REPRODUCTIVE BIOLOGY OF AMERICAN SHAD, *ALOSA SAPIDISSIMA*, IN THE MATTAPONI RIVER

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Presented to
The Faculty of the School of Marine Science
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In Partial Fulfillment
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Master of Science

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by
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APPROVAL SHEET

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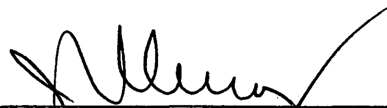

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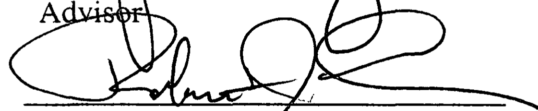

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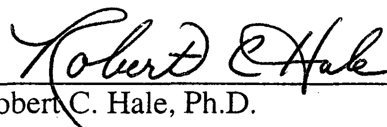

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ABSTRACT

The seasonal fecundity of American shad, *Alosa sapidissima*, from the Mattaponi River was calculated using estimates of spawning frequency, batch fecundity, and residence time. Spawning frequency was estimated using histological and macroscopic techniques. Histological techniques were more reliable and allowed the estimation of spawning frequency from animals containing migratory nucleus stage oocytes, hydrated oocytes, 1-day old postovulatory follicles, and 2-day old postovulatory follicles. Female shad spawn once every two to three days. Batch fecundity was estimated for 70 specimens collected in 2002 and 2003 using the gravimetric method. Batch fecundity, though highly variable, was positively linearly correlated with eviscerated weight (EW) and ranged from 12,700 to 81,000 eggs per batch. Relative batch fecundity ranged from 12.6 eggs/g EW to 68.3 eggs/g EW. Mean relative batch fecundity was 30 to 36 eggs/g EW. Residence time was obtained from a concurrent sonic tagging study and was 34 days. On average shad in the Mattaponi River release 11 to 17 batches per season based on mean spawning intervals of two to three days. Seasonal fecundity for an average virgin (4.96 years old and 1088g EW) was estimated to be between 380,000 and 550,000 eggs. This is 1.5 to 2 times higher than mean virgin fecundity reported in previous investigations that assumed determinate fecundity. Histology was also used in an attempt to elucidate the spawning strategy of American shad as either determinate or indeterminate. Histology indicates a possible determinate strategy with the level of remnant fecundity declining during the season and a reduction in the number of partially yolked oocytes in animals that have spawned several times. These results are confounded by the observation of animals containing significant remnant fecundity exiting the river in another study; and by the discrepancy between this study's seasonal fecundity estimates and those made assuming determinate fecundity. Therefore the hypothesis of indeterminate fecundity was not rejected.

REPRODUCTIVE BIOLOGY OF AMERICAN SHAD, *ALOSA SAPIDISSIMA*, IN THE MATTAPONI RIVER

INTRODUCTION

Life History

The American shad (*Alosa sapidissima*) is an anadromous clupeid native to the western North Atlantic Ocean. Shad spawn in both tidal and non-tidal freshwater in rivers stretching from St. John's River, Florida to Atlantic Canada (Bigelow and Schroeder 1953). Though shad of multiple populations mix in the ocean, they likely home to natal waters (Waters et al. 2000, Melvin et al. 1986). River-specific spawning traits are possible given the apparent reproductive isolation. Shad are known to be semelparous in the southern end of their range and mostly iteroparous in northern waters with the York River, Virginia spawning populations falling in the middle of the latitudinal gradient. This is thought to be an adaptation to variable thermal conditions and short growing seasons in northern nursery areas where recruitment failure may occur frequently enough to select for adults that spread reproductive effort out over several years. Consequently, southern fish are hypothesized to invest greater reproductive effort in their single spawning season. Thus, lifetime reproductive effort would be similar across latitudes despite the lack of repeat spawning in semelparous populations. Leggett and Carscadden (1978) introduced this hypothesis in a study in which they counted the standing stock of oocytes prior to spawning and assumed that all vitellogenic oocytes would be spawned. They found greater total fecundity relative to body size in southern semelparous populations. Thus, fecundity estimates are key in this possible example of intra-specific life history evolution.

Batch spawning and fecundity patterns

Batch spawners release their gametes in several spawning events occurring throughout the spawning season (Hunter and Macewicz 1985). Lehman (1953) noted that while shad in the Hudson River often contained as many as 500,000 eggs, only 30,000 appeared ripe at any one time. Recent findings confirm that American shad are batch spawners with asynchronous ovarian development (Mylonas et. al 1995, Olney et al. 2001). Mylonas et al. (1995) noted numerous size classes of oocytes in biopsied ovaries from shad that were tank spawned using gonadotropin-releasing hormone analogue. Individual animals released several batches over nine days. Olney et al. (2001) noted numerous developmental stages of oocytes in wild caught fish, as well as postovulatory follicles present simultaneously with advanced oocytes. Postovulatory follicles indicate previous spawning and advanced oocytes indicate groups of eggs soon to be spawned. Batch spawners may have determinate or indeterminate fecundity.

Determinate fecundity

The standing stock of oocytes at the onset of spawning represents likely annual fecundity and is depleted with each successive batch. Southern blue whiting (*Micromisistius australis*) (Pajaro and Macchi 2001) and Atlantic mackerel (*Scomber scombrus*) (Walker et al. 1994) are examples of batch spawners that exhibit determinate fecundity. Southern blue whiting, Atlantic mackerel, and other determinate batch spawners begin the spawning season with a measurable number of oocytes to be spawned. No new vitellogenisis occurs and most oocytes mature and are spawned during the season less an insignificant fraction that is resorbed. The duration of the

spawning season is generally too short for the completion of vitellogenesis in batch spawners with determinate fecundity. This pattern is most common in cool temperate and northern species where the thermal window for spawning is shorter. At the onset of spawning, vitellogenesis is generally well underway with an absence of early stage vitellogenic oocytes. Intermediate stages between the perinucleolar phase of primary growth and yolked oocytes are not usually evident. In some determinate spawners, such as the Atlantic mackerel, a gap between pre-vitellogenic oocytes and vitellogenic oocytes is not evident prior to spawning but becomes evident as spawning progresses.

Indeterminate fecundity

Batch spawners that exhibit indeterminate fecundity include jack mackerel *Trachurus symmetricus* (Macewicz and Hunter 1993), northern anchovy *Engraulis mordax* (Hunter and Macewicz 1985), swordfish *Xiphias gladius* (Arocha 2002), and Brazilian menhaden *Brevortia aurea* (Macchi and Acha 2000) among others. These species continually recruit pre-vitellogenic oocytes throughout the season and resorb the remaining yolked oocytes when spawning is terminated. Typically, spawning activity typically occurs over a protracted period such as in the five-month spawning season of the seatrout *Cynoscion nebulosus* (Brown-Peterson and Warren 2001). Some species, such as the vermilion snapper (Cuellar et al. 1996), spawn as many as 30-40 batches per season. In these species, ovaries contain a continuum of developmental stages of eggs from primary growth through fully mature at any given point in the spawning season. In species with indeterminate fecundity, the standing stock of oocytes at the beginning of the spawning season is not indicative of the likely annual fecundity. Instead, fecundity is

a function of batch size multiplied by the number of batches released in a season with batch size assumed to be constant. The number of batches spawned in a season may be estimated by deciphering the spawning frequency and duration of spawning. Spawning seasons for indeterminate spawners are typically protracted, thus allowing sufficient time for vitellogenisis to produce new yolked oocytes from unyolked oocytes.

American shad fecundity pattern

American shad exhibit asynchronous oocyte development with a continuous size distribution of oocytes in mature individuals and a single distinct batch of larger oocytes at each spawning event. Shad with advanced yolked oocytes have also been identified to contain postovulatory-follicles indicating that fish preparing to spawn had already spawned (Olney et al. 2001). Previous investigations of shad fecundity and life history evolution (Leggett and Carscadden 1978) assumed this species to have determinate seasonal fecundity but batch spawners with asynchronous ovarian development often have indeterminate fecundity as in the previous examples. Olney et al. (2001) observed that up to 70% of females shad exit the York River spawning grounds in a partially spent state. This indicates substantial atresia closing out the season, a strong indication of indeterminate fecundity in this stock. Additionally, Olney and McBride (2003) found that batch size did not vary significantly between one semelparous and two partially iteroparous populations.

Measuring spawning frequency

Histological post-ovulatory follicle method

Spawning frequency is seldom observed directly but is deduced by examining the condition of the ovaries in females on the spawning grounds. The most common method, the post ovulatory follicle method or histological method, is discussed in Hunter and Macewicz (1985). The proportion of females spawning on any given day is a function of the spawning frequency. This fraction can be inferred from histological sections of ovary by identifying whether fish have spawned one or two days prior to capture or if they were going to spawn on the day of capture. The age of the POF defines days since previous spawn. Fish containing oocytes that have completed final maturation are expected to spawn on the day of capture. If the onset of final maturation is identifiable then the fraction of fish that would have spawned the day following capture can be estimated as well, provided the fish sample is representative and catchability is equal between groups.

POFs are the remnant follicular envelopes of theca and granulosa cells that house the individual oocytes and provide connection to the vascular system via the ovigerous lamellae. The POF remains in the ovary after ovulation and spawning and is gradually resorbed. If the rate of degeneration of the POF can be described over time then it is possible to identify fish that have spawned in previous days and assign a time since last spawn. Using this information as well as assessment of maturation of unspawned oocytes, an estimate of spawning frequency can be obtained. For example, if fish spawn once every four days, then 25% of the sample population spawns each day. Any sample should yield 25% of fish identified as ready to spawn the following day, 25% fish prepared to spawn, 25% of fish containing day-old POFs, and 25% two day-old POFs.

Hunter and Macewicz (1985) describe a technique for aging POFs in northern anchovy (*Engraulis mordax*) using wild caught fish on the spawning grounds. This technique involves capturing fish in sequential samples over several days and observing the appearance of POFs over that time in histological sections. In any given sample of spawning fish, multiple ages of POFs will be encountered. Provided the spawners have a diel spawning rhythm, a pattern in appearance of POFs that allows the assignment of POF ages should be observed. For example, a sample taken from a population that spawns in the evening will contain 12hr, 36hr, and 60hr POFs in the morning. All should be sufficiently dissimilar to be assigned to the proper age category. Anecdotal evidence, personal communication with fishermen, suggests that shad do have a diel spawning pattern centered around dusk. Histology will confirm this. If shad do not have a diel spawning pattern then there is no means of estimating the time since spawn and POF aging will not be possible. Hunter and Macewiczs' (1985) technique for aging POFs and estimating spawning frequency has been used on many fish species that spawn pelagic or semi-pelagic unadorned eggs. Some of these species include seatrout, *Cynoscion nebulosus* (Brown- Peterson and Warren 2001), Atlantic mackerel *Scomber scombrus* (Walker et al. 1994), and jack mackerel, *Trachurus symmetricus* (Macewicz and Hunter 1993). POFs are similar in each species.

Fresh POFs are characterized by a distinct lumen, well defined cuboidal to columnar granulosa, and an outer layer of simple squamous thecal epithelium. The granulosa layer is generally very convoluted in fresher POFs and appears within the thecal layer. The theca often has visible capillaries evident by the presence of erythrocytes with strongly basophilic nuclei. As the POFs degenerate, the cell walls

between the granulosa become indistinct as nuclei become pycnotic. Pycnotic nuclei are generally apparent as being less basophilic and more diffuse than nuclei in fresh material. As granulosa are resorbed, the POF lumen shrinks and the layer of granulosa becomes less convoluted. Despite the similarities between species, Hunter and Macewicz (1985) recommend detailed analysis POF structure for each species of interest as POF degeneration rates vary across species and temperatures. Even within species, temperature plays a significant role in POF resorbtion rate. In Atlantic menhaden, *Brevortia tyrannus* (Fitzhugh and Hettler 1995) and seatrout (Brown-Peterson and Warren 2001) temperature increases of 10°C as much as doubled the rate of POF degeneration. At temperatures above 20°C, POF became indistinguishable beyond 36h whereas at temperatures around 15°C POFs persisted for >50h and could be aged to 48h before morphology became ambiguous.

Macroscopic hydrated oocyte method

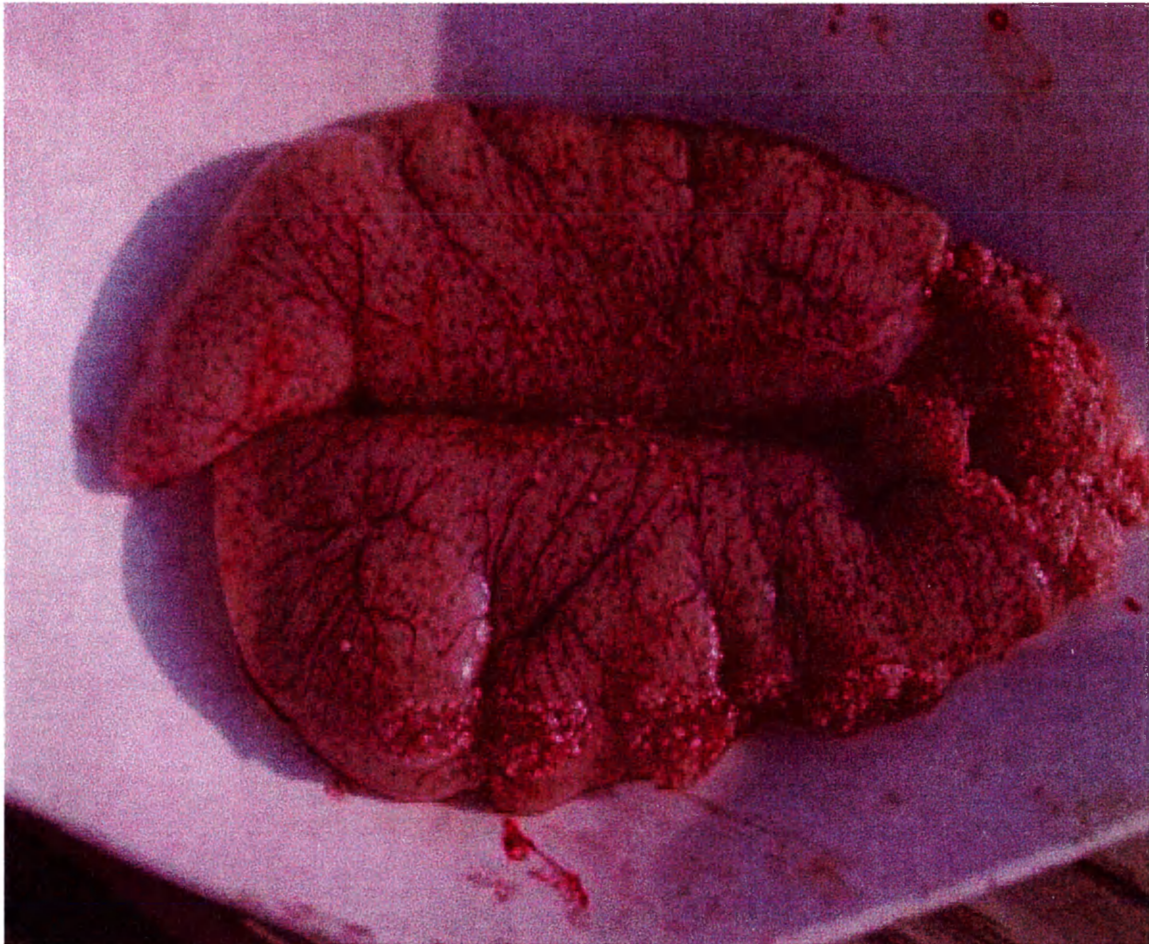
The hydrated oocyte method uses gross ovary anatomy to identify whether a fish is going to spawn on the day of capture. The proportion of fish spawning in any given day is equal to the assumed spawning frequency. Spawning fish are assumed to be those that contain hydrated oocytes. The hydrated oocyte method is not as robust an estimate of spawning frequency as the POF method because it relies on the identification of one spawning state in order to estimate the number of fish spawning each day, whereas the POF method generates multiple estimates of spawning frequency from one sample. However, the hydrated oocyte method does not require the extensive lab preparation that

the POF method requires. Furthermore, these data are sometime available for American shad as ancillary information collected in hatchery operations that use wild brood stock.

The hydrated oocyte method may provide reasonable estimates of spawning frequency if several criteria can be verified. These criteria are generally adapted from Hunter and Macewicz (1985). The first criterion is that hydrated oocytes must be identifiable in gross viewing. Hydrated shad eggs are translucent and stand out well among the smaller and opaque yolked oocytes (Figure 1). Secondly, fish should be collected during a time of day that spawning is not actively occurring. If this criterion is violated, some fish will have spawned before capture and may not be identifiable as spawners using gross anatomy. Fish must also be collected at the time when all females that will spawn that day can be identified. For this criterion to be achieved, hydration must not persist for greater than 24h. Otherwise, fish identified as ready to spawn may not actually be ready to spawn on the day of capture.

There are other potential sources of error when using the hydrated oocyte method. Hydrated fish may be more vulnerable to capture techniques and thus over represented in the sample. This can cause an overestimate of spawning frequency. Hydrated fish may also be patchily distributed, leading to biases in their catch and high variance on the estimate as catch rates of clustered animals could fluctuate greatly. Olney et al. (2001) estimated spawning frequency for American shad on the Pamunkey River, Virginia using the hydrated oocyte method on fish captured by the Virginia Department of Game and Inland Fisheries (VDGIF) in 1999 brood stock collections. The average over the entire season was estimated at 3.5 days. However, daily estimates ranged from every other day

Figure 1. Macroscopic appearance of hydrated ovaries. Note the presence of large translucent eggs.



to no spawning observed, an indication of contagious distribution of spawners. Also, variability may exist in the proportion of daily spawners if fish can arrest maturation.

Partially spent fish may be less likely to be clustered than actively spawning individuals (Hunter and Macewicz 1985; Brown-Peterson and Warren 2001). Brown-Peterson and Warren (2001) documented segregation between spawners and partially spent sea trout with partially spent fish being more uniformly distributed grounds. This suggests that spawning frequency estimates made from POFs are less likely to suffer from bias induced in the hydrated oocyte method by clustering of spawners.

OBJECTIVES

Leggett and Carscadden's (1978) classic paper regarding latitudinal variation in reproductive effort estimated fecundity without accounting for potential indeterminate fecundity in American shad. Olney and McBride (2003) questioned the assumption of determinate fecundity citing that shad are multiple spawners. They failed to detect a difference in batch fecundity between a southern semelparous population and two iteroparous stocks. In order to confirm the gradient in reproductive effort described by Leggett and Carscadden (1978), accurate estimates of seasonal fecundity are necessary and the question of determinate versus indeterminate fecundity requires resolution.. Seasonal fecundity estimates are derived from estimates of batch fecundity, spawning frequency, and residence time in multiple spawners. Seasonal fecundity has not been calculated previously for American shad. Olney and McBride (2003) began investigating these parameters by estimating batch fecundity and generating an estimate of spawning frequency from one year of Virginia Department of Game and Inland Fisheries (VDGIF) egg-taking data. The current study builds on this research by reporting additional estimates of batch size, evaluating methods of estimating spawning frequency, and utilizing a modified method of estimating spawning frequency. A new estimate of seasonal fecundity based on batch size, spawning frequency, and residence time is reported and compared to that reported in Leggett and Carscadden (1978). Finally, histologic data are used to address the assumption of indeterminate fecundity in American shad.

