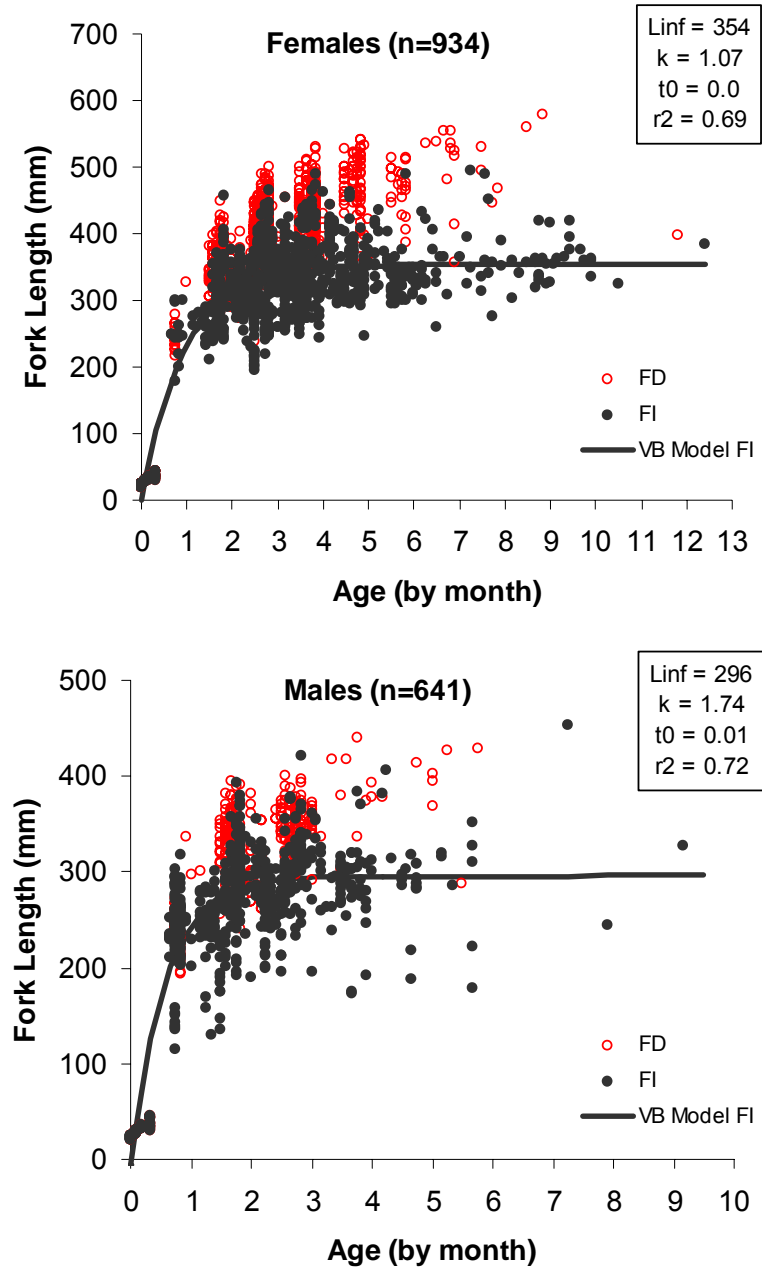


Figure 10: Female (top) and male (bottom) striped mullet length at age. Fishery dependent (FD, open red circles) and independent (FI, closed circles) samples are shown. Sample sizes ($n=$) are for FI data only. Data are fit to von Bertalanffy (VB) growth model using FI data only (solid lines). VB parameters are shown. Female and male growth curves were significantly different ($p < 0.05$).

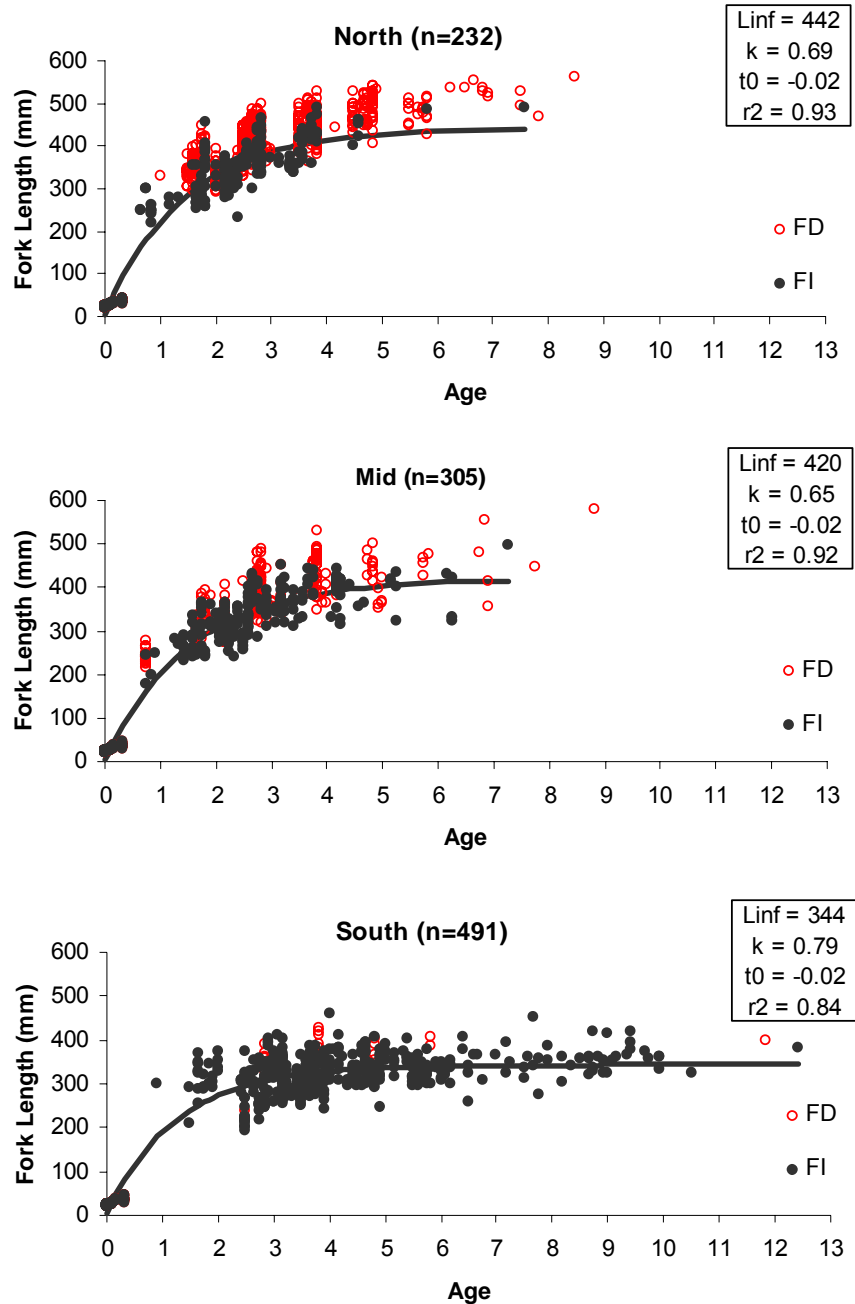


Figure 11: Female striped mullet length at age by sampling region. Fishery dependent (FD, open red circles) and independent (FI, closed circles) samples are shown. Data are fit to von Bertalanffy (VB) growth model using FI data only (solid lines). Sample sizes (n=) are for FI data only. VB parameters are shown. Growth curves from the south were significantly different from the north and mid regions ($p > 0.05$).

