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THE REPRODUCTIVE BIOLOGY OF THE GOOSEFISH,

LOPHIUS AMERICANUS

BY

CATALINA M. MARTINEZ

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

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MASTER OF SCIENCE THESIS

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ABSTRACT

The goosefish, *Lophius americanus*, is a commercially important fish species that is currently overfished. Little is known about the goosefish and the goal of this study was to provide a maturity schedule to assist fisheries managers in the development of an updated minimum catch size. My objectives were to describe gamete and gonad development by applying histochemical techniques to the analysis of gonadal tissue collected in several seasons from wild goosefish.

Gonadal tissue samples were collected during the winter, summer, and fall 1998 National Marine Fisheries Service (NMFS) survey cruises, and summer samples were also collected from the commercial fishery. Gonadal tissue samples were fixed in a buffered formalin solution, processed histologically, and stained with either hematoxylin and eosin as a general tissue stain, or osmium tetroxide to stain for lipids with eosin as a counterstain. Through microscopic examination of ovarian tissue, criteria were determined for maturity classification of oocytes. Seven stages of ovarian maturity were described using these criteria, and a maturity ogive was constructed to calculate length-at-maturity estimates.

As of the passage of Amendment 9 to the Northeast Multispecies Fishery Management Plan in January of 1998, the north Atlantic goosefish population has been managed by northern and southern regions, each with its own minimum catch size. The current minimum catch size for the northern region is 43.2 cm, and for the southern region is 53.3 cm.

The minimum catch size is an essential fisheries management tool, and is typically determined using estimates such as the mean length-at-maturity for female

fishes. The method used by NMFS to determine maturity is macroscopic examination of the gonad. Our current understanding of oocyte development in fishes indicates that these criteria can lead incorrectly to the assignment of immature females to the mature category, and the criteria used are also highly subjective. The most recent estimate of the L_{50} for female *L. americanus* is 44 cm (Almeida *et al.*, 1995) and macroscopic examination was the method used. The results from this maturity study indicate that the L_{50} for females is 57 cm, which is an alarming result given the current minimum catch sizes of 43.2 cm and 53.3 cm. The information provided by histological examination of gonads very likely offers a more accurate picture of maturity than the traditional method of macroscopic examination and, since successful management is the goal, this new maturity information should be considered.

Key Words: goosefish; *Lophius americanus*; sexual maturity; ovarian development; oogenesis; egg veil.

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PREFACE

An understanding of the reproductive biology of an organism is essential to the construction of an accurate maturity schedule, which, in turn, is necessary information for the effective management of the population. Efforts to manage the north Atlantic goosefish (*Lophius americanus*) population have been long in coming and have been hampered by the lack of reproductive information on this unusual but commercially important fish species.

It was for these reasons that I chose my research topic, with the main objective of my study being to provide information on the reproductive biology of *L. americanus*, to construct a maturity schedule to assist in the management of this species.

This thesis has been written in manuscript form and will be submitted to the *Journal of Fish Biology*.

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INTRODUCTION

Lophius americanus is predominantly a benthic fish, with a range extending from the Grand Banks and Gulf of St. Lawrence to Cape Hatteras, North Carolina (Bigelow and Schroeder, 1953). The bathymetric distribution of *L. americanus* extends from the shoreline (Bigelow and Shroeder, 1953; others) to approximately 840 m (Markle and Musick, 1974). The goosefish has been known to occur in temperatures ranging from 0-24 °C (Bigelow and Schroeder, 1953; and Armstrong *et al.*, 1992). Although spawning has not been witnessed, the North Atlantic goosefish is believed to spawn from late spring through early fall, depending upon latitude (Wood, 1982; Armstrong *et al.*, 1992).

The goosefish, *L. americanus* (sometimes referred to as monkfish or angler), was considered a “trash” fish until dwindling fish catches and rising prices of the more conventional fishery species shifted the determination of “food fish” to other species such as the goosefish (Armstrong *et al.*, 1992; Hartley, 1995). Prior to the targeted fishery, bycatch accounted for approximately 80% of goosefish landings (New England Fishery Management Council and the Mid-Atlantic Fishery Management Council, 1997). Bycatch currently accounts for approximately 70% of goosefish landings, with the other 30% resulting from the targeted fishery (New England Fishery Management Council and the Mid-Atlantic Fishery Management Council, 1997), which has been in existence for about two decades. Data from research vessel surveys show that relative abundance of *L. americanus* continues to fall steadily, while landings continue to rise (NEFSC, 1993). This is indicative of increased fishing pressure (Hartley, 1995), which is one of several factors contributing to the continuous decline of this North Atlantic lophiid. Immature goosefish were also targeted in what was termed a “pee-wee” fishery, with their tails

marketed as “drumsticks” (Almeida *et al.*, 1995; and Hartley, 1995). Goosefish livers were sold on the Japanese market for up to \$13.00 per pound (Hartley, 1995), which created additional incentive for the taking of immature fish.

Since the passage of Amendment 9 to the Northeast Multispecies Fishery Management Plan in January of 1998, the North Atlantic goosefish has been managed by northern and southern regions – each region with its own minimum catch size (Figure 1). In the northern region the minimum catch size is 43.2 cm and in the southern region the minimum catch size is 53.3 cm. The main reason for the difference in minimum catch size between regions is that the predominance of the directed fishery is in the southern region (New England Fishery Management Council and the Mid-Atlantic Fishery Management Council, 1997). Also, the dominant gear types used in each region and the selectivity of the gear played roles in the determination of catch sizes for the two regions (New England Fishery Management Council and the Mid-Atlantic Fishery Management Council, 1997). Such management policies are used in an attempt to minimize the impact of the fishery on immature fish, allowing a certain percentage of the population to reproduce at least once before being taken out of the reproductive population by the fishery. Thus, accurate maturity information is critical.

Over the course of the past decade, there have been three published reports indicating mean lengths at maturity for the goosefish *L. americanus* (Armstrong *et al.*, 1992; Almeida *et al.*, 1995; Hartley, 1995), with estimates ranging from 36-49 cm for female goosefish. The most recent estimate of mean length-at-maturity for female goosefish is 44 cm (Almeida *et al.*, 1995). The fish used for this estimate were collected from 1975-1993. Hartley (1995) reported mean length-at-maturity of 36 cm from female

fish collected from 1992-1993. Armstrong *et al.* (1992) published a mean length-at-maturity for female goosefish of 49 cm from fish collected from 1982-1985. Macroscopic examination of the gonad is the method traditionally used for maturity determination of fishes, and was the method used in all three reports. Through histological examination of the gonads, a more accurate picture of maturity can be obtained than from this traditional method, and the goal of this study is to provide such information to be used in the management of this North Atlantic lophiid.