MATERIALS AND METHODS

Specimen Collection

Adult American shad were obtained from the spawning grounds of the Mattaponi River in the spring of 2002 and 2003. Samples in 2002 were taken in two sessions of 72-hour round-the-clock collections; April 4th-7th and April 17th-20th, and by purchasing weekly samples from fishermen. Samples in 2003 were purchased from local gill-netters (Table 1).

In 2002, samples for batch fecundity estimation were obtained twice weekly from fishermen of the Mattaponi Indian reservation and from sub-samples in the 72h collections. Date and time of collection were selected and all specimens were collected using drift gill nets. Native American fishers typically used 5.00" to 5.25" stretched mesh nets. Fishers were instructed to fish in a traditional way and set aside the first 20 females that were encountered without regard for condition or size. Fish were generally collected between river mile 43 and 50 with time of day, wind direction, and tide dictating where it was possible to set a net. In 2003, weekly samples were purchased from fishers using the same protocol as in 2002 with one exception. They were instructed to fish before 1400 to avoid capturing fish that were running ripe or that had just completed the release of a batch.

Specimens used in POF aging and spawning frequency estimation were collected in two 72-hour round-the-clock sessions when water temperatures were 13-16°C and 22-26°C. Fish were taken in a drift gillnet, 4.875" stretched mesh, at four to six hour intervals for 72-h with a goal of capturing 10-20 females per session. The net was

Table 1. Collection data and numbers of American shad used in an evaluation of fecundity and spawning frequency, spring 2002-2003.

Date	Time Collected	Water Temp* (°C)	Air Temp* (°C)	Number of Females Collected	Source	Histology (Y/N)	Sampled for Batch Counts (#hydrated)	River Mile
3/28/02	evening	13	14	19	Purchased	Yes	Yes (1)	43
4/3/02	evening	14	26	18	Purchased	Yes	Yes (3)	47
4/4/02	1830	14	10	12	VIMS	Yes	No	51
4/4/02	2230	15	8	0	VIMS	Yes	No	52
4/5/02	230	14	6	0	VIMS	Yes	No	51
4/5/02	600	14	2	13	VIMS	Yes	No	50
4/5/02	1030	14	5	6	VIMS	Yes	No	50
4/5/02	1400	16	11	12	VIMS	Yes	No	51
4/5/02	1830	16	10	30	VIMS	Yes	No	50
4/5/02	2230	16	5	3	VIMS	Yes	No	51
4/6/02	600	14	1	12	VIMS	Yes	No	50
4/6/02	1100	15	3	6	VIMS	Yes	No	49
4/6/02	1400	15	6	16	VIMS	Yes	No	49
4/6/02	1900	17	2	13	VIMS	Yes	No	51
4/6/02	2300	14	-1	1	VIMS	Yes	No	51
4/7/02	600	13	-4	15	VIMS	Yes	Yes (9)	49
4/7/02	1100	14	5	6	VIMS	Yes	No	48
4/7/02	1400	16	8	10	VIMS	Yes	No	49
4/12/02	evening	16	20	20	Purchased	Yes	Yes (5)	45
4/17/02	1900	24	35	20	VIMS	Yes	No	50
4/18/02	530	23	22	17	VIMS	Yes	Yes (8)	50
4/18/02	1100	25	30	14	VIMS	Yes	No	49
4/18/02	1400	25	30	7	VIMS	Yes	No	47
4/18/02	1900	24	24	13	VIMS	Yes	No	49
4/19/02	530	23	21	21	VIMS	Yes	No	50
4/19/02	1100	25	31	4	VIMS	Yes	No	47
4/19/02	1400	25	32	18	VIMS	Yes	No	48
4/19/02	1900	24	23	27	VIMS	Yes	No	48
4/20/02	530	23	19	12	VIMS	Yes	No	51
4/20/02	1100	25	29	17	VIMS	Yes	No	51
4/25/02	afternoon	23	22	4	Purchased	Yes	Yes (0)	42
4/14/03	late morning	14	23	20	Purchased	No	Yes (4)	47
4/21/03	mid morning	17	23	15	Purchased	No	Yes (7)	51
4/24/03	mid morning	17	22	17	Purchased	No	Yes (6)	52
4/28/03	mid morning	20	27	9	Purchased	No	Yes (6)	43
5/2/03	mid morning	21	27	19	Purchased	No	Yes (11)	44
5/5/03	mid morning	19	15	20	Purchased	No	Yes (14)	51

*Air temperatures for collections purchased from the fishermen were approximated from the Richmond airport. Water temperature for collections purchased from the fishermen was from the hatchery at the Mattaponi reservation, near the fishing location.

allowed to soak for a maximum of one hour in each session before haul back and processing of specimens. Fishing occurred between river miles 51 and 46. Wind direction, tide height, and tide direction dictated the location of each set. Ancillary data recorded included air temperature, water temperature, and tide stage.

In the first 72-h round-the-clock collection, samples were obtained at approximately 0600, 1000, 1400, 1800, and 2130 (EST) for three consecutive days beginning at 1830 on April 4th. Two collections at 0200 failed to produce fish. A total of 155 mature female American shad were collected. Most daytime samples yielded the desired minimum of ten fish per sample. The 1030 and 1100 samples were limited to six fish per sample. A total of only four fish were collected in the samples attempted at 2230h.

In the second 72-h round-the-clock collection, beginning at 1900h on 17-April, 2002, samples were collected at dawn, midday, and dusk for a total of nine samples. Midday samples consisted of two collections, one at 1100 and one at 1400 because of low catch rates during the middle of the day. A total of 170 fish were collected from April 17th-20th.

Specimen Processing

For all specimens, total length (TL) and fork length (FL) were measured to the nearest millimeter. Eviscerated weight (EW) and gonad weight (GW) were measured to the nearest half-gram. Scales were removed for aging and identification of spawning marks. Scales from the specimens sampled for batch counts (second to last column, Table 1) were analyzed by a lab specialist following the techniques of Cating (1953).

Macroscopic gonad stage was noted as mature (but not actively spawning), hydrated, running ripe, or spent. The primary interest for macroscopic staging was identifying ripe fish for the hydrated oocyte method. Gonads were removed and at least one lobe placed in 10% neutral buffered formalin to be preserved for histological preparation. One lobe of hydrated ovaries suitable for batch size estimation was placed on ice and returned to the lab for batch counts. Each ovary placed in formalin was allowed to harden overnight and was then cut into multiple sections to ensure complete infiltration by the preservative.

Histological Preparation

Ovaries fixed in formalin were returned to the lab and prepared for standard paraffin histology. Ovary sub-samples were trimmed to fit within perforated cassettes. Cassettes were placed in DI water overnight to extract formalin. Tissue samples were then placed in 70% ethanol until infiltrated with paraffin. Paraffin infiltration was in an automatic tissue processor that dehydrates the tissue in organic solvents and then soaks the tissue in hot paraffin. Infiltrated tissue was manually imbedded in paraffin blocks and cut on a microtone to 6μm thickness and mounted on glass slides. Slides were stained with Harris' hematoxylin and eosin counter-stain (H&E). Hematoxylin induces a blue hue to basic structures while eosin imparts a pink to red hue to acidic cellular components.

Batch Counts

Hydrated ovaries placed on ice for batch counts were sub-sampled using the gravimetric method detailed in Lowerre-Barbieri and Barbieri (1993). Three 4 to 5 gram sub samples are taken from the hydrated ovary for this method. Location of the sub-sample within the ovary is unimportant (Olney et al. 2001). Oocytes were washed from the tissue in a sieve and placed in 2% neutral buffered formalin and allowed to become opaque and harden before enumeration.

Preserved eggs larger than 1.6mm were manually counted and recorded as eggs/gram ovary in three sub-samples. Each sub-sample was multiplied by the ovary weight to yield an estimate of the total number of mature oocytes present in the whole ovary. The final estimate of batch fecundity is an average of the three sub-samples.

Relative batch fecundity is reported as eggs/gram eviscerated weight. A linear regression was used to describe batch fecundity relative to somatic weight. Simple t-tests were used to compare relative batch fecundity between years and between repeat and virgin spawners within years. One-way analysis of variance was used to test for a difference of means between dates and ages within years.

Batch fecundity and relative batch fecundity were compared between histological characteristics that indicated spawning history within the spawning season. These were separated into groups that had POFs and groups without POFs. A two-sample t-test was used to test the difference of means between the two groups.

Spawning Frequency

Histological Method: Spawning frequency was estimated as the reciprocal of the proportion of the sample spawning each day. The number of spawners was determined

from histology. Histology was used to examine ovaries to identify oocytes in final maturation, a precursor to imminent spawning, and to age POFs. This allowed the assignment of females to categories such as ripe, one-day post spawn, and two-days post spawn.

Oocytes in final maturation were identified based on criteria in Wallace and Sellman (1981). The methodology of Hunter and Macewicz (1985) was used to develop age criteria for POFs for wild caught specimens. POF age criteria were developed by examining slides based on the time of day in which each specimen was collected in the 72-h samples and assigning the POFs found to an age category. Opportunistic collection of running ripe fish provided known age zero POFs as a starting point. Once POF age criteria were developed, slides were shuffled randomly and read twice without knowledge of the time of capture to determine if age could be verified without prior knowledge of the time since spawning. A chi- square test of symmetry (Hoenig et al. 1995) was used to test for systematic error between readings. Histological findings reported are from this second reading.

Mean spawning frequency and standard deviation were calculated for each sample because histology provides multiple estimates of the number of daily spawners in each sample and an attempt is made to characterize the variance on the mean fraction of daily spawners. Seasonal mean spawning frequency and standard deviation were calculated for spawning frequency estimates derived from each ovarian character. Spawning frequency estimates were compared by time of day for each structure used since the ovarian structures used to identify spawners change in appearance during the day. One-way

ANOVA was used to test for a difference in estimates of spawning frequency by time of day.

Hydrated Oocyte Method: Spawning frequency estimated by the hydrated oocyte method was also calculated as the reciprocal of the proportion of the sample spawning each day. Daily estimates of spawning frequency were produced for each collection in 2002 and 2003 and averaged to obtain a seasonal mean and standard deviation. Additionally, a seasonal average spawning frequency was calculated as the total number of ripe females divided by the total number of females captured in all collections.

Fish were identified macroscopically as hydrated based on the presence of large translucent eggs in the ovary. In the 72-hour samples, replicates were grouped by time of day. A chi-square analysis was used to compare the ability to identify spawners macroscopically and by histology.

The hydrated oocyte method was also used to estimate spawning frequency from egg-taking data from the Virginia Department of Game and Inland Fisheries (VDGIF). VDGIF collects females from the Mattaponi River's neighboring tributary, the Pamunkey River, for brood stock. Data were available from 1999, 2000, 2001, and 2002. VDGIF collects females for strip spawning in the evening. In these data, the daily number of hydrated fish was determined by VDGIF personnel. Fish that produce eggs with light abdominal pressure were recorded as ripe. The reciprocal of ripe/total catch is reported as the spawning frequency.

For all available data, daily observations in which less than 20% of the sample was hydrated were dropped as outliers and were assumed to indicate that spawning

activity was not occurring. Most excluded samples were collected at the beginning of the spawning season.

Seasonal Fecundity

Seasonal fecundity integrates the estimates of spawning frequency, batch fecundity, and spawning duration. Residence time on the spawning grounds was estimated by Olney et al. (in prep) from acoustic tagging trials in 2003. These data were used as a proxy for spawning duration under the assumption that females spawn during the entire stay on the spawning grounds. This assumption may not be valid for all rivers. However, on the York River, partially spent and spent female American shad with POFs appear in the main-stem of the York River well below the spawning grounds (Olney et al. 2001). This suggests that exit from the spawning grounds occurs abruptly after spawning ceases. Relative seasonal fecundity (eggs/gram EW/season) was reported as the daily egg production per gram female (relative batch fecundity X spawning frequency) times the residence time. This was expanded to seasonal fecundity (eggs/season) by multiplying by the average weight of females in the spawning stock with weight at age calculated from the specimens aged in this project.

Fecundity Pattern

Fecundity pattern was investigated using criteria (Table 2) and methods similar to those in Murua et al. (1998), Walker et al. (1994), and Fitzhugh et al (1993). The objective was to determine if shad release their entire standing stock of oocytes without

Table 2. Criteria for determining fecundity pattern in multiple spawners.

1. The distribution of oocyte types in the ovary remains continuous without a gap between immature and vitellogenic oocytes in species with indeterminate fecundity (Murua et al. 1998).

2. A constant level of remnant fecundity (number of vitellogenic oocytes) is maintained in species with indeterminate fecundity (Murua et al. 1998).

3. Fecundity estimates from potential annual fecundity measured prior to spawning (i.e. Leggett and Carscadden (1978) for American shad) and the seasonal fecundity calculated by estimating batch fecundity, spawning frequency, and spawning duration are not similar in species with indeterminate fecundity (Walker et al. 1994).

4. The prevalence and intensity of atresia increase at the end of the spawning season in species with indeterminate fecundity (Walker et al. 1994, Murua et al. 1998)

replacement or if they continually recruit new eggs into vitellogenesis while spawning. All types of oocytes were counted on all histological slides. Counts were made from five randomly selected fields of view of the slide at 40x magnification on a standard compound microscope using the SPOT image processing system. All oocytes for which 50% or more of the oocyte was visible in the field of view were counted before selecting a new field of view. The proportion of each type of oocyte to the total number of oocytes observed was recorded and plotted against gonadosomatic index (GSI). GSI and the proportion of each type of oocyte in the ovary were compared between specimens having no POFs visible, one age class of POF visible, and two age classes of POF visible using a one-way ANOVA.

RESULTS

Batch Fecundity

A total of 70 batches of hydrated eggs were counted from samples collected in 2002 and 2003 (N=26 and N=44 respectively) (Tables 3 and 4). Batch fecundity (hydrated eggs per ovary) ranged from 12,713 eggs to 81,372 eggs. Relative batch fecundity (hydrated eggs/gram EW) ranged from 12.6 eggs/g EW to 68.3 eggs/g EW. Relative batch fecundity was lower in 2003 ($P = 0.029$, Table 5). In 2002 and 2003, batch fecundity was positively correlated with EW though variance was high [(2002: batch fecundity = 32.1*EW + 4387, $r^2 = .10$, $P = 0.046$), (2003: batch fecundity = 20.1*EW + 11,501, $r^2 = 10$, $P = 0.057$)].

Specimens from the 2003 samples averaged one year older and 100 grams larger than in the 2002 samples ($P < 0.05$). Ages four, five, and six were represented in 2002 with ages five and six (the 1996 and 1997 year classes) predominating. Ages four-nine were represented in 2003 with ages five, six, and seven (year classes 1996-1998) predominating. Thus, the increase in mean age and weight in 2003 was due to the return of the 1996 and 1997 year classes in that year. Weight at age for age five and six was similar in both years so size and age data was pooled to generate the following regression: (eviscerated weight = 512*ln(age) + 268, $r^2 = 21$. $p < 0.05$) (Figure 2).

Mean batch fecundity was lower in 2003 (mean = 35,000) than in 2002 (mean = 39,000) ($P = 0.16$ two sample t-test) despite the larger average age and size of females collected. This is reflected in lower average relative batch fecundity in 2003 (36.2 hydrated eggs/g EW 2002, 29.7 hydrated eggs/g EW 2003, $P = 0.03$ two-sample t-test)

Table 3. Oocyte counts, ovary weight, batch fecundity, eviscerated weight (EW), relative batch fecundity (eggs per gram EW), gonadosomatic index (GSI), age, spawning history, and presence or absence of POFs of individual fish collected in 2002. NA = not aged due to scale damage or absence.

Date	Eggs per gram ovary	Ovaryweight	Batch Fecundity	Eviscerated Weight (EW)	Relative Batch Fecundity	GSI	Age	Spawning Marks	POFs (Y/N)
3/28/02	212.2	253.8	53,865.1	1,280.3	42.1	19.8	6	1	Y
4/3/02	114.3	351.6	40,205.2	1,101.8	36.5	31.9	5	0	Y
4/3/02	187.8	337.8	63,444.4	1,002.9	63.3	33.7	6	1	N
4/3/02	126.9	374.5	47,539.4	1,254.9	37.9	29.8	5	0	Y
4/7/02	110.7	489.0	54,132.1	1,222.5	44.3	40.0	5	1	N
4/7/02	112.9	323.0	36,479.5	994.0	36.7	32.5	6	1	Y
4/7/02	139.4	299.0	41,666.2	1,200.0	34.7	24.9	5	0	Y
4/7/02	173.1	199.5	34,524.7	733.0	47.1	27.2	4	0	Y
4/7/02	139.7	91.0	12,713.0	1,011.5	12.6	9.0	5	0	N
4/7/02	114.4	316.0	36,145.0	977.0	37.0	32.3	5	0	N
4/7/02	83.9	198.5	16,659.5	895.5	18.6	22.2	5	0	N
4/7/02	67.1	299.0	20,071.9	1,204.0	16.7	24.8	NA	NA	N
4/7/02	95.8	327.0	31,314.5	1,097.0	28.5	29.8	NA	NA	Y
4/12/02	79.7	226.0	18,013.9	1,000.4	18.0	22.6	5	0	N
4/12/02	130.3	450.5	58,713.3	1,258.0	46.7	35.8	5	1	Y
4/12/02	167.7	304.2	51,021.2	1,137.5	44.9	26.7	5	1	Y
4/12/02	180.5	232.0	41,884.3	1,093.0	38.3	21.2	6	1	Y
4/12/02	190.4	190.8	36,334.6	1,109.9	32.7	17.2	5	0	Y
4/18/02	139.2	199.0	27,707.2	1,194.0	23.2	16.7	NA	NA	N
4/18/02	239.4	229.5	54,950.6	1,204.5	45.6	19.1	NA	NA	Y
4/18/02	240.2	187.5	45,046.3	1,047.0	43.0	17.9	5	0	Y
4/18/02	279.7	155.0	43,357.6	1,056.5	41.0	14.7	6	2	Y
4/18/02	199.1	195.0	38,833.6	1,357.0	28.6	14.4	6	1	Y
4/18/02	250.8	164.0	41,127.1	1,023.0	40.2	16.0	5	1	N
4/18/02	151.1	185.0	27,949.0	1,038.0	26.9	17.8	NA	NA	Y
4/18/02	245.3	214.5	52,607.4	946.5	55.6	22.7	5	0	Y

Table 4. Oocyte counts, ovary weight, batch fecundity, eviscerated weight (EW), relative batch fecundity (eggs per gram EW), gonadosomatic index (GSI), age, and spawning history of individual fish collected in 2003. NA = not aged due to scale damage or absence.

