Reproductive Biology & Fecundity of Atlantic Menhaden

Final Report to the National Science Foundation

Science Center for Marine Fisheries

August 19, 2019

James Gartland, Robert J. Latour, and Wolfgang K. Vogelbein

Virginia Institute of Marine Science

William & Mary

P.O. Box 1346

Gloucester Point, Virginia 23062

Email addresses: J. Gartland – jgartlan@vims.edu, R.J. Latour – latour@vims.edu, W.K Vogelbein – wolf@vims.edu

Corresponding author - J. Gartland, Tel: (804) 684-7546, Fax: (804) 684-7110

Key words: Atlantic menhaden, Reproductive biology, Annual fecundity, Indeterminate batch spawning, Mid-Atlantic Bight, Linear mixed effects models

Contents

Introduction

Background

Marine teleost fishes display a wide range of reproductive strategies that have evolved to maximize fitness in response to selective pressures exerted by both biotic and abiotic factors. The majority of exploited marine fishes are gonochoristic, iteroparous, oviparous species (Murua and Saborido-Rey 2003, Lowerre-Barbieri *et al*. 2011a). Within this framework, however, size- and age-at-maturity, spawning seasonality and location, and spawning mode are species-specific. Spawning mode is typically classified into three distinct categories: namely, determinate total spawning, determinate batch spawning, and indeterminate batch spawning (Hunter and Goldberg 1980, Hunter *et al*. 1985, Lowerre-Barbieri *et al*. 2011a). Species inhabiting cold-temperate and boreal environments tend to be determinate spawners, meaning that all gametes destined to be spawned in a given year undergo maturation prior to the onset of the spawning season and are subsequently released in either a single event (i.e., total spawning) or multiple events (i.e., batch spawning) over a short timeframe (Pavlov *et al*. 2009). Fitness is maximized by releasing all reproductive products in concert with some short-term, favorable environmental condition (e.g., spring bloom). Most temperate and subtropical fishes, however, exhibit indeterminate batch spawning (Hunter *et al*. 1985, Murua and Saborido-Rey 2003). Spawning seasons are typically protracted, mature ova and sperm are released in several batches, and immature gametes are continually recruited to maturity and spawned throughout the season. Indeterminate batch spawning enables individuals to overcome the limitations of body cavity volume on gamete production and increases the probability that at least some offspring encounter favorable environmental conditions, thereby increasing fitness (Sadvoy 1996).

Quantifying the reproductive potential of an exploited marine species is an essential component of the stock assessment and management process, as this information yields insight into sustainable levels of harvest and the capacity of the stock to recover from overexploitation (Lowerre-Barbieri 2009). Most stock assessments in the US characterize reproductive potential via measures of spawning stock biomass, typically characterized as the total biomass of mature

fish or aggregate biomass of mature females only. It is likely, however, that annual fecundity measured as total egg production is a more accurate representation of reproductive potential, given typical nonlinearities in the relationship between fish size and reproductive output (Marshall 2009, Morgan *et al*. 2009, Lowerre-Barbieri *et al*. 2011b). Indeed, a number of stock assessments have begun to incorporate estimates of annual egg production as indicators of reproductive potential in lieu of spawning stock biomass (e.g., SEDAR 2015, SEDAR 2018). The methodology employed to generate these fecundity estimates varies depending on the reproductive biology, and particularly the spawning mode, of the stock of interest. Failure to properly characterize reproductive biology and spawning mode often results in the misapplication of methods meant to quantify fecundity, which in turn can result in the over- or under-estimation of the annual fecundity of a stock (Hunter *et al*. 1985, Brown-Peterson *et al*. 2017).

Atlantic menhaden (*Brevoortia tyrannus*) are a gonochoristic, iteroparous, oviparous teleost that comprise a single stock ranging from Nova Scotia to Northern Florida (Higham and Nicholson 1964, Lynch *et al*. 2010). This species supports the largest commercial fishery, by volume, on the US East Coast; approximately 76% of the annual harvest is supplied to a reduction facility located in Reedville, VA, while the remaining 24% is taken as part of a smaller-scale bait fishery (SEDAR 2015). The status of the Atlantic menhaden stock is assessed using the Beaufort Assessment Model (BAM), and estimates of annual fecundity are used to quantify reproductive potential and develop biological reference points in these assessments. Specifically, population-level egg production is compared with that expected at the threshold fishing mortality rate to determine whether the stock is overfished. A fecundity-at-length relationship is coupled with length-at-age information to yield fecundity-at-age at the beginning of the fishing season. These data are then combined with abundance-at-age and maturity information and summed across ages to estimate annual egg production.

While the assessment approach for Atlantic menhaden has moved beyond the use of spawning stock biomass as a measure of reproductive potential, there are a number of concerns with the fecundity-at-length relationship used to estimate annual fecundity in past assessments, and re-

evaluation of the fecundity of this stock was defined as a high-priority research recommendation following the most recent benchmark assessment (SEDAR 2015). Specifically, the fecundity-at-length information used in the past was developed based on sampling that occurred between 1956 and 1981 and was restricted to the coastal ocean in the vicinity of Beaufort, NC during fall (Higham and Nicholson 1964, Dietrich 1979, Lewis *et al*. 1987). Annual egg production has been shown to exhibit plasticity in response to variability in both biotic and abiotic factors (Brown-Peterson and Warren 2001), and as such a contemporary evaluation of Atlantic menhaden fecundity from throughout a broad range of the stock is warranted. Further, while evaluation of Atlantic menhaden ovary samples collected in the 1950s suggested that this species may exhibit indeterminate batch spawning, the approach used to quantify the aforementioned fecundity-at-length relationship employed methodology associated with determinate total spawning species (Higham and Nicholson 1964, Lewis *et al*. 1987). Mischaracterization of spawning mode has been shown to impact population-level fecundity estimates on the order of 100-1000 fold (Brown-Peterson *et al*. 2017). Further, efforts to quantify the reproductive biology and fecundity of two congener species, the Gulf menhaden (*Brevoortia patronus*) and the Brazilian menhaden (*Brevoortia aurea*), found that both exhibit characteristics consistent with indeterminate batch spawning (Macchi and Acha 2000, Brown-Peterson *et al*. 2017). The designation of Gulf and Brazilian menhaden as indeterminate batch spawners was due, in part, to the use of gonad histology in the respective investigations, a technique that was not widely implemented when the fecundity of Atlantic menhaden was originally assessed.

Objectives

The objective of this investigation was to generate a contemporary evaluation of female Atlantic menhaden reproductive biology that represented a broad spatiotemporal spawning range, and subsequently yield updated estimates of fecundity using methodology that is consistent with the spawning mode of this species. Specifically, we:

- Collected female Atlantic menhaden across all seasons and from throughout the Mid-Atlantic Bight.

- Implemented standard gonad histology techniques to assess ovarian maturity phases, provide insight into spawning mode, and yield information on spawning seasonality, interval, and frequency.
- Counted ova to estimate fecundity using methodology consistent with the spawning mode of this species.
- Modeled fecundity as a function of fish length and coupled this information with size-at-age, spawning frequency, and maturity data to yield age-specific estimates of annual fecundity.

Methods

Sample Collection

Female Atlantic menhaden were collected between Cape Cod, MA and Cape Hatteras, NC from 2013-2018 (Figure 1). Samples acquired in April, May, October, and November were provided by the Northeast Area Monitoring and Assessment Program (NEAMAP) Mid-Atlantic/Southern New England Trawl Survey (Bonzek at al. 2008), while those from the summer months (i.e., June, July, and August) were collected in conjunction with port sampling of purse seine boats at the Omega Protein reduction facility in Reedville, VA. Female Atlantic menhaden also were acquired from this purse seine fleet in November 2017. Samples collected during the months of December and January were derived from the winter bottom trawl and gillnet fisheries that operate off of the coast of New Jersey.

Atlantic menhaden were processed for biological data at the time of capture on NEAMAP, while those derived from industry sources were held on ice for up to 24h prior to the collection of these data. For each fish, fork length (FL; mm), whole weight (g), macroscopic sex and maturity phase, and total ovary weight (0.001g) were recorded. Maturity phases were classified following Brown-Peterson *et al*. (2017). Ovaries were preserved in either 10% neutral buffered formalin or Normalin; samples preserved in Normalin were rinsed in tap water and transferred to formalin within one week. Gonadosomatic index (GSI) was calculated for each female fish by:

$$GSI = \left(\frac{OW}{(W-OW)}\right) * 100 \quad (1)$$

where W is whole fish weight and OW is total ovary weight.

