Sperm Acquisition and Storage Dynamics Facilitate Sperm Limitation in the Selectively Harvested Blue Crab, *Callinectes sapidus*

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ABSTRACT

Selective harvest of male blue crabs *Callinectes sapidus* can reduce the operational sex ratio and alter male mating history and behaviors, reducing the quantity of sperm females acquire during mating. Females mate shortly after molting to maturity, storing sperm at least two years, and fertilize multiple broods of eggs. We combined field surveys, a mark-recapture experiment, and modeling in the most extensive study of sperm storage and use after mating in blue crabs evaluating: 1) spatiotemporal patterns in sperm quantity acquired during mating, 2) the pattern and rate of decline of sperm during storage, 3) the quantity of sperm used for fertilization, and 4) the potential for sperm limitation. We also explored the spatial extent of spawning in Chesapeake Bay in comparison to the spawning sanctuary. Female crabs acquired up to $3x10^9$ sperm, but sperm stores declined by 90–95% in the first 1–2 months after mating. Sperm quantity differences between first and second year spawners indicated use of 4 sperm egg^{-1} during fertilization. Approximately 15% of spawning females were in their second spawning season, and remaining sperm stores were indicative of sperm limitation resulting in a 5–10% decrease in reproductive output of the spawning stock. The current spawning sanctuary encompassed 98% of ovigerous females and 100% of females with evidence of prior spawning. Although many females do not experience sperm limitation prior to harvest or natural mortality, reductions in the reproductive output of second-year spawners likely limits population resilience to inter-annual variation in spawning stock biomass.

Keywords: Sperm limitation, Population dynamics, Reproductive output, Selective harvest, *Callinectes sapidus*

1 1. INTRODUCTION

2 Sustaining fisheries as vital components of coastal economies requires detailed information on
3 the impacts of fishery removals on the mortality and life history of fished species. In some
4 fisheries, selective harvest of certain sexes or size classes can have unintended negative
5 consequences for populations and ecosystems that may need to be accounted for in management
6 (Garcia et al. 2012, Ogburn 2019). For example, selection that truncates the age structure of
7 fished populations can increase instability of populations due to nonlinear effects of altered
8 competition, mortality rates, and/or life history (Anderson et al. 2008). In addition, short-lived
9 species (generally <10 years longevity) are vulnerable to fishery collapse (Pinsky et al. 2011)
10 and require management regimes that can respond to the short-term fluctuations in abundance
11 common to these species (Adams 1980, King & McFarlane 2003). To avoid unintended
12 consequences of selective fisheries for short-lived species, it is critical to understand fully the
13 impacts of harvest on fished populations.
14
15 The blue crab *Callinectes sapidus* is an example of a short-lived crustacean critical to estuarine
16 and coastal ecosystems that supports valuable commercial and recreational fisheries along the
17 US Atlantic and Gulf coasts. It plays important ecological roles in food webs, structures benthic
18 communities, and forms critical links between benthic productivity and higher trophic levels
19 (Baird & Ulanowicz 1989, Martin et al. 1989, Eggleston et al. 1992, Clark et al. 1999). Blue crab
20 fisheries are characterized by numerous small-scale operations harvesting crabs by pot, trotline,
21 scrape, or other gear (Kennedy et al. 2007). Long-term declines in harvests along the US Atlantic
22 coast, and the recognition that at least some populations were undergoing recruitment
23 overfishing, prompted shifts in management from effort control including a seasonal marine

protected area (sanctuary, Fig 1.) designed to protect spawning stock (Lambert et al. 2006b), toward spawning stock biomass targets and thresholds (Lipcius et al. 2002, Miller et al. 2011, Colton et al. 2014, Ogburn & Habegger 2015). This new management strategy requires detailed information on the status and trends of the crab population, and in particular of the mature female spawning stock. It has also shifted additional harvest effort towards male crabs (Semmler 2016), which has the potential to alter sex ratios and reduce reproductive output through sperm limitation (Hines et al. 2003, Sato & Goshima 2006, Ogburn et al. 2014, Pardo et al. 2017).

Sperm limitation occurs when females cannot achieve their potential lifetime reproductive output because they receive insufficient sperm during mating (Pennington 1985, Pitnick 1993). In populations of harvested crabs, reductions in sperm stores obtained during mating events occur in sites (Pardo et al. 2017) and time periods (Sato et al. 2010, Ogburn et al. 2014) of high fishing intensity targeting males, and when small males provide fewer sperm per mating event than large males (Sato et al. 2006). The female blue crab stores sperm from a single mating period following a functional terminal molt to maturity (Van Engel 1958). Individuals in Chesapeake Bay may have a lifetime reproductive potential of up to 8 broods in two or more years fertilized from that single mating period (Hines et al. 2003, Dickinson et al. 2006, Darnell et al. 2009). Mechanisms for reduced sperm stores include males mating too frequently to fully recover between subsequent mating events (Kendall et al. 2002, Sato & Goshima 2006, Sato et al. 2010), males allocating fewer sperm per mating event to maximize future mating opportunities (Rondeau & Saint-Marie 2001, Sato & Goshima 2006), or rarely an operational sex ratio so extreme that females fail to find a mate (Rowe & Hutchings 2003, Rains et al. 2018). The single mating period of female blue crabs, combined with long-term sperm storage and production of

multiple broods, make them particularly susceptible to sperm limitation (Austin 1975, Morgan et al. 1983, Paul & Paul 1992).

Blue crab fisheries also likely affect the reproductive output of female blue crabs by truncating the age distribution. In Chesapeake Bay, archaeological samples spanning the previous three millennia suggest that intensive modern harvests reduce the frequency of large, likely older, crabs (Rick et al. 2015). Today's fishers harvest many mature female crabs prior to spawning, targeting them during the fall and spring migrations from low salinity juvenile habitats to high salinity spawning grounds (Van Engel 1958, Epifanio 1995, Aguilar et al. 2005). Harvesting mature females in Maryland is illegal for recreational fishers but legal for commercial fishers throughout the summer; and non-egg-bearing females are legal in Virginia (Miller et al. 2011). A winter dredge fishery also targeted mature female crabs for many years, but it is currently under moratorium (Miller et al. 2011). Females that do survive to spawn are protected from harvest in the spawning stock sanctuary (Fig. 1) during summer when it is in place, but the sanctuary is open to harvest during the spring and fall (Miller et al. 2011, Corrick 2018). The limited data available suggest that 85% of mature female blue crabs do not survive from their first to second spawning season, and the associated lost reproductive output is accounted for in stock assessment (Lambert et al. 2006a, Miller et al. 2011). However, the reproductive contribution of females that do survive to a second or perhaps third spawning season is most likely affected by initial sperm acquisition and storage levels and remains poorly known.

Gaps in our understanding of reproductive biology have led to differing perspectives on the potential for sperm limitation in the Chesapeake Bay blue crab fishery. Models focused on

individual lifetime reproductive output indicate that some females surviving to their second spawning season may be sperm-limited due to reduced sperm quantities obtained during mating, potentially lowering population reproductive output (Ogburn et al. 2014). In contrast, individual-based models focused on estimating population averages suggested that sperm limitation would only occur at much higher male harvest rates than currently observed due to inability of some females to find mates (Rains et al. 2018). Improving our understanding of reproductive biology and interactions with female age distribution is critical for resolving these differences and predicting the effects of selective harvest on population-level reproductive output.