Date	Eggs per gram ovary	Ovaryweight	Batch Fecundity	Eviscerated Weight (EW)	Relative Batch Fecundity	GSI	Age	Spawning Marks
4/14/03	222.3	231.0	51,358.7	1,409.5	36.4	16.4	6	2
4/21/03	95.4	499.0	47,587.8	1,453.5	32.7	34.3	7	2
4/21/03	130.8	436.0	57,047.2	1,337.5	42.7	32.6	5	0
4/21/03	151.0	539.0	81,371.5	1,191.5	68.3	45.2	7	3
4/21/03	77.7	375.0	29,128.7	1,401.0	20.8	26.8	6	0
4/21/03	124.4	224.5	27,927.8	1,307.0	21.4	17.2	7	3
4/21/03	62.5	406.0	25,395.2	1,396.0	18.2	29.1	8	3
4/21/03	103.7	263.0	27,277.9	838.0	32.6	31.4	5	0
4/24/03	147.5	144.1	21,250.3	1,158.9	18.3	12.4	7	2
4/24/03	165.3	301.3	49,802.2	897.8	55.5	33.6	5	0
4/24/03	133.8	218.3	29,199.4	1,032.5	28.3	21.1	5	0
4/24/03	114.2	354.9	40,540.3	1,398.9	29.0	25.4	7	2
4/24/03	269.2	107.9	29,043.3	1,178.5	24.6	9.2	8	3
4/24/03	63.2	473.4	29,936.4	1,272.6	23.5	37.2	5	0
4/28/03	129.5	269.2	34,853.2	1,097.0	31.8	24.5	6	1
4/28/03	79.4	490.0	38,912.9	1,379.3	28.2	35.5	7	2
4/28/03	214.1	203.8	43,635.2	1,269.3	34.4	16.1	7	2
4/28/03	124.7	245.3	30,599.5	1,302.4	23.5	18.8	6	1
4/28/03	131.0	285.5	37,403.7	1,404.2	26.6	20.3	7	2
4/28/03	128.5	278.1	35,738.0	861.5	41.5	32.3	5	0
5/2/03	111.4	137.3	15,293.3	811.6	18.8	16.9	5	0
5/2/03	57.9	359.9	20,839.3	1,344.7	15.5	26.8	6	1

Table 4 continued.

Date	Eggs per gram ovary	Ovaryweight	Batch Fecundity	Eviscerated Weight (EW)	Relative Batch Fecundity	GSI	Age	Spawning Marks
5/2/03	132.0	259.8	34,292.2	1,110.9	30.9	23.4	5	0
5/2/03	72.6	418.9	30,401.2	1,235.7	24.6	33.9	5	0
5/2/03	89.9	279.8	25,149.3	1,254.4	20.0	22.3	NA	NA
5/2/03	111.2	302.8	33,670.6	1,162.1	29.0	26.1	5	0
5/2/03	144.1	371.1	53,491.5	1,090.8	49.0	34.0	7	2
37743	89.2	162.8	14,519.0	1,032.0	14.1	15.8	6	2
37743	100.9	478.4	48,266.3	1,758.9	27.4	27.2	6	2
37743	119.8	291.0	34,870.1	1,101.4	31.7	26.4	4	0
37743	82.6	312.8	25,836.1	1,096.4	23.6	28.5	4	0
37746	108.0	239.7	25,895.6	1,060.5	24.4	22.6	5	0
37746	124.0	138.2	17,133.7	862.4	19.9	16.0	NA	NA
37746	170.3	219.8	37,435.8	1,245.7	30.1	17.6	5	0
37746	200.3	187.7	37,588.3	1,203.0	31.2	15.6	7	3
37746	250.4	153.6	38,468.9	1,294.0	29.7	11.9	6	2
37746	252.6	195.8	49,462.9	1,471.4	33.6	13.3	9	4
37746	114.4	378.1	43,257.5	1,179.2	36.7	32.1	6	2
37746	98.3	240.5	23,647.8	1,169.7	20.2	20.6	NA	NA
37746	172.4	224.8	38,760.8	866.4	44.7	25.9	5	0
37746	157.5	271.1	42,688.5	1,198.8	35.6	22.6	6	1
37746	227.9	158.4	36,101.3	1,178.5	30.6	13.4	NA	NA
37746	160.2	140.5	22,508.1	1,105.0	20.4	12.7	5	0
37746	147.8	299.5	44,258.3	1,064.2	41.6	28.1	6	0
37746	141.9	165.9	23,538.1	1,121.9	21.0	14.8	6	2

Table 5. Relative batch fecundity (eggs per gram eviscerated weight) by date for 2002 and 2003. Mean relative fecundity between years is significantly different (p=0.029)

2002

Mean relative batch fecundity = 36.18

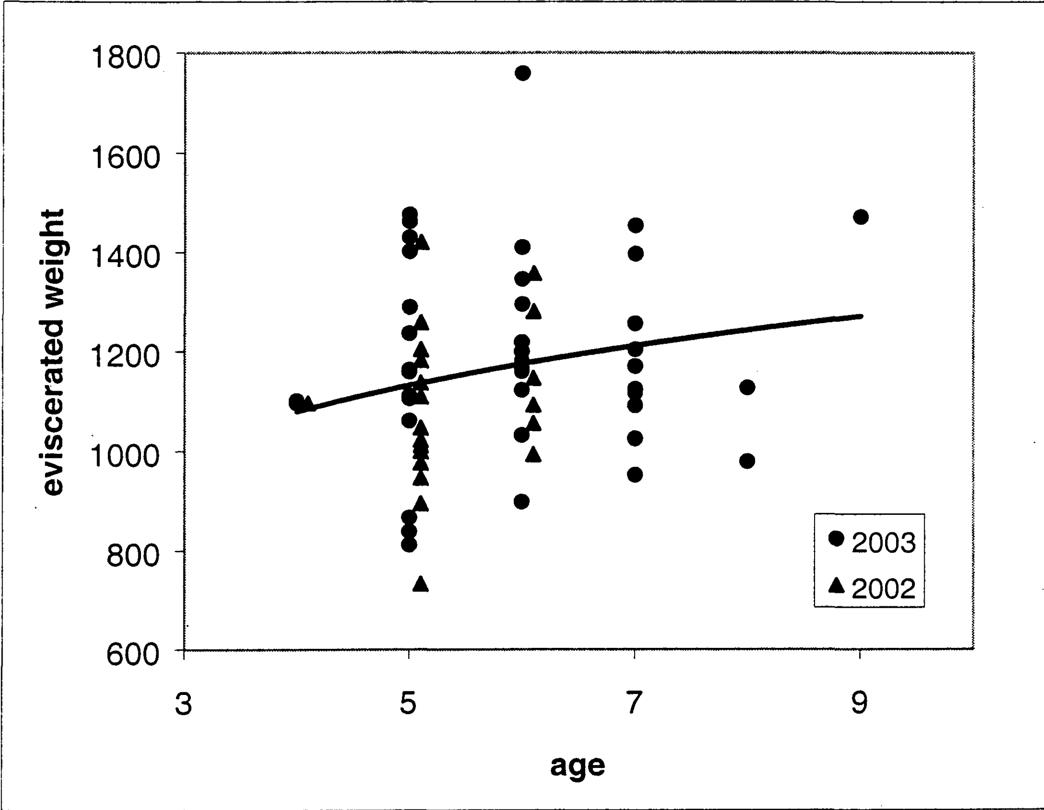
Date	N	Relative Batch Fecundity	SD
28-Mar	1	42.1	*
3-Apr	3	45.9	15.0
7-Apr	8	30.7	12.3
12-Apr	5	36.1	11.5
18-Apr	8	38.0	10.9
25-Apr	0	*	*

2003

Mean relative batch fecundity = 29.69

Date	N	Relative Batch Fecundity	SD
14-Apr	0	*	*
21-Apr	7	33.8	17.5
24-Apr	6	29.9	13.1
28-Apr	6	31.0	6.4
2-May	11	25.8	9.7
5-May	14	30.0	8.1

Figure 2. Evicscerated weight (EW) at age for shad capture in drift gill nets on the spawning grounds of the Mattaponi River, Virginia in 2002 and 2003. Regression is of pooled 2002 and 2003 data. (eviscerated weight = 512*ln(age) + 268, $r^2 = 21$. $p < 0.05$)



1800
1600
1400
1200
1000
800
600
3
5
7
9
eviscerated weight
age
2003
2002

In 2002, there was a significant difference detected between relative batch fecundity in repeat (41 hydrated eggs/g EW, n = 10) and virgin (32 hydrated eggs/g EW, n = 11) spawners (two sample t-test, p = 0.08, Table 6) though small sample sizes render this finding suspect. There was no significant difference in relative batch fecundity between ages [one-way ANOVA by age, p = 0.59, (Table 7)] or between repeat (mean = 30.3 hydrated eggs/g EW, n = 24) and virgin (mean = 31 hydrated eggs/g EW, n = 18) spawners in 2003 (Table 6). There was no difference detected in relative batch fecundity between age five specimens from 2002 and 2003 (Table 7). Age six specimens had higher relative batch fecundity in 2002 than in 2003 (two sample t-test, p = 0.033, Table 5). The mean GSI was identical in each year (23.9). Batch fecundity was weakly positively correlated with GSI (Figure 3). The mean GSI declined by date within each year [(2002, one-way ANOVA by date df = 3, f = 3.9, p = 0.016), (2003, one-way ANOVA by date, df = 4, f = 3.1, p = 0.026)], (Table 8). The number of hydrated eggs per gram of ovary was greater in 2002 than in 2003 (p=0.06). Hydrated eggs per gram ovary was highest on the last sample date in 2002 and 2003 (one-way ANOVA by date, p = 0.001 and 0.007 respectively). The number of hydrated eggs per gram ovary was negatively correlated with GSI (Figure 4).

Mean relative batch fecundity in 2002 was 25% higher in fish in which POFs were detected. Mean relative batch fecundity in specimens that contained POFs was 39.6 eggs/gram EW (n = 17). Mean relative batch fecundity in specimens without POFs was 29.7 eggs/gram EW (n = 9). However, mean relative batch fecundity was not significantly different between the two groups (two sample t-test, p = 0.11).

Table 6. Mean relative batch fecundity (Eggs/ g EW) for specimens grouped by the number of spawning marks in 2002 and 2003.

Spawning Marks	Number	Mean Relative Batch Fecundity	SD
0	11	32.3	11.5
1	9	41.8	13.4
2	1	41.2	

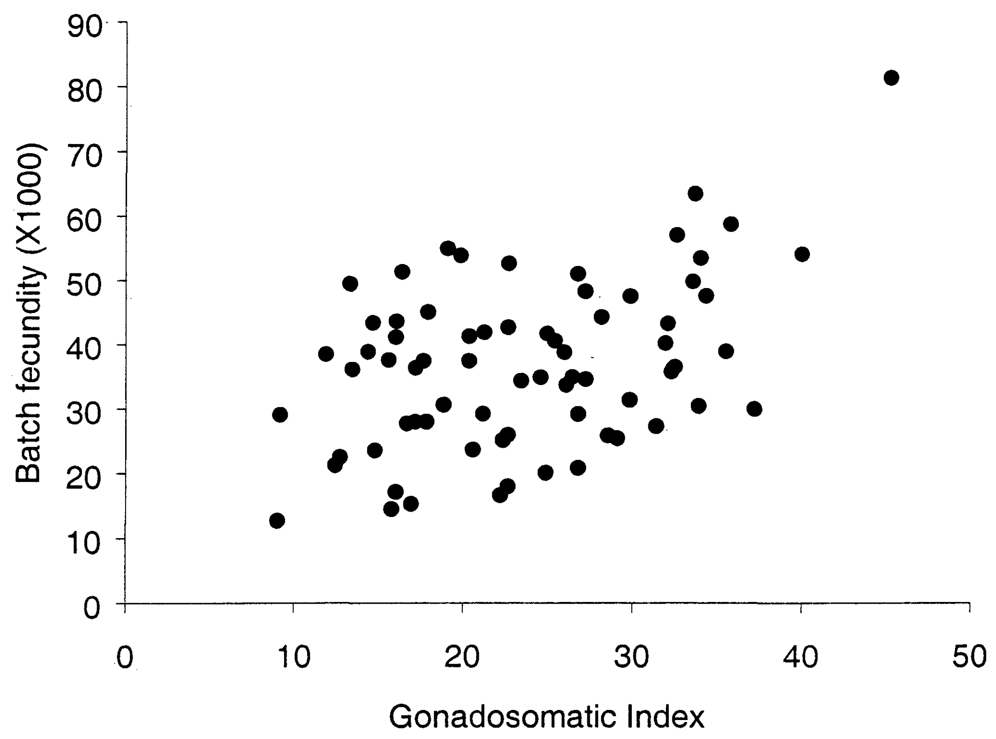
Spawning Marks	Number	Mean Relative Batch Fecundity	SD
0	18	31.4	10.1
1	4	26.6	9.0
2	3	29.5	9.0
3	6	33.4	18.4
4	1	33.6	

Table 7. Mean relative batch fecundity (Eggs/g EW) for each age group in 2002 and 2003.

Age	Number	Mean Relative Batch Fecundity	SD
4	1	28.5	
5	14	34.8	13.3
6	6	41.7	11.6

Age	Number	Mean Relative Batch Fecundity	SD
4	2	28.5	13.5
5	14	32.0	10.4
6	12	28.7	8.7
7	11	34.3	14.7
8	2	21.9	4.6
9	1	34.6	

Figure 3. A scatterplot of batch fecundity and gonadosomatic index (GSI) of American shad in the Mattaponi River in 2002 (N = 26) and 2003 (N = 44).



Batch fecundity (X1000)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Gonadosomatic Index

Table 8. Summary of gonadosomatic index (GSI) estimates by date within year of hydrated females used for batch fecundity counts.

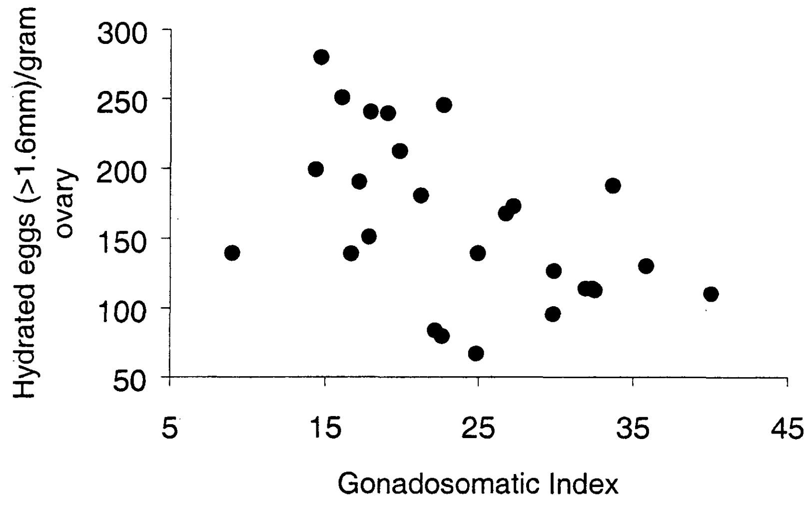
2002
One-Way ANOVA
(df = 3,F = 3.9,p = 0.016)

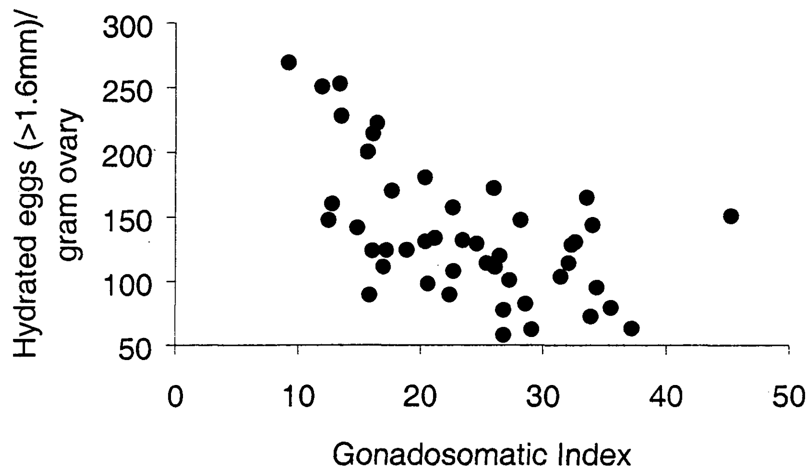
Date	N	Mean GSI	StDev
3-Apr	3	31.8	1.9
7-Apr	9	27.0	8.6
12-Apr	5	24.5	7.1
18-Apr	8	17.4	2.7

2003
One Way ANOVA
(df = 4,F = 3.1,p = 0.026)

Date	N	Mean GSI	StDev
21-Apr	7	31.0	8.4
24-Apr	6	23.0	11.2
28-Apr	6	24.6	7.8
22-Apr	11	25.5	5.8
25-Apr	14	19.1	6.3

Figure 4. Scatterplots of hydrated oocyte density versus gonadosomatic index. Hydrated oocyte density is negatively correlated with GSI.


2002
Hydrated eggs (>1.6mm)/gram ovary
300
250
200
150
100
50
5
15
25
35
45
Gonadosomatic Index


2003
Hydrated eggs (>1.6mm)/ gram ovary
300
250
200
150
100
50
0
10
20
30
40
50
Gonadosomatic Index

General Histology

Seven oocyte types (Table 9) and four age classes of POFs were noted in sections. Primary growth, partially yolked, and yolked oocytes were present in most sections (Figure 5A). Primary growth oocytes were small (<200μm), stain darkly basophilic, and have no yolk vesicles, lipid vesicles, or cortical alveoli. Partially yolked oocytes (200-900μm) varied from light basophilic structures with clear lipid vesicles and the beginnings of yolk accumulation to larger and more eosinophilic with yolk accumulated around the center of the egg. Yolked oocytes (900-1400μm) were large and had eosinophilic yolk throughout, except for a ring of clear lipid vesicles around the periphery of the egg bordering the zona radiata. Sections from fish undergoing final maturation contained oocytes in various stages of nuclear migration termed germinal vesicle migration (GVM) or migratory nucleus stage. The migratory nucleus stage was recognized as three stages; early migratory nucleus (EMN), intermediate migratory nucleus (MN), and late migratory nucleus (LMN) (Figure 6). EMN stage was characterized by the nucleus being displaced toward the periphery of the egg, but the yolk being largely unchanged compared to yolked oocytes. MN stage was characterized by the displacement of the nucleus further toward the periphery of the oocyte and by the yolk beginning to coalesce into large but still mostly small, richly eosinophilic globules. EMN and MN stage were generally slightly larger (1300-1500μm) than yolked oocytes. LMN stage eggs were typified by the coalescence of yolk into large pale staining plates as if hydrated. The size increase between MN and LMN is dramatic. However, the dehydration process for paraffin histology caused the LMN stage oocytes to shrink considerably due to the extraction of water and lipid. Late germinal vesicle migration

Table 9. Description of basic oocyte types observed in American shad.

1. Primary Growth (PG): These are small with a large central nucleus (germinal vesicle) and dark basophilic cytoplasm. The germinal epithelium is not yet pronounced and consists of a single layer of simple squamous epithelia. The chorion is not yet developed. No yolk vesicles are visible. Multiple nucleoli are visible within the nucleus.

2. Partially Yolked (PY): This category combines the earliest vitellogenic stage through most yolk accumulation. These exhibit a ring of basophilic cytoplasm surrounding the germinal vesicle. In some, only clear lipid vesicles are present in the cytoplasm. As they mature eosinophilic yolk vesicles appear among the lipid vesicles and proliferate throughout the ooplasm. The germinal vesicle is centrally located. The chorion is evident and the germinal epithelium is now distinguishable as two separate layers, a cuboidal granulosa layer and squamous theca layer.

3. Yolked (Y): The germinal vesicle is still centrally located. Vitellogenesis is generally complete with the entire volume of the cell filled with eosinophilic yolk globules. Lipid vesicles line the periphery of the oocyte lying just below the chorion.