Reproductive Biology

Ovarian tissues from each female Atlantic menhaden were processed using standard histological techniques (Prophet 1992). Specifically, these tissues were fixed for a minimum of one week in 10% neutral buffered formalin prior to histological preparation. The ovaries of a given fish were removed from formalin, blotted dry with a paper towel, and weighed (0.001g) to yield preserved total ovary weight (PW). Atlantic menhaden ovaries were shown to be homogenous with respect to oocyte abundance and size distribution throughout each ovary and between left and right ovaries (Higham and Nicholson 1964). As such, a cross section of approximately 5 mm was removed from the central region of either the left or right ovary, placed in a sample cassette, and rinsed for 24h in tap water. These samples were then dehydrated in a series of graded ethanols and embedded in paraffin. Each sample was cross-sectioned at 4 µm using a benchtop rotary microtome, and the resulting section was mounted on a glass slide and subsequently stained with hematoxylin and eosin.

Ovarian sections were evaluated at 80x magnification using a dissecting microscope. For a given sample, all egg stages present in the ovary were recorded, and reproductive phase was assigned based on the most advanced egg stage. Reproductive phases were classified as immature, early developing, developing, spawning capable (with an actively spawning subphase), regressing, and regenerating (Brown-Peterson *et al*. 2011). Further, the presence of oocyte maturation (OM; i.e., germinal vesicle migration and germinal vesicle breakdown) and post ovulatory follicles (POF) was noted for all fish classified as spawning capable or actively spawning. The diversity of egg stages found in the ovary sections of spawning capable fish was used to characterize female Atlantic menhaden as total or batch spawners. Spawning seasonality (SS) was quantified by identifying those months in which spawning capable or actively spawning female Atlantic menhaden were encountered and by evaluating monthly mean GSI (Brown-Peterson *et al*. 2017).

Two approaches were used to identify whether the reproductive biology of female Atlantic menhaden exhibited characteristics consistent with determinate or indeterminate batch spawning. First, the size-frequency (i.e., diameter-frequency) distribution of ova in a fish collected early in the spawning season was compared with that of a fish collected late in the season. Determinate spawners typically display a notable gap in the size-frequency distribution between immature and mature oocytes, and the relative abundance of mature ova usually declines as the spawning season progresses. In contrast, indeterminate spawners show a more continuous size-frequency distribution and a relatively consistent abundance of mature eggs throughout the spawning period (Brown-Peterson *et al*. 2017). These size-frequency distributions were obtained by counting and measuring all ova in a 0.02 g – 0.03 g subsample of preserved ovary (PS), and subsequently scaling these data to the whole ovary using the ratio of PW/PS. Atlantic menhaden spawning mode was also evaluated by comparing annual fecundity estimates generated using methodology appropriate for determinate spawning species with those estimates resulting from the approach associated with indeterminate spawning for two spawning capable fish collected at the beginning of the spawning season (Brown-Peterson *et al*. 2017).

Annual Fecundity

Given that the evaluation of female Atlantic menhaden reproductive biology yielded strong evidence that this species exhibits indeterminate batch spawning (see 'Results & Discussion' section), efforts to quantify annual fecundity followed the methodology associated with this spawning mode. Specifically, relative batch fecundity (RBF) was estimated and combined with information on spawning frequency, size-at-age, and maturity-at-age to yield estimates of annual fecundity-at-age.

RBF was quantified for each fish in the spawning capable gonad phase and actively spawning sub-phase using the oocyte size-frequency method (Hunter *et al*. 1985). In this approach, the oocytes in the most advanced stage of maturity, typically third-stage vitellogenic (VTG3), represent the cohort of ova to be released in the proximate batch and batch fecundity (BF) is estimated by counting these ova. Note that while many studies have quantified BF using the

hydrated oocyte method (e.g., Macchi and Acha 2000, Brown-Peterson *et al*. 2017), such an approach was not possible in this investigation, given that no hydrated oocytes were observed. The oocyte size-frequency method, albeit more labor-intensive, yields results comparable to those derived from the hydrated oocyte method (Hunter *et al*. 1985).

The first step in quantifying RBF for Atlantic menhaden involved identifying the size-distribution of VTG3 ova in spawning capable fish, as VTG3 represents the most advanced egg stage in this phase. Four spawning capable fish were selected from throughout the defined spawning season, and the size-frequency distribution of ova was quantified for each using the methods described above. These distributions were then combined into a single size-frequency distribution, and Gaussian curves were fitted to this distribution to identify the number of modal groups present, as well as the size range associated with each group. The lower bound on the diameter of VTG3 ova (i.e., minVTG3) was defined as the mean diameter of the largest modal group minus one standard deviation. Batch fecundity (BF) was then quantified for each spawning capable and actively spawning Atlantic menhaden by counting all ova larger than minVTG3 in a 0.02 g – 0.03 g subsample of preserved ovary and scaling these counts to the whole ovary using the ratio of PW/PS. RBF was estimated from BF by:

$$RBF = \frac{BF}{W-OW} \tag{2}$$

This process was repeated so as to yield two estimates of RBF for each fish.

Linear mixed effects (LME) models were applied to evaluate the relationship between RBF and Atlantic menhaden size (Zuur *et al*. 2009). Specifically, two competing model forms were considered:

$$\log(\boldsymbol{RBF}) = \beta_1 * \boldsymbol{L} + \gamma * \boldsymbol{F} + \boldsymbol{\varepsilon} \quad \text{where } \gamma \sim N(0, \sigma^2_{fish})$$
$$\boldsymbol{\varepsilon} \sim N(0, \sigma^2) \tag{3}$$

and

$$\log(\boldsymbol{RBF}) = \gamma * \boldsymbol{F} + \boldsymbol{\varepsilon} \quad \text{where } \gamma \sim N(0, \sigma^2_{fish})$$
$$\boldsymbol{\varepsilon} \sim N(0, \sigma^2) \tag{4}$$

where ***RBF*** was the vector (*n* x 1) of RBF estimates for the *n* individual spawning capable or actively spawning Atlantic menhaden, ***L*** was the design matrix (*n* x 1) for the fixed-effect of fork length, β_1 was the coefficient associated with fork length, ***Z*** was the design matrix (*n* x 1) for the random effect of fish, γ was the estimate of the random effect (normally distributed with zero mean, variance σ^2_{fish}), and $\boldsymbol{\varepsilon}$ (*n* x 1) was the error vector (distributed normally with zero mean, variance σ^2). Note that fish was included as a random effect given that there were two estimates of RBF for each individual fish. The most supported model was identified using Akaike information criterion (Akaike 1973, Burnham and Anderson 2002).

Atlantic menhaden spawning interval (SI) was quantified using both the OM and POF methods (Hunter and Macewicz 1985). Spawning interval based on the OM method was calculated as

$$SI_{OM} = \frac{1}{\left(n_{OM}/n_{SC}\right)} \tag{5}$$

where n_{OM} was the number of spawning capable fish with oocytes undergoing OM and n_{SC} was the number of spawning capable fish. Interval based on the POF method was:

$$SI_{POF} = \frac{1}{\left(n_{POF}/n_{SC}\right)} \tag{6}$$

where n_{POF} was the number of spawning capable fish containing POFs. Estimates of spawning frequency (*SF*) for each approach were then generated by:

$$SF_{OM} = \frac{SS}{SI_{OM}} \tag{7}$$

and

$$SF_{POF} = \frac{SS}{SI_{POF}} \tag{8}$$

Annual fecundity-at-age *a* in year *i* (AF_{ai}) was estimated as:

$$AF_{ai} = RBF * WT_{ai} * SF * PM_{ai} \tag{9}$$

where WT_{ai} and PM_{ai} represented weight-at-age and maturity-at-age for female Atlantic menhaden at the beginning of the fishing year *i* and were obtained from current efforts related

to the 2019 benchmark assessment for this species. Spawning frequency was represented by SF_{OM}, SF_{POF}, and the mean of these two values ($SF_{\bar{x}}$) to characterize the uncertainty in the number of batches of ova spawned by Atlantic menhaden over the course of a spawning season. This approach yielded upper (using SF_{OM}), lower (using SF_{POF}), and mean (using $SF_{\bar{x}}$) values of AF_{ai} for this stock.

All statistical analyses were performed using the R software program (v3.3.2, R Core Team 2016). Package 'mixtools' was used to fit Gaussian curves to the full egg size-frequency distribution when identifying the minimum egg diameter associated with the proximate batch, while the package 'lme4' was accessed to model RBF.

Results & Discussion

Sample Collection

A total of 336 female Atlantic menhaden was collected from the nearshore waters of the Mid-Atlantic Bight and Chesapeake Bay from 2013-2018. Of these, 154 fish were derived from the NEAMAP Trawl Survey while 182 were provided by the commercial fishing industry. The sample size in this investigation is comparable with that used to characterize the reproductive biology and fecundity of Gulf menhaden (337 fish; Brown-Peterson *et al*. 2017) as well as Brazilian menhaden (315 fish; Macchi and Acha 2000). Note that while macroscopic determination of sex has been shown to be problematic for some *Brevoortia* species (Brown-Peterson *et al*. 2017), only 5 of the 341 fish (1.47%) classified as female macroscopically were found to be male upon histological examination. The relatively low error rate in macroscopic sex determination for Atlantic menhaden is likely due to the larger body and gonad size of this species. Further, while it has been shown that sex determination is more difficult when specimens have been frozen, all fish in this investigation were processed either at the time of capture or after having been held on ice.