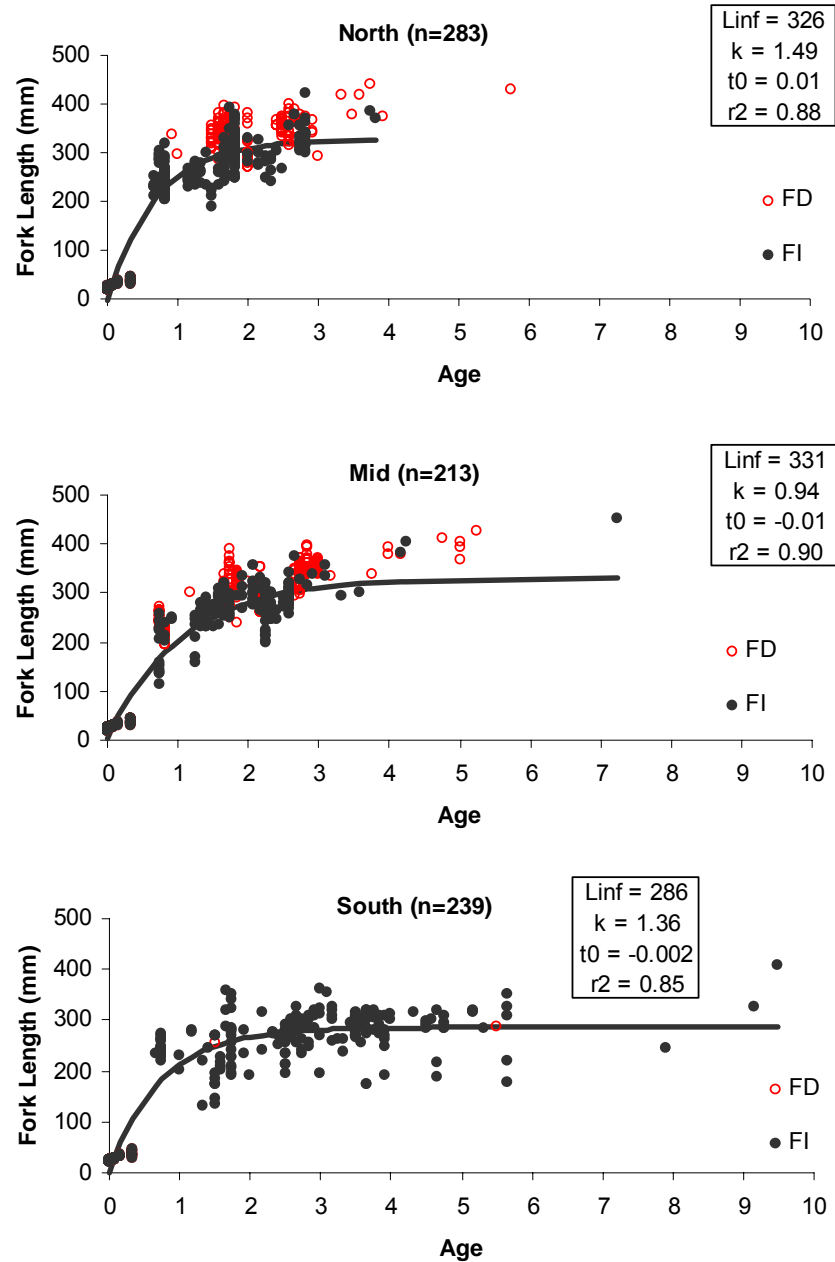


Figure 12: Male striped mullet length at age by sampling region. Fishery dependent (FD, open red circles) and independent (FI, closed circles) samples are shown. Data are fit to von Bertalanffy (VB) growth model using FI data only (solid lines). Sample sizes (n=) are for FI data only. VB parameters are shown. Growth curves from the south were significantly different from the north and growth curves from the north were significantly different from the mid regions ($p > 0.05$).

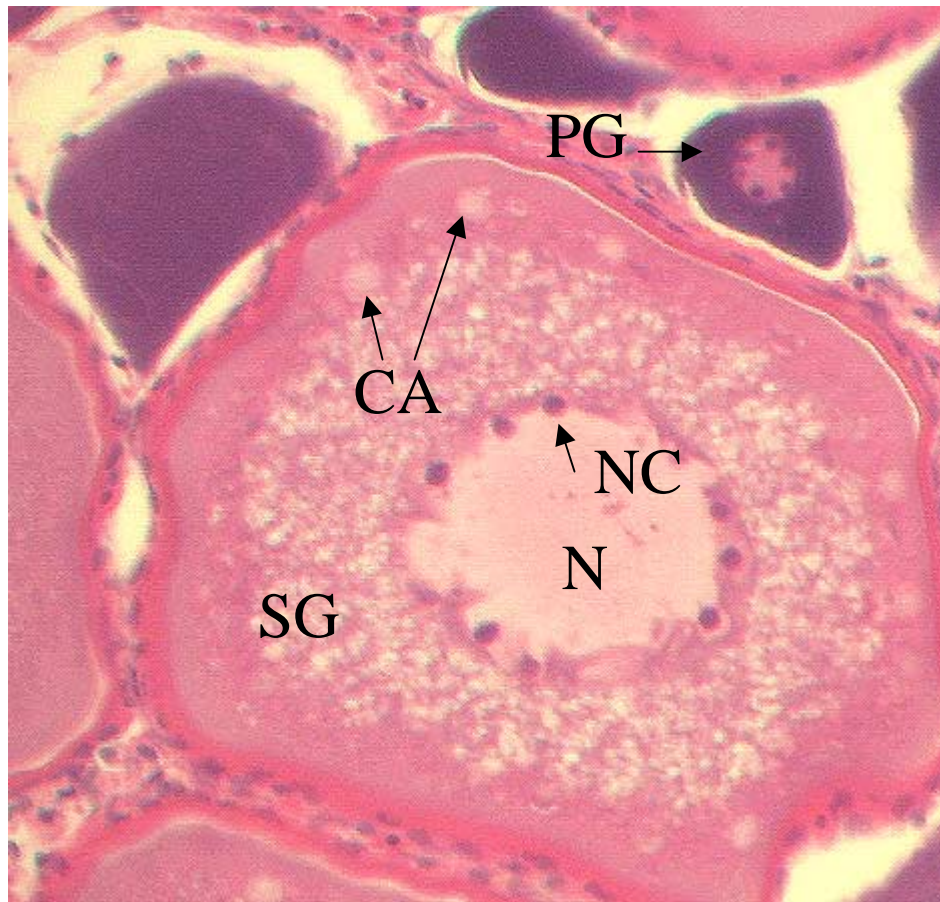


Figure 13: Striped mullet pre-vitellogenic stage oocytes. Secondary (SG) and primary (PG) growth oocytes are shown. Cortical alveoli or granules (CA), oocyte nucleus (N), and nucleoli (NC) are shown.

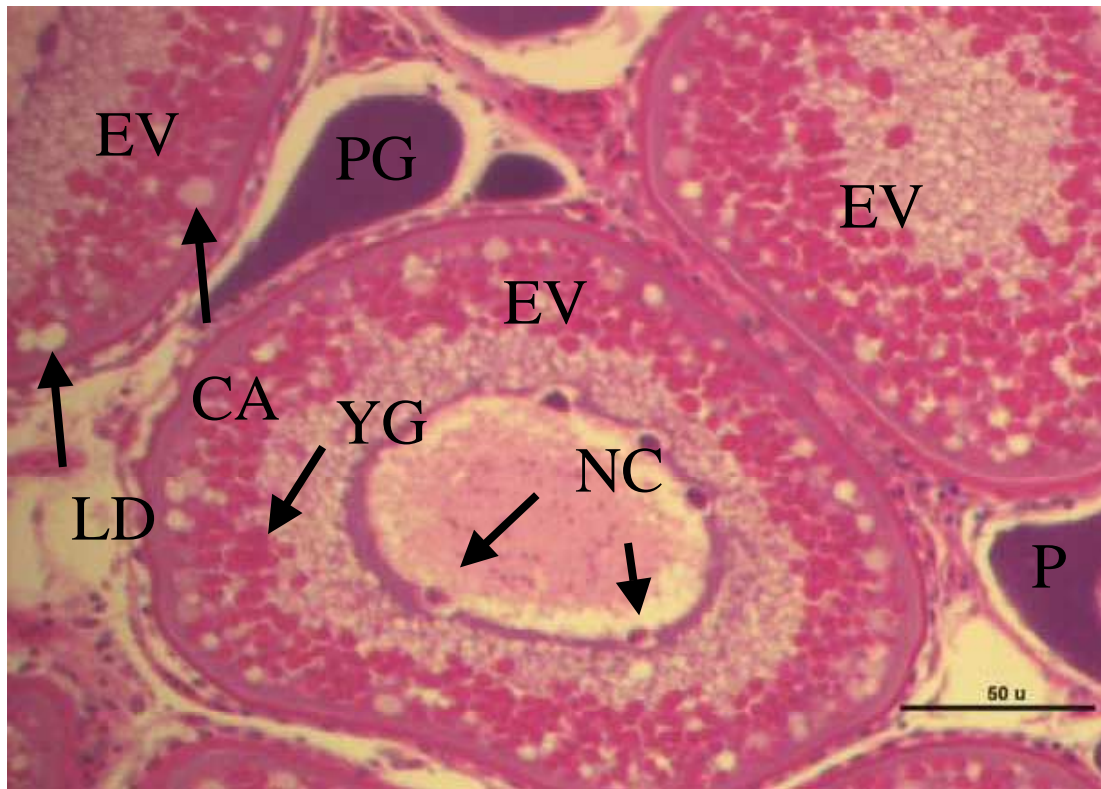


Figure 14: Striped mullet early vitellogenic growth oocytes (EV) with some primary growth oocytes (PG). Cortical alveoli (CA), nucleoli (NC), lipid droplets (LD), and yolk globules (YG) are shown.