The only histological information on the ovarian morphology and oocyte development in *L. americanus* comes from Armstrong *et al.* (1992) in which 33 ovaries were examined for correlation of histological determination of maturity with macroscopic determination. Histological studies have been conducted on the gonads of *Lophiomus setigerus* (Yoneda *et al.*, 1998a, b), and *Lophius piscatorius* (Fulton, 1898; Afonso-Dias & Hislop, 1996). Through the work done on *L. setigerus* and *L. piscatorius*, it has been determined that oocyte development in the Lophiids is similar to that of other teleosts in that the oocytes progress through several consistent stages similar to those described in Bromage and Cumaranatunga (1988) and Tyler and Sumpter (1996) (Afonso-Dias & Hislop, 1996; Yoneda *et al.*, 1998a, b). It was also determined that the ovarian structure of Lophiids was unique among teleosts and that the ovary was uniform throughout the length of the organ (Afonso-Dias & Hislop, 1996; Yoneda *et al.*, 1998a, b).

Very little is known about the reproductive biology of the goosefish, *L. americanus*, and the goal of this study was to provide information for a maturity schedule to assist fisheries managers in the development of an updated minimum catch size. The

objectives were to describe gamete and gonad development through the application of histochemical techniques to the analysis of gonadal tissue.

MATERIALS AND METHODS

Source of Samples

There were two sources for the collection of goosefish samples. The majority of the samples were collected during trawl survey cruises conducted by the National Marine Fishery Service (Woods Hole, MA division) aboard the *R/V Albatross IV*. Winter (1998) and Fall (1998) samples were collected for this study during NMFS bottom trawl survey cruises which sampled from George's Bank to Cape Hatteras (from approximately $40^{\circ}54'$ N – $71^{\circ}51'$ W to $36^{\circ}34'$ N – $74^{\circ}47'$ W), and from depths of approximately 14-200 fathoms (approximately 28-400 m) (Fishermen's Report, February 1997) (Figure 2). Fish collected during winter and fall bottom trawl survey cruises were caught with a standardized #36 Yankee flat trawl (Fishermen's Report, February 1997). Tows lasted approximately 30 minutes, and the codend and upper belly of the trawl were lined with 1/2 inch mesh to retain young-of-the-year fishes (Fishermen's Report, February 1997). Most summer samples were collected during the NMFS 1998 Summer Scallop Survey Cruise, which also sampled from George's Bank to Cape Hatteras, but sampled from depths of approximately 15-63 fathoms (approximately 30-126 m) (Fishermen's Report, July-August 1998). Fish were caught with a standard eight foot New Bedford type scallop dredge, and tows lasted fifteen minutes (Fishermen's Report, July-August 1998).

The other source of samples were collected during late summer, 1998, and were collected by the F/V *Laura Lynn*, a commercial trawler that docks in Point Judith, Rhode Island. The commercial trawler collected fish westward of the closed Nantucket region with a three-inch mesh whiting trawl with a 160 foot sweep. All fish obtained from the F/V *Laura Lynn* were of sub-legal size (less than 43 cm total length), but none less than 28 cm were caught with the commercial trawl.

Sampling Procedures

Tissue sample and data collection

During the NMFS cruises, the following information was collected from goosefish caught aboard the R/V *Albatross IV*. Total length, total weight, and gonad weight were measured, and macroscopic determination of sex and maturity was recorded. Total weights were not recorded during the first leg of the summer 1998 NMFS cruise, and some gonad weights were also not recorded during the fall NMFS cruises.

Gonadal tissue samples were collected and placed in pre-filled vials of 10% neutral buffered formalin. If the gonads were ribbon-like and not discernible as male or female tissue, the entire paired gonads were placed in the pre-filled vials of formalin solution. Approximately 24 hours later, the tissue in formalin solution was transferred to pre-filled vials of 70% ethanol, where they were stored for later histological analysis. The sample vials were pre-numbered and the vial numbers corresponding to each fish were recorded on the sample data sheets.

During the summer and fall NMFS cruises, if fish were so small that collectors were unsure of gonad location, the fish were frozen whole for later dissection. This did not occur during the winter cruises, so no samples were obtained from fish less than 28

cm total length. At the end of each cruise, goosefish data sheets from each trawling station were photocopied and collected so that the data could be used in this study.

The fish collected on the commercial trawler *Laura Lynn* were stored on ice until the boat docked, and trips lasted approximately 3-4 days. The fish were then transferred to a cooler and stored on ice until sampling took place (within one or two days). Information recorded was total length and weight of each fish, gonad weight, and macroscopic determination of sex. Due to the time lapse between fish collection and fish sampling, gonads were discolored, so no attempt was made to determine stage of maturity macroscopically. Gonad samples were collected and preserved in the manner described for sample collection aboard the *R/V Albatross IV*. Vertebrae were not collected for age determination from the fish obtained from the commercial fishery.

Histological processing of samples

Small mid-sections of gonad tissue were processed for histological examination. This processing consisted of an ethanol dehydration series, clearance in xylene, and infiltration of tissue by paraffin. The samples were then embedded in paraffin blocks and sectioned at 5 μm . Sections were then adhered to glass slides and stained with hematoxylin and eosin (Manual of Histological Staining Methods of the Armed Forces, 1968). Osmium tetroxide was also used to stain for lipids and eosin was used as a counterstain (Manual of Histological Staining Methods of the Armed Forces, 1968).

Microscopic examination

Observations were made with a Nikon Eclipse I600 microscope, and images were collected using a Spot-100 digital camera and a PowerMac 8600/200. Criteria were determined for maturity classification of oocytes with Tyler and Sumpter (1996) and

maturity criteria for summer flounder (Merson *et al.*, in review) used as references. Six stages of oocyte development were determined. Seven stages of ovarian development were defined by the developmental stage of the most advanced oocytes within the ovary.