Atlantic menhaden sampled as part of this investigation ranged in size from 170 mm FL to 330 mm FL (Figure 2). The size range of fish included in the previous investigation of Atlantic

menhaden fecundity ranged from 180-360 mm FL (Lewis *et al*. 1987). The difference in the maximum size between these two studies may be due to differences in the availability of larger fish between the time periods (1950s-1970s v. 2010s) or in sampling methods. Fish were collected from all months of the year with the exception of February, March, and September. Monthly sample sizes ranged from a single fish collected during April to 105 sampled during October (Figure 3). Sample sizes from the remaining months ranged between 19 and 52 female Atlantic menhaden.

Reproductive Biology

Histological preparations of ovarian tissue were evaluated for each of the 336 female Atlantic menhaden collected in this investigation, and this study represents the first to assess the reproductive biology of this species via gonad histology. The ovaries of 42 fish were found to be in the immature phase, 51 were early developing, 64 were developing, 44 were spawning capable, 17 were in the actively spawning sub-phase of the spawning capable phase, 14 were regressing, and 104 were in the regenerating phase. It is worth noting that, of the 17 fish in the actively spawning sub-phase, none of the ovarian tissues were found to contain hydrated oocytes. Eleven of these fish were undergoing OM, with late germinal vesicle migration often as the most advanced stage, and six possessed POFs that were less than 24 h old, indicating that spawning was likely to occur or had occurred within 24 h, respectively (Hunter and Macewicz 1985, Fitzhugh and Hettler 1995). Note that the absence of hydrated oocytes in these Atlantic menhaden was not surprising, given that spawning is thought to occur between midnight and 2am, hydration occurs over an approximate 6 h timeframe (Fitzhugh and Hettler 1995), and all collections of fish for this investigation occurred during daylight hours.

For each of the female Atlantic menhaden in the developing, spawning capable, and actively spawning phases (i.e., representing 125 fish), the presence of ova in the most advanced stage of development was accompanied by oocytes in all earlier stages of development. For example, while the ovary of an Atlantic menhaden in the spawning capable phase was characterized by the presence of numerous VTG3 ova, many stage 1 and stage 2 vitellogenic eggs, along with oocytes in the cortical alveolar stage, were also present (Figure 4). These observations indicated

that oocyte development is asynchronous in Atlantic menhaden and confirms that this species exhibits batch spawning (Brown-Peterson *et al*. 2011). Batch spawning was postulated for this species based on macroscopic assessments of ovaries collected in the late 1950s (Higham and Nicholson 1964), and two congeners of Atlantic menhaden, Gulf menhaden and Brazilian menhaden, were shown to exhibit characteristics associated with batch spawning (Macchi and Acha 2000, Brown-Peterson *et al*. 2017).

Atlantic menhaden spawning seasonality was assessed by evaluating monthly mean GSI and the percentage of female fish in the spawning capable phase of gonad development throughout the year. Monthly mean GSI showed clear peaks from October to December (Figure 5), and the percentage of spawning capable fish was highest during these months as well (Figure 6). While GSI showed a slight increase in May and spawning capable fish also were present during this time, sample size for this month was small. Specifically, only 22 fish were collected during May and, of those, only one was spawning capable. As such, the estimate of Atlantic menhaden spawning season was restricted to the October – December period (i.e., 92 days) to generate a conservative estimate of season length. The presence of slightly elevated mean GSI in May and the occurrence of a spawning capable fish, along with previous observations of Atlantic menhaden larvae along the coast during the spring and early summer in the Mid-Atlantic (Nelson *et al*. 1977), indicates that the realized spawning season is likely longer than that presented here. Further, the lack of larger Atlantic menhaden from collections during summer months, perhaps due to topping-off in Chesapeake Bay during reduction fleet operations and subsequent sampling from the top of the fish hold during port sampling, may have introduced some bias in this estimate of spawning season duration. Additional collections for these months, as well as those from which samples were not acquired for this investigation (i.e., February, March, and September), are ongoing, and estimates of spawning seasonality will be updated in the future.

The size-frequency distributions of oocytes in the ovaries of spawning capable Atlantic menhaden collected near the beginning (i.e., October) and end (December) of the spawning season were evaluated to characterize the spawning mode of this species (Figure 7). The lack of

a distinct gap between immature and mature ova in each of these distributions was consistent with indeterminate spawning. In addition, the relative abundance of mature eggs did not decline late in the spawning season, which serves as evidence of continual recruitment of oocytes into maturity throughout the season and suggests that this species exhibits indeterminate spawning. Further, annual egg production for two specimens collected early in the spawning season was quantified first using methodology appropriate for determinate batch spawners (i.e., count all mature ova), followed by that associated with indeterminate batch spawning (i.e., combine RBF, fish size, and spawning frequency). For each fish, annual fecundity estimates derived under the assumption of indeterminate batch spawning were nearly double those associated with determinate batch spawning (specimen 1: 1,163,770 eggs v. 538,567 eggs; specimen 2: 937,634 eggs v. 535,096 eggs), indicating that the standing stock of mature ova at the start of the spawning season was insufficient to supply the demand over the course of the spawning period, and that indeterminate spawning is likely. Finally, it is worth noting that mean GSI values remained elevated throughout the duration of the spawning season, a hallmark of indeterminate batch spawning species.

Given the above evidence, it is likely that Atlantic menhaden exhibit indeterminate batch spawning, and this finding is consistent with the determinations of spawning mode for both Gulf menhaden and Brazilian menhaden (Macchi and Acha 2000, Brown-Peterson *et al*. 2017). Indeed, most warm temperate and subtropical fishes are indeterminate batch spawners (Hunter *et al*. 1985, Murua and Sabrido-Rey 2003), and Atlantic menhaden appear to be no exception. This investigation represents the first to use empirical information on the reproductive biology of Atlantic menhaden to classify the spawning mode of this species.

Annual Fecundity

RBF was quantified for 61 Atlantic menhaden, which represented all fish in either the spawning capable phase of maturity or the actively spawning sub-phase, and included individuals ranging from 220 mm FL – 330 mm FL. The oocyte size-frequency method was used to quantify batch fecundity for each of these fish, and analysis of the oocyte size-frequency distribution generated from egg-diameter measurements of four spawning capable Atlantic menhaden

supported evidence of five Gaussian curves (Figure 8). The mean diameter of the most advanced stage of ova was 551 µm with a standard deviation of 98 µm. As such, the lower bound of the diameter of the most advanced egg stage in these fish (i.e., minVTG3) was 453 µm, and all ova $\geq$ 453 um in diameter were counted to yield estimates of batch fecundity. Note that the previous investigations of Atlantic menhaden fecundity counted all eggs greater than 350 µm in diameter under the assumption that this species exhibits determinate total spawning (Higham and Nicholson 1964, Dietrich 1979, Lewis *et al*. 1987). Further, alternative approaches to quantifying minVTG3 (e.g., mean – 2*sd) in this investigation would have altered the resulting batch fecundity and RBF estimates.

Estimates of batch fecundity ranged from 8484 – 363576 eggs/batch, while RBF values varied from 28 – 734 eggs/g ovary-free body weight. RBF was modeled using mixed effects models (3) and (4) above. AIC favored model (4) and the coefficient of the fixed-effect Length was not significant (p=0.12) in model (3), indicating no relationship between RBF and Atlantic menhaden size. Indeed, RBF calculations are intended to remove the effect of fish size and facilitate comparisons of batch fecundity for individuals of differing size (Brown-Peterson *et al*. 2017). The mean, bias-corrected RBF for the Atlantic menhaden stock was 236.92 eggs/g ovary-free body weight (Figure 9). The high variability in both batch fecundity and RBF observed in this investigation was not unexpected, given that batch sizes are influenced by a myriad of both biotic and abiotic factors (Hunter *et al*. 1985, Macchi and Acha 2000, Brown-Peterson *et al*. 2017), and the estimate of mean RBF for Atlantic menhaden fell within the ranges reported for both Gulf menhaden and Brazilian menhaden.