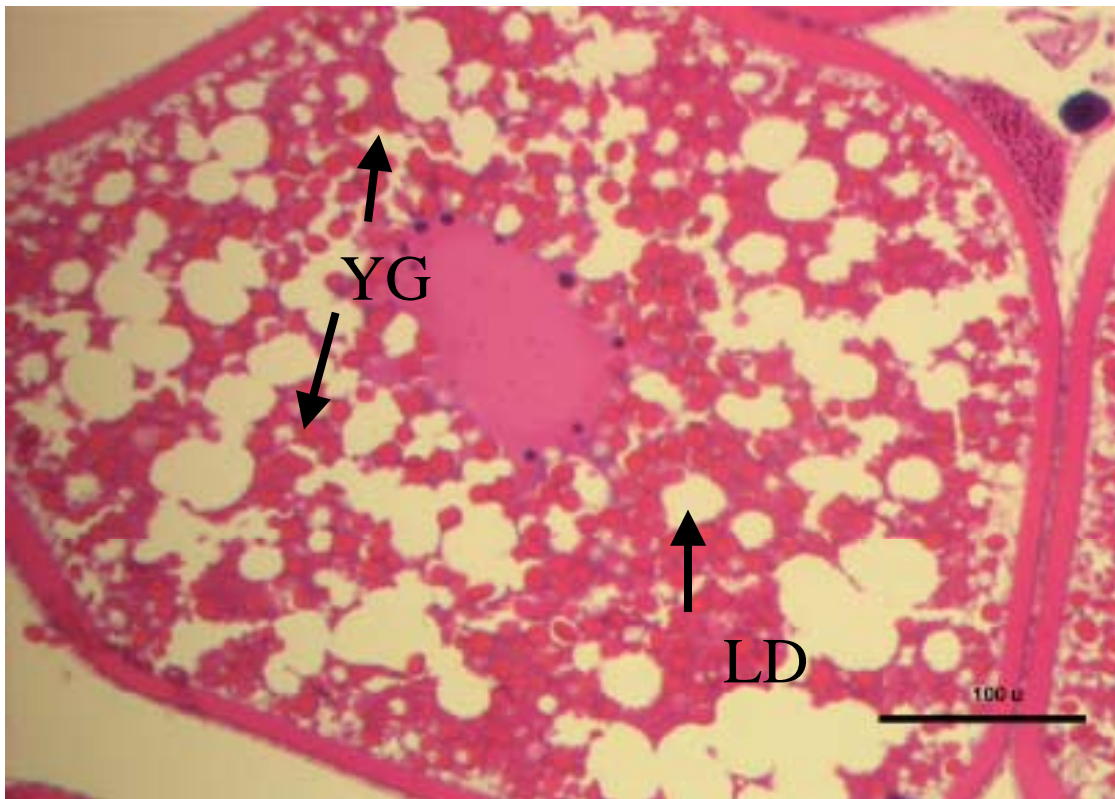


Figure 15: Striped mullet late vitellogenic oocyte. Large lipid droplets (LD) and yolk globules (YG) are shown.

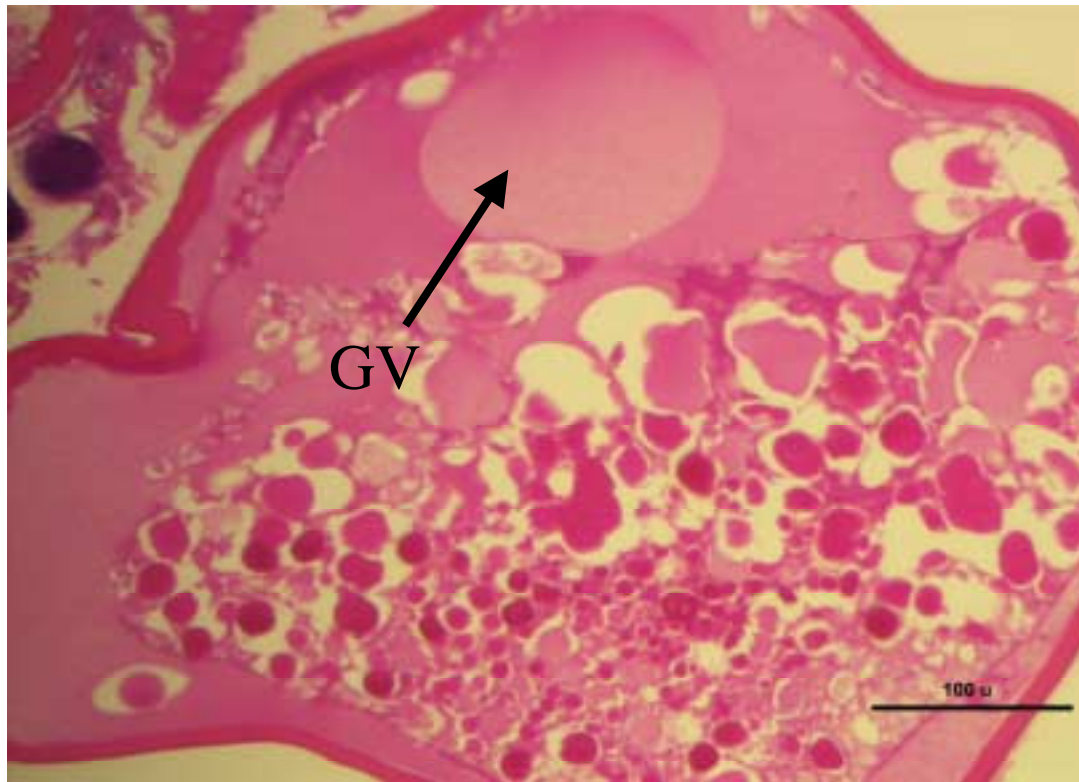


Figure 16: A striped mullet oocyte undergoing final oocyte maturation (FOM). The lipid droplets and yolk globules are coalescing and the germinal vesicle (GV) or nucleus has migrated to the oocyte periphery. This oocyte is one of a few which has not yet ovulated (released from follicle cells).

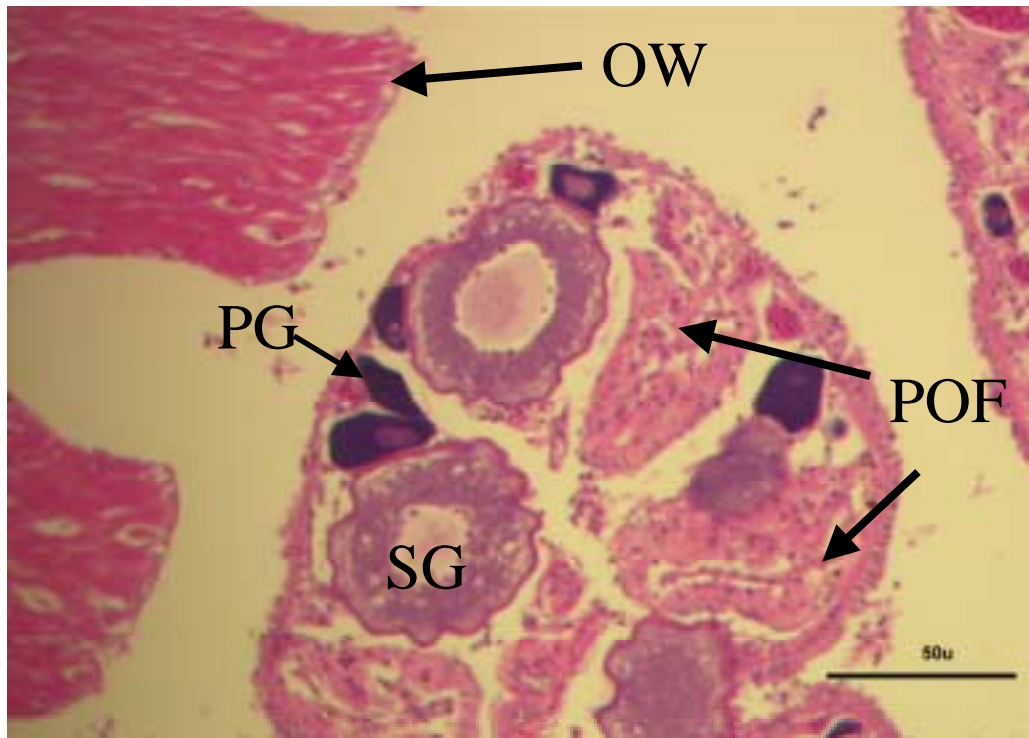


Figure 17: A post-spawned female striped mullet ovarian cross section. Common post spawned ovarian characteristics such as post-ovulatory follicles (POF) and the presence of a thick gonadal wall (OW) are depicted. Secondary (SG) and primary (PG) growth oocytes are shown.