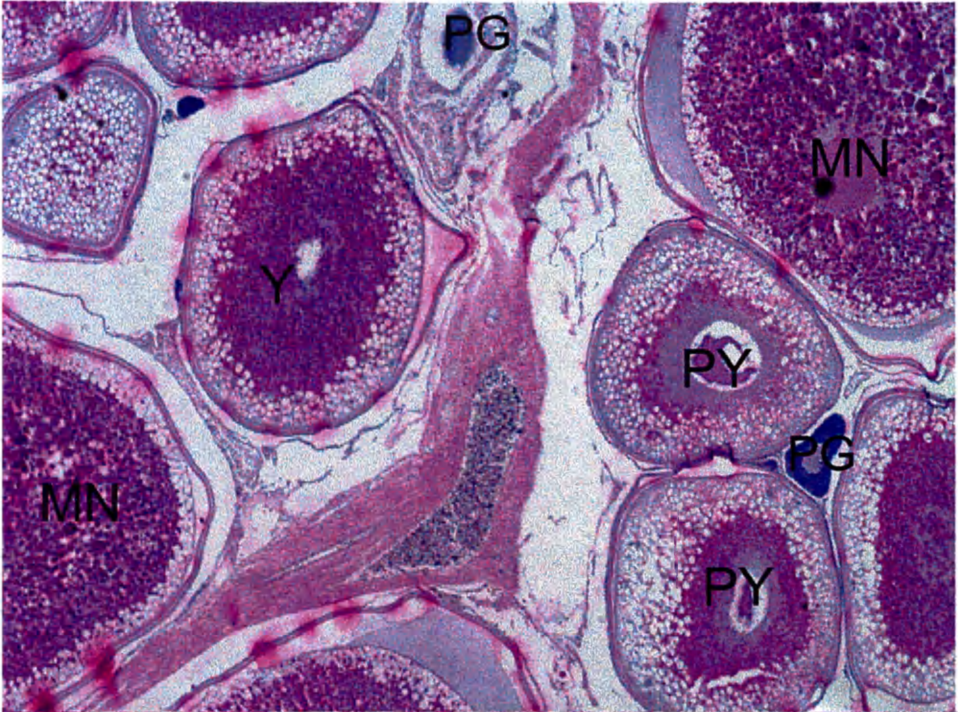
4. Early migratory nucleus stage (EMN). The germinal vesicle is displaced slightly off center in sections. The oocyte otherwise looks like a yolked oocyte.

5. Migratory nucleus stage (MN). The migratory nucleus stage marks the onset of final maturation. The germinal vesicle is displaced toward the periphery of the cell. At the onset of this stage, the yolk globules resemble those of yolked oocytes but may be slightly coalesced. Once the germinal vesicle reaches the periphery of the oocyte, the yolk begins to coalesce.

6. Late migratory nucleus stage (LMN). The germinal vesicle has reached the periphery of the egg. The yolk has mostly coalesced into large pale staining globules and oocyte diameter has increased.

7. Hydrated (H): The germinal vesicle is no longer evident. Yolk has coalesced into large pale staining globules.

Figure 5. Histologic appearance of basic oocyte types. PG = primary growth, PY = partially yolked, Y = yolked, MN = migratory nucleus stage, H = hydrated


PG
MN
Y
PY
PG
MN
PY

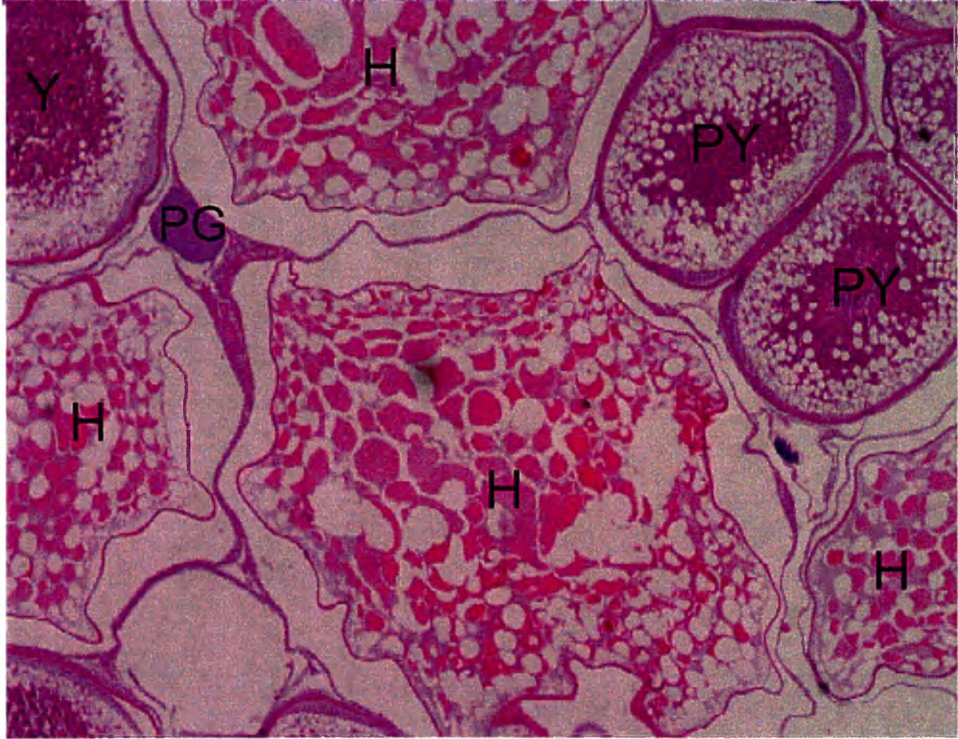
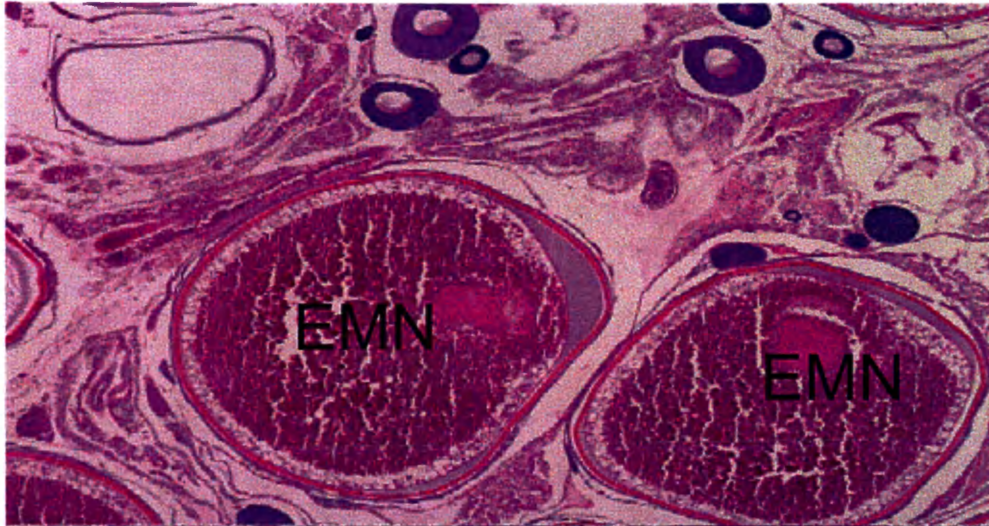
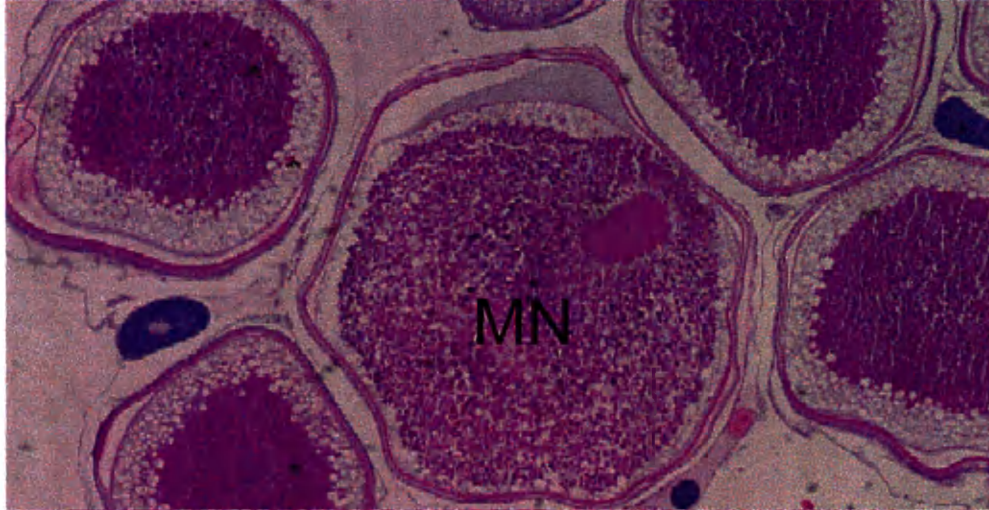
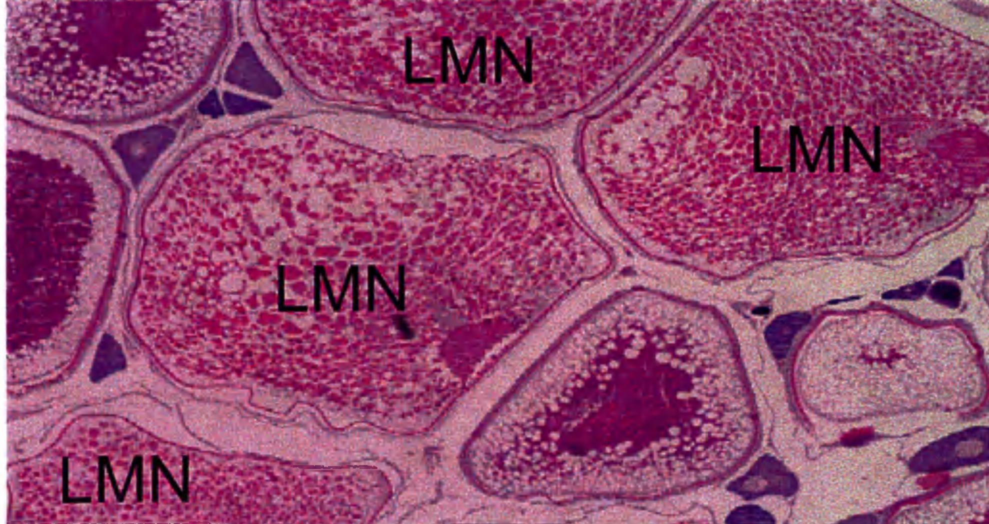

Y
H
PY
PG
PY
H
H
H

Figure 6. Phases of nuclear migration leading to final maturation. EMN = early migratory nucleus, MN = migratory nucleus, LMN = late migratory nucleus. In EMN and MN oocytes the germinal vesicle (GV) moves progressively toward the periphery of the oocyte with slight coalescence of the yolk. In LMN oocytes the GV has reached the periphery of the oocyte and the yolk has coalesced into larger, paler globules.


EMN
EMN


MN


LMN
LMN
LMN
LMN

was distinguishable from hydrated stage oocytes by the presence of the not-yet dissolved nucleus at the apex of the cell. Ripe ovaries contained hydrated oocytes and ovaries that had undergone very recent ovulation contained new POFs (Figure 7). Hydrated oocytes stained pale pink and often shrunk substantially due to the extraction of lipid and water in processing (Figures 5B, 7A). No germinal vesicle was evident. New POFs had a large central lumen with a very convoluted granulosa layer (Figures 8A and 9A). The cells of the granulosa and theca layers were little changed in appearance from the follicles still enveloping oocytes and had prominent apical basophilic nuclei. As the POFs deteriorated the granulosa cells hypertrophied and stained with less intensity (see following section).

Alpha and beta atretic oocytes were also observed (Figure 10). In alpha atresia, the zona radiata dissolves and the granulosa cells invade the yolk appearing to phagocytize the cell contents. The granulosa hypertrophy and begin to present large vacuoles in sections. In oocytes undergoing beta atresia, the yolk has been resorbed and all that remains are pale basophilic remnants of the granulosa cells, usually possessing large vacuoles.

POF Aging

POFs were divided into four age categories with age based on the observation that spawning is centered around dusk, especially later in the season based on the timing of appearance of running ripe specimens. The physical appearance of POFs was similar in fish during both 72 hour collection although POFs appear to have degraded more rapidly

Figure 7. Histologic appearance of ripe, running ripe, and recently spawned ovaries. (A) Ripe/Hydrated. (B) Running ripe. An ovulated hydrated oocyte is visible as is a new POF. (C) New POFs are extremely abundant

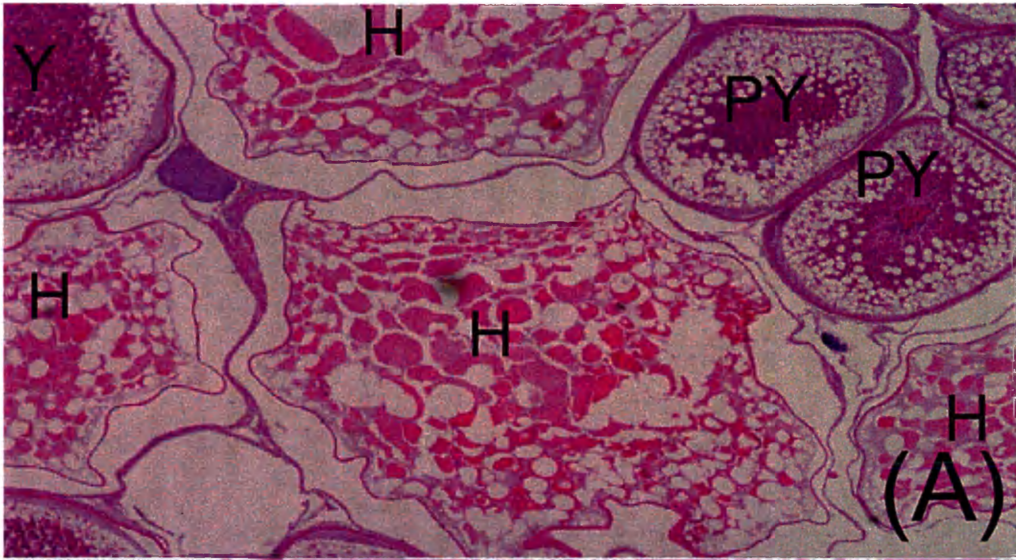
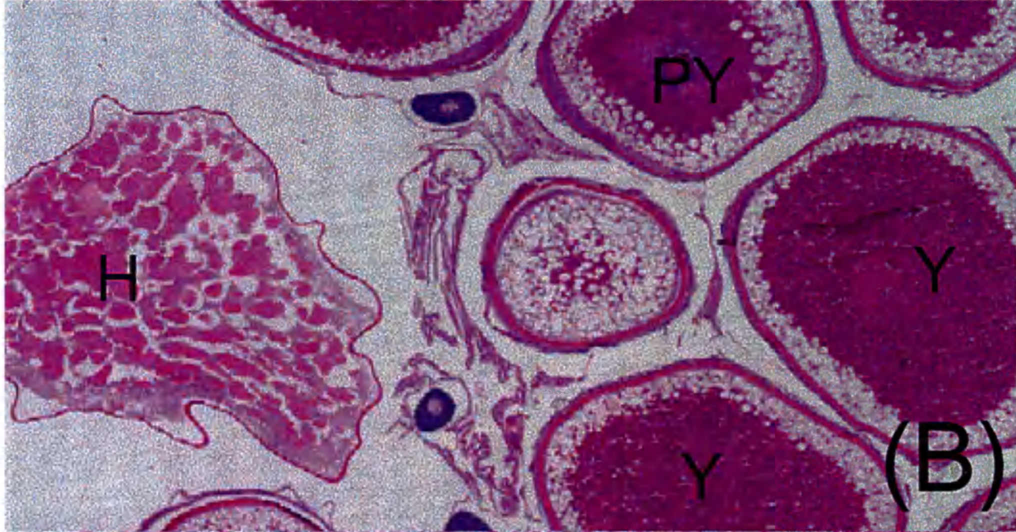
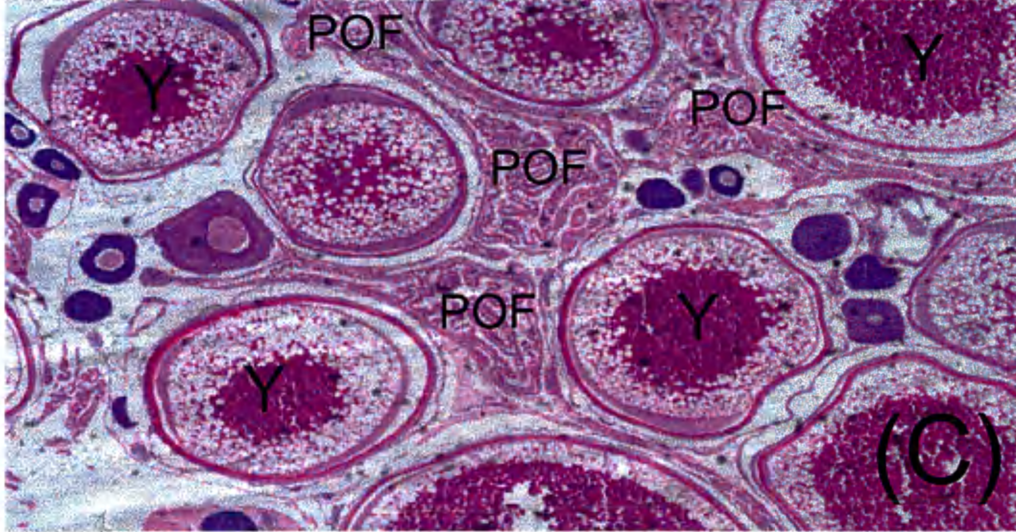

Y
H
PY
PY
H
H
H
(A)

PY
H
Y
Y
(B)

POF
Y
Y
POF
POF
POF
Y
Y
(C)

Figure 8. General appearance of POFs through the first 24hours of degeneration. (A) New POF. 40x. (B) 12hour POF. 40x. (C) 24hour. 40x.