While spawning interval was calculated using the OM and POF methods both monthly and for the full spawning season (Table 1), only those generated using data across the entire season were considered due to relatively low monthly sample sizes of spawning capable and actively spawning fish. The OM method indicated that female Atlantic menhaden spawn once every 5.5 days during the spawning season, while the POF method was used to estimate a 10.2 day spawning interval. The discrepancy in these estimates of spawning interval are typical (Macchi and Acha 2000, Brown-Peterson *et al*. 2017). It is likely that the OM method underestimates the

spawning interval, perhaps due to the aggregation of animals in the actively spawning subphase just prior to a spawning event, while the POF method likely overestimates the interval, since rapid degradation of POFs in warm water environments can make these structures difficult to distinguish from *beta*-stage atresia. In an effort to ameliorate these biases, a mean spawning interval is usually calculated using the estimates derived from each of these methods. As such, the mean spawning interval for Atlantic menhaden was determined to be 7.86 days. While the mean spawning interval is longer than the 3.2 day interval estimated for Gulf menhaden (Brown-Peterson *et al*. 2017), it is consistent with the 8.0 day interval of the Brazilian menhaden (Macchi and Acha 2000), a species with a similar life history to that of the Atlantic menhaden.

Given a 92 day spawning season, it is expected that a female Atlantic menhaden would spawn an average of 11.72 times during that season (i.e., mean spawning frequency). Spawning frequency derived using the POF method was 9.02 spawns/season, while that estimated with the OM method was 16.73 spawns/season. These spawning frequency estimates were used to characterize uncertainty in the age-specific annual fecundity for this species.

Estimates of age-specific annual fecundity for Atlantic menhaden spanning age-0 to age-6+ were provided to the Chair of the Atlantic States Marine Fisheries Commission (ASMFC), Atlantic menhaden Stock Assessment Subcommittee (SAS) for the 2019 benchmark assessment for this stock. These estimates were generated using Equation (9) where *RBF* was 236.92 eggs/g ovary-free body weight, *SF* was 11.70 spawns/season, and where WT_{ai} and PM_{ai} were the weight at age *a* and proportion of fish mature at age *a* for a given year *i* at the start of the fishing year (i.e., March 1). Uncertainty in these annual fecundity estimates was characterized by substituting *SF* with SF_{POF} and SF_{OM} to yield lower and upper bounds on these fecundity estimates. An example of these fecundity estimates for 2015 is provided (Table 2). Note that when compared with the age-specific annual fecundity estimates generated from the fecundity-at-length relationship used previously for this species (Lewis *et al*. 1987), increases in age-specific annual fecundity ranged from 554.7% - 680.8%, with a mean increase of 623.5%. **These updated estimates of age-specific annual fecundity were accepted by the Chair of the ASMFC,**

Atlantic menhaden SAS and incorporated into the BAM used in the 2019 benchmark stock assessment for this species.

Updated estimates of annual fecundity for Gulf menhaden using methodology consistent with the spawning mode of the species resulted in increases on the order of 1100%-2300% (Brown-Peterson *et al*. 2017), indicating that the results of this investigation are not unreasonable. The ongoing collections of female Atlantic menhaden during spring, summer, and early fall months will likely increase the annual fecundity estimates of this species further, and perhaps yield results more similar to those observed for the Gulf menhaden. These additional collections may also support the generation of age-specific estimates of *SF*. Given that many species exhibit variability in spawning season, spawning interval, and therefore spawning frequency with age (Lowerre-Barbieri *et al*. 2011b, Fitzhugh *et al*. 2012, Brown-Peterson *et al*. 2017), characterizing these ontogenetic changes for Atlantic menhaden would yield even greater insight in to the reproductive biology and annual fecundity for this species.

Acknowledgements

The authors would like to acknowledge the staff of the NEAMAP Trawl Survey and Captain James A. Ruhle Sr. of the *F/V Darana R* for sampling Atlantic menhaden from this survey. Captains Leif and Steven Axelsson of the *F/V Dyrsten*, Captain Kevin Wark of the *F/V Dana Christine*, and Peter Himchak and Montgomery Diehl of Omega Protein, Inc. deserve thanks for providing Atlantic menhaden samples from the commercial fishing industry. Mr. Himchak's efforts to deliver fish to VIMS from the New Jersey ports were greatly appreciated. Melanie Chattin, Melanie Kolacy, Cameron Ward, and Debra Gauthier provided valuable assistance with histological preparations and in quantifying batch fecundity. This investigation was funded by the National Science Foundation Science Center for Marine Fisheries (SCeMFiS) under NSF award 1266057 and through membership fees provided by the SCeMFiS Industry Advisory Board.

References

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. In: Petrov, B.N. and F.Csaki (eds). Second international symposium on information theory. Akademiai Kiado, Budapest, p267-281.

Bonzek, C.F., J. Gartland, R.A. Johnson and J.D. Lange. 2008. NEAMAP Near Shore Trawl Survey: peer review documentation. Atlantic States Marine Fisheries Commission. Alexandria, VA. 143p.

Brown-Peterson, N.J. and J.W. Warren. 2001. The reproductive biology of spotted seatrout, *Cynoscion nebulosus*, along the Mississippi Gulf Coast. *Gulf of Mexico Science* 1:61-73.

Brown-Peterson, N.J., D.M Wyanski, F. Sabrido-Rey, B.J. Macewicz and S.K. Lowerre-Barbieri. 2011. A standardized terminology for describing reproductive development in fishes. *Marine and Coastal Fisheries* 3:52-70.

Brown-Peterson, N.J., R.T. Leaf, A.M. Schueller and M.J. Andres. 2017. Reproductive dynamics of Gulf menhaden (*Brevoortia patronus*) in the northern Gulf of Mexico: effects on stock assessments. *Fishery Bulletin* 115:284-299.

Burnham, K.P and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer. New York.

Dietrich, C.S. 1979. Fecundity of Atlantic menhaden, *Brevoortia tyrannus*. *Fishery Bulletin* 77:308-311.

Fitzhugh, G.R. and W.F. Hettler. 1995. Temperature influence on postovulatory follicle degeneration in Atlantic menhaden, *Brevoortia tyrannus*. *Fishery Bulletin* 93:568-572.

Fitzhugh, G.R., K.W. Shertzer, G.T. Kellison and D.M. Wyanski. 2012. Review of size- and age-dependence in batch spawning: implications for stock assessment of fish species exhibiting indeterminate fecundity. *Fishery Bulletin* 110:413-425.

Higham, J.R. and W.R. Nicholson. 1964. Sexual maturation and spawning of Atlantic menhaden. *Fishery Bulletin* 63:255-271.

Hunter, J.R and S.R Goldberg. 1980. Spawning incidence and batch fecundity in northern anchovy, *Engraulis mordax*. *Fishery Bulletin* 77:641-652.

Hunter, J.R., N.C. Lo and R.J. Leong. 1985. Batch fecundity in multiple spawning fishes. *NOAA Technical Report NMFS, 36,* 67-77.

Hunter, J.R. and B.J. Macewicz. 1985. Measurement of spawning frequency in multiple spawning fishes. *NOAA Technical Report NMFS, 36,* 79-94.

Lewis, R.M, D.W. Arenholtz and S.P. Epperly. 1987. Fecundity of Atlantic menhaden, *Brevoortia tyrannus*. *Estuaries* 10: 347-350.

Lowerre-Barbieri, S.K. 2009. Reproduction in relation to conservation and exploitation of marine fishes. In: Jamieson, B. (ed). Reproductive biology and phylogeny of fishes (agnathans and bony fishes). London: CRC Press. p372–394.

Lowerre-Barbieri, S.K., K. Ganias, F. Saborido-Rey, H. Murua and J.R Hunter. 2011a. Reproductive timing in marine fishes: variability, temporal scales, and methods. *Marine and Coastal Fisheries* 3:71-91.

Lowerre-Barbieri, S.K., N.J. Brown-Peterson, H. Murua, J. Tomkiewicz, D.M. Wyanski and F. Saborido-Rey. 2011b. Emerging issues and methodological advances in fisheries reproductive biology. *Marine and Coastal Fisheries* 3:32-51.

Lynch, A.J., J.R. McDowell and J.E. Graves. 2010. A molecular genetic investigation of the population structure of Atlantic menhaden (*Brevoortia tyrannus*). *Fishery Bulletin* 108:87-97.

Macchi, G.J. and E.M. Acha. 2000. Spawning frequency and batch fecundity of Brazilian menhaden, *Brevoortia aurea*, in the Rio de la Plata estuary off Argentina and Uruguay. *Fishery Bulletin* 98:283-289.

Marshall, T.M. 2009. Implementing information on stock reproductive potential in fisheries management: the motivation, challenges and opportunities. In: Jakobsen, T., M.J. Fogarty, B.A. Megrey and E. Moksness (eds). Fish reproductive biology. Chichester, UK: Wiley-Blackwell Scientific Publications. p395-420.