The mature female spawning migration complicates efforts to study the blue crab spawning stock and its susceptibility to sperm limitation and age truncation. The prevailing paradigm, developed based on data from Chesapeake Bay and North Carolina, is that female blue crabs migrate to and remain near the mouths of estuaries for larval hatching (e.g. Van Engel 1958, Tankersley et al. 1998, Aguilar et al. 2005). This idea was recently called into question as too narrow a definition of spawning habitat for the species due to observations of offshore spawning on the continental shelf in other areas (Gelpi et al. 2009, Ogburn & Habegger 2015). Ogburn & Habegger (2015) proposed a new definition of blue crab spawning habitat based on reproductive status: spawning areas are areas containing ovigerous females and non-ovigerous females with both developing ovaries and egg remnants providing evidence of spawning events. This new definition is consistent with spatiotemporal patterns of spawning crab abundance within estuaries (e.g. Van Engel 1958, Tankersley et al. 1998, Rittschof et al. 2010) in the coastal ocean off the South Atlantic Bight (Dudley and Judy 1971, Rittschof et al. 2010, Ogburn & Habegger 2015), and 20–50 km offshore of Louisiana in the Gulf of Mexico (Gelpi et al. 2009). It is also supported by

observations of tagged animal movements (Carr et al. 2004) and stable isotope data (Gelpi et al. 2013) which indicated that females move to high salinity habitats (whether in estuaries or offshore) and remain there during production of the first to last brood. These findings point to a need to re-evaluate the current spawning sanctuary in Chesapeake Bay to determine whether it adequately protects the spawning stock.

In this study, we conducted field surveys and a mark-recapture experiment to evaluate the effects of selective harvest on reproductive output of the Chesapeake Bay blue crab spawning stock. The specific objectives were: 1) to explore spatial patterns in sperm acquired by recently-mated females prior to the spawning migration, 2) to document trends in the quantity of sperm stored by female crabs from shortly after mating to post-migratory crabs in the second spawning season, 3) to estimate the quantity of sperm used for fertilization, and 4) to explore spatial patterns in the reproductive status of the spawning stock at the peak of the summer spawning season. We apply the study results to address issues in blue crab fisheries management, including evaluating the extent to which the reproductive output of the Chesapeake Bay spawning stock is reduced due to sperm limitation, the proportion of mature females that survive to spawn in a second year, and the spatial extent of spawning habitat relative to the spawning sanctuary.

2. MATERIALS & METHODS

2.1. Sperm acquired by recently-mated females

To understand spatial and temporal patterns in the quantity of sperm and seminal fluid obtained during mating, recently-mated, non-ovigerous, female crabs were collected at multiple sites in the Chesapeake Bay in 2013, 2014, and 2015. In 2013, groups of crabs were collected at seven

sites (Fig. 1, Table 1) between 21 August and 10 September during the peak period of maturation and mating (Hines 2007). Carapace width (CW) and carapace length (CL) were recorded. Crabs were initially determined to be recently-mated by maturity (triangular abdomen for immature females and rounded abdomen for mature females), by molt stage following Smith and Chang (2007) and references therein (A–D, with A or B indicating recently molting and mating), and by carapace color (relatively clean and white carapace indicating recent molting and mating, relative to yellow or brown discoloration) (Fig. 2). Note that the carapace coloration scale is relative and the timing of discoloration following molting is poorly known. Crabs were then dissected in the laboratory by removing the carapace and evaluated for the presence of hardened seminal fluid (sperm plug) (e.g. Jivoff et al. 2007), percent fullness of spermathecae based on visual estimation of seminal fluid volume as in Hines et al. (2003), and ovarian condition (stage 0–4) as in Hines et al. (2003) and corresponding with Hard's stage I-V (Hard 1945). Spermathecae were dissected out and weighed individually, then preserved individually in 70% ethanol for sperm counting. Only crabs with hardened sperm plugs, which degrade within approximately two weeks after mating in captivity (Wolcott et al. 2005), and an ovary stage of 0 or 1 were used for analyses to ensure crabs were recently-mated (N=8–12 per site) (Table 1).

Sampling was expanded in fall 2014 to 15 sites throughout the Chesapeake Bay, and some lower bay sites were sampled again in early summer 2015 (Table 1). This sampling effort was designed to be representative of the females that would make up the majority of the spawning stock in July 2015. In fall 2014, groups of 20 crabs were obtained from 15 sites (Fig. 1) throughout the Chesapeake Bay from 8 September – 29 October (Table 1). For most sites, crabs were subsampled from batches of crabs tagged as part of a mark-recapture experiment (see below). In

early summer 2015, groups of 20 recently-mated crabs were collected from Fishing Bay, Potomac River, Rappahannock River, and York River (Fig. 1) from 25 June – 21 July. Crabs were dissected and evaluated as described above. Subsamples of 4–5 crabs (fall 2014) or 8–10 crabs (early summer 2015) were randomly selected for sperm counts. Only recently-mated crabs (those with a hardened sperm plug) were used for analyses, but ovary stage ranged from 1–2 for 2014 crabs and 1–3 for 2015 crabs.

Sperm count

Sperm were removed from spermathecae and counted following Ogburn et al. (2014). Spermathecae and associated preservative were emptied into a Petri dish and the spermathecal membrane was separated from the spermatophores and sperm plug and rinsed with 70% ethanol. Spermatophores and the sperm plug were finely chopped (<1 mm) and poured with associated preservative into a 16 mL glass PYREX Dounce homogenizer and the total volume was recorded. Fuller spermathecae weighing more than 1 g were diluted to 1 g of spermathecal weight per 100 ml ethanol. Samples were homogenized to separate individual sperm cells for counting. Three replicated drops of homogenate were examined one at a time on a Petroff-Hausser Counting Chamber (5x5 grid) at 400x magnification under a phase contrast microscope. Sperm were counted in all 25 grid cells. Total sperm count was calculated as the average of the three replicates of: Sperm count = (Number of sperm cells/Volume of counting chamber) x Dilution x 2, where volume was 0.00002 ml, the dilution was the volume (in ml) of homogenate, and the multiplier of two was used because only one of the two spermathecae was analyzed. Analyzing a single spermatheca reduced processing time and should not introduce bias (Duluc et al. 2005, Rodgers et al. 2011).

2.2. Sperm decline during storage and fertilization

The decline of stored sperm following mating was assessed by counting stored sperm in cohorts of crabs sampled at different time points after mating. Information on time after mating was obtained from the large-scale mark-recapture experiment and from crabs determined to be first or second year spawners based on estimated ages of the barnacle *Chelonibia testudinaria* (see below for details). For the mark-recapture experiment, batches of about 500 recently-mated crabs were obtained from commercial crabbers at 12 sites throughout the Chesapeake Bay (subsamples of 20 crabs were retained from these batches plus three additional sites as described above for evaluation of sperm stores after mating) (Fig. 1). Up to 500 crabs were tagged at each site with white vinyl disk tags with the words 'Reward', 'Extra reward possible if crab kept and frozen', and contact information listed on the front of the tag. The back of the tag requested information including tag number, site of recapture, date of recapture, and water depth. Tags were attached using stainless steel wire, and tags and the tagging method are described in detail in Corrick (2018). Crabs were recaptured by commercial and recreational crabbers and reported by telephone or web form. Recaptured crabs were frozen after capture and later transferred to Smithsonian Environmental Research Center (SERC) for analysis. Interactions between the fishery and reproductive stock (e.g. exploitation rate, fishery composition) are reported separately (Corrick 2018). Time at large was calculated as the difference between release and recapture dates and grouped by month. No crabs were recaptured and retained from 2–5 months after tagging due to seasonal closure of the fishery, or more than 12 months following tagging. Crabs were later dissected and evaluated following the methods detailed above for reproductive status and sperm count.