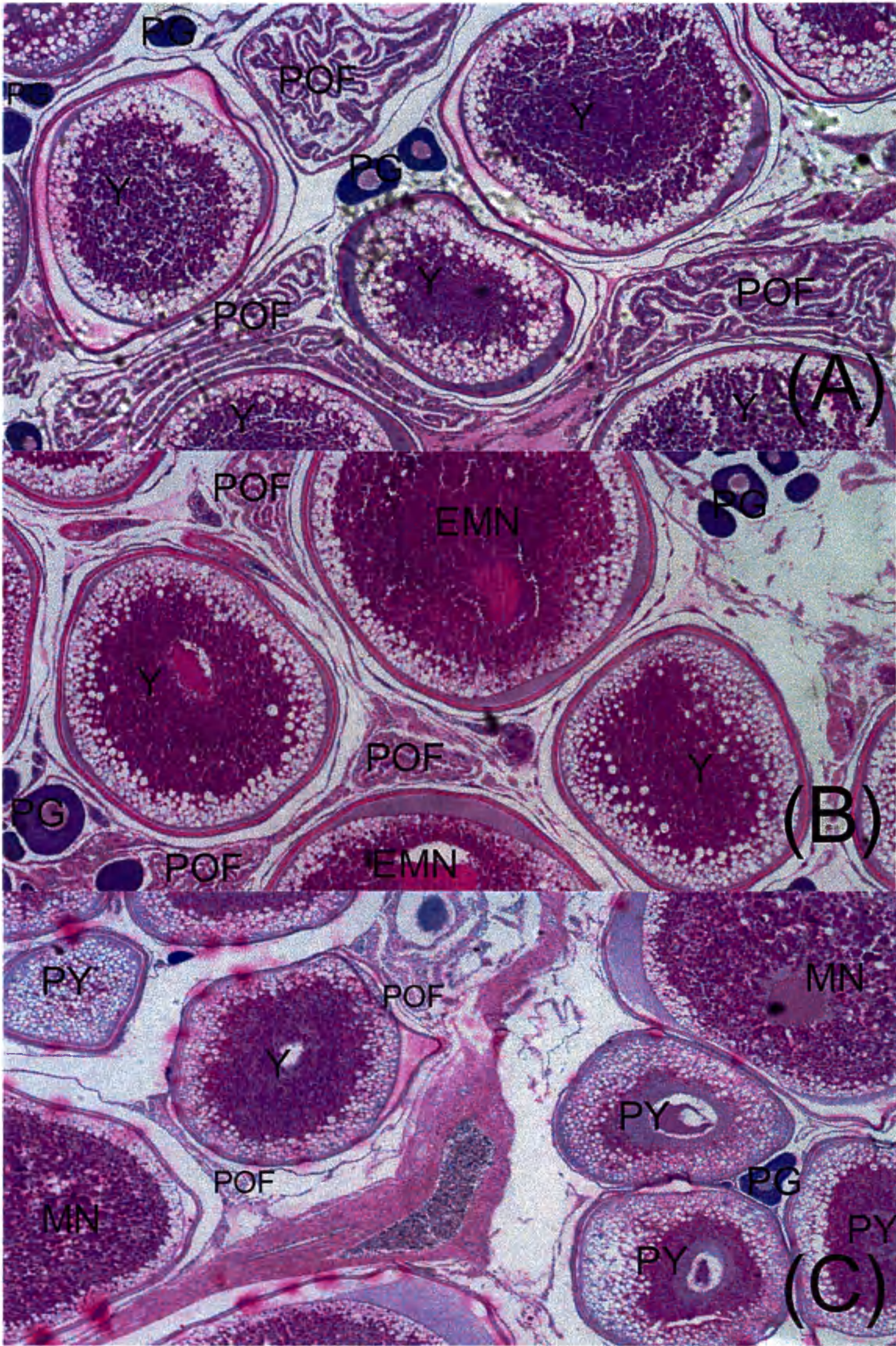
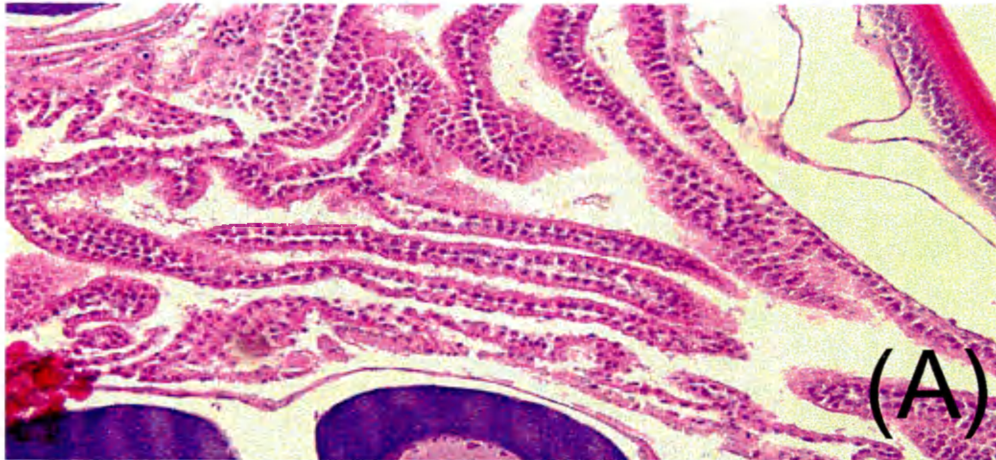
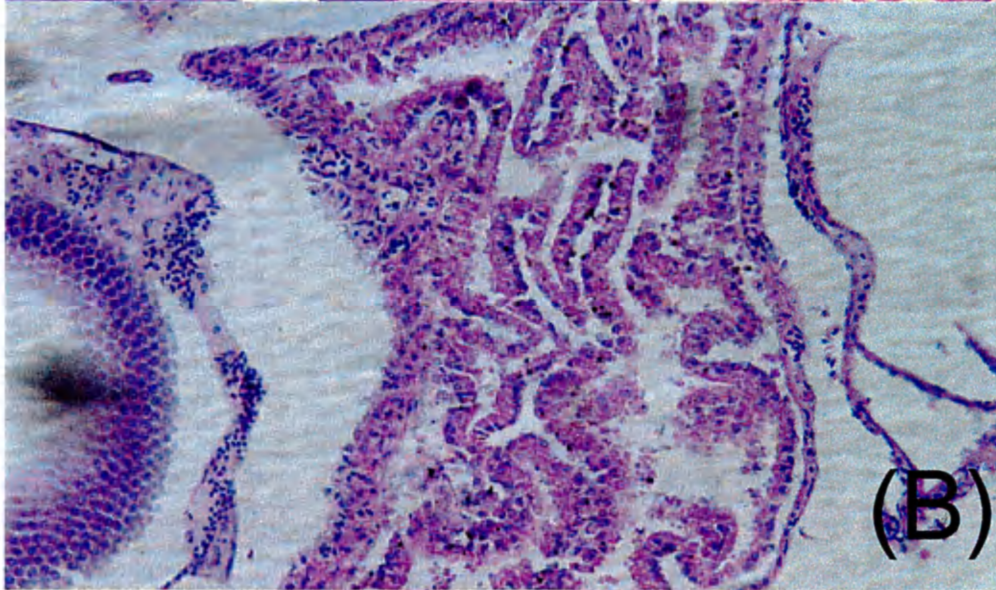

PG
POF
PG
Y
PG
Y
Y
POF
POF
Y
Y
(A)
POF
EMN
PG
Y
POF
Y
PG
POF
EMN
(B)
PY
POF
MN
Y
PY
POF
PG
MN
PY
PY
(C)

Figure 9. New and one-day POFs at higher magnification. 200x (A) New POFs. (B) 12-hour POFs. (C) 24-hour POFs


(A)


(B)

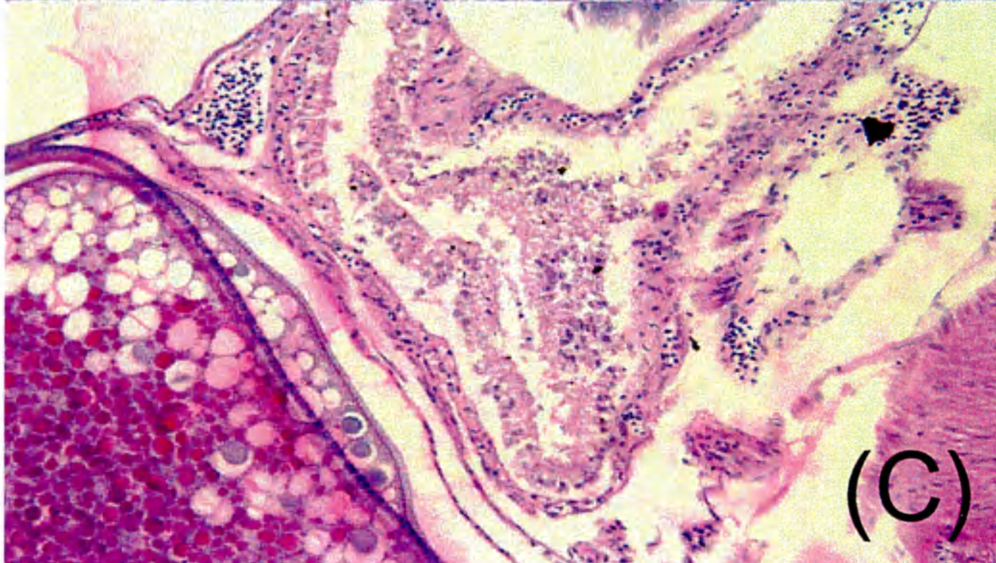
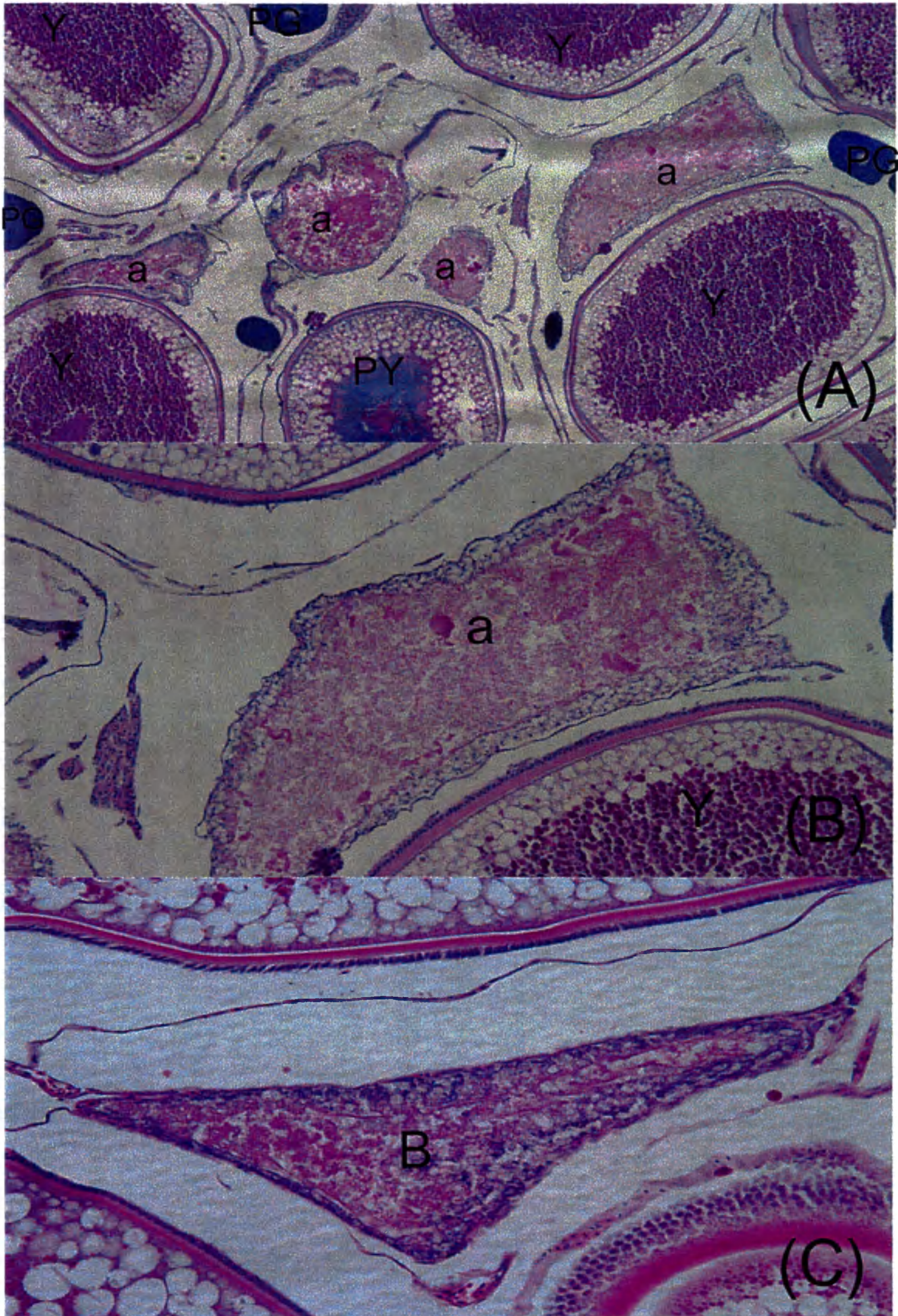

(C)

Figure 10. Appearance of atretic oocytes. Alpha (α)and Beta (β) atresia. (A) 40x alpha atresia. (B) 100x alpha atresia.
(C) 200x beta atresia


Y
PG
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a
PG
a
a
a
Y
Y
PY
(A)
a
Y
(B)
B
(C)

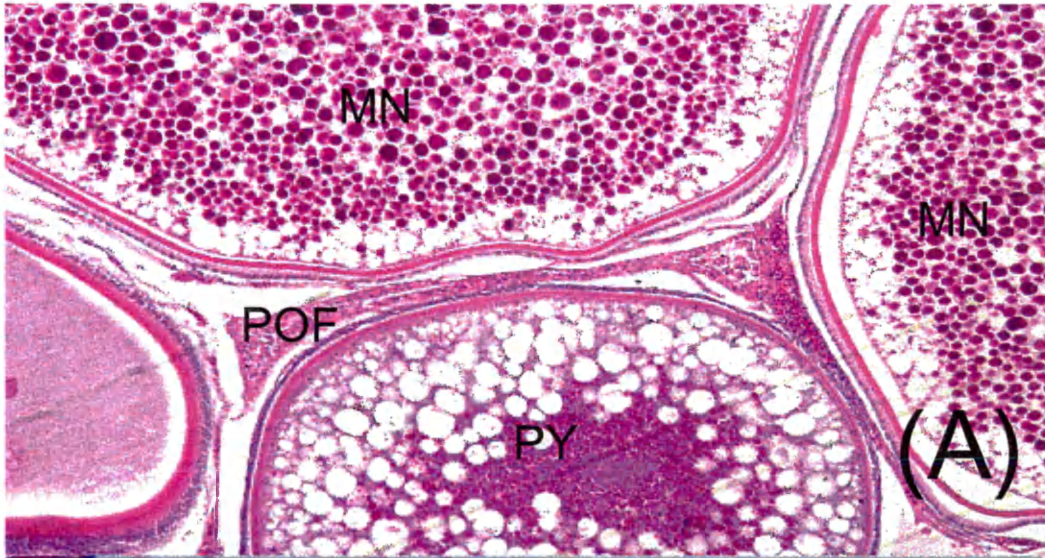
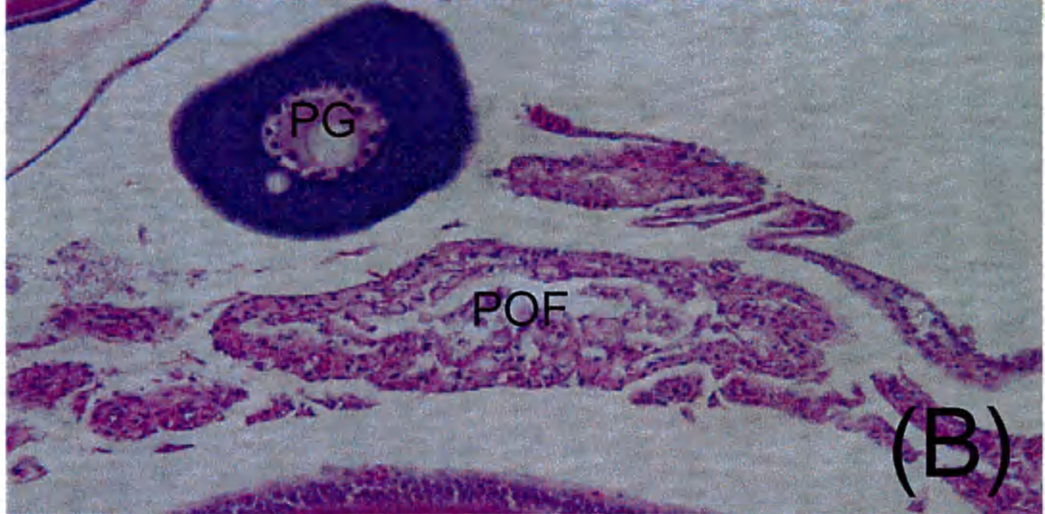
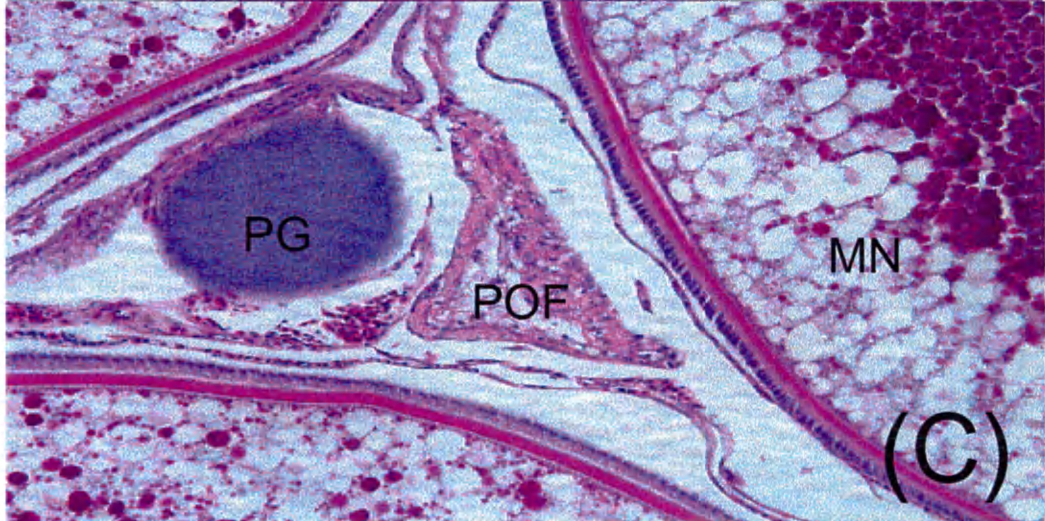
in warm water than in cooler. For example, POFs assumed to be 24-h old in samples collected at temperatures above 20°C resembled POFs assumed to be 36-h old in samples collected at temperatures below 20°C. Similar criteria for aging were applied across all temperatures. However criteria were adjusted to account for more rapid deterioration, at higher temperatures (>20°C).

New POFs: New POFS indicate that spawning has occurred within twelve hours. New POFs (Figures 8A and 9A) have a large and convoluted central lumen. At higher magnification, the granulosa have a very distinct cuboidal shape with well-defined borders and prominent round basophilic nuclei.

One-day POFs: One-day POFs indicated that spawning occurred within 12-24hours. One-day POFs show a range of appearance as they degrade. They range from one-half to one-quarter the size of new POFs. The central lumen is reduced in size and less convoluted. The granulosa layer is still recognizable but becomes increasingly ragged and the granulosa cells begin to hypertrophy. By the end of the day the lumen is still obvious but has lost most of its folding. The granulosa cells have lost the distinguishing columnar to cuboidal shape and stain with less intensity. POFs are classified as 1day up to the point where the lumen is barely evident (Figures 8B&C and 9B & C). At higher temperatures, the lumen was fully collapsed by 24-h post spawn. At temperatures above 20°C one-day old POFs included those resembling figure 11A.

Two-day POFs. These indicate that spawning occurred within 24-48hours. These range in size from two to three times larger than primary growth oocytes to roughly the size of primary growth oocytes. The lumen has little to no folding if at all evident. Staining affinity is much weaker than in fresh POFs. The nuclei of some of the granulosa

Figure 11. Appearance of POFs including one-day(high temperature), Two-day, and older POFs. (A). one-day POF at high temperature, newer two-day POF at lower temperature. 100x. (B). Two day POF, older. 200x. (C). 2 day POF at high temperature, older than two day POF at lower temperature. 200x.


MN
MN
POF
PY
(A)

PG
POF
(B)

PG
POF
MN
(C)

cells are still evident. Most of the individual granulosa are difficult to distinguish from one another. In the most degraded POFs classified as two-day, the granulosa and theca layers are reduced to a pale eosinophilic strip of tissue invaded with dark basophilic staining erythrocytes and occasionally macrophages (Figure 11). At temperatures above 20°C, POFs classified as two-days old included POFs resembling Figure 11c.