Morgan, M.J., H. Murua, G. Kraus, Y. Lambert, G. Marteinsdottir, C.T. Marshall, L. O'Brien and J. Tomkiewicz. 2009. The evaluation of reference points and stock productivity in the context of alternative indices of stock reproductive potential. *Canadian Journal of Fisheries and Aquatic Sciences* 66:404-414.

Murua, H. and F. Saborido-Rey. 2003. Female reproductive strategies of marine fish species in

the North Atlantic. *Journal of Northwest Atlantic Fishery Science* 33:23-31.

Nelson, W. R., M. C. Ingham and W. E. Schaaf. 1977. Larval transport and year-class strength of Atlantic Menhaden, *Brevoortia tyrannus*. *Fishery Bulletin* 75:23–41.

Pavlov, D., N.G. Emel'yanova and G.G. Novikov. 2009. Reproductive Dynamics. In: Jakobsen, T., M.J. Fogarty, B.A. Megrey and E.Moksness (eds). Fish reproductive biology. Chichester, UK: Wiley-Blackwell Scientific Publications. p48-69.

Prophet E. 1992. Laboratory methods in histotechnology. American Registry of Pathology. Washington, DC.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.R-project.org/.

Sadvoy, Y.J. 1996. Reproduction of reef species. In: Polunin, N.V.C and C.M. Roberts (eds). Reef fisheries. London: Chapman and Hall. p15-59.

SEDAR. 2015. SEDAR 40 – Atlantic Menhaden Stock Assessment Report. SEDAR. North Charleston, SC. 643p.

SEDAR. 2018. SEDAR 63 – Gulf Menhaden Stock Assessment Report. SEDAR. North Charleston, SC. 352p.

Zuur, A.F., E.N. Ieno, N.J. Walker, A.A. Saveliev and G.M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York.

Tables

Table 1. Estimates of spawning interval (SI) in days for female Atlantic menhaden collected from 2013-2018. Monthly estimates of SI using both the oocyte maturation (SI_{OM}) and post ovulatory follicle (SI_{POF}) methods are given, and estimates aggregated across the spawning season are indicated in bold font. Sample sizes of spawning capable female Atlantic menhaden by month are provided, and the number of fish undergoing OM or possessing POFs are indicated in parenthesis. Note that, if no fish were observed undergoing OM or containing POFs for a given month, SI was undefined (UND).

Month	SI_{OM}	SI_{POF}	Sample Size (OM Count/POF Count)
May	1.00	UND	1(1/0)
Oct	6.60	6.60	33(5/5)
Nov	UND	UND	11(0/0)
Dec	3.20	16.00	16(5/1)
Overall	**5.55**	**10.17**	**61(11/6)**

Table 2. Estimates of age-specific annual fecundity for age-1 to age-6+ Atlantic menhaden in 2015 using the fecundity-at-length relationship used in past stock assessments (Lewis *et al*. 1987) and estimates derived from this investigation (Gartland *et al*.). Mean, minimum (min.), and maximum (max.) estimates of annual fecundity were derived using mean spawning frequency ($SF_{\bar{x}}$), spawning frequency based on the post ovulatory follicle method (SF_{POF}), and spawning frequency based on the oocyte maturation method (SF_{OM}). The percent change quantifies the change in estimates of age-specific annual fecundity between Lewis *et al*. (1987) and this investigation where $SF_{\bar{x}}$ was used to represent spawning frequency.

	Annual Fecundity				
Age	**Lewis et al. 1987**	**Gartland et al. Mean**	**Gartland et al. Min.**	**Gartland et al. Max.**	**Percent Change**
1	3,770	27,350	21,076	39,086	+625%
2	37,765	270,490	208,437	386,555	+616%
3	98,782	771,287	594,344	1,102,240	+681%
4	137,741	1,044,859	805,156	1,493,198	+659%
5	173,022	1,132,786	872,911	1,618,854	+555%
6+	180,394	1,271,904	980,114	1,817,666	+605%

Figures

Figure 1. Distribution of sites from which female Atlantic menhaden were sampled by the NEAMAP Trawl Survey (orange) and commercial purse seine, trawl, and gillnet fisheries (green) from 2013-2018.

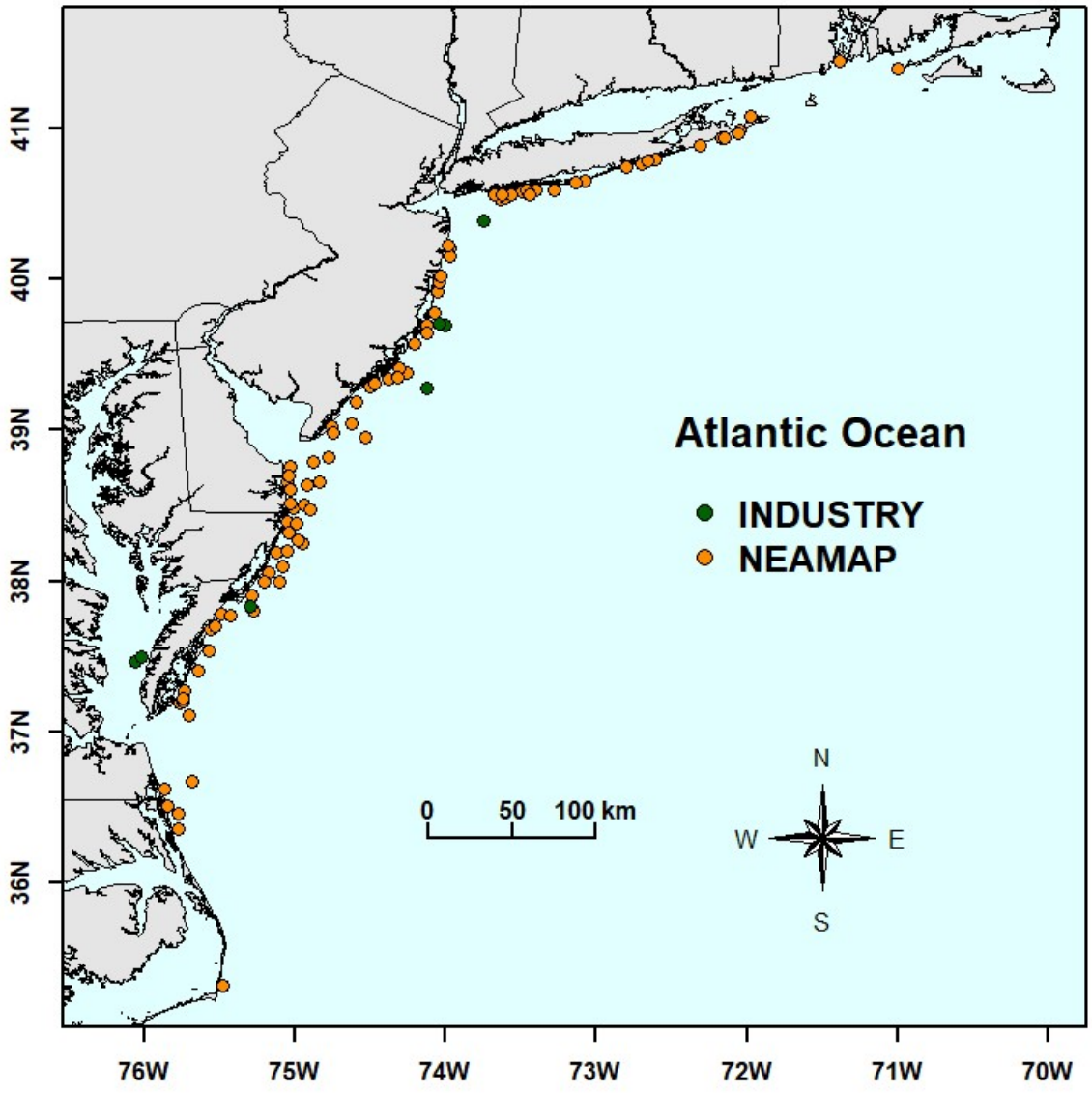


Figure 2. Length-frequency distribution of female Atlantic menhaden collected from 2013-2018 to characterize the reproductive biology and fecundity of this species.