Sperm use during fertilization was estimated by calculating the difference in sperm count between recaptured crabs in Apr/May (6–7 months after tagging) prior to their first spawning season and crabs estimated to be in their second spawning season. Crabs estimated to be in their second spawning season (fouled with large barnacles *Chelonibia testudinaria* – see below) were obtained from the Chesapeake Bay Multispecies Monitoring and Assessment Program (ChesMMAP; see details below) in July 2015–2016. None of the crabs from the mark-recapture experiment or ChesMMAP samples were older than the second spawning season. We assumed that crabs in their first spawning season had already undergone initial sperm declines following mating as observed in Walcott et al. (2005) and the present study, produced an average of three broods during the interval between the beginning of the first and second spawning seasons based on Dickinson et al. (2006), and experienced no additional loss of sperm due to degradation or other causes. The number of sperm used to fertilize each was calculated using a brood size of $3x10^6$ eggs (Hines 1982, Prager et al. 1990).

2.3. Reproductive status of the spawning stock

The spatial distribution and reproductive status of the blue crab spawning stock were evaluated at the height of the spawning season. Crabs were collected in 2015–2017 during July sampling of the fishery independent ChesMMAP survey (Fig. 3, Fig. A1). Stratified random sampling (N=80 sites per year) was conducted from the *R/V Bay Eagle* using a 13.9-m bottom trawl (15.2 cm mesh net and 7.6 cm mesh cod end) (Bonzek et al. 2015). Tows were 20-min in duration at a speed of 1.54 ms^{-1}. All mature female crabs encountered were frozen upon collection and later transferred to SERC for evaluation of reproductive status.

We evaluated crabs for reproductive status using external and internal characteristics. External measures and indicators included CW, CL, sexual maturity, molt stage, presence of external egg mass (ovigerous females), presence of egg remnants on pleopods of non-ovigerous females, and presence and size of the barnacle *C. testudinaria*. The largest barnacle on each crab was measured for the maximum rostro-carinal length, which was used to estimate barnacle age (Ewers-Saucedo et al. 2015). These measurements were used to infer whether female crabs with barnacles were in their first (barnacle <10 mm) or second year (barnacle >15 mm). Internal characteristics included the presence of a sperm plug, spermathecae fullness, and ovarian condition. Differences in the sperm quantity of first and second year spawners were evaluated using the non-parametric Mann-Whitney Rank Sum Test due to small samples sizes. Differences in reproductive status across the sampled salinity range were evaluated by grouping crabs into salinity bins (<15, 16–20, 21–25, 26–30, 31–35) and comparing spermathecae weight and percent fullness using non-parametric Kruskal-Wallis one-way Analysis of Variance on Ranks due to unequal sample sizes and unequal variance. All statistical analyses were conducted using SigmaPlot 12.3 (Systat Software, Inc.).

3. RESULTS

3.1. Sperm acquired by recently-mated females

Recently-mated female crabs varied substantially in the quantity of stored sperm and seminal fluid, with higher values tending to occur in the upper Chesapeake Bay. Percent fullness of spermathecae in recently-mated females varied from 51.0 ± 5.8% (Mean ± Standard Error here and throughout the manuscript) to 68.9 ± 4.6% in late summer 2013, from 62 ± 11% to 92 ±

4.0% in fall 2014, and from 67.0 ± 6.3% to 80 ± 4.2% in early summer 2015, with no apparent spatial pattern. Sperm quantity in recently-mated females varied from $7.7x10^8 \pm 1.6x10^8$ in the Little Choptank River to $3.0x10^9 \pm 3.7x10^8$ in the Chester River in late summer 2013 ($N = 7$ sites). There was not a significant linear relationship between sperm count and latitude ($p = 0.236$) at these same seven sites, although the highest sperm counts were observed for crabs at the highest latitudes in upper Chesapeake Bay (Fig. 4a). A significant linear relationship ($y = 7x10^8x - 3x10^{10}$, $R^2 = 0.523$, $p = 0.002$) was observed between sperm count and latitude for fall 2014 ($N = 15$ sites), when sperm quantity in recently-mated females varied from $6.4x10^8 \pm 8.1x10^7$ in Fishing Bay to $3.0x10^9 \pm 5.6x10^8$ in the Middle River (Fig. 4b). Spermathecal weight, used as a proxy for quantity of seminal fluid, varied from 2.76 ± 0.36 g in the Little Choptank River to 5.28 ± 0.55 g in the Gunpowder River in late summer 2013 and from 2.21 ± 0.33 g in the Nanticoke River to 5.35 ± 0.69 g in the West River in fall 2014. For sites sampled in both fall 2014 and summer 2015, there was a significant interaction between site and year, with the Rappahannock and York rivers having higher sperm counts in 2014, but there were no differences among years in Fishing Bay or the Potomac River (Fig. A2).

3.2. Sperm decline during storage and fertilization

The quantity of stored sperm in crabs tagged during fall 2014 declined dramatically in the first weeks following tagging, then remained unchanged into summer 2015. Recaptured crabs generally had 1–2 orders of magnitude fewer stored sperm than crabs subsampled at the time of tagging (Fig. 5a). The relationship between spermatheca fullness and stored sperm was non-linear, declining dramatically as spermatheca fullness dropped below 60% for crabs tagged in brackish tributary habitats (Fig. 5b, $y = 17.18 + 0.0459x + 0.0009x^2 + 0.000001x^3$, $F = 172$, $p <$

0.001). Data for higher salinity areas at Pungoteague Creek and Bradford Bay were analyzed separately because they are in spawning areas and recaptured crabs appeared to have fertilized broods of eggs as indicated by several recaptured individuals with very low numbers of stored sperm relative to recently-mated crabs (Fig. 5c). In the first two weeks after tagging, recaptured crabs had an average of 9.8 ± 11.3% (range = 2.0% to 38.5%) of the stored sperm of recently-mated crabs from the same date and site where tagging took place (Fig. 6a). By one month after tagging, sperm stores of recaptured crabs declined to 5.5 ± 3.9% of that of crabs collected at the time of tagging. After overwintering when the crab fishery reopened (March), crabs were estimated to contain 6.3 ± 4.8% of initial sperm stores at six months after tagging and 5.0 ± 4.5% after seven months. By the time fertilization was expected to begin, about eight months after the date of tagging (approximately May), female crabs still stored 4.8 ± 3.0% of the original sperm stores, and 5.5 ± 4.1% at 9-11 months after tagging. From one month after tagging, there was no trend in the quantity of stored sperm ($R^2 = 0.002$, $p = 0.758$).