Older POFs: Older POFs were very small pale eosinophilic structures with little form or obvious cell structure. They were often triangular and smaller than primary growth oocytes (Figure 11c). When temperatures exceeded 20°C any POF more degraded than that depicted in Figure 11c was assigned as older. Older POFs were similar to the beta atretic oocytes previously described. However, the beta atretic oocytes tended to be more basophilic and had larger vacuoles in the remaining granulosa cells.

Timing of spawning and final maturation

The onset of final maturation (the migration of the nucleus and subsequent hydration of the oocyte) was detected in thin sections up to 36 hours before spawning assuming that spawning activity peaks at dusk. The presence of EMN and MN stage oocytes indicates that spawning should occur the day following capture. The presence of LMN and hydrated oocytes indicates that spawning was likely during the day of capture. New POFs indicate that spawning has very recently occurred. EMN stage oocytes were most prevalent in early and late morning samples and dropped in prevalence later in the day as maturation of that batch progressed (Table 10, Figure 12). MN stage oocytes peaked in prevalence in mid-late afternoon specimens. LMN oocytes began to appear in the late evening and were prevalent in morning samples. Figure 13 is a timeline of final

Table 10. Average percent frequency of samples containing oocyte types by time of day over each 72-hour sampling period. (EMN = early migratory nucleus, MN = migratory nucleus, LMN = late migratory nucleus, H = hydrated, NP = new POF)

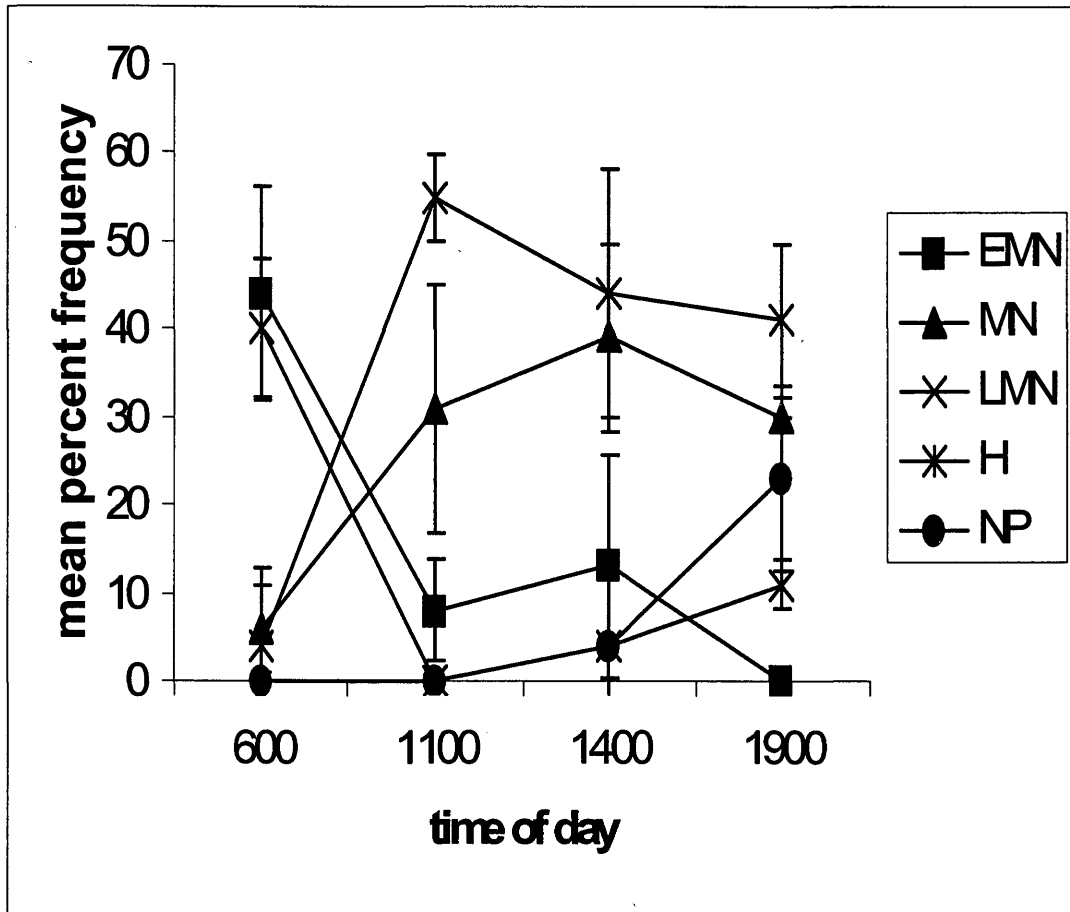
April 4-7th (water temperature = 13-16°C, sunrise 05:50 EST, sunset 18:35 EST)

Time of Day	EMN	MN	LMN	H	New POF
0600	27	22	3	32	5
1000	39	33	0	29	0
1400	13	39	7	18	21
1800	14	27	23	7	25

April 17-20th (water temperatures = 22-26°C, sunrise 05:30 EST, sunset 18:50EST)

Time of Day	EMN	MN	LMN	H	New POF
600	44	6	40	4	0
1100	8	31	0	55	0
1400	13	39	4	44	4
1900	0	30	11	41	23

Figure 12. Mean frequency of occurrence of ovaries containing EMN, MN, LMN, hydrated oocytes, and new POFs by time of day for April 17th – 20th. EMN and LMN both peak in the morning and then drop during the day. MN and hydrated increase in prevalence as EMN and LMN decline. New POFs appear as hydrated ovaries become less prevalent and signal the occurrence of spawning.



mean percent frequency
70
60
50
40
30
20
10
0
600
1100
1400
1900
time of day
EMN
MN
LMN
H
NP

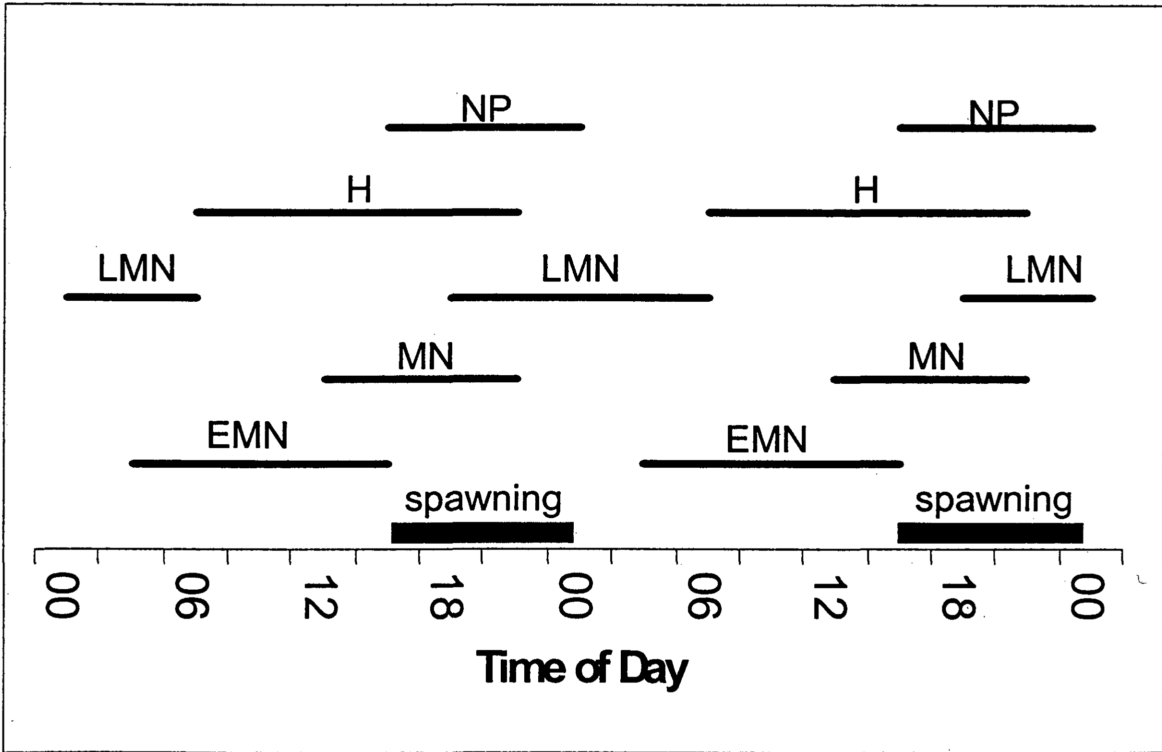
maturation, based on the April 17th-20th data, beginning with the onset of nuclear migration. The timeline represents two consecutive 24-hour periods. One complete cohort of eggs is depicted from 03h on day one to 00h at the end of day two. A cross section at any time represents the stages of oocytes that would be encountered in a sample taken at that time of day. A similar pattern was apparent but with more over-lap between stages in the April 4th-7th collections based on the analysis of the frequencies in Table 10.

Judging from histological data, spawning occurs in the afternoon and extends through the night and tapers off near daybreak early in the season (Figure 13). Spawning shifted toward dusk later in the season and did not continue until morning (Table 10). In both sample periods, new POFs appeared in the 2PM and 6PM samples and were generally absent in morning samples (Table 10). Twenty-one percent of females collected in the early afternoon in the April 4th-7th collection possessed new POFs while only four percent of females collected at that time in the April 17th-20th collection possessed new POFs. On April 4-7th, hydrated fish were most prevalent in the dawn and mid-morning samples. This pattern shifted on April 17th-20th with the proportion of hydrated fish peaking in the middle of the day and afternoon.

Histological reading consistency

A chi-squared test of symmetry (Hoenig et al. 1995) of the 155 histological slides from the April 4th-7th collection and the 170 histological slides from the April 17th-20th collection failed to detect a pattern of disagreement between readings in classifying oocytes and POFs [(p-value = 0.65 and 0.91 respectively), (Table 11 and 12)]. Stage

Figure 13. Schematic timeline of final maturation in American shad on the Mattaponi River beginning with the onset of nuclear migration. A cross section at any time represents the stages of oocytes that would be encountered in a sample taken at that time of day. Early nuclear migration (EMN). Nuclear migration (MN). Late nuclear migration (LMN). Hydrated (H). New POFs (NP).



NP
NP
H
H
LMN
LMN
LMN
MN
MN
EMN
EMN
spawning
spawning
00
06
12
18
00
06
12
18
00
Time of Day

Table 11. Reading to reading consistency (percent agreement and χ^2 test of symmetry) for the April 4th-7th, 2002 collections (N =155 slides). The χ^2 statistic was calculated for oocytes and POFs separately because it is impossible to confuse POFs and oocytes. Thus each table is treated as two 4X4 tables. Observations in agreement are in bold.

Reading 1

Reading 2	EMN	MN	LMN	H	NewPOF	1day	2day	3day
EMN	**20**	5	0	0				
MN	6	**29**	6	0				
LMN	0	3	**15**	0				
H	0	0	0	**29**				
NewPOF					**26**	0	0	0
1day					0	**44**	5	0
2day					0	6	**47**	7
3day					0	0	6	**29**

Table 12. Reading to reading consistency (percent agreement and χ^2 test of symmetry) for the April 17th-20th, 2002 collections (N =170 slides). The χ^2 statistic was calculated for oocytes and POFs separately because it is impossible to confuse POFs and oocytes. Thus each table is treated as two 4X4 tables. Observations in agreement are in bold.

Reading 1

Reading 2	EMN	MN	LMN	H	NewPOF	1day	2day	3day
EMN	**23**	3	0	0				
MN	4	**39**	2	0				
LMN	0	1	**19**	0				
H	0	0	0	**57**				
NewPOF					**15**	0	0	0
1day					0	**50**	10	0
2day					0	5	**43**	9
3day					0	0	13	**29**

assignment never differed by more than one class. For example, one reading never reported EMN while the other indicated LMN.

Overall, agreement between the two readings was good, although disagreement was higher for older POFs in the April 17th-20th collection (Table 12). There was also a notable disagreement between readings for LMN and MN stage oocytes in the April 4th-7th collections. All errors occurred in the evening collections when LMN first appeared in early April. Since this was the time of day when LMN oocytes first appeared, many were likely at a transition point between MN and LMN and were difficult to assign. Provided error is consistent in each direction, this error should not have affected estimates of spawning frequency. The EMN+MN category was rarely confused with the LMN+H+NewPOF category. Likewise, NewPOFs were never confused with one-day-old POFs and one-day-old POFs were infrequently confused with two-day-old POFs.

Spawning frequency

Histological Method

Each sample generated four or five estimates of spawning frequency depending on the presence of older POFs. The separate estimates were based on: (1) fish beginning final maturation that will spawn in the day following capture (EMN +MN); (2) ripe fish {LMN+hydrated(H)+new POFs(NP)}; (3) fish containing one-day-old POFs; (4) fish containing two-day-old POFs; (5) and fish containing older POFs when water temperatures were below 20°C (Table 13). Values reported for each observation are the estimated number of days between spawns. The value is calculated as the total number of

Table 13. Spawning frequency of American shad based on analysis of histological data. Each observation in the table is the estimated average number of days between each spawning event (reciprocal of the number of females possessing the stated structure divided by the number of females in the sample) with the number of females possessing the specified structure observed in the ovary noted in parentheses. Days till spawn is the number of fish observed with the specified structure divided by the number of females in the. EMN = early migratory nucleus stage, MN = migratory nucleus stage, LMN = late migratory nucleus stage, H = hydrated, NP = new POFs. Row means and column means include the standard deviation in brackets. N = 133 for April 4-7th. N = 159 for April 17-20th.

April 4-7th

N	date	time	EMN+MN	LMN+H+NP	1day POF	2day POF	older	row mean [stdev]
13	5-Apr	600	1.3(10)	6.7(2)	1.6(8)	2.6(5)	1.9(7)	2.5[2.2]
12	6-Apr	600	4.0(3)	1.7(7)	4.0(3)	4.0(3)	2.0(6)	3.1[1.2]
15	7-Apr	600	2.1(7)	2.1(7)	3.0(5)	5.0(3)	2.1(7)	2.9[1.3]
12	5-Apr	1400	2.4(5)	1.7(7)	4.0(3)	2.4(5)	5.9(2)	3.3[1.7]
16	6-Apr	1400	2.0(8)	2.0(8)	5.3(3)	2.7(6)	5.3(3)	3.4[1.7]
10	7-Apr	1400	1.4(7)	3.3(3)	2.5(4)	2.5(4)	3.3(3)	2.6[0.8]
12	4-Apr	1800	2.4(5)	1.7(7)	2.4(5)	2.4(5)	2.0(6)	2.2[0.3]
30	5-Apr	1800	2.5(12)	1.9(16)	2.7(11)	2.0(15)	3.8(8)	2.6[0.7]
13	6-Apr	1800	2.2(6)	1.9(7)	3.3(4)	3.3(4)	4.3(3)	3.0[1.0]
		column mean [stdev]	2.3[1.2]	2.6[1.6]	3.1[1.1]	3.0[1.0]	3.4[1.5]	2.9[1.7]

April 17-20th

N	date	time	EMN+MN	LMN+H+NP	1day POF	2day POF	row mean [stdev]
20	18-Apr	530	2.2(9)	2.0(10)	2.5(8)	2.2(9)	2.2[0.2]
17	19-Apr	530	1.7(10)	3.4(5)	1.7(10)	4.3(4)	2.8[1.2]
14	20-Apr	530	2.8(5)	1.8(8)	3.5(4)	2.8(5)	2.7[1.0]
13	18-Apr	1100	2.6(5)	1.9(7)	2.6(5)	2.2(6)	2.3[0.5]
21	19-Apr	1100	2.1(10)	1.9(11)	3.5(6)	2.3(9)	2.5[0.7]
18	20-Apr	1400	1.6(11)	2.6(7)	2.0(9)	2.6(7)	2.2[0.5]
27	17-Apr	1900	2.5(11)	1.7(16)	3.9(7)	1.8(15)	2.5[1.1]
12	18-Apr	1900	2.4(5)	1.7(7)	3.0(4)	2.4(5)	2.4[0.7]
17	19-Apr	1900	2.4(7)	1.9(9)	2.8(6)	2.1(8)	2.3[0.4]
		column mean [stdev]	2.3[0.5]	2.1[0.5]	2.8[0.8]	2.5[0.6]	2.4[0.7]

fish in the sample divided by the number of fish in the sample containing the specified ovarian characteristic. A higher value should be interpreted as a lower spawning frequency since it represents more days between spawns and fewer spawns per season. In Table 13, the row mean is the average spawning frequency for each sample. The column mean is the average spawning frequency from each histological characteristic across samples. An overall mean is listed in the lowest rightmost cell and is an average of all individual estimates. In the following, each 72-h sampling period in April 2002 is treated separately.

April 4-7th: Histological analysis yielded 45 individual estimates of spawning frequency, ranging from 1.3 to 6.7 days. The highest estimated spawning frequency was once every 1.3 days from the EMN+MN category in a sample collected on April 5th, 2002 as 0600h. The lowest estimated spawning frequency was once every 6.7 days LMN+H+NP category for the same sample. The highest sample mean spawning frequency was once every 2.2 days for the April 4th sample collected at 1800. The lowest sample mean spawning frequency was once every 3.4 days for the April 5th sample collected at 1400. The overall mean is once every 2.9 days. The average spawning frequency (column mean in Table 13) estimated from POFs was lower than the average estimated from all other histologic categories (ANOVA, $p = 0.04$).

April 17th-20th (Table 13b): Results in this second 72-h collection were similar but less variable than those in the first sampling period. Thirty-six individual estimates of spawning frequency ranged from 1.6 days (EMN+MN, 20-Apr 1400h) to 4.3 days (two-day old POFs, 19-Apr 0530h). The highest estimated sample mean spawning frequency was once every 2.2 days for the April 20th sample at 1400. The lowest

estimated sample mean spawning frequency was once every 2.8 days from the April 19th sample at 0530. The overall mean was once every 2.4 days. As with the collections taken on April 4th-7th, the average estimates of spawning frequency (column means, Table 13) were lower when estimated based on POFs than when based on other histologic characters (one way ANOVA, $p = 0.06$).

In most collections, the sum of the percent frequency of fish that would spawn the day following capture (EMN + MN) and the day of capture (LMN+H+new POFs) was between 0.9 and 1.0. This implies that most fish were going to spawn within two days of capture. Additionally, 70% of specimens that contained migratory nucleus stage oocytes (MN) also contained one-day-old POFs. Similarly, 66% of specimens containing late migratory nucleus oocytes (LMN), hydrated oocytes, and new POFs contained two-day-old POFs. Sixty –six to seventy percent of animals collected were directly observed containing structures indicating that spawns occurred within 48hrs of one another. No ovary simultaneously contained EMN or MN and hydrated oocytes or hydrated oocytes and 1 day POFs. Therefore, spawning is not more frequent than once every two days.

Mean estimated spawning frequency was 2.9, 3.1, and 2.6 days in morning, midday, and evening samples respectively across histological characteristics. For each ovarian characteristic except "older POFs", a one-way ANOVA found no significant difference between times of day. For "older POFs" in the April 4th-7th collection, spawning frequency estimates were significantly lower in midday and evening samples; once every 4.8 and 3.9days respectively, a likely result of poor detection of older POFs beyond 60 hours.

Table 14 summarizes the spawning frequency estimates listed in Table 13 grouped by time of day. The fraction of the sample spawning daily showed no trend over one month of sampling, ranging from 35% to 46% on any given day (Table 15).