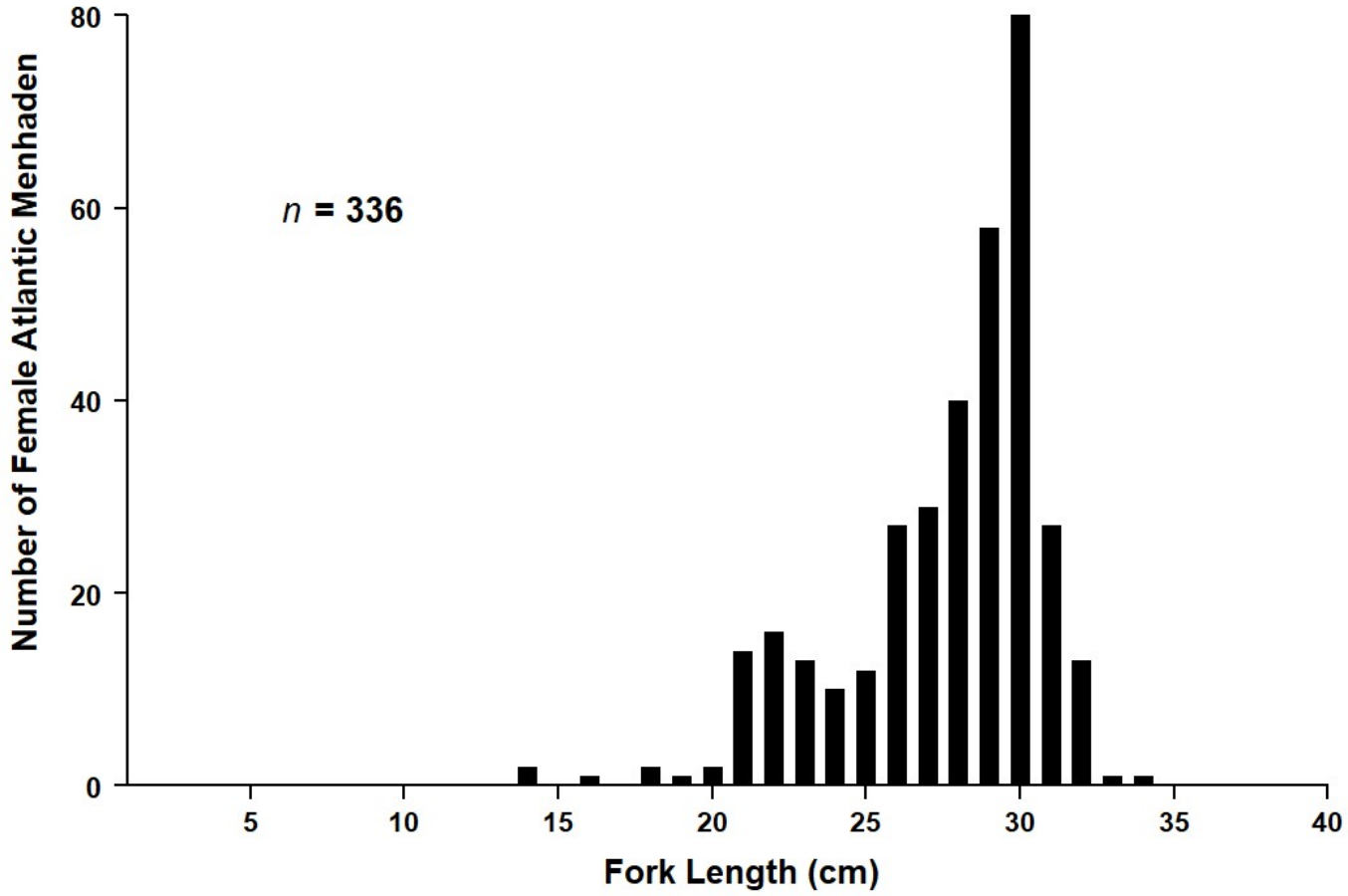


Figure 3. Number of female Atlantic menhaden collected monthly from 2013-2018 to characterize the reproductive biology and fecundity of this species.

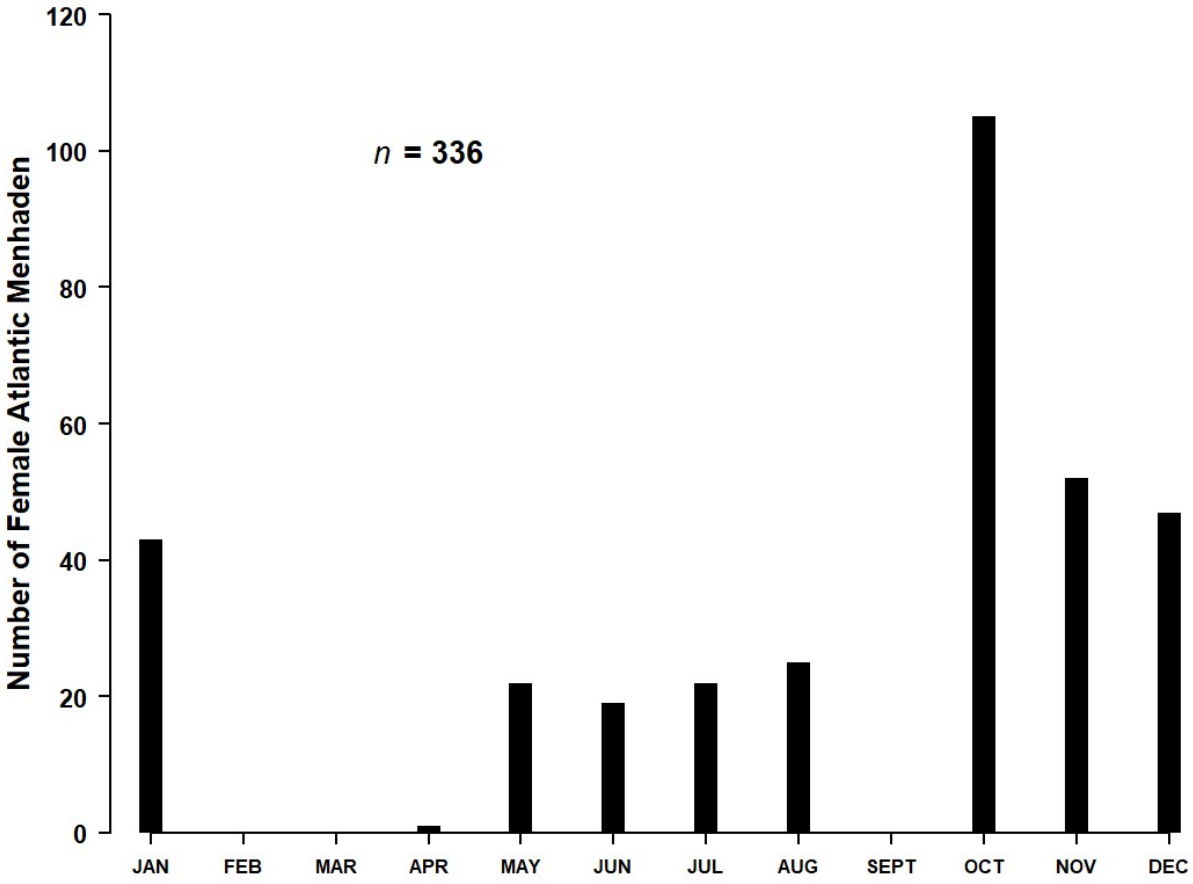


Figure 4. Histological gonad section (80x magnification) of a 295mm fork length, spawning capable Atlantic menhaden collected from the coastal ocean of New Jersey in October 2017. Asynchronous oocyte development characteristic of batch spawning was evident. PG = primary growth, CA = cortical alveolar, VTG1 = first stage vitellogenesis, VTG2 = second stage vitellogenesis, VTG3 = third stage vitellogenesis, OVW = ovarian wall.