Sperm quantity continued to decline from the first to the second spawning season in crabs aged using barnacle size, providing an estimate of sperm use during fertilization. Barnacle rostro-carinal length varied from 1–23 mm, corresponding to estimated ages of 31–719 days, with older barnacles tending to be found on crabs closest to the ocean (Fig. 3c). Crabs identified as first year spawners (barnacles <10 mm or <313 days old) had sperm stores varying from 1.1×10^7 to 4.5×10^7 with a mean of $2.5 \times 10^7 \pm 4.1 \times 10^6$ (Fig. 6b). The sperm quantity of second year spawners (barnacles >15 mm or >469 days old) was significantly lower ($p = 0.014$), ranging from 5.9×10^6 to 2.9×10^7 with a mean of $1.5 \times 10^7 \pm 2.6 \times 10^6$. Using a sperm quantity of $5.2 \times 10^7 \pm 7.0 \times 10^6$ for recaptured crabs just prior to their first spawning season (crabs tagged in Sep/Oct and recaptured

in Apr/May) as a starting point and assuming no additional sperm loss due to degradation or other causes, $3.7x10^7$ sperm were used on average for fertilization during the first spawning season and through early July of the second spawning season. Assuming a brood size of $3x10^6$ eggs and three broods fertilized during this time period, an estimated four sperm were used to fertilize each egg ($1.2x10^7$ sperm per brood). Using this same brood size, crabs in their second spawning season had sufficient sperm stores to produce 0-2 additional full broods with a mean of 1±1 additional broods. Second year spawners make up 15% of the spawning stock (Miller et al. 2011). This result suggests a 5% reduction in population reproductive output if second year spawners had already produced one brood of eggs before being sampled in July and could produce one additional brood (two out of a potential three broods in the second year), or a 10% reduction if they had not fertilized a brood yet in their second year (one out of three broods).

Simulations of the lifetime reproductive output of individual mature female blue crabs suggest that some individuals are likely sperm-limited in their second spawning season. Crabs obtaining $3x10^9$ sperm at the time of mating have sufficient sperm to fertilize their assumed lifetime output of six broods of $3x10^6$ eggs across a wide range of sperm:egg ratios if there is no loss of sperm to other causes (Table 2). As the number of sperm obtained during mating decreased, and the loss of sperm prior to fertilization increased (50% loss observed by Wolcott et al. [2005] and 95% loss observed in the present study), the number of full broods that could be fertilized with remaining sperm stores declined. At the values estimated from the present study, 95% loss of sperm before fertilization of the first brood and a sperm:egg ratio of 4:1, lifetime reproductive output dropped from 6 broods to 2 broods as the quantity of sperm obtained at the time of mating declined from $3x10^9$ to $5x10^8$ sperm.

3.3 Spatial extent of the spawning stock

In July at the peak of the spawning season, mature female blue crabs were most abundant at ChesMMAP survey sites in Virginia waters of Chesapeake Bay. Mature female crab relative abundance varied from 0–39 crabs tow^{-1} (Fig 3a). Reproductive status also advanced from the upper to lower bay (Fig 3b), with the fraction of crabs with sperm plugs declining and the fraction of ovigerous crabs and crabs with egg remnants from a recent spawning event increasing with increasing salinity (Fig 7a). Spermathecae weight ($H = 100.45$, $p < 0.001$) and percent fullness ($H = 80.96$, $p < 0.001$) also decreased with increasing salinity (Fig 7b,c, Fig. A1). Of the 186 ovigerous females collected, 97.8% were collected south of the northern extent of the spawning sanctuary, and 100% (94) of females with egg remnants were collected within or along the eastern or western boundaries of the sanctuary (Fig. 3c).

4. DISCUSSION

The quantity of sperm in recently-mated female blue crabs was highly variable within Chesapeake Bay, with the highest values in the low salinity upper bay and lower values near the bay mouth. This result, observed in both 2013 and 2014, is suggestive of strong variation in the amount of sperm acquired at the time of mating, and is consistent with a prior small-scale study which also sampled only recently-mated females (Ogburn et al. 2014). The sperm stores of upper bay females of $3x10^9$ sperm were equivalent to those of wild females collected during times of high operational sex ratios (Ogburn et al. 2014), in places with low crab exploitation (Hines et al. 2003), and in laboratory experiments using males prevented from re-mating until they were deemed ‘fully recovered’ from a previous mating (Kendall et al. 2002, Carver et al. 2005). Rains

et al. (2016) observed lower mean sperm stores in the upper Chesapeake Bay ($3.2x10^8$ sperm in the Chester River and $4.7x10^8$ in the Choptank River), but low average spermathecae fullness of these collections suggests that many of the crabs may have already lost a substantial quantity of sperm after mating. Lower bay females had sperm stores equivalent to wild females collected during a period of low operational sex ratio (Ogburn et al. 2014) and with females in laboratory experiments that mated with males that previously engaged in multiple mating events with insufficient time to fully recover (Kendall et al. 2002). Estimating the operational sex ratio and mating history of males was beyond the scope of the present study, but there can be substantial temporal and spatial variation in exploitation of male blue crabs with some exploitation rates exceeding 0.5 per month (Semmler 2016). This suggests that the likelihood of females receiving insufficient sperm during mating is not uniformly distributed in space and time, but likely occurs in association with intensive selective harvest targeting males.

The decline in the quantity of sperm from mating to the time of expected first reproduction by tagged female blue crabs in the field greatly exceeded that observed for captive crabs. Sperm stores of recaptured crabs declined by 95% within the first month after tagging compared to the mean sperm quantity of crabs subsampled from the same batch that was tagged. Sperm declines of 49% in about three months were observed in a laboratory study in North Carolina (Wolcott et al. 2005), and declines of 66% were estimated for a field survey in Chesapeake Bay (Rains et al. 2016). The causes of these differences are unclear, but crabs in the North Carolina laboratory experiments were well fed and movement was restricted, which could have improved the condition of crabs in ways that resulted in smaller sperm declines. For the field study (Rains et al. 2016), the expected initial sperm quantity after mating was not well known and may have

been underestimated compared to the present study in which the expected initial concentration was inferred from subsamples of the crabs from the same collections.

Surprisingly, sperm quantity did not appear to continue declining after the first month even though first reproduction was not expected to occur until five or more months later. Crabs were tagged in fall and were expected to fertilize their first brood the following spring (Aguilar et al. 2005, Hines 2007). This result suggests that sperm decline prior to fertilization primarily occurs during the time of breakdown of the sperm plug. If there is additional sperm decline during storage after the first month, it is less than the variability observed in this study. Rains et al. (2006) observed a different pattern in sperm decline relative to spermatheca fullness (log-linear), but the majority of their crabs apparently had low spermatheca fullness, a point at which sperm decline is rapid and roughly linear (mean % fullness ranged from 11.0 – 38.1%), rather than the more stable values we observed above 50% fullness (Fig. 5).

The quantity of sperm used to fertilize each brood was inferred by comparing the quantity of sperm remaining for crabs assessed to be in their first or second spawning seasons. We estimated that approximately four sperm were used per fertilized egg, assuming that all remaining sperm were viable. Sperm viability was not evaluated in the present study. This 4:1 sperm:egg ratio is within the range of other Arthropoda including 1:1 ratios for some *Drosophila* species (Bressac et al. 1994) and as low as 7:1 for the snow crab *Chionoecetes opilio* (Sainte-Marie & Lovrich 1994). However, it is lower than prior estimates for *C. sapidus* that ranged from 8:1 based on simulations of sperm decline and use for spawning (Ogburn et al. 2014) to 30:1 based on initial levels of sperm received during mating (Hines et al. 2003). Focused laboratory experiments of

sperm use in females of known mating and spawning history would be valuable for confirming the sperm:egg ratio we inferred from field-collected crabs.