The Image Pro Plus (version 4.0) program was used for analysis of oocyte diameters. Oocyte diameters were calculated by taking the mean of the maximum and minimum diameter of those oocytes that had been sectioned through the nucleus. Foucher and Beamish (1980) found this method to be representative of the true oocyte diameter. Oocytes in the stage of final oocyte maturation were measured whether a nucleus was apparent or not since the process of germinal vesicle breakdown may have occurred by this stage.

Three ovarian samples from each stage of ovarian development were analyzed from the summer samples, with the exception of stage five. There were only two ovaries in the fifth stage of development and so analysis was limited to this number. Three fields were captured per ovary and all oocytes present that had been sectioned through the nucleus were measured in each field to obtain information on the distribution of oocyte diameters present. These same ovarian samples were used to determine the percent frequency of occurrence of oocyte developmental stages at each ovarian stage.

DATA ANALYSIS

Length at first maturity

The object of this analysis was to determine the length at which 50% (L_{50}) and 95% (L_{95}) of the fish sampled were reproductively mature. A sample size of 185 was used for females and included all ovarian samples from the winter, summer, and fall. The

gonads of all fish were examined microscopically, and the determination of reproductive maturity was made. Fish were categorized as either mature or immature. The samples from all three seasons were also analyzed individually for both the L_{50} and the L_{95} .

The random component of the data was binomial and non-linear, and once represented graphically, fit a logistic curve. Thus logistic regression analysis was used to model the probability of a fish of a particular length being part of the mature population. The following logistic regression function was used for the model:

$$\text{Log}[p(x)/(1-p(x))] = \alpha + \beta x \quad (1)$$

with p = probability, α = y-intercept, and β = slope.

The suitability of this model was analyzed with the Wald Chi-square goodness of fit test, as well as the Chi-squared statistic for the maximum log likelihood functions. The parameters α and β were estimated using the Logistic function in SAS (version 6.10), and the model can be treated as a generalized linear model.

RESULTS

Differentiation of Gonadal Tissue

Differentiated gonadal tissue was evident in fish as small as 12 cm total length. An early sign of ovarian differentiation was the outpocketing of the ovigerous lamellae, forming cyst-like structures containing clusters of developing oogonia (Figure 3(a)). Differentiation of testicular tissue was indicated by the formation of seminiferous tubules that contained small clusters of developing spermatogonia (Figure 3 (b)).

Oocyte Development

Six stages of oocyte development were determined through microscopic examination of samples and were defined as follows.

Pre-primary growth oocytes were defined as the first stage of oocyte development and these darkly staining oocytes consisted of a thin layer of ooplasm surrounding a very large nucleus. Stage 1 oocytes were typically less than 100 μm in diameter.

In a primary growth oocyte, or developmental stage 2, the nucleus appeared smaller in relation to the amount of ooplasm present and multiple nucleoli were typically apparent (Figure 4 (a), (b)). One to several spherical lipid droplets were often observed in the ooplasm of primary growth oocytes (Figure 4 (b)). The lipid inclusions appeared as non-staining spheres in the ooplasm when sections were stained with hematoxylin and eosin (Figure 4 (a)), and stained black with osmium tetroxide (Figure 4 (b)). The mean diameter of oocytes in developmental stage 2 was $201 \pm 3 \mu\text{m}$.

The ooplasm of oocytes in developmental stage 3 contained many spherical lipid droplets and/or cortical alveoli, but no yolk protein granules, and the follicle layer had become clearly visible (Figure 4 (c), (d)). The diameter of these pre-vitellogenic secondary growth oocytes had increased to a mean of $300 \pm 8 \mu\text{m}$.

Small yolk protein granules (which stained pink with hematoxylin and eosin) could be seen among lipid globules at the periphery of the ooplasm in early stage 4 oocytes (Figure 4 (e)). The mean diameter of early stage 4 oocytes was $320 \pm 7 \mu\text{m}$. As vitellogenesis progressed, more yolk protein granules entered the oocytes and coalesced to form larger yolk spheres which migrated towards the center of the ooplasm.

Eventually the ooplasm appeared to be filled with these larger yolk globules and the oocytes had grown considerably in size, reaching a mean diameter of $610 \pm 31 \mu\text{m}$ (Figure 4 (g)).

During final oocyte maturation (developmental stage 5), yolk protein globules coalesced in the ooplasm forming a homogeneous pink fluid, within which one or two large lipid globules were typically present (Figure 4 (i)). The nucleus was absent in these oocytes, as germinal vesicle breakdown may have already occurred. The diameter of the stage 5 oocytes had increased to a mean of $1027 \pm 26 \mu\text{m}$.

The sixth stage of oocyte development is the post-ovulatory follicle (Figure 4 (k), (l)). A lumen was sometimes observed in the post-ovulatory follicle (Figure 4 (k)), but was often absent.

Ovarian Development

Seven stages of ovarian development were determined through microscopic examination and were defined by the developmental stage of the most advanced oocytes within the ovary (Table 1).

Ovarian developmental stage 0 was the earliest sign of ovarian differentiation with outpocketing of the ovigerous lamellae forming finger-like projections containing clusters of developing oogonia (Figure 3 (a)). A stage I ovary consisted of nests of oogonia, with one or two darkly staining pre-primary growth oocytes developing at the terminal position within each cluster (Figure 5 (a)).

In a stage II ovary, several primary growth oocytes were present within each cluster and nests of oogonia could be seen at the basal portion of the clusters (Figure 5(b)).

A stage III ovary consisted of several primary growth oocytes with one or two pre-vitellogenic secondary growth oocytes per cluster (Figure 5 (c)). Primary growth oocytes were present at each stage of ovarian development in the ovaries examined (Figure 6), and represented the most abundant stage present within the ovaries at each stage (Figure 7).

Yolk protein granules became apparent in the peripheral ooplasm of the most advanced oocytes in stage IV ovaries – an obvious indication of vitellogenesis (Figure 5 (d)). The yolk protein granules increased in number and fused to form larger spheres (globules), which appeared to fill the ooplasm of late stage IV ovaries (Figure 5 (f)).

As ovarian maturation progressed, a small proportion of oocytes increased in diameter, and by late ovarian developmental stage IV there was a clear demarcation in size of this small group of oocytes (Figure 7). By stage V this group of oocytes had increased in diameter by several magnitudes (Figure 7).