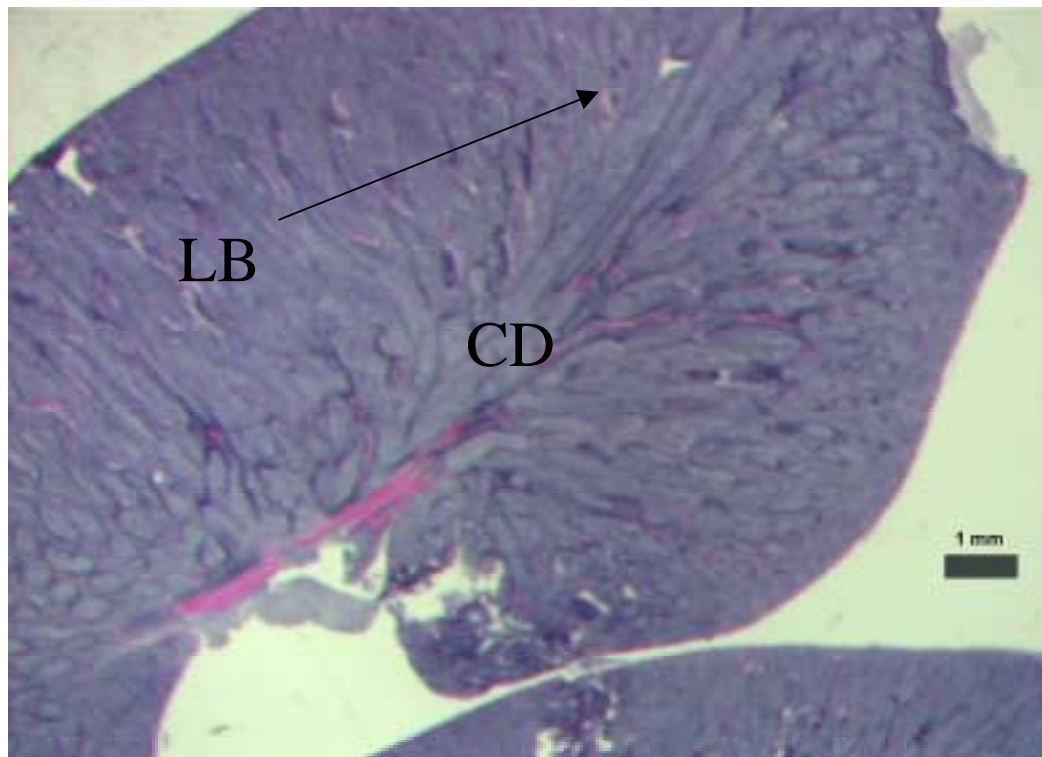


Figure 18: A cross section of a male striped mullet testis in late maturation. Lobules (LB) and collecting ducts (CD) are filled with spermatozoa.

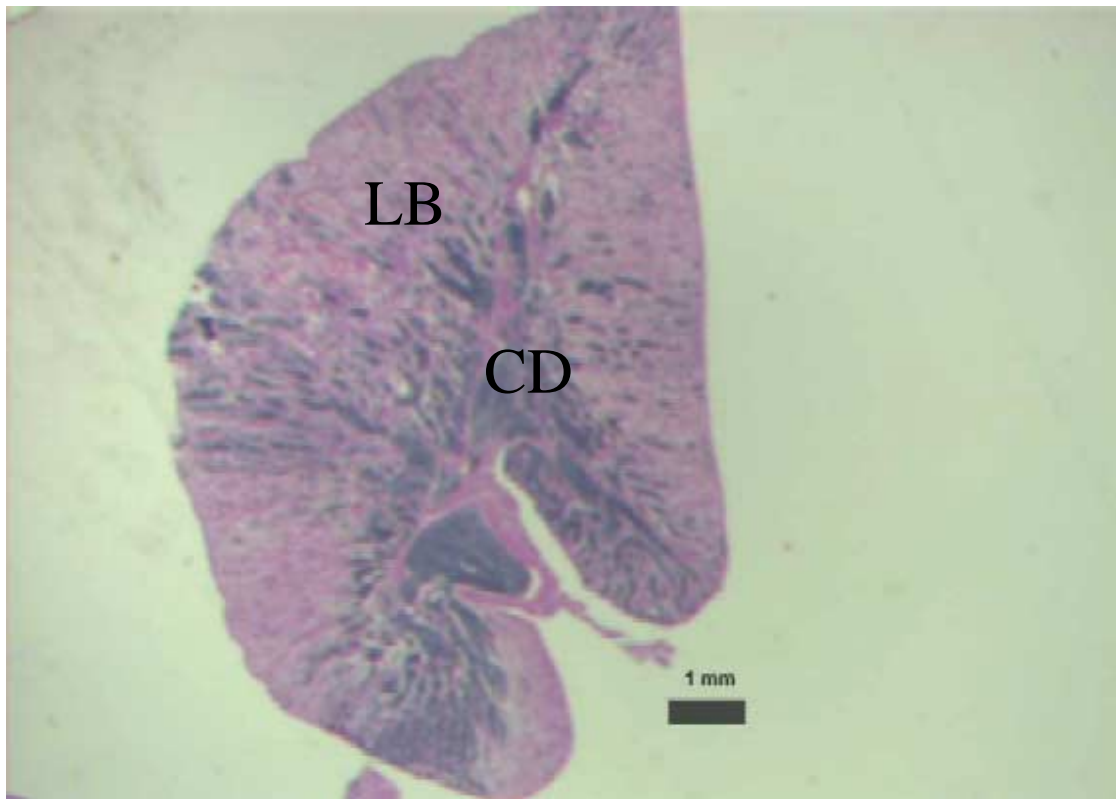


Figure 19: A cross section of a spent male striped mullet testis. Collecting duct (CD) hold residual spermatozoa, but lobules (LB) are mostly empty.

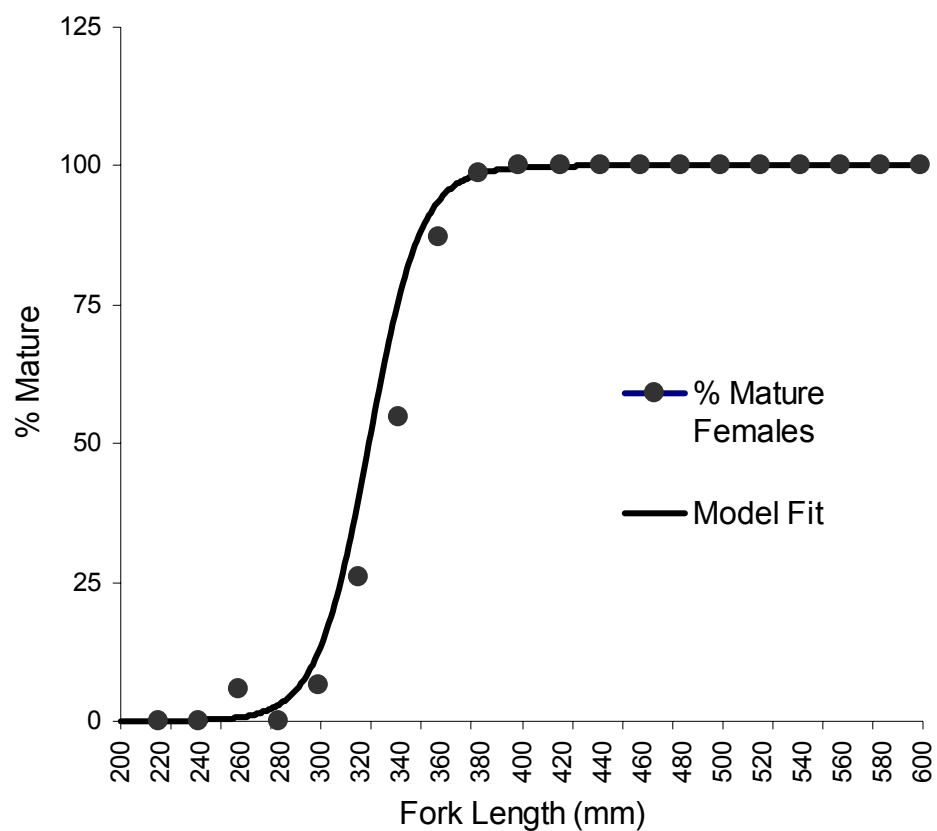


Figure 20: Percentage of female striped mullet (n=1011) mature by 20 mm length classes. Fish were collected between October and December. Data fit using logistic regression (solid line).