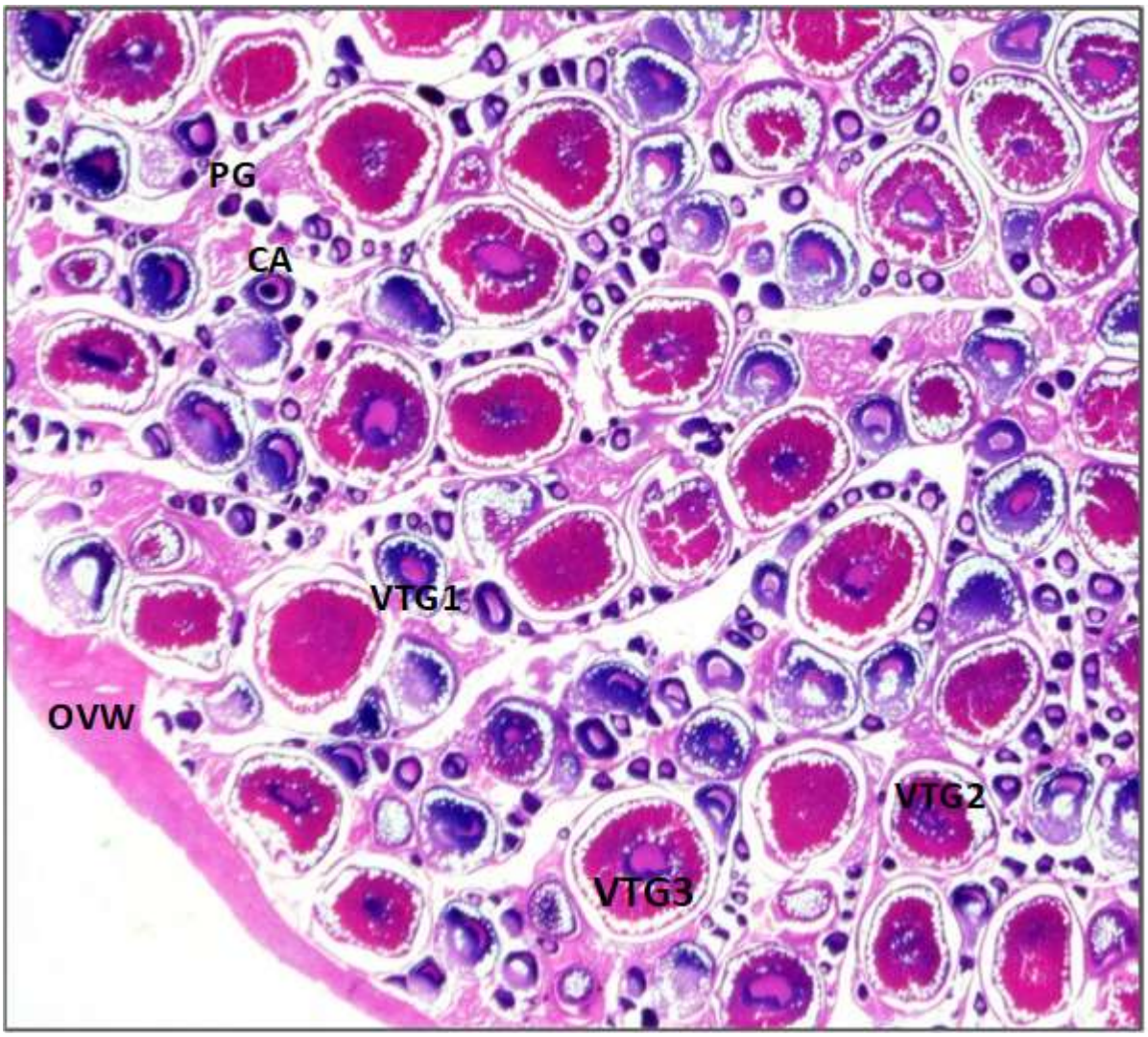


Figure 5. Mean monthly gonadosomatic index (GSI) for female Atlantic menhaden collected from 2013-2018. Error bars represent standard errors of the means.

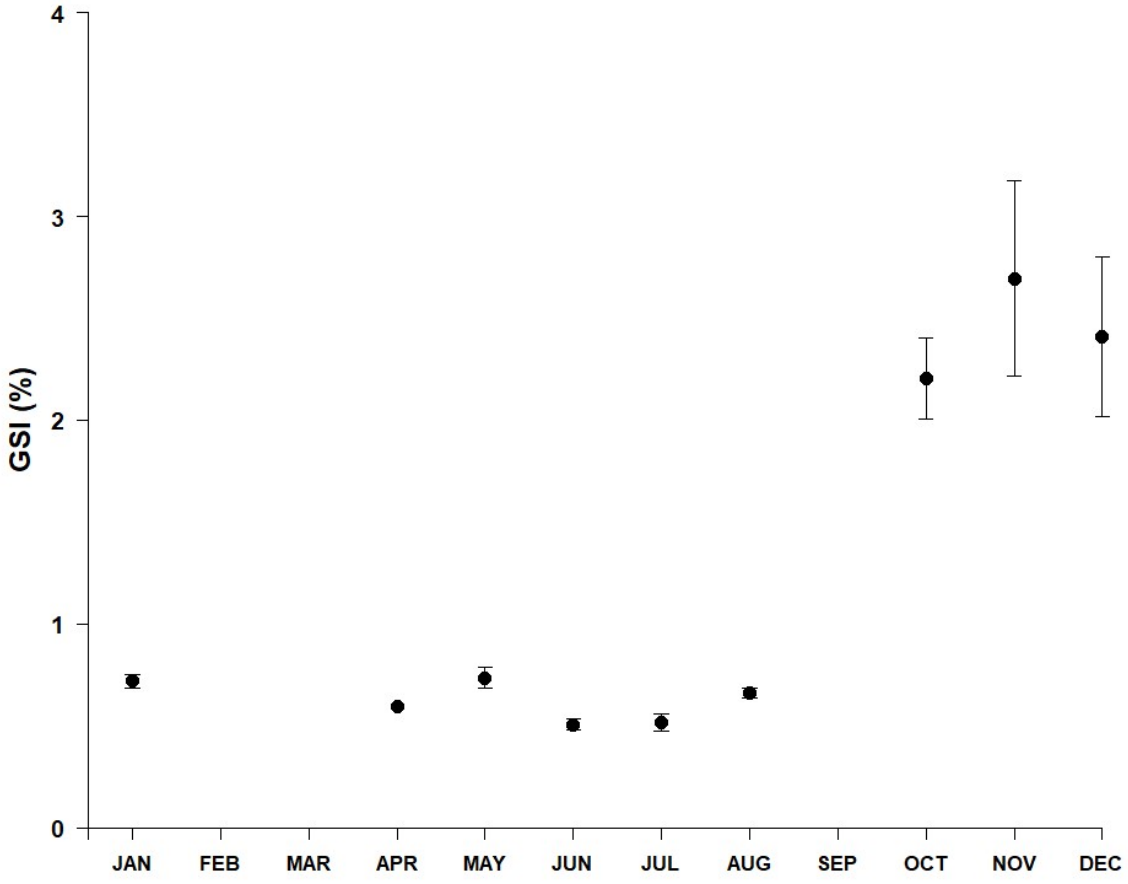


Figure 6. Monthly percentage of female Atlantic menhaden in the spawning capable gonad phase (including the actively spawning sub-phase). Fish were collected from 2013-2018 in an effort to characterize the reproductive biology and fecundity for this species.

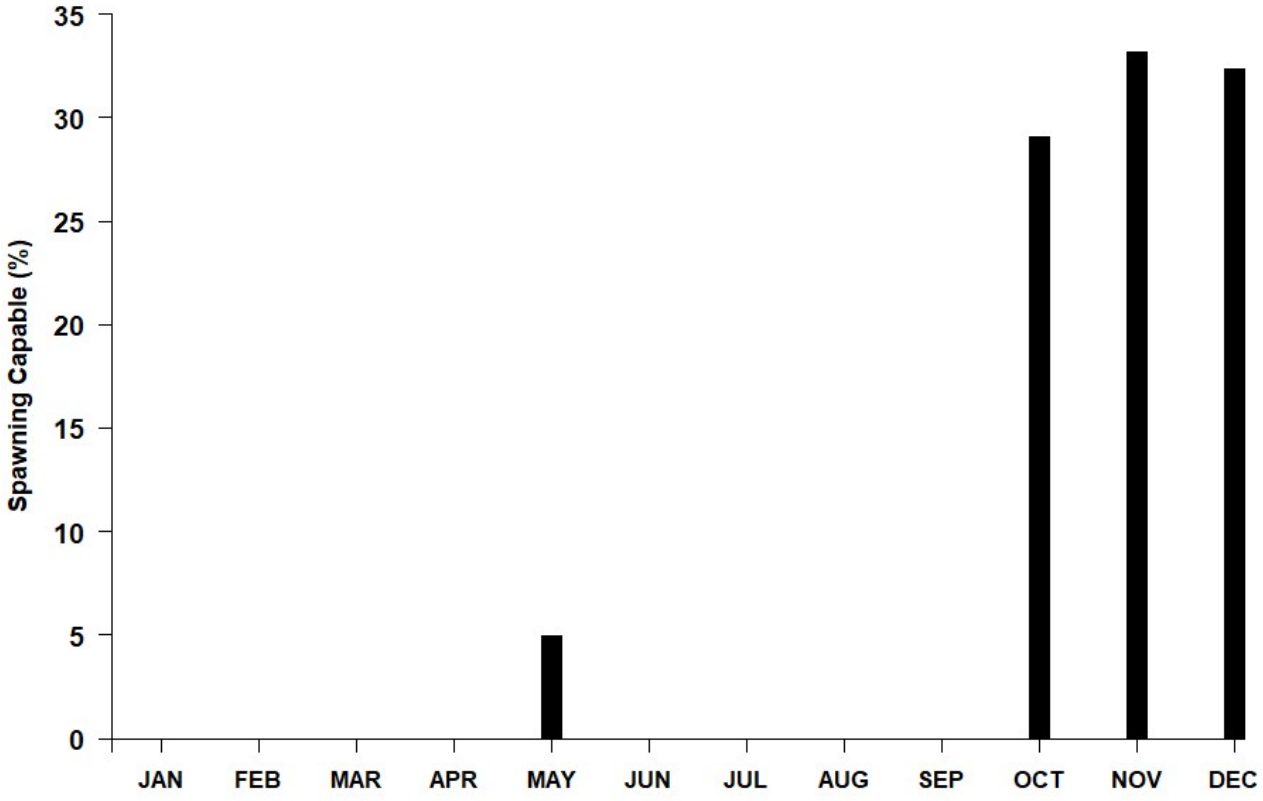
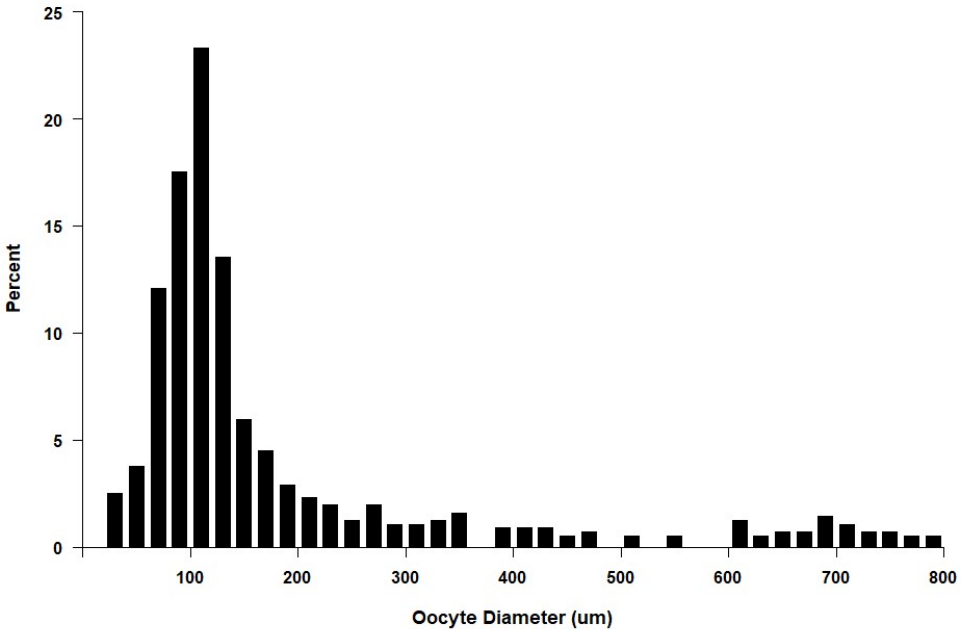