Final oocyte maturation (FOM) was apparent in stage V ovaries, whereby the yolk protein globules and lipid droplets in the ooplasm of the maturing oocytes had coalesced, and germinal vesicle breakdown (GVB) had probably occurred since the nucleus was no longer observed (Figure 5 (g)). As the ovary developed, the epithelial lining of the ovigerous and non-ovigerous lamellae secreted a thick mucogelatinous matrix that would eventually fill the lumen and form the egg veil. This gelatinous material could be seen surrounding the oocyte clusters in stage V ovaries (Figure 5 (g)), and also in late stage IV ovaries (Figure 5 (f)).

Atretic follicles were present in post-spawning ovaries (developmental stage VI), and several primary growth oocytes were typically present within each cluster of post-spawning ovaries (Figure 5 (h)). Secondary growth oocytes were also present in all post-spawning ovaries in various stages of development (Figure 6). Maturing oocytes in the size range 300-500 μm were present in stage VI atretic ovaries, and this size range was consistent with that of vitellogenic oocytes (Figure 7).

Secondary growth oocytes were evidenced by cytoplasmic inclusions such as lipid droplets, cortical alveoli, and yolk protein globules, and the recruited oocytes were larger than the other oocytes in the clusters. The presence of clearly recruited secondary growth oocytes was indicative of a mature or maturing ovary, and was the criteria used to determine the maturity status of the ovaries for this study. For maturity classification purposes, all ovaries in developmental stages 0, I, and II were considered immature since there were no secondary growth oocytes present. All ovaries in developmental stages III, IV, V, and VI were considered mature since secondary growth oocytes were present.

Seasonality of ovarian stages

In all seasons, ovarian developmental stage II occurred with the most frequency (Table 2). The only ovarian developmental stages represented in the winter samples were stages II and IV (Table 2). The majority of the summer and fall ovarian samples were found to be immature (73% and 63%, respectively) (Table 2). Mature ovaries in a pre-spawning state (stage V) were found only in the fish collected during the summer months, and in a very low number (Table 2). 13% of the summer ovarian samples and

15% of the fall samples included atretic follicles (stage VI), and thus were in a post-spawning condition (Table 2).

Length at maturity

Lengths at which the ovaries of fish were determined to be mature were clearly demarcated in winter samples, with all fish over 55 cm total length in the fourth stage of ovarian development (Figure 8). No fish less than 25 cm total length were included in the sample collection from the winter months (Figure 8). Some very large length classes included immature fish in both the summer and the fall samples (Figure 8). 38% of the summer samples and 80% of the fall samples from fish between 56-65 cm total length were immature, and 25% of the fall samples from fish between 66-75 cm total length were immature (Figure 8). All fish greater than 75 cm total length were mature in all seasons (Figure 8). It was determined that the length at which 50% of the fish from all seasons were considered mature (the L_{50}) was 57 cm and the L_{95} was calculated as 63 cm. (Figure 9). A breakdown by season of the calculations for the L_{50} and the L_{95} can be found in Table 3, with the largest L_{50} of 53 cm calculated for the winter samples and the largest L_{95} of 59 cm calculated for the fall samples.

DISCUSSION

The present histological study examined in detail for the first time the ovarian structure and stages of oocyte and ovarian development in *L. americanus* during the winter, summer, and fall seasons. Observations were made on ovaries of fish collected through survey cruises conducted by the National Marine Fisheries Service (Woods Hole, MA), and also from fish collected by a commercial trawling vessel. Histological criteria

were defined for the purpose of differentiating immature ovaries from those that were maturing or mature, and an updated mean length at maturity was calculated from this information. Gonadal differentiation and gamete and gonad development are described below, and management implications are discussed.

Through histological examination it has been determined that stages of ovarian development in *L. americanus* corresponded to stages described for *L. setigerus* by Yoneda *et al.* (1998a, b). It was also determined that the process of oocyte development in the goosfish is similar to that of other teleosts in that the oocytes progress through several consistent stages similar to those described in Bromage and Cumaranatunga (1988) and Tyler and Sumpter (1996). The major developmental events are oogenesis, primary growth, lipid inclusion/cortical alveolus stage, vitellogenesis, maturation, and ovulation.

The ovaries of the goosfish are fused at their posterior ends and form a long, wide, flattened tube connected to a thin mesovarium. The fusion of the two ovarian lobes is an adaptation for the production and expulsion of gelatinous egg masses and is known to occur in Lophiiform species (Fulton, 1898; Rasquin, 1958; Armstrong *et al.*, 1992; Hartley, 1995; Afonso-Dias and Hislop, 1996) and some Scorpaeniform species (Fishelson, 1977, 1978; Erickson & Pikitch, 1993; Koya *et al.*, 1995). Most other teleosts have two distinct organs (Fulton, 1898; Rasquin, 1958; Armstrong *et al.*, 1992; Hartley, 1995; Afonso-Dias and Hislop, 1996). This long confluent, specialized organ is coiled up in the abdominal cavity of the goosfish and is sometimes mistaken for the intestine (personal observation). The dimensions of the goosfish ovary vary with size of fish and

time of year (Fulton, 1898), with a marked increase in size prior to spawning due to the production of the egg veil.

The lumen of the goosfish ovary is lined with epithelial cells that cover ovigerous and non-ovigerous sections of the ovary (Afonso Dias and Hislop, 1996). Stalk-like oocyte clusters protrude from the ovigerous wall of the ovary and are covered by this epithelial layer that can be one to several layers thick (ovigerous lamellae epithelium). The facing ovarian wall is non-ovigerous, thus the oocyte clusters develop from one side of the ovary only. The ovarian epithelium is responsible for the production of the mucogelatinous material that eventually fills the lumen and develops into the egg veil.

The stalk-like clusters that protrude from the ovigerous lamellae of the goosfish ovary contain oocytes in a gradation of developmental stages. The most developed oocytes within each cluster are located at the terminal position suggesting a location dependent recruitment of oocytes into secondary growth. Although several oocytes per cluster may be recruited into secondary growth, only one oocyte per cluster becomes fully mature and is spawned.