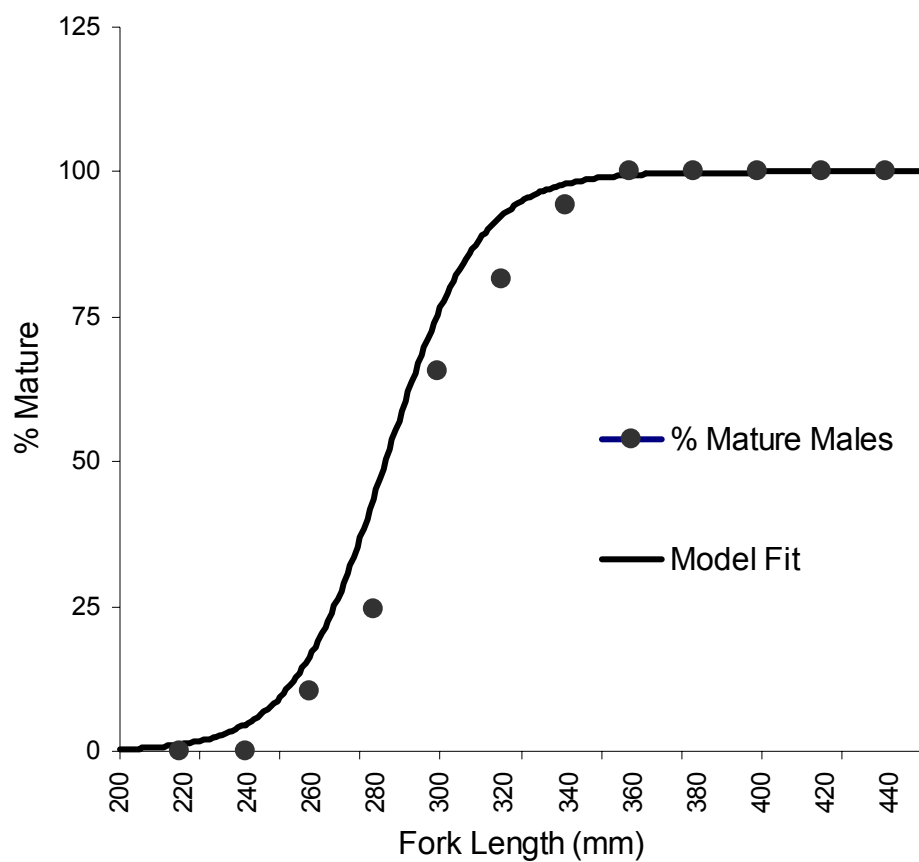


Figure 21: Percentage of male striped mullet (n=556) mature by 20 mm length bins. Fish were collected between October and December. Data fit using logistic regression (solid line).

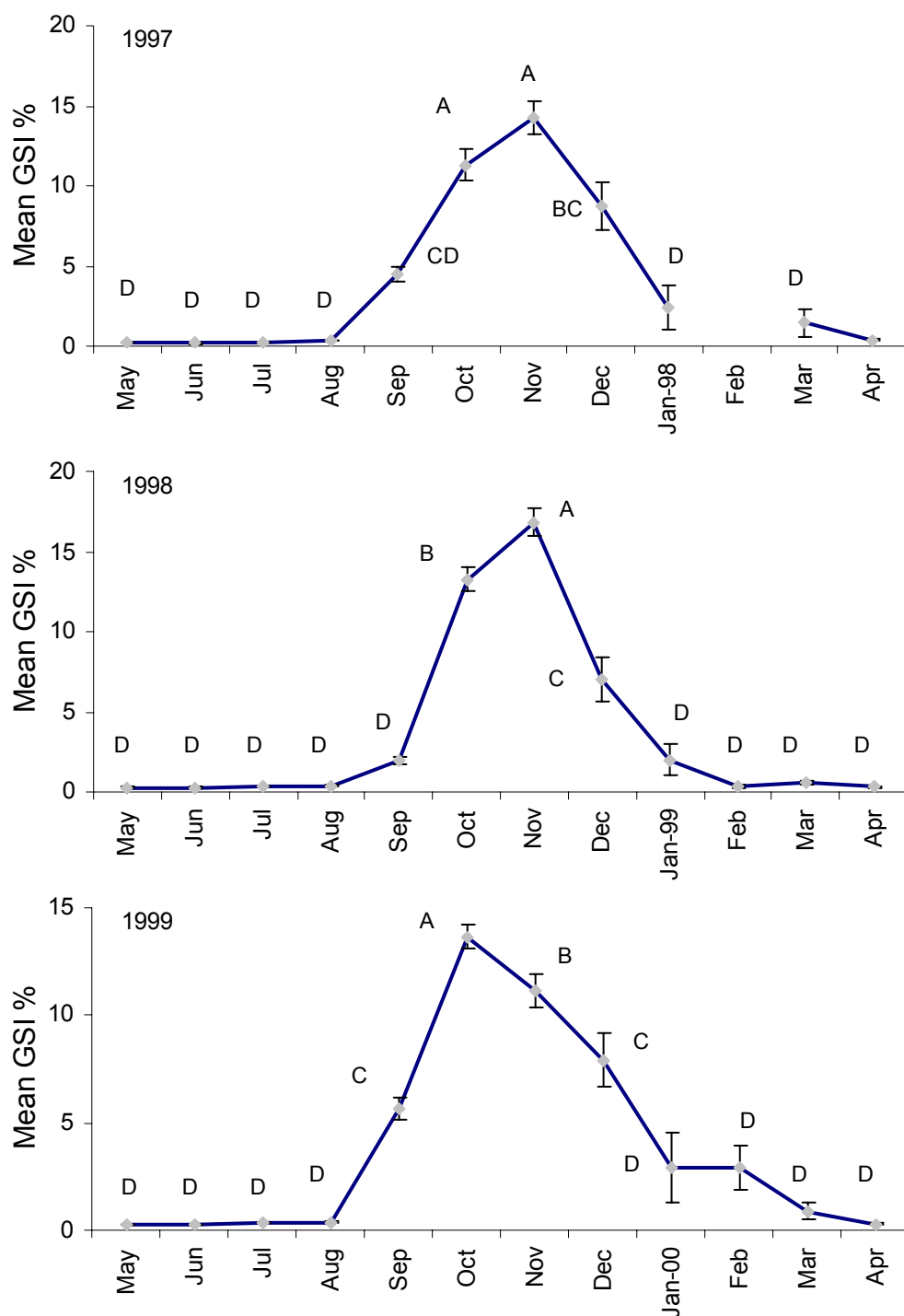


Figure 22: Female striped mullet monthly mean (\pm standard error) gonadosomatic indices (GSI) for the 1997 (top), 1998 (middle) and 1999 (bottom). Means tested using 1-way ANOVA, Duncan's multiple range tests ($P=0.05$).