Females that were assessed as having survived to their second spawning season only had sufficient sperm stores remaining to produce 0-2 additional full broods of eggs. Unfortunately, none of our tagged female crabs were recaptured and retained in the second summer after tagging, preventing us from determining sperm stores of known second-year spawners. Instead, we assessed whether crabs were likely first or second year spawners based on the estimated age of *C. testudinaria* barnacles on the carapace. In the present study, about 15% of mature female crabs with *C. testudinaria* had barnacles in their second year, consistent with tagging studies suggesting 15% of mature females in the winter dredge survey survive to spawn in a second year (Lambert et al. 2006a). This result suggests that estimating barnacle age from rostro-carinal length provides a reasonable estimate of the ratio of first to second year spawners, although we acknowledge that the barnacle growth rate we used was from a laboratory study (Ewers-Saucedo et al. 2015) and growth may vary seasonally in the wild. For females in their second spawning season, sperm stores were only sufficient for fertilizing 33-67% of the expected second year brood production (one or two out of three broods). Sperm limitation thus results in a 5-10% reduction in reproductive output of the spawning stock. This finding is consistent with the hypothesis that some individuals are likely to be sperm-limited if they survive to their second spawning season (Ogburn et al. 2014) and potentially consistent with observed declines in clutch volume in captive crabs (Darnell et al. 2009), but differ from studies that based findings on crabs with sperm stores representative of the population average (Rains et al. 2016, 2018). Rains et al. (2018) argue that sperm limitation is a population level process, but this assumption is unrealistic

because it ignores the reality that sperm limitation and any subsequent decline in reproductive potential occurs at the level of the individual female (Ogburn 2019). Reduced reproductive output of many second-year spawners, as inferred from results of the present study, likely works in tandem with age truncation due to harvest to further shift the annual reproductive output of the population towards production by single year class. Increased reliance on reproductive output of a single year-class of females may make the Chesapeake Bay blue crab population and fishery less resilient.

A recent model investigation of the potential for fishery-induced sperm limitation in Chesapeake Bay (Rains et al. 2018) makes several assumptions that should be re-evaluated in light of the results of the present study. 1) The quantity of sperm each female received at mating should be reduced by 90-95% prior to fertilization of the first brood of eggs (this study) rather than 50% Rains et al. 2018). 2) The quantity of sperm each female receives should be used to calculate a female's individual reproductive output, and this value should be summed across all females to calculate population-level reproductive output (Ogburn 2019). 3) An alternative metric, such as the proportion of individual females with 25% reduction in reproductive output, would provide additional information for interpreting the extent to which fishery-induced sperm limitation may occur. Nevertheless, we agree with Rains et al. (2018) that the sex ratio of the population is a poor indicator of fertilization success. It is the operational sex ratio at the time and site of mating (Ogburn et al. 2014), male mating history (Kendall and Wolcott 1999, Kendall et al. 2001, 2002, Hines et al. 2003) and mating behaviors (Kendall and Wolcott 1999, Pardo et al. 2016), which determine the quantity of sperm obtained during mating. Incorporating our new data into the Rains et al. (2018) model and stock assessment models (Miller et al. 2011) could yield valuable

insights into the effects of sperm limitation on reproductive output of the Chesapeake Bay blue crab population.

The spatial distribution of females of varying reproductive status matched expected patterns closely, indicating that the existing spawning sanctuary is appropriate for protecting the spawning area. There was a clear progression of reproductive status and decline of spermathecae weight with increasing salinity, and all females at salinities >30 were either ovigerous or had egg remnants indicating prior spawning. Spatially, reproductive status progressed from recently-mated females with sperm plugs in the upper bay and in tributaries of the lower bay to a clearly-defined spawning area containing both ovigerous females and females with egg remnants indicating recent spawning. The spawning area as defined by reproductive status began just south of the northern border of the spawning sanctuary and continued to the bay mouth. This result supports the hypothesis that the spawning sanctuary is effective at protecting spawning female crabs during the peak spawning season in summer when the sanctuary is in place (Lipcius et al. 2003, Lambert et al. 2006a,b). Mature females are available to the fishery during spring and fall when the sanctuary area is open to harvest, so females protected in summer may still not achieve their full lifetime reproductive potential. In addition, our results indicate that there is little or no protected spawning corridor for crabs moving south into the spawning area from Maryland, the Potomac River, or Virginia tributaries, and most females harvested before reaching the sanctuary may not reproduce at all. This result, supported by the large proportion of tag recaptures in the few months following tagging, suggests substantial loss of reproductive output due to age truncation. There are no fishery-independent surveys occurring outside the mouth of Chesapeake Bay during the peak spawning period in summer, making it difficult to use mature female

reproductive status to evaluate the appropriateness of the southeastern border of the spawning sanctuary from existing data.

Selective harvest, typically selecting for large males or restricting harvest of females, is a relatively common feature of crustacean fisheries that can alter sex ratios and have follow-on effects on reproductive output (Ogburn 2019). For example, sperm limitation has been observed in field studies on fished populations of snow crabs (Rondeau and Sainte-Marie 2001), king crabs (Sato et al. 2005, 2006, 2007), coconut crabs (Sato et al. 2010, Sato 2011), and lobsters (MacDiarmid & Butler 1999). Mating experiments support field observations that selective harvest can lead to sperm limitation, with sex ratio manipulation and selective removal of large males reducing fertilization success and/or reproductive output of female spiny lobster *Jasus edwardsii* (MacDiarmid & Butler 1999), snow crab *Chionoecetes opilio* (Rondeau & Sainte-Marie 2001), and an unfished stone crab species *Hapalogaster dentata* (Sato & Goshima 2006). Such selective harvest may exert the strongest effects on the quantity of sperm obtained during mating and lifetime reproductive output for species like blue crabs that have internal fertilization and a single brief mating period (Ogburn 2019). Selective harvest is also a mechanism for protecting females from age truncation to achieve greater lifetime reproductive output, although banning harvest of females rarely maximizes population-level reproductive output (Clark & Tait 1982). Future studies and management efforts should investigate spatiotemporal hotspots of selective harvest for shifts in the operational sex ratio and reductions in female sperm stores, and evaluate tradeoffs between age truncation and sperm limitation to maximize reproductive output and fishery resilience.

5. SUMMARY

The present study is the most comprehensive assessment to date of the dynamics of sperm storage and use after mating for free-living blue crabs under selective harvest. We found strong spatial and some temporal variability in sperm stores obtained during mating and hypothesize that these may be related to spatio-temporal patterns in male exploitation. The quantity of stored sperm declined by at least an order of magnitude between mating and fertilization of the first brood, much more than expected from laboratory studies. Yet, most mature female crabs obtained enough sperm to support at least one full season of brood production, and many had at least enough sperm for a second season. We infered that a 5–10% reduction in reproductive output of the blue crab spawning stock in Chesapeake Bay occurs due solely to sperm limitation of females that survive through a second spawning season. Incorporating this information into population models and stock assessments should provide an improved understanding of potential impacts of sperm limitation on population dynamics. We also found that the current spawning sanctuary is likely providing effective protection for spawning crabs during the spawning season, but that it offers little protection to females migrating from low salinity areas to the sanctuary. Under the current selective harvest regime, many female blue crabs in Chesapeake Bay do not achieve their full lifetime spawning potential even when they survive to a second spawning season. Tradeoffs between the effects of sperm limitation and age truncation on population reproductive output deserve more explicit consideration in future stock assessments and management strategies.