In order to classify very small juvenile fish as male or female, it was necessary to determine the point at which an undifferentiated gonad had begun to differentiate into ovarian or testicular tissue. The sex-specific characteristics of germ cells in *L. americanus* were apparent in fish as small as 12 cm total length. Differentiation of testicular tissue was indicated by the formation of seminiferous tubules that contained developing spermatogonia. An early sign of ovarian differentiation was the outpocketing of the ovigerous lamellae, forming finger-like projections surrounding clusters of

developing oogonia. Early ovarian development was identifiable in the muskellunge (*Esox masquinongy*) at 13.8 cm total length (Lin, *et al*, 1997) and at 14 cm total length in *Salaria pavo* (Patzner and Kaurin, 1997). In the muskellunge, it was determined that gametogenesis occurred earlier in females than in males, and it appears that this may also be the case in *Salaria pavo* (Patzner and Kaurin, 1997). It was not possible to make such a determination for *L. americanus* from this study due to the small number of samples collected at this very early stage of development.

In *L. americanus* the process of oogenesis culminated in stage I ovaries which consisted of pre-primary growth oocytes (less than 100 μm in diameter) and nests of oogonia at the basal position of each cluster. As maturation progressed, oocytes grew considerably in size which resulted in primary growth oocytes with a mean diameter of 201 μm . The first appearance of primary growth oocytes was indicative of a stage II ovary. Stage II ovaries from this study were consistent with the “virgin” ovarian stage in *L. piscatorius* from the Afonso-Dias and Hislop study (1996), and with the “immature” stage in the ovaries of *L. setigerus* in the study by Yoneda *et al.* (1998b). All ovaries in developmental stages 0, I, and II were considered immature.

At ovarian developmental stage III, one or two oocytes located at the terminal position of each cluster were recruited into secondary growth. The start of secondary growth was evidenced by the accumulation of lipids in the ooplasm, and by the demarcation of size of recruited oocytes to those remaining in primary growth. The pattern of lipid accumulation in secondary growth oocytes was indicative of exogenous induction (of external origin) in that small lipid droplets accumulated around the periphery of the ooplasm during early lipid induction and then the droplets apparently

migrated towards the nucleus. Wallace and Selman (1996) determined that lipid inclusions may be derived from vitellogenin and may thus be part of the vitellogenic growth phase of oocyte development. Holland *et al.* (in press) considered the appearance of small lipid droplets to be the start of secondary growth in the oocytes of striped bass. Thus all fish with ovaries in developmental stage III were considered to be maturing, and were included in the mature category for analysis. Those non-staining spheres present in the ooplasm of secondary growth oocytes stained with hematoxylin and eosin that were osmium tetroxide negative, were tentatively considered to be cortical alveoli.

Vitellogenic stage IV ovaries from this study were consistent with the condition of ovaries designated “developing” in *L. piscatorius* (Afonso-Dias and Hislop, 1996) and *L. setigerus* (Yoneda *et al.*, 1998b). Stage IV ovaries were considered to be maturing in this study and were included in the mature category for analysis, as were ovaries in developmental stage V. Ovulation was not apparent in any of the samples.

It was determined from this study that apparent vitellogenesis occurred throughout the winter, summer, and fall seasons, with ovaries in a state of late maturation (late-stage IV) found during the winter and summer months. Post-spawning ovaries were observed during the summer (13%) and fall (15%), but none were present in the winter samples. Maturing oocytes were present in all post-spawning ovaries, with the mean diameter of the largest oocytes being 446 μm , which was approximately the mean diameter of those present in mid-stage IV ovaries (409 μm).

Although the current understanding of reproduction in the goosefish is that it spawns once per season, some evidence exists from this study and from the study on *L.*

setigerus by Yoneda *et al.* (1998) that alternative hypotheses may need to be considered. These hypotheses and the evidence to support them are discussed below.

Oocytes in various stages of secondary growth were present in all post-spawning ovaries from this study during the summer and fall, and these oocytes were not being resorbed. Afonso-Dias and Hislop (1996) came to the conclusion that it was unlikely that *L. piscatorius* spawned more frequently than once per year because the remaining oocytes in post-spawning ovaries from their study appeared to be degenerating. This was not the case in post-spawning ovaries of *L. americanus* from this study. Developing vitellogenic oocytes were present in 100% of the post-spawning ovaries from the summer samples and in 25% of the post-spawning ovaries from the fall samples, indicating the possibility that *L. americanus* spawns more frequently than once per season. This evidence was also apparent in *L. setigerus* in the study by Yoneda *et al.* (1998a), and the conclusion was that females that had spawned previously would probably mature and spawn again in the same season. It was determined by Koya *et al.* (1994) that the rockfish *Sebastes macrochir* (another fish species that spawns gelatinous egg veils) was a multiple spawner by the presence of post-ovulatory follicles and maturing oocytes in ovaries of several fish examined. Several frogfishes (also Lophiiformes) are said to shed their eggs in veils in several batches during a spawning season (Ray, 1961; Pietsch & Grobaker, 1987), and this possibility should be explored for the goosefish.

Ovaries in a very developed state were present in the winter *L. americanus* samples. Some of these ovaries had developed a thick mucus layer and oocytes nearest the lumen were nearing final maturation. This, coupled with the occurrence of vitellogenic oocytes in post-spawning ovaries, may provide indirect evidence for the

alternative hypothesis of an extended period of recrudescence in *L. americanus*. The secondary growth oocytes in maturing post-spawned ovaries may reach an arrested stage of development at some critical size/point, or they may develop very slowly over the course of a year. The examination of spring samples may have helped to clarify these hypotheses, but unfortunately it was not possible to collect spring samples for this study. Both hypotheses warrant further investigation.

Macroscopic examination of the gonad is the method traditionally used by NMFS for maturity determination of fishes. These criteria describe how the ovary or testis might look at various stages of maturation such as the color of the organ, extent of vascularization, and other obvious visual signs such as the presence of milt or oocytes. This study suggests that these criteria can lead incorrectly to the assignment of immature females to the mature category. The most recent estimate of mean length-at-maturity (L_{50}) for female *L. americanus* is 44 cm (Almeida *et al.*, 1995). Determinations of maturity for the Almeida *et al.* (1995) study were made by macroscopic examination according to staging criteria described by Burnett *et al.* (1989) during cruises conducted by the National Marine Fisheries Service (NMFS). These criteria are currently used during the NMFS cruises and are not specific to goosefish. In Almeida *et al.* (1995), all stages other than immature were classified as mature, thus all ovaries classified as developing and resting were included in the mature category. When comparing the histological classification of ovarian developmental stage with that of the macroscopic staging by NMFS, 85% (60 out of 71 fish) of the immature stage II ovaries were classified as resting or developing – thus would be included in the mature category. The determination of maturity would be shifted to the left by including in a mature category

such a large proportion of fish classified as immature by my histological criteria. This may partially explain the difference between the 44 cm L_{50} determined by Almeida *et al.* (1995) and that of the 57 cm L_{50} determined from this study.