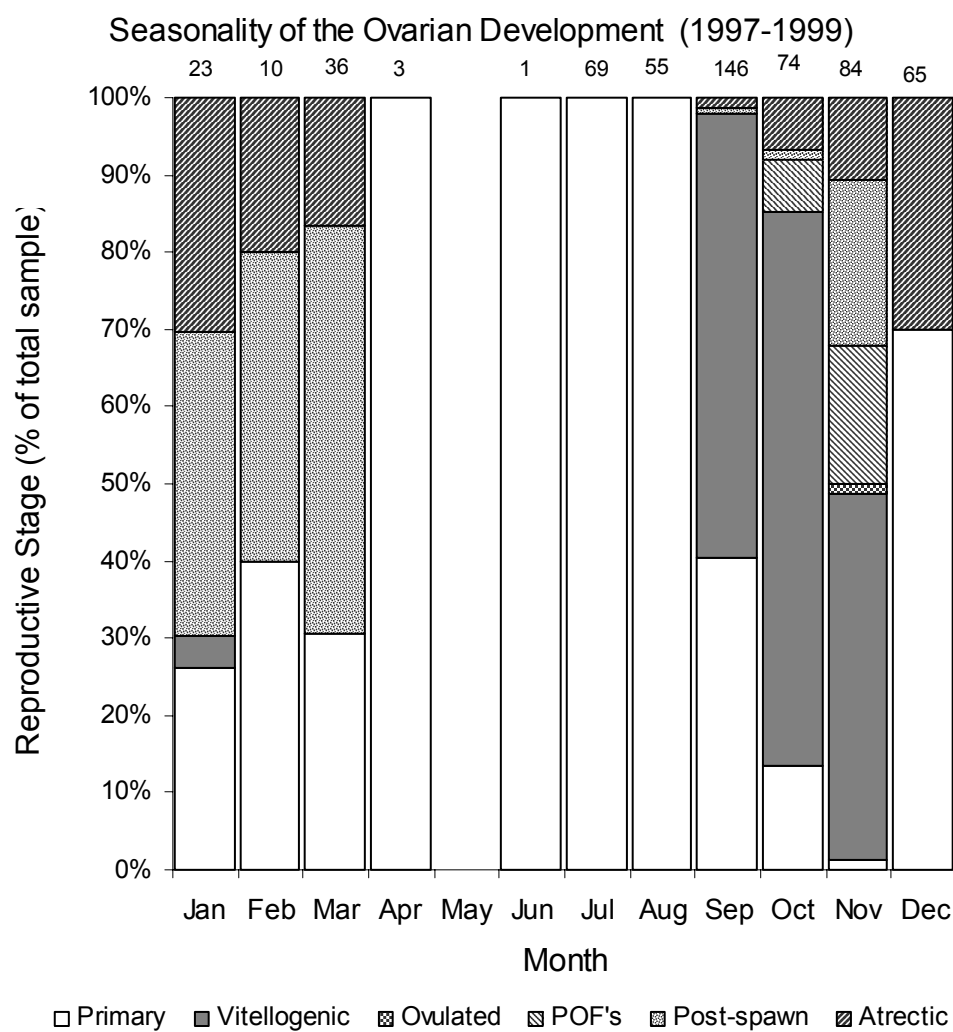


Figure 23: Relative frequency (%) of striped mullet ovarian maturity by month. Data represents pooled samples from 1997-1999. Sample sizes are given above each monthly frequency bar. POF's = post-ovulatory follicles.

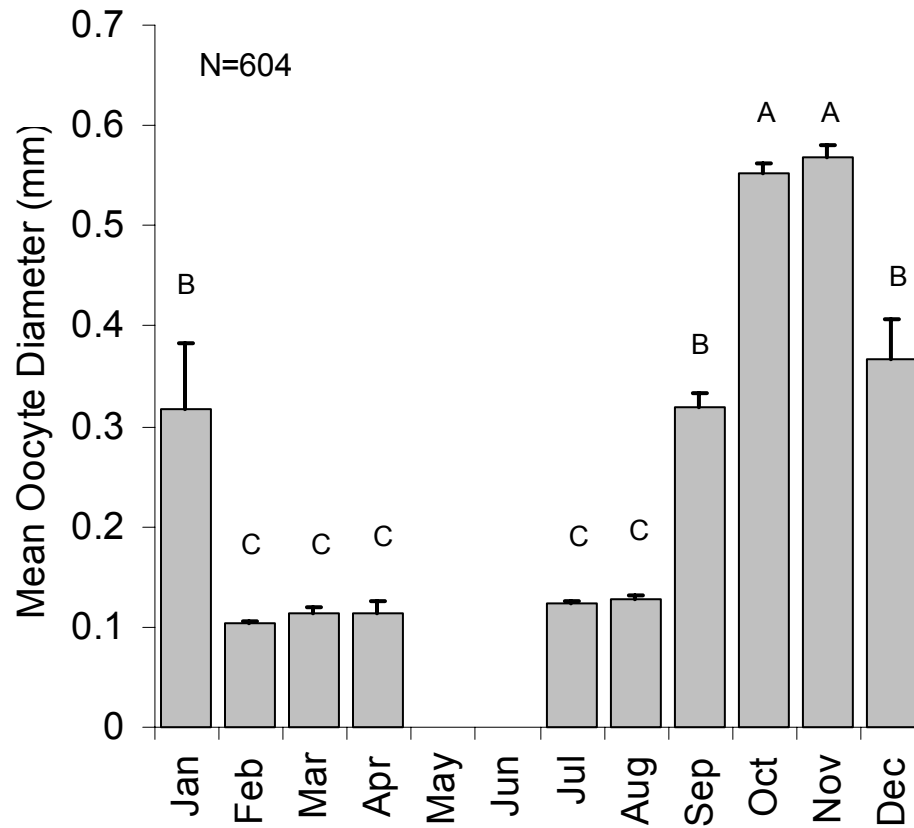


Figure 24: Striped mullet monthly mean oocyte diameters (+/- standard error). Means tested using 1-way ANOVA, and Duncan's multiple range tests (P=0.05). Data pooled for all years sampled 1997-2000.

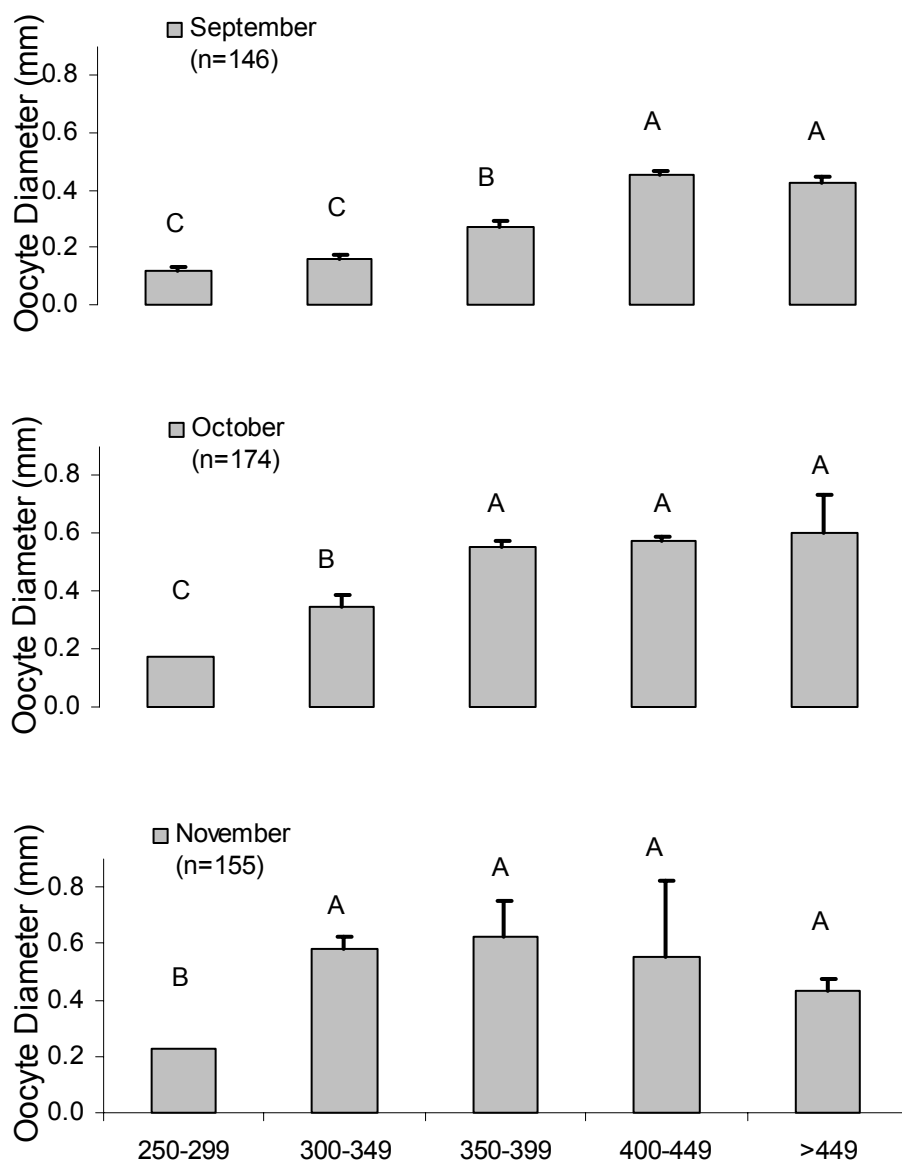


Figure 25: Striped mullet monthly mean oocyte diameters (\pm standard error) by size class (fork length, mm). Means tested within each month using 1-way ANOVA, and Duncan's multiple range tests ($P=0.05$). Data pooled for all years sampled 1997-2000.