Hydrated Oocyte Method

The average spawning frequency for the 2002 samples estimated using the macroscopic hydrated oocyte method was 2.8±0.8 days (Table 16a) when daily estimates were averaged. When the total number of spawners was divided by the total number of females collected in 2002 the estimated spawning frequency is 2.7 days in 2002. Collections from March 28th and April 3rd were excluded from the seasonal estimates because less than 20% of females in the sample were recorded as "ripe" and it was assumed that spawning was not yet fully underway. The fraction ripe in morning samples was significantly higher than from the evening samples even with the outliers, March 28th and April 3rd, excluded (Table 17)

In 2003 (Table 16b) the average spawning frequency was 2.4±1.4 days. The estimated spawning frequency for the season was 2.1 days when calculated as the total number of spawners divided by total catch of females for the season. No samples were collected after noon in 2003.

The data from 1999-2002 egg taking by the Virginia Department of Game and Inland Fisheries (VDGIF) are provided in Tables 18-21. Estimates ranged from 3.0 to 4.0 days, with no significant difference detected between years. No trend is present by date within years when outliers are removed. Most outliers were low estimates that occurred early in the season when spawning was assumed not fully underway.

Table 14. Spawning frequency by time of day (mean and standard deviation). For each time period and classification N=3. The bottom row is the p value for a one-way ANOVA run by time of day.

April 4th-7th

time	EMN+MN	LMN+H+NP	1day POF	2day POF	older POFs	mean
morning	2.5(1.4)	3.5(2.7)	2.9(1.2)	3.9(1.2)	2.0(0.1)	2.9(0.2)
midday	1.9(0.5)	2.4(0.9)	3.9(1.4)	2.5(0.1)	4.8(1.3)	3.1(0.4)
evening	2.4(0.2)	1.8(0.1)	2.8(0.4)	2.5(0.6)	3.4(1.2)	2.6(0.4)
p	0.73	0.49	0.42	0.14	0.04	0.24

April 17th-20th

time	EMN+MN	LMN+H+NP	1day POF	2day POF	mean
morning	2.3(0.7)	2.3(0.9)	2.7(1.2)	3.1(0.9)	2.6(0.4)
midday	2.2(0.6)	2.2(0.5)	2.7(0.7)	2.5(0.2)	2.4(0.1)
evening	2.4(0.0)	1.7(0.9)	3.4(0.6)	2.2(0.3)	2.5(0.1)
p	0.89	0.46	0.59	0.20	0.48

Table 15. Fraction of specimens containing the specified structure in each collection with the samples from the 72-h round the clock collections, April 4-7th and April 17-20th, pooled.

Date	N	EMN+MN	LMN+H+NP	1day POFs	2day POFs	Older POFs	Mean
28-Mar	19	0.53	0.37	0.47	0.21	0.21	0.36
3-Apr	18	0.50	0.33	0.55	0.27	0.38	0.41
4-Apr to 7-Apr	156	0.44	0.39	0.32	0.34	0.29	0.35
12-Apr	20	0.70	0.25	0.60	0.35	0.40	0.46
17-Apr to 20-Apr	170	0.44	0.48	0.35	0.40	no data	0.41
25-Apr*	4	1.00	0.00	0.25	0.50	no data	0.44

*April 25th was not included in any analysis because of the small number of specimens in the sample.

Table 16. Spawning frequency as estimated by the macroscopic hydrated oocyte method in 2002 and 2003. Tables include the number of females collected, the number of females identified macroscopically as hydrated, the fraction hydrated, and the spawning frequency in days calculated from the fraction hydrated.

(A) 2002 collections*. The mean daily spawning frequency is the mean of the "Spawning Frequency" column. 1/fraction ripe is the reciprocal of the mean fraction ripe. Total/#Ripe is the sum of "number collected" divided by the sum of the "number ripe". Standard deviation is in parentheses where means were calculated.

Date	Time Collected	Number Collected	Number Hydrated	Fraction Hydrated	Spawning Frequency
3/28*	evening	19	1	0.05	19.0
4/3*	evening	18	3	0.17	6.0
4/04 to 4/07	morning	59	21	0.36	2.8
4/04 to 4/07	midday	56	15	0.27	3.7
4/04 to 4/07	evening	40	13	0.33	3.1
4/12	evening	20	5	0.25	4.0
4/17 to 4/20	morning	50	22	0.44	2.3
4/17 to 4/20	late morning	36	20	0.56	1.8
4/17 to 4/20	mid afternoon	23	10	0.43	2.3
4/17 to 4/20	evening	61	24	0.39	2.5
		Sum	Sum	Mean	Mean
		345	130	0.38(0.15)	2.8(0.75)
			Total/#hydrated	1/(mean fraction hydrated)	
			2.7	2.7	

*3/28 and 4/03 were not included in calculations

(B) 2003 collections. The mean daily spawning frequency is the mean of the "Spawning Frequency" column. 1/fraction ripe is the reciprocal of the mean fraction ripe. Total/#Ripe is the sum of "number collected" divided by the sum of the "number ripe". Standard deviation is in parentheses where means were calculated

Date	Time Collected	Number Collected	Number Hydrated	Fraction Hydrated	Spawning Frequency
4/14	late morning	20	4	0.20	5.00
4/21	mid morning	15	7	0.47	2.14
4/24	mid morning	17	6	0.35	2.83
4/28	mid morning	9	6	0.67	1.50
5/2	mid morning	19	11	0.58	1.73
5/5	mid morning	20	14	0.70	1.43
		Sum	Sum	Mean	Mean
		100	48	0.49(0.18)	2.44(1.36)
			Total/#hydrated	1/ (mean fraction hydrated)	
			2.08	2.04	

Table 17. Spawning frequency estimates obtained using the macroscopic hydrated oocyte method grouped by time of day. The mean fraction hydrated and average spawning frequency are calculated for morning (0530-1100) and afternoon (1400-1900) samples with the standard deviation reported in parentheses.

Date	Time	N	Number Hydrated	Fraction Hydrated	Spawning Frequency
18-Apr	530	17	8	0.47	2.13
19-Apr	530	21	6	0.29	3.50
20-Apr	530	12	7	0.58	1.71
5-Apr	600	13	2	0.15	6.67
6-Apr	600	12	6	0.50	2.00
7-Apr	600	15	6	0.40	2.50
18-Apr	1100	14	8	0.57	1.75
20-Apr	1100	17	9	0.53	1.89
5-Apr	1400	12	3	0.25	4.00
6-Apr	1400	16	4	0.25	4.00
7-Apr	1400	10	3	0.30	3.33
19-Apr	1400	18	7	0.39	2.57
28-Mar	1700	19	1	0.05	19.00
3-Apr	1700	18	3	0.17	6.00
12-Apr	1700	20	5	0.25	4.00
4-Apr	1830	12	6	0.49	2.04
5-Apr	1830	30	9	0.30	3.33
6-Apr	1900	13	2	0.15	6.67
17-Apr	1900	20	11	0.55	1.82
18-Apr	1900	13	4	0.31	3.25
19-Apr	1900	27	9	0.33	3.00
				Mean Fraction Hydrated	Average Spawning Frequency
			Morning	0.44(0.15)	2.77(1.68)
			Afternoon*	0.31(0.15)	4.63(4.37)
			Afternoon	0.32(0.11)	3.46(1.30)

*Include the 3/28 and 4/03 data.

Table 18. Daily catch by the Virginia Department of Game and Inland Fisheries for broodstock collection on the Pamunkey River, Virginia 1999. Collections with less than 20% hydrated were excluded from calculations.

Date	Number Hydrated	Total Number of Females	Percent Hydrated	Spawning Frequency
16-Mar	0	1	0.0	0.0
19-Mar	0	80	0.0	0.0
23-Mar	0	76	0.0	0.0
25-Mar	2	106	1.9	53.0
27-Mar	0	70	0.0	0.0
28-Mar	13	186	7.0	14.3
29-Mar	16	237	6.8	14.8
30-Mar	24	259	9.3	10.8
31-Mar	50	252	19.8	5.0
1-Apr	60	260	23.1	4.3
2-Apr	137	355	38.6	2.6
3-Apr	48	258	18.6	5.4
4-Apr	83	253	32.8	3.1
5-Apr	122	266	45.9	2.2
6-Apr	64	298	21.5	4.7
7-Apr	67	197	34.0	2.9
8-Apr	49	240	20.4	4.9
10-Apr	0	13	0.0	0.0
11-Apr	57	167	34.1	2.9
13-Apr	18	79	22.8	4.4
14-Mar	19	84	22.6	4.4
15-Apr	37	196	18.9	5.3
16-Apr	46	213	21.6	4.6
17-Apr	53	137	38.7	2.6
21-Apr	38	120	31.7	3.2
22-Apr	60	193	31.1	3.2
23-Apr	0	55	0.0	0.0
24-Apr	59	187	31.6	3.2
25-Apr	51	112	45.5	2.2
26-Apr	42	99	42.4	2.4
29-Apr	0	56	0.0	0.0
4-May	39	93	41.9	2.4
5-May	75	141	53.2	1.9
6-May	52	111	46.9	2.1
7-May	36	80	45.0	2.2
8-May	0	8	0.0	0.0
	Sum	Sum	Mean	Mean
	1227	3685	34.5 (9.98)	3.2(0.98)
		Total #Females/#hydrated	1/Fraction Hydrated	
		3.0	2.9	

Table 19. Daily catch by the Virginia Department of Game and Inland Fisheries for broodstock collection on the Pamunkey River, Virginia 2000. Collections with less than 20% hydrated were excluded from calculations.

Date	Number Hydrated	Total Number of Females	Percent Ripe	Spawning Frequency
10-Mar	16	137	11.7	8.6
13-Mar	27	133	20.3	4.9
14-Mar	6	177	3.4	29.5
15-Mar	24	213	11.3	8.9
16-Mar	42	198	21.2	4.7
25-Mar	15	273	5.5	18.2
26-Mar	99	412	24.0	4.2
27-Mar	78	257	30.4	3.3
28-Mar	58	240	24.2	4.1
29-Mar	65	251	25.9	3.9
30-Mar	72	345	20.9	4.8
31-Mar	73	353	20.7	4.8
1-Apr	62	305	20.3	4.9
2-Apr	80	279	28.7	3.5
3-Apr	54	232	23.3	4.3
4-Apr	80	287	27.9	3.6
5-Apr	65	229	28.4	3.5
6-Apr	53	308	17.2	5.8
8-Apr	11	96	11.5	8.7
9-Apr	52	191	27.2	3.7
10-Apr	36	269	13.4	7.5
15-Apr	36	136	26.5	3.8
16-Apr	44	173	25.4	3.9
19-Apr	39	135	28.9	3.5
20-Apr	31	124	25.0	4.0
22-Apr	40	137	29.2	3.4
24-Apr	55	179	30.7	3.3
26-Apr	11	56	19.6	5.1
27-Apr	30	110	27.3	3.7
29-Apr	17	87	19.5	5.1
30-Apr	12	67	17.9	5.6
1-May	16	75	21.3	4.7
2-May	10	44	22.7	4.4
	Sum	Sum	Mean	Mean
	1208	4825	25.2(3.34)	4.0(0.55)
		Total #Females/#hydrated	1/Fraction Hydrated	
		4.0	4.0	

Table 20. Daily catch by the Virginia Department of Game and Inland Fisheries for broodstock collection on the Pamunkey River, Virginia 2001. Collections with less than 20% hydrated were excluded from calculations.

Date	Number Hydrated	Total Number of Females	Percent Ripe	Spawning Frequency
28-Mar	4	215	1.9	53.8
30-Mar	22	267	8.2	12.1
31-Mar	22	275	8.0	12.5
1-Apr	10	217	4.6	21.7
8-Apr	154	639	24.1	4.1
9-Apr	109	444	24.5	4.1
11-Apr	162	466	34.8	2.9
12-Apr	160	397	40.3	2.5
13-Apr	152	362	42.0	2.4
14-Apr	60	207	29.0	3.5
16-Apr	76	280	27.1	3.7
17-Apr	48	138	34.8	2.9
19-Apr	17	115	14.8	6.8
20-Apr	56	143	39.2	2.6
21-Apr	67	143	46.9	2.1
22-Apr	77	196	39.3	2.5
23-Apr	52	132	39.4	2.5
25-Apr	14	46	30.4	3.3
26-Apr	21	97	21.6	4.6
27-Apr	36	103	35.0	2.9
29-Apr	11	55	20.0	5.0
30-Apr	10	70	14.3	7.0
1-May	19	63	30.2	3.3
22-Apr	40	97	41.2	2.4
24-Apr	55	124	44.4	2.3
26-Apr	11	45	24.4	4.1
27-Apr	30	80	37.5	2.7
29-Apr	17	70	24.3	4.1
30-Apr	12	55	21.8	4.6
1-May	16	59	27.1	3.7
2-May	10	34	29.4	3.4
	Sum	Sum	Mean	Mean
	1398	4142	33.3(7.90)	3.2(0.77)
		Total #Females/#hydrated	1/Fraction Hydrated	
		3.0	3.0	

Table 21. Daily catch by the Virginia Department of Game and Inland Fisheries for broodstock collection on the Pamunkey River, Virginia 2002. Collections with less than 20% hydrated were excluded from calculations.

Date	Number Hydrated	Total Number of Females	Percent Ripe	Spawning Frequency
18-Mar	15	186	8.1	12.4
21-Mar	10	213	4.7	21.3
26-Mar	29	262	11.1	9.0
27-Mar	45	284	15.8	6.3
28-Mar	80	487	16.4	6.1
29-Mar	58	398	14.6	6.9
30-Mar	101	473	21.4	4.7
1-Apr	113	461	24.5	4.1
2-Apr	122	451	27.1	3.7
4-Apr	187	481	38.9	2.6
5-Apr	91	308	29.5	3.4
7-Apr	50	256	19.5	5.1
8-Apr	61	278	21.9	4.6
9-Apr	84	202	41.6	2.4
10-Apr	123	381	32.3	3.1
12-Apr	93	314	29.6	3.4
13-Apr	68	337	20.2	5.0
14-Apr	156	482	32.4	3.1
15-Apr	72	331	21.8	4.6
16-Apr	31	153	20.3	4.9
17-Apr	71	240	29.6	3.4
19-Apr	46	161	28.6	3.5
20-Apr	50	153	32.7	3.1
21-Apr	21	80	26.3	3.8
22-Apr	45	151	29.8	3.4
23-Apr	33	123	26.8	3.7
24-Apr	25	118	21.2	4.7
25-Apr	8	72	11.1	9.0
4/27/02*	9*	9*	100.0*	1.0*
30-Apr	48	155	31.0	3.2
	Sum	Sum	Mean	Mean
	1698	5997	28.1(5.62)	3.7(0.90)
		Total #Females/#hydrated	1/Fraction Hydrated	
		3.5	3.6	

*4/27 was excluded from calculations.

Macroscopic versus histologic approaches in the hydrated oocyte method

The average fraction of females identified as hydrated and ready to spawn was lower when using macroscopic methods (0.36) than when using histology (0.48) in 2002 collections (two sample t-test, $p < 0.05$) (Table 22). In comparisons between morning and afternoon/evening samples, the histological and macroscopic methods are not different in morning collections (0.46 and 0.44 respectively). In afternoon samples, the macroscopic method yielded 31% hydrated females and the histological method yielded 49% hydrated females (two sample t-test, $p < 0.05$). A chi-square test confirmed that macroscopic and microscopic methods to identify spawners are not interchangeable in the afternoon (Table 23). However, there was no difference in estimates generated by the two methods for samples collected in the morning.

Seasonal Fecundity

To facilitate comparison to estimates of seasonal fecundity reported in Leggett and Carscadden (1978), seasonal fecundity was calculated based on age and spawning history and the predicted eviscerated weight (see Batch Fecundity section) for a female of each age. Two values of relative batch fecundity were used (one from 2002 and one from 2003, Table 4) since these values were different between years. A range of spawning frequency estimates was used that represents the overall means calculated in this study (2.9 and 2.4 days, Table 12) and the value (3.5 days) reported in Olney and McBride

Table 22. Comparison of spawning frequency estimates calculated using the hydrated oocyte method when histology (Histo) and macroscopic (Macro) criteria are used to identify spawners from 2002 collections.

Date	time	N	Number of Spawners Macro	Number of Spawners Histo	Fraction Spawning Macro	Fraction Spawning Histo	Spawning Frequency Macro	Spawning Freqency Micro
28-Mar	1700	19	1	7	0.05	0.37	19.00	2.71
3-Apr	1700	18	3	6	0.17	0.33	6.00	3.00
4-Apr	1830	12	6	7	0.49	0.57	2.04	1.75
5-Apr	600	13	2	2	0.15	0.15	6.67	6.67
5-Apr	1400	12	3	7	0.25	0.58	4.00	1.72
5-Apr	1830	30	9	16	0.30	0.53	3.33	1.89
6-Apr	600	12	6	7	0.50	0.58	2.00	1.72
6-Apr	1400	16	4	8	0.25	0.50	4.00	2.00
6-Apr	1900	13	2	7	0.15	0.54	6.67	1.85
7-Apr	600	15	6	7	0.40	0.47	2.50	2.13
7-Apr	1400	10	3	3	0.30	0.30	3.33	3.33
12-Apr	1700	20	5	9	0.25	0.45	4.00	2.22
17-Apr	1900	20	11	12	0.55	0.60	1.82	1.67
18-Apr	530	17	8	8	0.47	0.47	2.13	2.13
18-Apr	1100	14	8	8	0.57	0.57	1.75	1.75
18-Apr	1400	7	4	4	0.57	0.57	1.75	1.75
18-Apr	1900	13	4	8	0.31	0.62	3.25	1.63
19-Apr	530	21	6	6	0.29	0.29	3.50	3.50
19-Apr	1400	18	7	7	0.39	0.39	2.57	2.57
19-Apr	1900	27	9	15	0.33	0.56	3.00	1.80
20-Apr	530	12	7	7	0.58	0.58	1.71	1.71
20-Apr	1100	17	9	9	0.53	0.53	1.89	1.89

	Mean Macr (stdev)	Mean Histo (stdev)	Mean Macro (stdev)	Mean Histo (stdev)
All	0.36(0.16)	0.48(0.12)	3.95(3.69)	2.34(1.11)
Morning	0.44(0.15)	0.46(0.16)	2.77(1.63)	2.69(1.71)
Afternoon	0.31(0.15)	0.49(0.11)	4.63(4.37)	2.14(0.55)

Table 23. Contingency tables for comparing the macroscopic and histologic methods of identifying spawners for the hydrated oocyte method.

All 2002 Samples		
	Spawner	Not Spawner
Macroscopic	123	233
Histologic	170	186
	χ^2=12.8	$p < 0.0001$
2002 Morning Samples		
	Spawner	Not Spawner
Macroscopic	52	69
Histologic	54	67
	χ^2=0.067	p =0.796
2002 Afternoon/Evening Samples		
	Spawner	Not Spawner
Macroscopic	71	164
Histologic	116	116
	χ^2=17.9	$p < 0.0001$

(2003). The possible seasonal fecundities calculated from the range of parameter values reported here are summarized by age in Figure 14.

The mean virgin (no spawning marks detected on scales) spawning in 2002 and 2003 was 4.96 years old and weighed 1088g. Virgin seasonal fecundity calculated from a residence time of 34d (Olney et al. in prep), spawning frequencies of 2.9 and 2.4 days (this study), and relative batch fecundities of 30 and 36 eggs/ g EW (this study) ranged from 382,600 eggs to 554,800 eggs.