Several stage II ovaries (as well as other stages of ovarian development) included the appearance of a fibrous substance at the terminal position of the clusters, but this material was not identifiable. This fibrous material was also witnessed by Yoneda (personal communication) during the work for the 1998 studies (Yoneda *et al.*, a, b), but was not identified. With the exclusion of the stage II ovaries with the questionable fibrous material, the L_{50} for this study was determined to be 54 cm and the L_{95} was determined to be 59 cm. Since there was no sign of maturity in these stage II ovaries they were included in the final calculations, with the L_{50} and the L_{95} calculated as 57 cm and 63 cm, respectively.

The majority of ovaries from fish collected for the winter, summer, and fall seasons were immature (63%, 73%, and 63%, respectively), and many fish larger than the current L_{50} of 44 cm had ovaries in an immature stage of development. The results of this study indicate that the mean length at maturity for *L. americanus* is 57 cm, which is much larger than previously thought. Therefore the current minimum catch sizes of 43.2 cm for the northern Atlantic region and 53.3 cm for the southern Atlantic region probably excludes a large proportion of the population from ever spawning. Since successful management is the goal, this new maturity information should be considered in the future management of *L. americanus* in the attempt to minimize the impact of the commercial fishery on the population.

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Table 1. Description of the most advanced oocytes and composition of ovary at various developmental stages in the goosfish.

Ovarian Stage	Description	Mean (+SE) diameter of largest oocytes (μm)
0	Tissue consists of primary oogonia only	—
I	Primary oocytes developing at terminal position of clusters with nests of oogonia at basal position of clusters; primary oocytes consist of a very large nucleus and little darkly staining ooplasm.	<100
II	Several primary growth oocytes are present within each cluster and all appear to be similar in size; these oocytes have a smaller nucleus than primary oocytes and more ooplasm is present; several non-staining spherical lipid droplets may be present in the ooplasm; multiple nucleoli may be apparent.	201 (3)
III	Several primary growth oocytes are present within each cluster with one or two pre-vitellogenic secondary growth oocytes located at the terminal position of the clusters; ooplasm of pre-vitellogenic oocytes contains many non-staining spherical lipid droplets and/or cortical alveoli.	300 (8)
Early-IV	Several primary growth oocytes are present within each cluster with one or two vitellogenic oocytes located at the terminal position of the clusters; a few to many small yolk protein globules, which stain pink, are present in the periphery of the ooplasm of vitellogenic oocytes.	320 (7)
Mid-IV	Several primary growth oocytes are present within each cluster with one or two vitellogenic oocytes located at the terminal position of the clusters; yolk protein globules are numerous in the ooplasm of the vitellogenic oocytes.	409 (8)
Late-IV	Several primary growth oocytes are present within each cluster with one or two vitellogenic oocytes located at the terminal position of the clusters;	610 (31)

yolk protein globules appear to fill the ooplasm of the vitellogenic oocytes;
a blue staining mucus matrix may be present around oocyte clusters and
within the lumen of the ovary.

- V Several primary growth oocytes are present in each cluster with one or two 1027 (26)
mature oocytes located at the terminal position of the clusters; final oocyte
maturation is apparent in the mature oocytes in that yolk protein globules
and lipid droplets have coalesced; germinal vesicle breakdown may be
apparent; a blue staining mucus matrix is present around oocyte clusters
and within the lumen of the ovary.
- VI Several primary growth oocytes are present within each cluster with one or 446 (6)
two secondary growth oocytes located at the terminal position of the
clusters; atretic follicles are apparent; vitellogenic oocytes are typically
present.
-

Table 2. Seasonal differences in frequency (%) of stages of ovarian development in female goosefish.

Ovarian Stage	Winter (N)	Summer (N)	Fall (N)
0	0	7	7
I	0	17	19
II	63	49	37
III	0	4	7
IV	37	9	15
V	0	1	0
VI	0	13	15

Table 3. Calculations of the L_{50} and the L_{95} by season.

Season	N	L_{50}	L_{95}
Winter	24	53	54
Summer	135	50	54
Fall	25	49	59

Figure 1. Map of northern and southern management areas for goosefish. Source: New England Fishery Management Council and the Mid-Atlantic Fishery Management Council, 1997, Amendment 9 of the Northeast Multispecies Fishery Management Plan.

Figure 2. Map of the trawling regions for the National Marine Fishery Service Groundfish Survey Cruise. Source: National Marine Fisheries Service, 1997, Fishermen's Report.

Figure 3. Photomicrographs of transverse sections through differentiated gonadal tissue of the goosfish, *L. americanus*. (a) Early differentiation of ovarian tissue. (b) Early differentiation of testicular tissue.

Figure 4. Photomicrographs of transverse sections through ovarian tissue stained with hematoxylin and eosin (columns 1 and 3) and with osmium tetroxide and hematoxylin. (columns 2 and 4) depicting oocyte development in the goosfish, *L. americanus*. (a), (b) Primary growth oocytes with lipid droplets in the ooplasm. Lipid droplets appear as non-staining spheres when stained with hematoxylin and eosin (a), and stain black with osmium tetroxide (b). (c), (d) Early secondary growth oocytes (pre-vitellogenic). Lipid droplets have increased in number and appear to move from the periphery of the ooplasm towards the nucleus. Multiple nucleoli can be seen and the follicular layer has become apparent. (e), (f) Early vitellogenic oocytes. Yolk protein granules stain pink with hematoxylin and eosin (e) and first appear as a ring at the periphery of the ooplasm. A ring of lipids can also be seen moving from the periphery towards the nucleus where they appear to be fusing into larger droplets (f). (g), (h) Late vitellogenic oocytes. Ooplasm appears to be filled with yolk protein globules and lipids. (i), (j) Final oocyte maturation. Yolk and lipid globules have coalesced and the nucleus is no longer apparent as germinal vesicle breakdown has occurred. (k), (l) Atretic follicles.