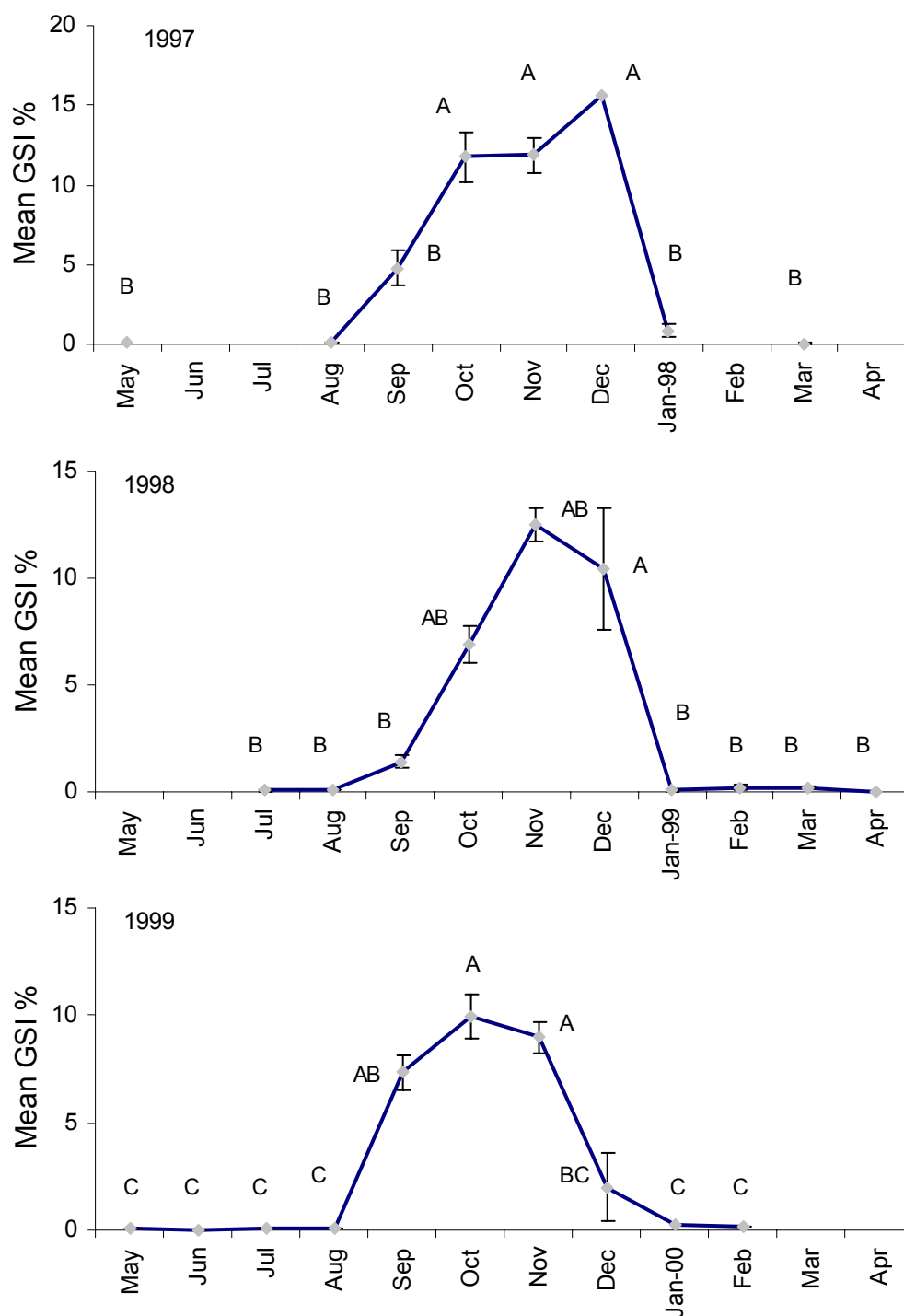


Figure 26: Male striped mullet monthly mean (\pm standard error) gonadosomatic indices (GSI) for the 1997 (top), 1998 (middle) and 1999 (bottom). Means tested using 1-way ANOVA, Duncan's multiple range tests ($P=0.05$).

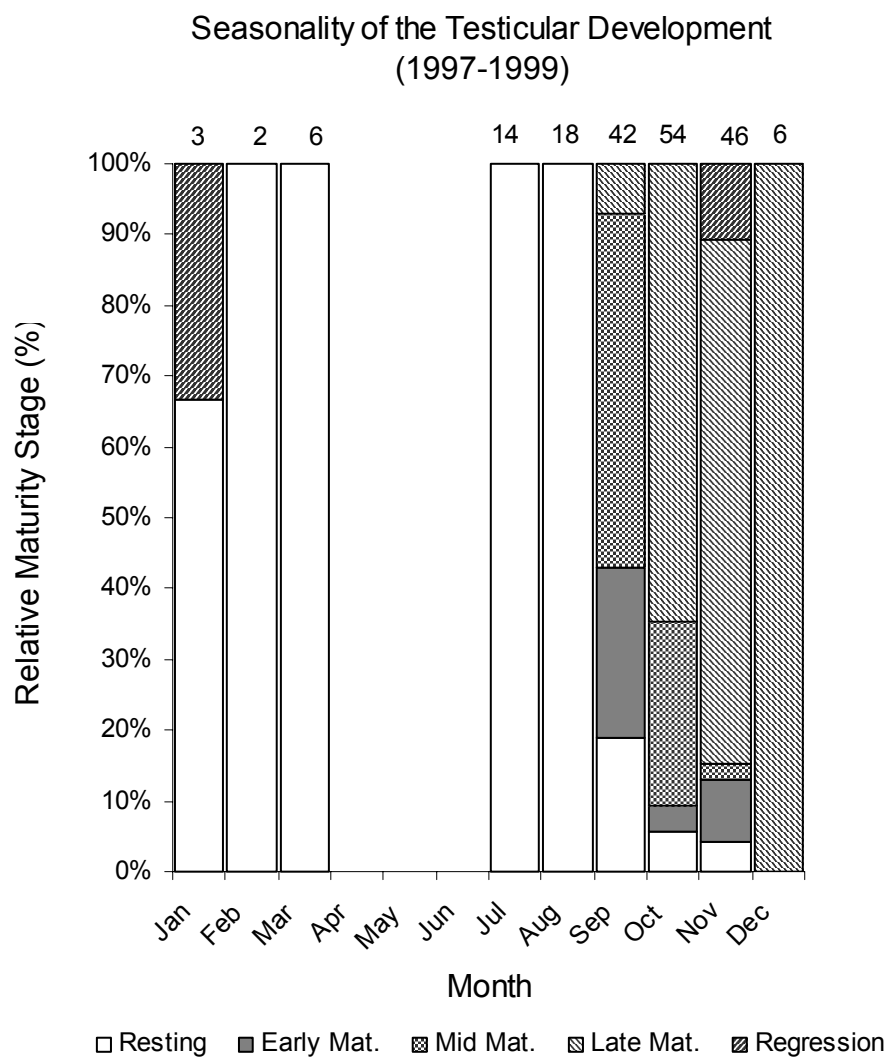


Figure 27: Relative frequency (%) of striped mullet testicular maturity by month. Data represents pooled samples from 1997-1999. Sample sizes are given above each monthly frequency bar.