Figure 7. Size-frequency distribution of all oocytes present in a spawning capable female Atlantic menhaden collected in (a) October off of the coast of New York and (b) December off of the coast of New Jersey. The fish collected in October was 285 mm fork length (FL), while that sampled in December was 300 mm FL.

(a)



(b)

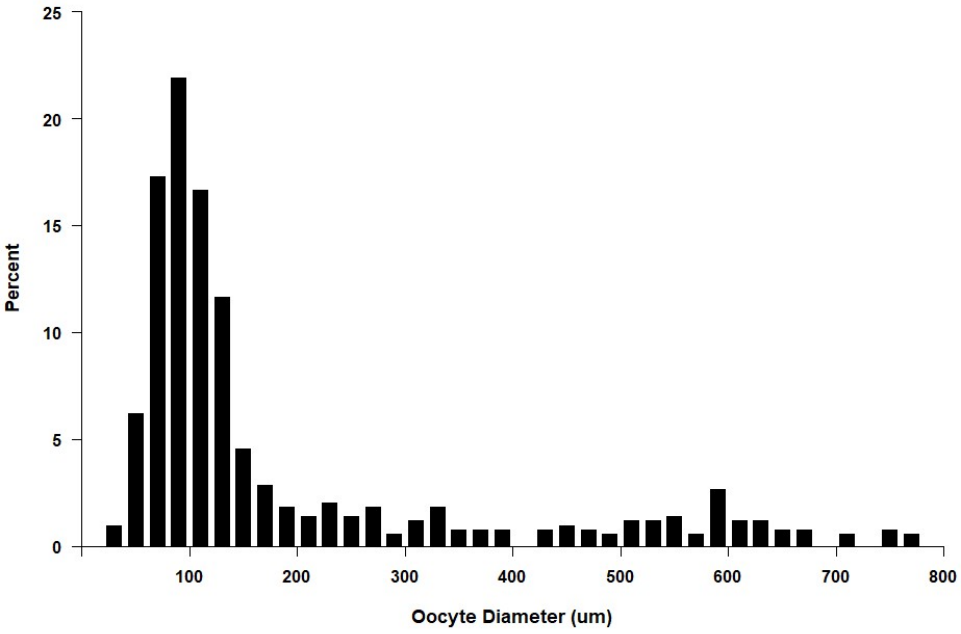


Figure 8. Size-frequency distribution of all oocytes present in the ovaries of four spawning capable female Atlantic menhaden collected from October – December. Model development supported the presence of five Gaussian curves within this distribution. The mean egg diameter of the largest group was 551 µm with a standard deviation of 98 µm. The lower bound for the size of third stage vitellogenic eggs (minVTG3) was 453 µm (i.e., 551 µm – 98 µm).

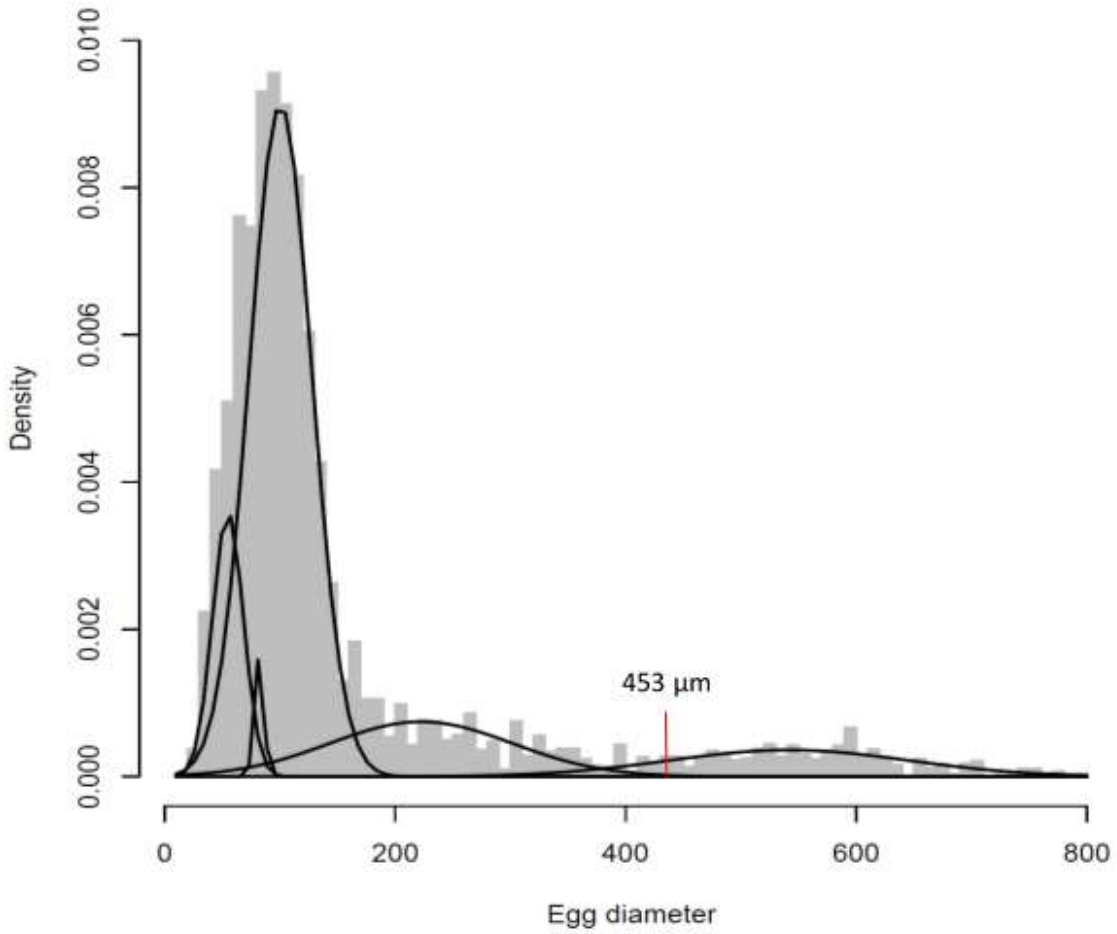


Figure 9. Plot of relative batch fecundity (RBF; eggs/g ovary-free body weight) on female Atlantic menhaden fork length for 61 fish with gonads either in the spawning capable phase or actively spawning sub-phase. Mean bias-corrected RBF for the Atlantic menhaden stock was 236.92 eggs/g ovary-free body weight.