Figure. 5. Photomicrographs of transverse sections through ovarian tissue depicting ovarian developmental stages in the goosfish, *L. americanus*. (a) Stage I ovary. (b) Stage II ovary. (c) Stage III ovary. (d) Early stage IV ovary. (e) Mid-stage IV ovary. (f) Late stage IV ovary. (g) Stage V ovary. (h) Stage VI ovary. Arrowheads indicate atretic follicles. All panels were stained with hematoxylin and eosin. ld, lipid droplet; mm, mucogelatinous material; N, non-ovigerous lamellae; O, ovigerous lamellae.

Figure 6. Percent frequency of occurrence of oocyte developmental stages within the ovary of the goosfish. *MM mucogelatinous material.

Figure 7. Percent frequency of oocyte diameters within the ovary at each developmental stage in the goosefish.

Figure 8. Percent frequency of occurrence of ovarian developmental stages at length for the goosefish.

Figure 9. Maturity ogive for the goosefish. Proportion mature at length based on logistic regression analysis (SAS, version 6.10). The curve was calculated for the raw data, and the points represent the proportion of mature fish at each 1 cm length class.

APPENDIX A. Gonadosomatic Index (I_G) and the Goosefish

Introduction

The gonadosomatic index (I_G) is another means of assessing the reproductive state of a fish, and is typically the ratio of gonad weight to total body weight. Monthly or seasonal changes in the I_G can be used along with other information such as incidence of maturity to determine the timing and duration of the spawning season. Although spawning has not been witnessed in *L. americanus*, the spawning season is believed to be late spring through early fall, according to latitude (Armstrong et al, 1992; Wood, 1982).

Materials and Methods

Data collection

Total body weight as well as other information was recorded from goosefish caught aboard the *R/V Albatross IV* during the NMFS cruises. Total weights were not recorded during the first leg of the summer 1998 NMFS cruise, and some gonad weights were also not recorded during the fall NMFS cruises. The fish collected on the commercial trawler *Laura Lynn* were stored on ice until the boat docked, and trips lasted approximately 3-4 days. The fish were then transferred to a cooler and stored on ice until sampling took place (within one or two days), at which time information such as total body weight was recorded.

Data analysis

The I_G was calculated as

$$I_G = W_g/W_t \times 100$$

where W_g is the weight of the gonad and W_t is the total body weight of the fish.

Also calculated was

$$W_g/TL \times 100$$

where TL is the total length of the fish.

Results

Mean gonadosomatic index and ovarian developmental stage

The mean I_G was very low (<3%) through developmental stages 0 and I for the summer and fall samples, and remained low through developmental stage II for all seasons (Figure 10). The mean I_G remained below 5% for all ovarian developmental stages for the fall samples. In winter samples, mean I_G peaked at 10% during ovarian stage IV (Figure 10). In summer samples, the mean I_G peaked at 23% during stage V (ovarian maturation), and fell once again to below 5% during the post-spawning ovarian stage VI (Figure 10).

Mean gonadosomatic index and length classes

The mean I_G increased slightly with an increase in total fish length up to the length class 46-55 cm in all seasons (Figure 11). There was a significant increase in mean I_G for the winter samples starting in the 56-65 cm length class and continued through the 66-75 cm length class (Figure 11). The summer samples demonstrated a slight increase in mean I_G as length class increased, but dropped slightly in the 76-85 cm length class (Figure 11). The mean I_G did not fluctuate much throughout the length classes of the fall samples (remained <4%) (Figure 11).

Mean ratio of gonad weight to total length for each ovarian developmental stage for all seasons.

The mean ratio of gonad weight to total length remained very low through ovarian developmental stage II for all seasons and increased to above 300 g/cm during stage III for the summer and fall seasons (Figure 12). The winter samples peaked at close to 900 g/cm during stage IV, and the summer samples peaked at approximately 2050 g/cm during the fifth stage of ovarian development (Figure 12). For both the summer and fall seasons, the mean gonad weight to total length dropped below 300 g/cm in post-spawning ovaries (stage IV) (Figure 12).

Discussion and Conclusion

Due to the variation in volume of gut contents between fish, the gutted body weight is often preferred when calculating the gonadosomatic index (I_G) for many fish species. The stomachs of the goosefish often contain large quantities of food (Afonso-Dias and Hislop, 1996; personal observation), which may cause total body weight to be misleading. Unfortunately it was not possible to measure gutted body weights during the collection of samples for this study and so total body weights have been used in the calculations of I_G . Due to the potential for the I_G to be misleading, the ratio of gonad weight to total length was also calculated to assess the usefulness of such a ratio as a designation of maturity in the field.

When compared against ovarian developmental stage, the trends in both the mean I_G and the ratio of gonad weight to total length were very similar for all seasons, with a

clear increase beginning at developmental stage III. Stage III is the point at which an ovary was considered mature in this study, so it would be interesting to determine whether a field ratio of gonad weight to total length would correspond to this analysis, and whether this could potentially become a useful field indicator.

The mean I_G remained below 5% during the fall season regardless of grouping, indicating that the animals were in a post-spawning or immature state at this time of the year. During the winter and the summer months, mean I_G increased in the larger animals in the ovarian stages indicative of a pre-spawning state, with the highest I_G occurring in animals between 56-75 cm in the winter months. 38% of the winter fish collected had ovaries with vitellogenic, maturing oocytes, and all fish caught during the winter months were collected from the southern management region for goosefish, which is below 41° latitude. It has been suggested that spawning is latitude dependent in *L. americanus* (Armstrong *et al*, 1992; Wood, 1982), which might explain the very high mean I_G in the larger fish during the winter months. Fish with the highest I_G 's (12, 15, 19, and 23%) caught during late summer (August) were also collected in the southern management region for goosefish. This information appears to contradict the latitude dependence of *L. americanus* spawning behavior, although the possibility exists that this species spawns multiple times during a reproductive season. Very few gonad weights were recorded for the fall samples, so the fall samples were not compared with the other seasons, as they were not appropriately represented.

From the samples collected for this study, it appears that maturing fish occur in the winter and summer months but not during the fall. Since maturing ovaries were found during the winter months, and fully mature ovaries in a pre-spawning state were

found only during the summer, this information supports the previously held belief that *L. americanus* spawns during the summer months.

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Figure 10. Mean gonadosomatic index and ovarian developmental stages during all seasons for the goosefish.

Figure 11. Mean gonadosomatic index at length during all seasons for the goosefish.