Indeterminate versus determinate fecundity

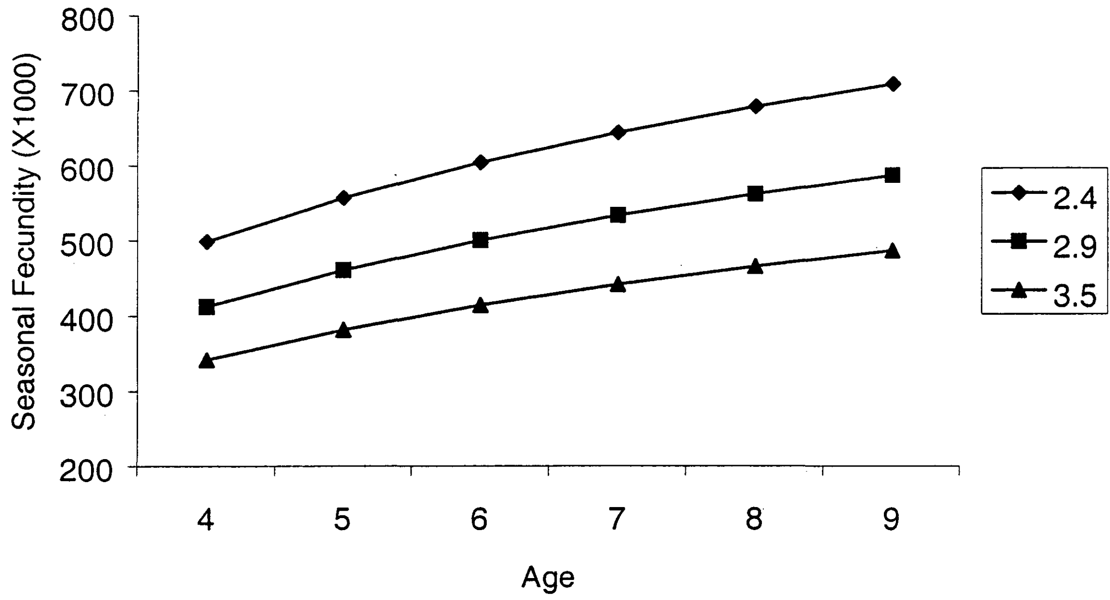
On average, ovarian composition did not change from one date to the next within samples. However, the fraction of specimens lacking partially yolked oocytes increased by date (Table 24). The gonadosomatic index and the proportion of partially yolked and yolked oocytes relative to the total number of oocytes counted in histological sections were lower in ovaries that contained one or more classes of POF (Table 25). The proportion of primary growth oocytes increased with increasing numbers of POF age classes.

The proportion of partially yolked oocytes to total oocytes in the ovary was negatively correlated with GSI with 30% of specimens with a GSI below 10.99 having no partially yolked oocytes. (Table 26, Figure 15). As previously noted, GSI declined by date.

On average the prevalence of atresia dropped by 50% from March 28th to April 20th in 2002 (Table 27). The intensity of atresia also declined significantly by seasons end. Ten of 383 females observed in 2002 on the spawning grounds were classified as

Figure 14. Seasonal fecundity at age estimated for American shad in 2002 and 2003 on the Mattaponi River, Virginia using spawning frequency (2.4, 2.9, 3.5 days), relative batch fecundity (30 and 36 eggs/g EW), and residence time (34 days). [seasonal fecundity at age N = (residence time/spawning frequency) * (relative batch fecundity * weight at ageN)].

relative batch fecundity = 36 mature eggs/gram EW



relative batch fecundity = 30 mature eggs/gram EW

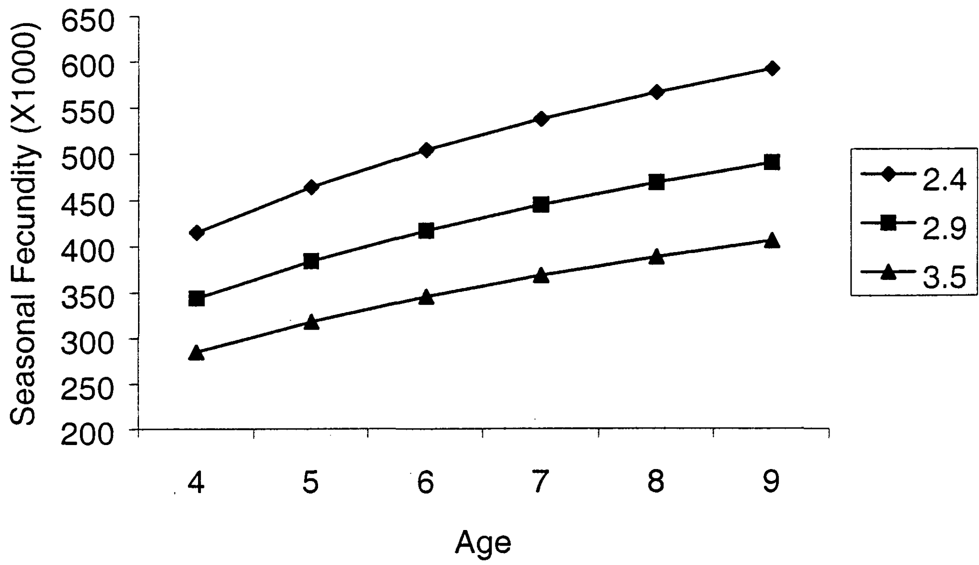


Table 24. Percent composition of oocyte type in American shad ovaries by date. Columns PG, PY, and Y are the mean (standard deviation in parentheses) proportion, expressed as a percentage, of that oocyte type to all oocyte types observed in ovaries in that sample. (PG = primary growth, PY = partially yolked, Y = yolked). "Fraction with no PY" is the fraction of ovaries in the sample in which no partially yolked (PY) oocytes were observed in five random microscope views. Columns PG, PY, and Y do not sum to 100% because columns expressing the proportion of migratory nucleus stages, hydrated oocytes, and atretic oocytes are not shown.

Date	N	PG	PY	Y	Fraction with no PY
28-Mar	19	51(10)	10(7)	18(6)	0.05
3-Apr	20	44(16)	9(6)	14(6)	0.05
4-Apr	14	54(10)	12(8)	14(4)	0.07
5-Apr	65	54(12)	11(7)	15(6)	0.05
6-Apr	48	49(13)	13(6)	16(6)	0.00
7-Apr	28	51(11)	9(6)	15(8)	0.11
12-Apr	20	51(11)	10(8)	16(8)	0.20
17-Apr	20	64(12)	8(6)	12(6)	0.15
18-Apr	61	62(11)	10(6)	12(6)	0.16
19-Apr	60	62(17)	9(9)	13(10)	0.13
20-Apr	29	64(14)	8(7)	12(7)	0.21

Table 25. Comparison of gonadosomatic index (GSI) and percent composition of oocyte types in American shad ovaries by the number of POF ages classes detected in ovaries. (PG = primary growth oocytes, PY = partially yolked oocytes, Y = yolked oocytes)

Number of POF age classes	Number of ovaries in class	Mean GSI	Mean PG	Mean PY	Mean Y
0	42	19.6(5.6)	43(10)	17(8)	19(7)
1	215	18.5(6.1)	56(12)	10(7)	14(6)
2	122	13.2(6.1)	60(15)	8(7)	12(7)
3	3	9.7(2.6)	80(11)	0(0)	2(1)

Table 26. The prevalence of PY in ovaries grouped by gonadosomatic index (GSI). Column PY is the average proportion of partially yolked oocytes to total oocytes in ovaries within the specified range of GSI (standard deviation in parantheses).

GSI	N	PY	Fraction of Specimens with no PY
0-10.99	65	6(6)	0.31
11 to 20.99	227	11(7)	0.09
21-30+	91	11(7)	0.01

Figure 15. Scatter plot of the proportion of partially yolked oocytes to total oocytes observed versus gonadosomatic index (GSI).

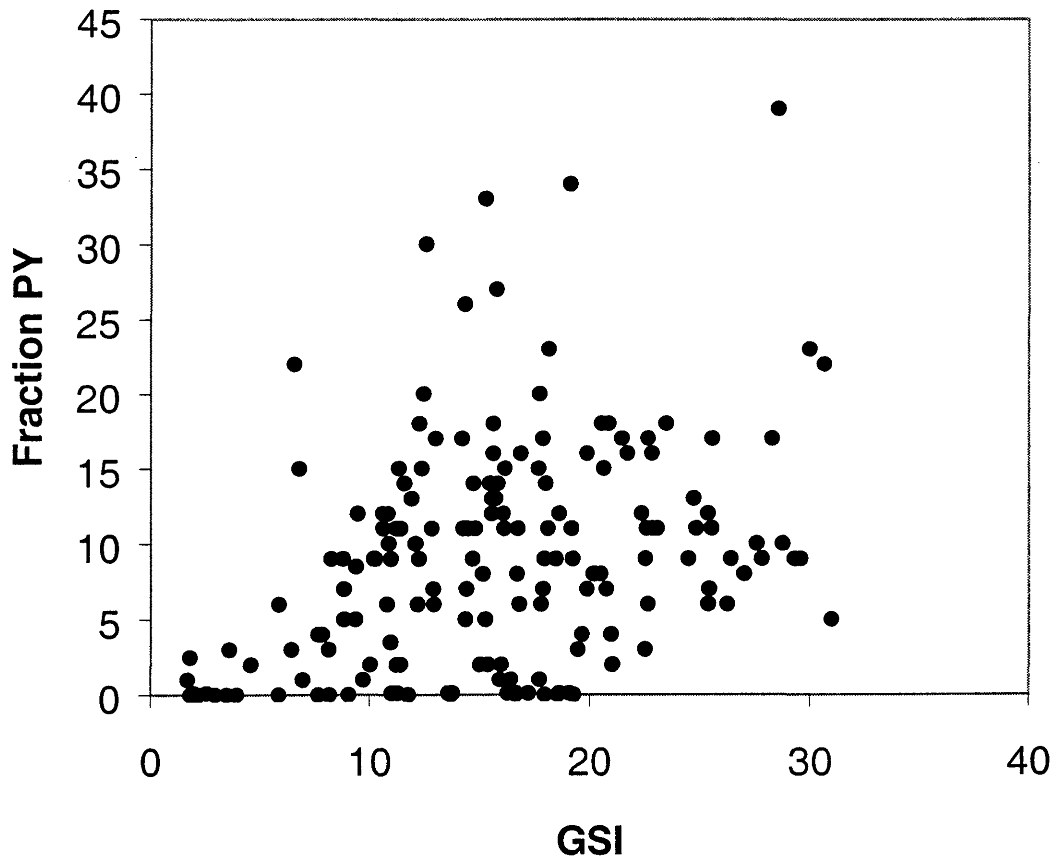

45
40
35
30
25
20
15
10
5
0
Fraction PY
0
10
20
30
40
GSI

Table 27. The prevalence and intensity of alpha (α) atretic oocytes by date. Prevalence of atresia is the fraction of animals in which any alpha atresia was detected. Intensity of atresia is the fraction of animals in which greater than one percent of oocytes observed were alpha atretic.

Date	N	Prevalence of atresia	Intensity of atresia
28-Mar	19	50	15
3-Apr	20	45	30
4-Apr	14	57	14
5-Apr	65	45	18
6-Apr	48	42	18
7-Apr	28	40	25
12-Apr	20	25	5
17-Apr	20	25	5
18-Apr	61	43	0
19-Apr	60	28	2
20-Apr	29	21	0

spent based on a GSI below 3, the absence of vitellogenic oocytes, and the presence of numerous POFs of two or more age classes.

DISCUSSION

Relative batch fecundity (eggs/g EW) showed no trend by date in either 2002 or 2003. Even as the relative size of ovaries (GSI) declined, relative batch fecundity remained constant with a similar number of eggs being produced for each gram of female biomass (Figure 4). The finding supports the assumption that batch fecundity is constant through the spawning season and that one value of batch fecundity or relative batch fecundity can be applied to seasonal fecundity estimates for a given season. In this study temporal coverage of the spawning season was truncated in both years. Thus, there remains a possibility that some difference in batch fecundity exists at either end of the season that was not detected in this study. Importantly, the observation that GSI declined significantly over the period of collection in both years suggests that batch fecundity estimates in this study reflect both new arrivals to the spawning grounds and fish that had released many batches previous to capture.

Relative batch fecundity was significantly lower in 2003 than 2002 (Table 5). Differing age structure of samples between years does not explain the difference, as mean relative fecundity did not vary significantly between ages. The only difference between years was the age 6 specimens in 2002 that had a higher relative fecundity than any other age group collected in either year. A rapid increase in temperature in 2002 may have resulted in greater egg production in each batch in compensation for the shortened spawning season. Were this speculation valid, the length of the spawning season could vary with annual temperature trends, while annual relative fecundity would remain stable.

A time series of batch fecundity estimates over varying annual conditions might provide more insight on this possibility.

Batch fecundity was highly variable, possibly resulting from varying spawning histories of fish in each sample. Nichol and Acuna (2001) found that yellowfin sole that lacked POFs had smaller batches than animals that had POFs, therefore having recently spawned. A similar pattern appears likely for American shad on the Mattaponi River. In 2002, mean relative fecundity was 25% higher in specimens that possessed POFs. although the differences were non-significant ($p = 0.11$).

Histological methods provide multiple estimates of spawning frequency and improve confidence in spawning frequency estimates. Each sample in this study generated a minimum of four estimates of spawning frequency using histology. Estimates were obtained from the large and easily identified oocytes undergoing final maturation (EMN, MN, LMN, and H) and from POFs. Any bias caused by differing gear vulnerability due to maturity stage was corrected as this method utilized ripening, ripe, and partially spent animals in spawning frequency estimates. Each estimate yielded similar values of spawning frequency with estimates from POFs being slightly but significantly lower. In this study, estimates of spawning frequency obtained using POFs were within one day of estimates obtained using EMN, MN, LMN, and H oocytes. Therefore, none were discarded as unreliable though there is a small discrepancy between categories. All are sufficiently similar to conclude that spawning frequency is between two and three days.

The spawning pattern of wild caught shad inferred in this study using histology differs from the spawning pattern observed directly in tank-spawned shad in Mylonas et

al. (1995). Shad stimulated to spawn in captivity using GnRH-A implants release eggs on two consecutive days followed by two days rest. The histological analysis suggests that wild caught specimens release a single batch followed by one to two days rest. Final maturation takes 36 to 48 hours to progress from the onset of nuclear migration to ovulation and spawning. Therefore ovaries should be observed to simultaneously contain migratory nucleus stage oocytes, tomorrow's batch, and hydrated oocytes, today's batch, if wild shad release batches on consecutive days. Of 383 ovaries section for histology in 2002, none simultaneously contained migratory nucleus stage and hydrated oocytes. A similar difference in spawning pattern was observed between controls and GnRH-A implanted yellowtail flounder, *Pleuronectes ferrugineus* (Larsson et al. 1997). Controls spawned once every 2.12 days and GnRH-A implant treatment specimens spawned every 1.28 days. The consecutive daily spawns observed in tank-spawned shad and yellowtail flounder may be an artifact of the hormone treatment required to induce final maturation and ovulation in captivity.

POFs, particularly older POFs, may not be detected as reliably as the larger oocytes. The estimate of spawning frequency from older POFs was significantly lower in midday and afternoon samples than in morning samples (Table 14), possibly indicating that these were becoming undetectable beyond 60hrs. Wilson and Neiland (1994) found that estimates of spawning frequency in red drum, *sciaenops ocellatus,* varied from one spawn every three days to one spawn every 80 days when attempting to use POFs. Their estimate of spawning frequency for zero-day specimens (corresponds to this study's LMN+H+NP category) was one spawn every 2-4 days. They concluded that their

estimates from zero-day ovaries were more reasonable than estimates from POFs citing that POFs were not as easily detected.

The macroscopic hydrated oocyte method did not accurately identify all spawners during afternoon and dusk periods. Animals that had already released that day's batch were missed macroscopically. Therefore, estimates of spawning frequency based on evening egg taking collections are likely underestimates. However, estimates of spawning frequency for collections for this study taken during the morning, when spawning was not occurring, agreed with histological results. This finding renders opportunistic sampling such as evening brood-stock collection of American shad ineffective for estimating spawning frequency. Furthermore, previous estimates of spawning frequency based on such collections (Olney et al. 2001) are subject to error.

Residence time is likely a reasonable proxy for spawning duration in the York River stock. The migration route is short and most spawning is presumed to occur within 30-40 river miles (Bilkovic et al. 2002) of the location of the listening station placed at the entrance to the spawning grounds by Olney et al. (in prep). Histological findings in this study suggest that spawning begins soon after arrival on the spawning grounds. Nearly all specimens collected in 2002 were ready to spawn within one day or had spawned. Fish abruptly exit the spawning grounds once spawning ceases. Of the nine specimens collected that were classified as spent, all contained recent (< 24h old) POFs. Additionally, Olney et al. (2001) recorded specimens containing recent POFs in the York estuary well below the spawning grounds which suggests rapid exit following the cessation of spawning.

Seasonal fecundity estimated here is higher than that reported in Leggett and Carscadden (1978). Estimates of virgin seasonal fecundity ranged from 380,000 eggs to 550,000 eggs, values that are approximately 1.5 to 2.1 times greater than the virgin fecundity reported for the York River by Leggett and Carscadden (1978). Since the average age of virgins in the two studies is similar, the difference in fecundity estimates is likely methodological.

The fecundity estimates obtained by counting the standing stock of oocytes prior to spawning probably missed some very early vitellogenic oocytes resulting in an underestimate of seasonal egg production. In fish that do not contain POFs, there is no gap in the distribution of oocyte maturity classes between primary growth (PG) and yolked oocytes (Y). Histology reveals very early vitellogenic oocytes in these samples. These oocytes resemble the perinucleolar stage of primary growth (PG) in size but contain lipid vesicles, cortical alveoli and a two-layer germinal epithelium and the precursor to the zona radiata. Wallace and Selman (1981) observed that oocytes showing these characteristics have exceeded the "critical size" and are ready for the gonadotropin dependant growth phase, or vitellogenesis. These could contribute to fecundity but not be enumerable by the methods in Leggett and Carscadden (1978) and Massman (1963).

The hypothesis of indeterminate fecundity in American shad is not rejected. Recalling the four criteria for determining fecundity pattern, the data in this study do not point fully to either strategy. A gap appears to develop between primary growth and later stages as the season progresses. This is evidenced by the decrease in the prevalence of PY oocytes in ovaries as GSI declined and by the increase in the number of ovaries lacking PY oocytes as the season progressed (Tables 24 and 26, Figure 15). Remnant

fecundity appears to decrease by date as evidenced by the decline in GSI by date. Intensity and prevalence of atresia also declined by date on the spawning grounds (Table 27). These findings suggest a determinate fecundity pattern in which the standing stock of eggs is spawned without replacement. This study's estimates of seasonal fecundity based on estimates of batch fecundity, spawning frequency, and duration do not agree with estimates of fecundity for the same stock that counted potential annual fecundity prior to spawning (Leggett and Carscadden 1978). Furthermore, these data do not account for the partially spent fish documented exiting the river by Olney et al. (2001). It seems likely that American shad in the York River spawn out the standing stock of oocytes without replacement, but the time at which replacement ceases occurs sometime after shad enter the spawning grounds and begin spawning. As such, they should be treated as having indeterminate fecundity when estimating seasonal egg production.

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