ACKNOWLEDGEMENTS

The authors would like to thank R. Aguilar, C. Corrick, M. Goodison, K. Heggie, M. Kramer, W. McBurney, P. Roberts, and R. Semmler for their assistance with field collections, crab tagging, and technical assistance in the lab. Special thanks are offered to the many Chesapeake Bay watermen who assisted with field collections and both commercial and recreational crabbers who called in recaptures of tagged crabs and saved them frozen for later analysis. We are grateful to R. Latour and J. Gartland at VIMS for samples and environmental data obtained during ChesMMAP surveys. The manuscript was improved following the helpful suggestions of four anonymous reviewers. Funding for this work was provided by the NOAA Saltonstall-Kennedy Grant Program award #NA14NMF4270048. M. Ogburn was partially supported on a Postdoctoral Fellowship from the Smithsonian Institution Fellowship Program and M. Jones was supported on an internship funded by the Smithsonian Women's Committee.

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Table 1. The number of recently-mated mature female blue crabs *Callinectes sapidus* assessed for sperm quantity by sample site and year. Sites not sampled in a given year are indicated as NS. Sites arranged from north to south.

Site	2013	2014	2015
Sassafras River	NS	5	NS
Gunpowder River	9	NS	NS
Middle River	NS	5	NS
Chester River	10	NS	NS
Eastern Bay	NS	5	NS
South River	NS	5	NS
West River	NS	5	NS
Little Choptank River	8	5	NS
Patuxent River	NS	5	NS
Fishing Bay	NS	5	10
Nanticoke River	NS	5	NS
Potomac River	12	4	10
Pocomoke River	NS	5	NS
Pungoteague Creek	NS	5	NS
Rappahannock River	9	5	10
York River	NS	5	10
Lower Chesapeake Bay	9	NS	NS
James River	9	5	NS

Table 2. Variation in simulated lifetime brood production of mature female blue crabs *Callinectes sapidus*. Simulations included different quantities of sperm obtained at the time of mating, percent loss of sperm between the times of mating and fertilization of the first brood, and sperm:egg ratios required for successful fertilization. Values in table are number of broods that could be completely fertilized assuming a brood size of $3x10^6$ eggs.

	Sperm:Egg Ratio					
Scenario	1	4	10	25	80	100
3x10^9 at the time of mating						
No loss	6	6	6	6	6	6
50% loss prior to brood 1	6	6	6	6	6	5
95% loss prior to brood 1	6	6	5	2	0	0
1x10^9 at the time of mating						
No loss	6	6	6	6	4	3
50% loss prior to brood 1	6	6	6	6	2	1
95% loss prior to brood 1	6	4	1	0	0	0
5x10^8 at the time of mating						
No loss	6	6	6	6	2	1
50% loss prior to brood 1	6	6	6	3	1	0
95% loss prior to brood 1	6	2	0	0	0	0

Figure captions

Figure 1. Map of study site including sampling sites and recaptures of tagged blue crabs *Callinectes sapidus*. In fall 2014, crabs were caught, tagged, and released at most sampling sites (squares) as part of a mark-recapture experiment, and subsamples were retained for lab analysis. At other sites (triangles), crabs were collected for lab analyses only. Circles indicate recapture sites of tagged crabs analyzed in the present study. Circle color indicates the number of months the crabs were at large between tagging and recapture. State abbreviations are Virginia (VA), Maryland (MD), Pennsylvania (PA), Delaware (DE), and New Jersey (NJ).

Figure 2. Female blue crabs *Callinectes sapidus* with ventral carapace ranging from clean white coloration indicating recent molting (A), to yellow (B) and brown (C) discoloration, indicating increasing time since molting.

Figure 3. July 2015–2017 sampling sites for the Chesapeake Multispecies Monitoring and Assessment Program. A) The number of mature female blue crabs *Callinectes sapidus* captured at each site. B) The proportion of each sample that was non-ovigerous with a sperm plug (Plug), non-ovigerous without a sperm plug (NonOvig), ovigerous (Ovig), or with egg remnants on the pleopods (Remnants). The hatched area indicates the spawning stock sanctuary that functions as a seasonal marine protected area. C) The estimated age in months of the oldest barnacle *Chelonibia testudinaria* on the carapace of any crab collected at each site.

Figure 4. Relationship between the count of stored sperm in recently-mated female blue crabs *Callinectes sapidus* and latitude of collection in A) 2013 and B) 2014. Error bars represent standard error of the mean.

Figure 5. Counts of stored sperm for mature female blue crabs *Callinectes sapidus* in a mark-recapture experiment. Panels represent (A) all crabs subsampled at the time of tagging (black triangles) or after tagging and recapture (white triangles) relative to percent fullness of spermathecae, (B) only crabs tagged in brackish water tributaries ($N = 88$, $R^2 = 0.842$, $p < 0.001$), and (C) only crabs tagged in high salinity sites in Pungoteague Creek and Bradford Bay ($N = 15$, $R^2 = 0.828$, $p < 0.001$). Crabs from high salinity sites were separated out for analysis due to the potential for recaptured crabs to have used some sperm to fertilize a brood of eggs prior to recapture. The line in (B) represents the best fit line for the relationship between percent fullness and sperm counts.

Figure 6. Decline of sperm after mating for female blue crabs *Callinectes sapidus*. A) Counts of stored sperm for recaptured mature female crabs relative to sperm counts of crabs subsampled at the time of tagging in the mark recapture experiment. Time in months (x-axis) indicates the approximate time that crabs were at large in the field between tagging and recapture. B) Mean count of stored sperm for female crabs *C. sapidus* assessed to be in their first or second spawning seasons based on the estimate age of barnacles *Chelonibia testudinaria* fouling the carapace. Error bars indicate standard error.

Figure 7. Relationships between salinity and reproductive data of mature female blue crabs *Callinectes sapidus* collected in July 2015-2017 in the Chesapeake Multispecies Monitoring and

Assessment Program survey. A) Reproductive status was assessed as non-ovigerous with a hardened pink sperm plug (P), non-ovigerous without a sperm plug (NO), ovigerous (O), or with egg remnants on the pleopods (R). B) Spermatheca weight. C) Spermatheca percent fullness. Box plots indicated the median (black line), 25^{th} to 75^{th} percentiles (box), 10^{th} to 90^{th} percentiles (whiskers), and remaining values (black dots). Letters indicated statistically-significant differences among groups based on Kruskal-Wallis Analysis of Variance on Ranks and post hoc tests.

Figure 1.