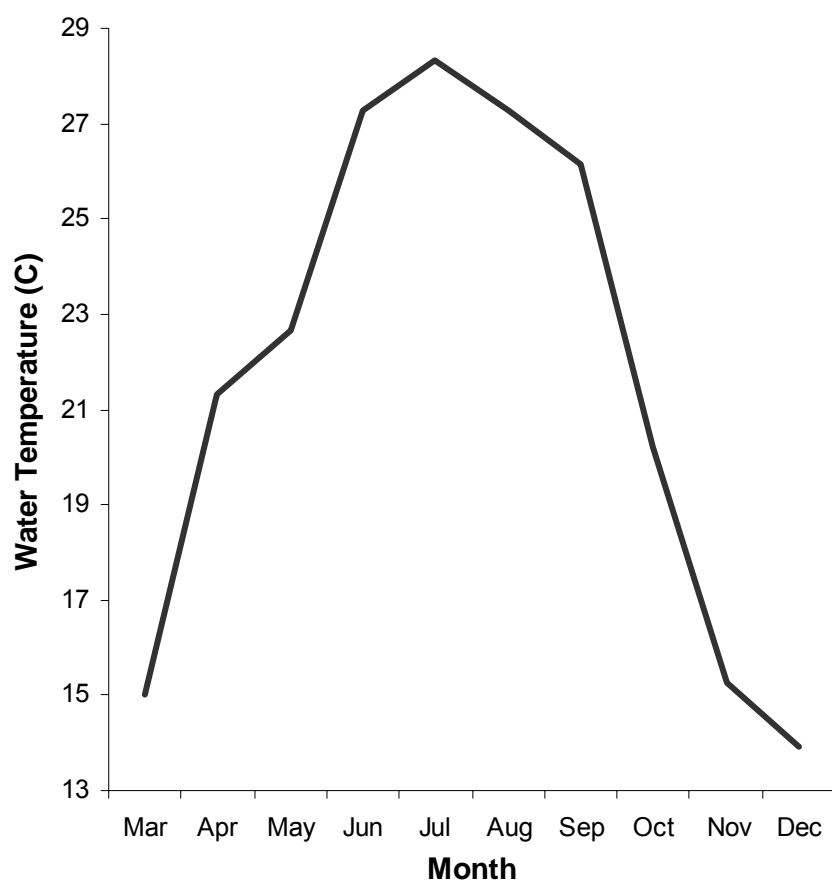


Figure 28: Mean monthly water temperature (C) as measured between 1997 and 1999. Water temperatures were taken at the time of mullet collections throughout the North Carolina sampling area.

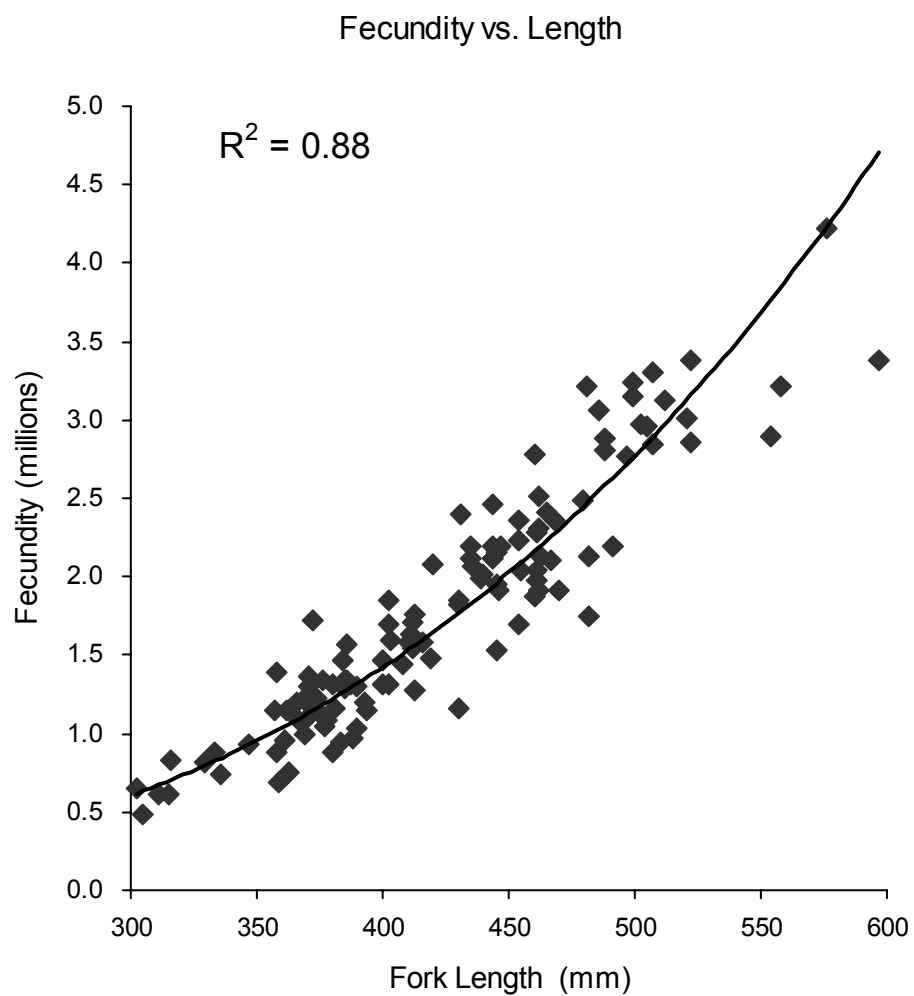


Figure 29: Female striped mullet fecundity to fork length (mm) relationship (n=120).

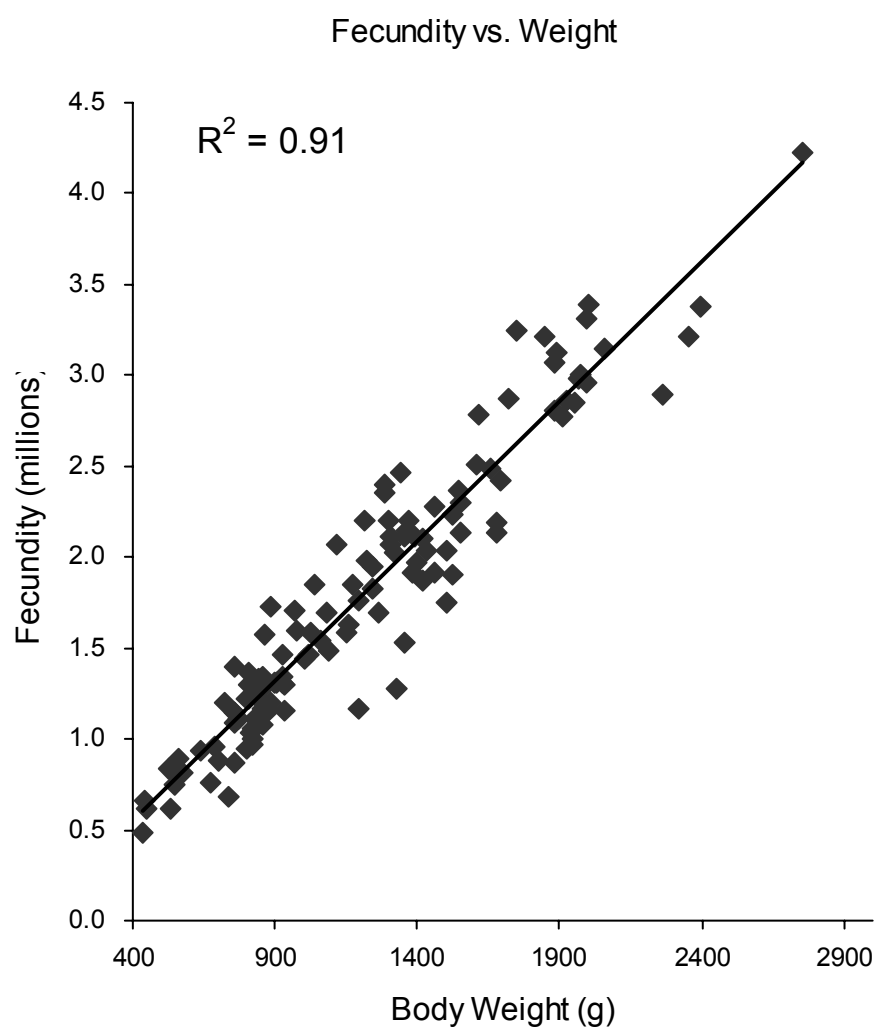


Figure 30: Female striped mullet fecundity to body weight (g) relationship (n=120).