Figure 12. Mean ratio of gonad weight to total weight for each ovarian developmental stage for all seasons for the goosefish.

APPENDIX B. Reproductive Strategies – a discussion

Teleosts, like most fishes and vertebrates, typically reproduce sexually. Fishes exemplify a wide range of reproductive strategies, with some considered unique to the animal kingdom (Hoar and Randall, 1969). These strategies range from the broadcast spawning of eggs and spermatozoa into the open water as in many pelagic species, to the mouthbrooding of cichlid fishes which show a higher degree of parental care, to the bearing of live young as in some species of sharks and the killifishes (Merron, 1988). There are several common modes of reproduction, with oviparity being the most common amongst vertebrates (Merron, 1988). Oviparous fish species release gametes into the water column, where fertilization takes place (Hoar and Randall, 1969; Merron, 1988). Ovoviviparous fishes bear live young, but are aplacental and incubate fertilized eggs within the oviduct, whereas truly viviparous species are placental (Hoar and Randall, 1969; Merron, 1988). Most fishes are dioecious, with eggs and spermatozoa formed in separate individuals. External fertilization is predominant in these fishes, with fertilization taking place immediately following the expulsion of gametes into the surrounding water (Hoar and Randall, 1969). Other common reproductive strategies in teleosts include sequentially hermaphroditic species which change sex when conditions or body size favor one sex over the other, and the self-fertilizing or gamete trading strategies of simultaneous hermaphrodites (Demski, 1987). Some deep-sea ceratoid anglerfishes represent a very unusual strategy not observed in other teleosts. The male, being only a fraction the size of the female, becomes parasitic by forming a permanent bond between his mouthparts and the female's body (Demski, 1987). Demski (1987)

suggests that the male relinquishes control of reproductive function with this permanent bond, and Pietsch (1976) has demonstrated histologically that this fusion causes a confluence of blood systems through which hormonal cues can pass, possibly controlling reproduction.

Although spawning behavior of the goosefish has not been witnessed, it is believed that external fertilization is the mode of reproduction, as no intromittent organ has been found in males (Dahlgren, 1928). In the viviparous halfbeak, a modified male anal fin (termed an andropodium) transfers sperm bundles (spermatozeugmata) to the female (Meisner and Burns, 1997). Goosfish demonstrate no such modification, and histological examination of the testes shows no spermatozeugmata present (Afonso-Dias and Hislop, 1996; personal observation). Prior to the discovery of an alternative mode of reproduction in an antennariid anglerfish by Pietsch and Grobecker (1980), all female members of the Lophiiformes were thought to expel nonadhesive, mucoid egg rafts or veils. The alternative strategy found in the antennariid anglerfish *Antennarius caudimaculatus*, involves the carrying of eggs on the external surface of the body (Pietsch and Grobecker, 1980), which is a level of parental care not otherwise observed in Lophiids.

Morphological differences exist in the reproductive biology of fishes according to reproductive strategy, with some adaptations being unique in the world of fishes. The reproductive biology of pediculate fishes such as the goosfish is unique among teleosts, and the massive gelatinous egg rafts produced by these unusual animals are developed within a very specialized ovary. It is this unique adaptation, along with the plight of the north Atlantic goosfish that caused me to become interested in studying this unusual

species in the first place. I do hope that the information provided by this study can be used by fisheries managers to help the north Atlantic population in some way.

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APPENDIX C. Problems encountered

Sample collection

The logistics of collecting gonadal tissue samples from a predominantly deepwater fish with unusual reproductive anatomy were difficult. Since the only way these fish are caught with any frequency is through trawling, I had to find a way to collect samples via this method. Fortunately NMFS conducts seasonal groundfish survey cruises during which they trawl repeatedly and goosefish are a common species in their hauls. I was very fortunate to have had the cooperation of NMFS (Woods Hole, MA) for without their hard work this study would not have been possible.

I would just like to point out a few of the problems that were encountered through the collection of these samples in the hope that this information might be helpful in the future. NMFS agreed to collect goosefish gonad tissue samples and fix and preserve them aboard the ship by using pre-filled 20 ml vials of fixative (10% neutral buffered formalin) and preservative (70% EtOH) that I supplied them. I also supplied them with a logbook and written sampling instructions that included the approximate size of tissue sample to be placed in each vial (1 cm width). This worked very well with the ovarian tissue, but the testicular tissue did not fix properly and thus was not used in the manuscript. This was partially the result of some very large testicular tissue samples being placed in the 20 ml vials of fixative, thus were not fixed properly. Another problem with fixation may have been that testicular tissue is a dense tissue that requires a higher fixation solution volume than ovarian tissue. If I were to do this again I would use larger vials and be more specific in the wording of my instructions.

The other problem encountered during the collection of gonadal tissue samples was that intestine was often mistaken for ovaries in the goosfish. During the first leg of the summer 1998 cruises, 56 out of 127 tissue samples collected were not gonad tissue, but instead were intestinal tissue. A list of these fish was given to NMFS so that they could remove them from their maturity data sets.

Testicular tissue

While embedding testicular tissue I encountered the problem that the sperm from the mature males stuck to the forceps and scalpel during histological preparation. Although each instrument was wiped with an ethanol soaked paper towel between tissue samples, sperm was observed on to the outside of many immature testicular samples when examined microscopically, as well as on a few of the ovarian sample slides. My guess is that it came from the instruments. By re-embedding each sample with a much more careful technique to ensure that mature sperm were not carried over, this problem could have been corrected, but many of the testicular tissue samples had not been fixed properly and so it would not have been worth the effort.

Methods

The testicular tissue samples were prepared histologically according to the same method as the ovarian tissue samples, were stained with hematoxylin and eosin, and were examined microscopically. Most tissue samples were damaged from poor fixation so the presence/absence of spermatogonia, spermatocytes, and spermatozoa were recorded only.

Results

It appeared that spermatozoa were present in the lumen of fish as small as 28 cm total length (Figure 13). Fish whose testes were filled with mature spermatozoa and had

no spermatocytes present were considered to be in spawning condition and were found only during the summer months (Figure 13). All testes from fish collected during the winter months and all testes from fish above 26 cm total length collected during the fall had spermatocytes and spermatozoa present (Figure 13).

Figure 13. Percent frequency of occurrence of germ cell developmental stages in the testes of the goosefish within length classes.

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