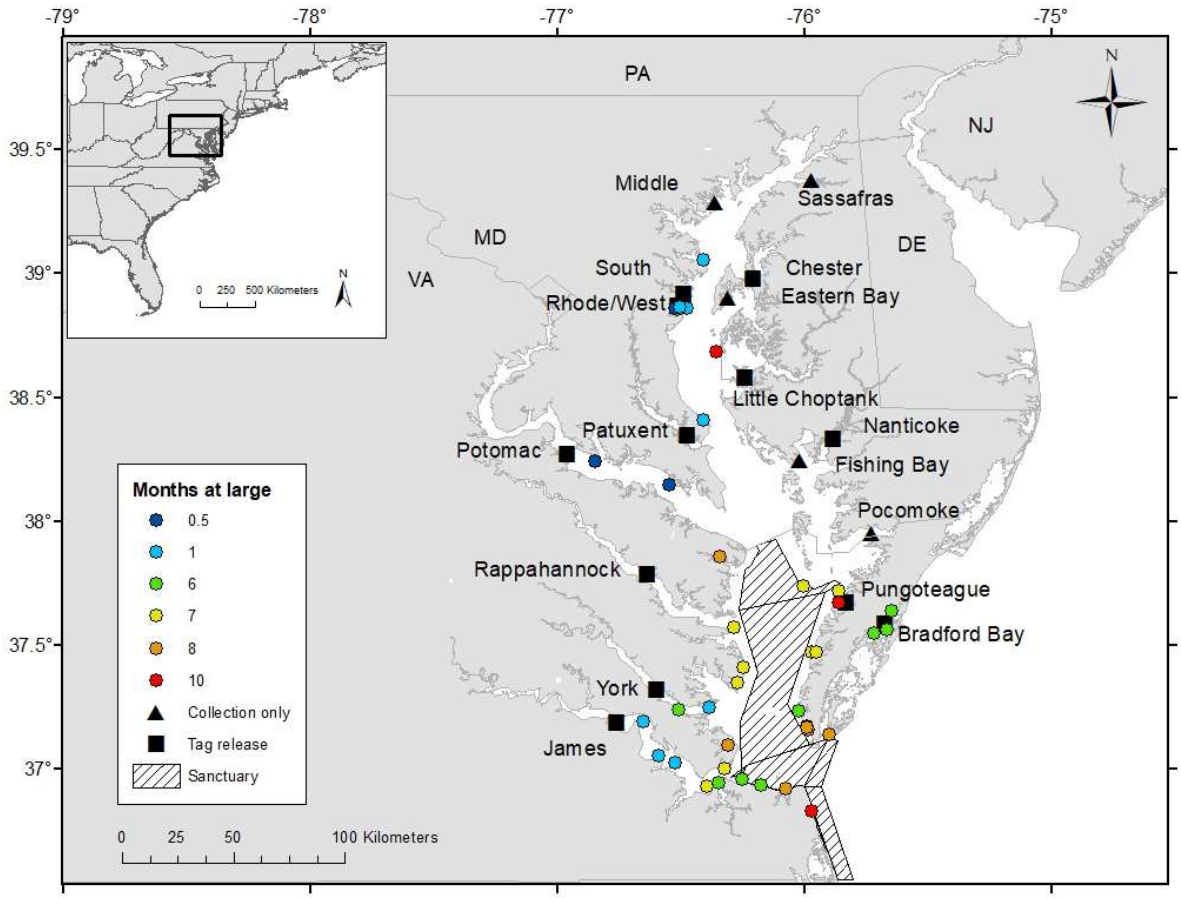
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Figure 2.

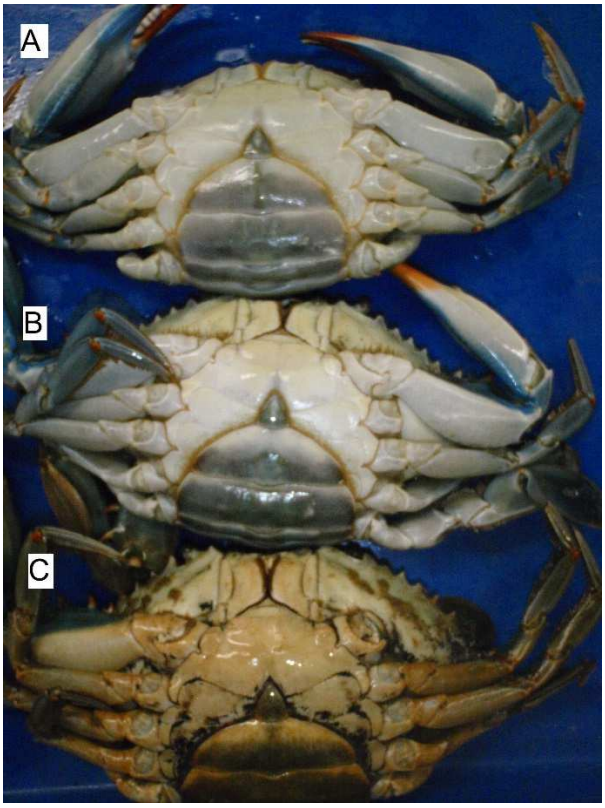


Figure 3.

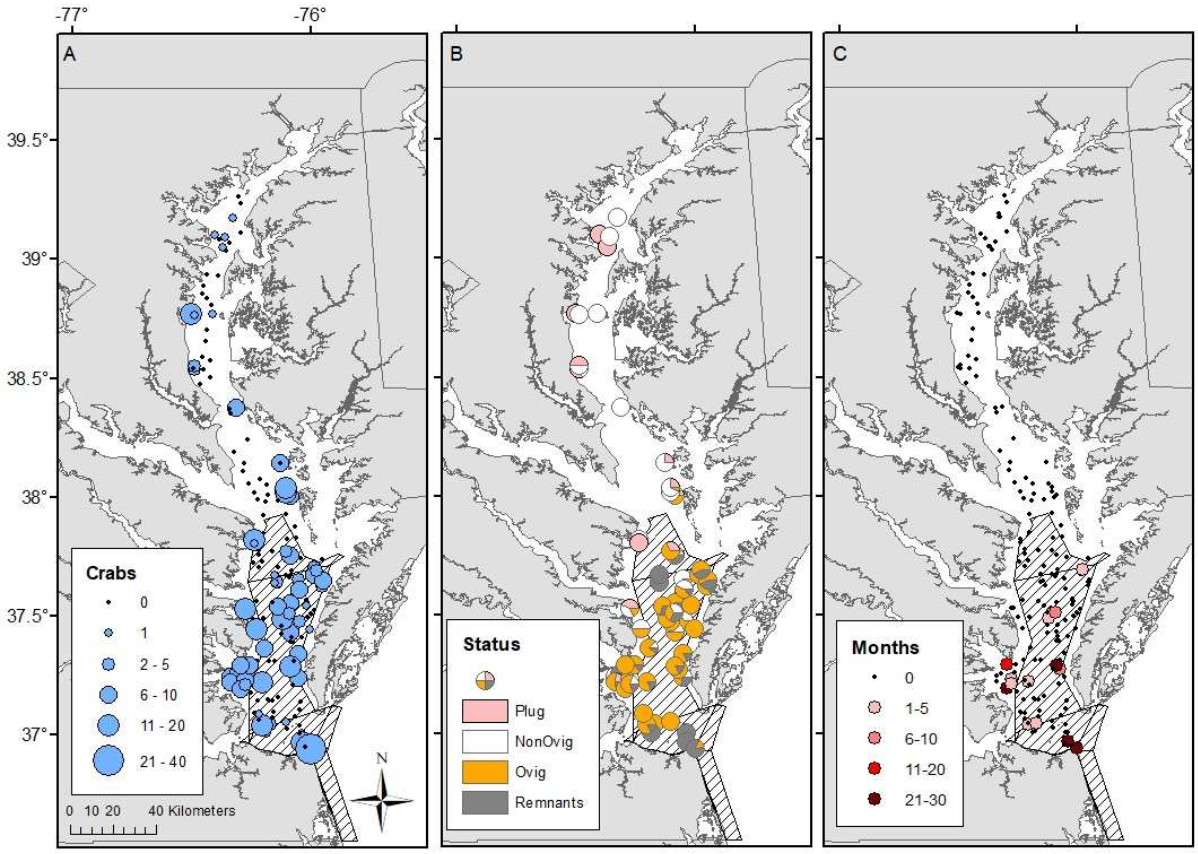

-77°
-76°
A
B
C
39.5°
39°
38.5°
38°
37.5°
37°
Crabs
0
1
2 - 5
6 - 10
11 - 20
21 - 40
0 10 20 40 Kilometers
N
Status
Plug
NonOvig
Ovig
Remnants
Months
0
1-5
6-10
11-20
21-30

Figure 4.

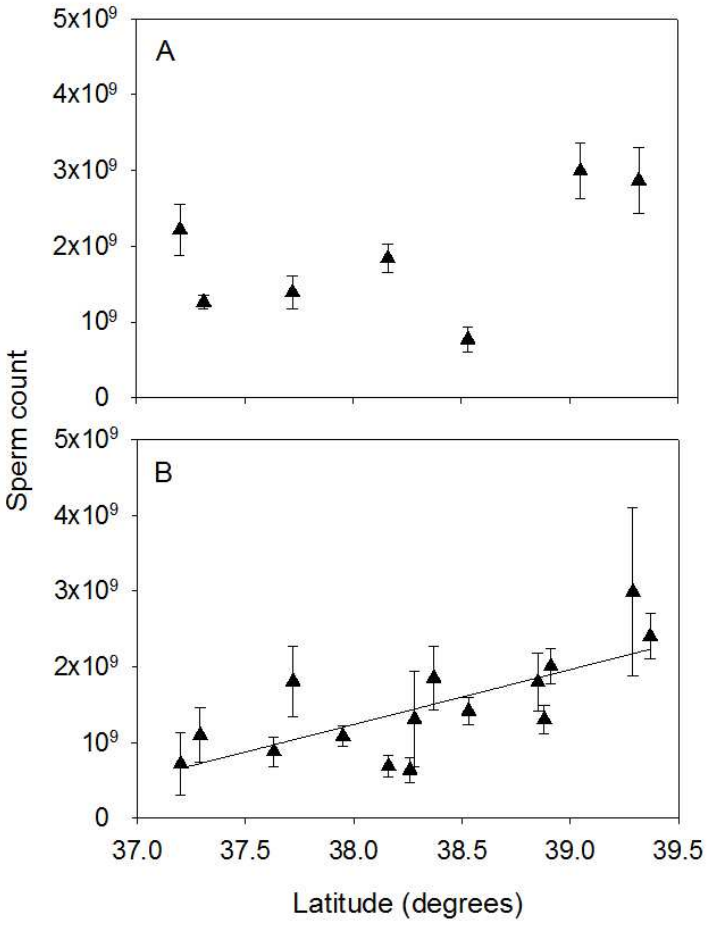

A
B
5x10⁹
4x10⁹
3x10⁹
2x10⁹
10⁹
0
Sperm count
37.0
37.5
38.0
38.5
39.0
39.5
Latitude (degrees)

Figure 5.

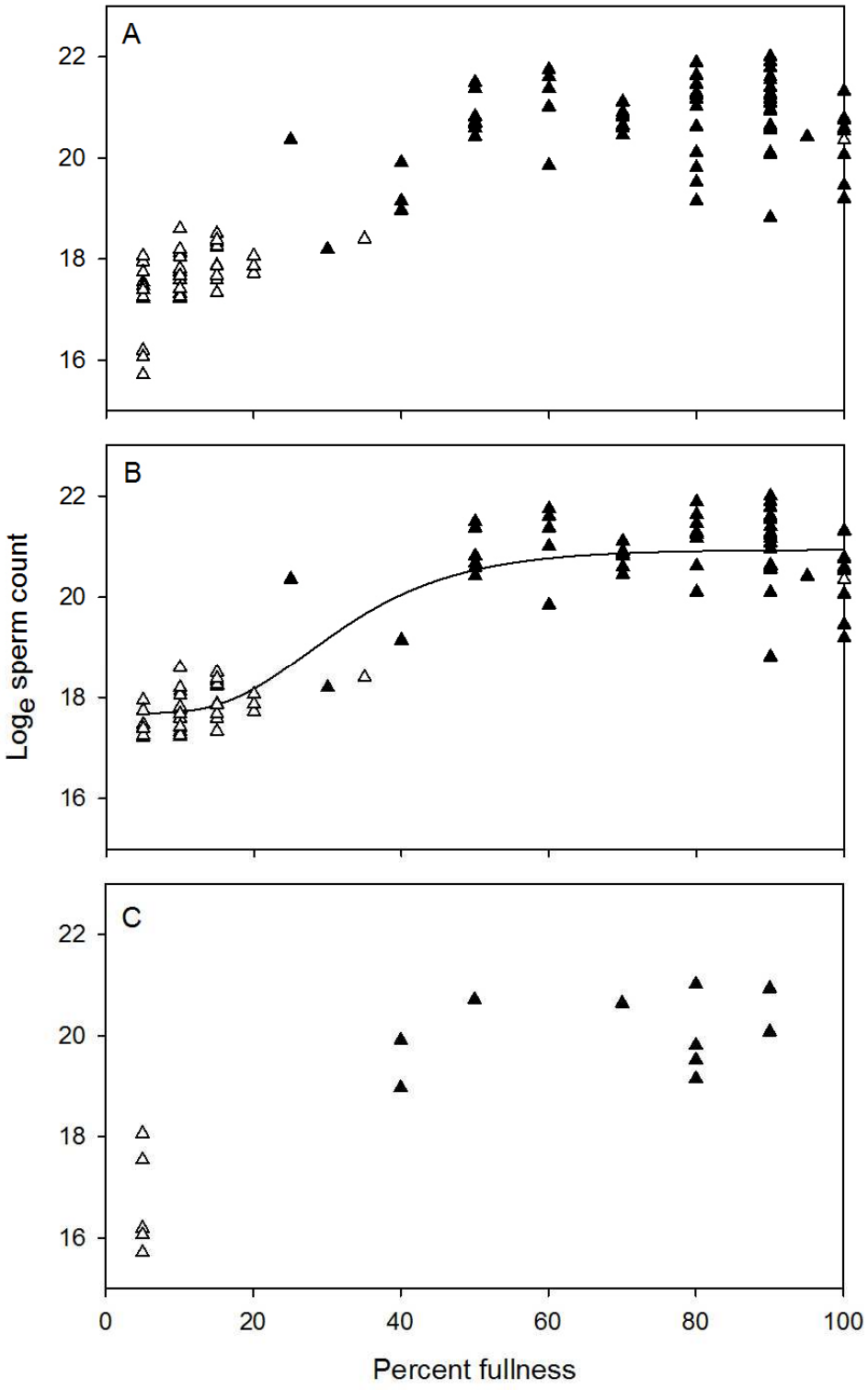

A
B
C
22
20
18
16
0
20
40
60
80
100
Log$_e$ sperm count
Percent fullness

Figure 6.

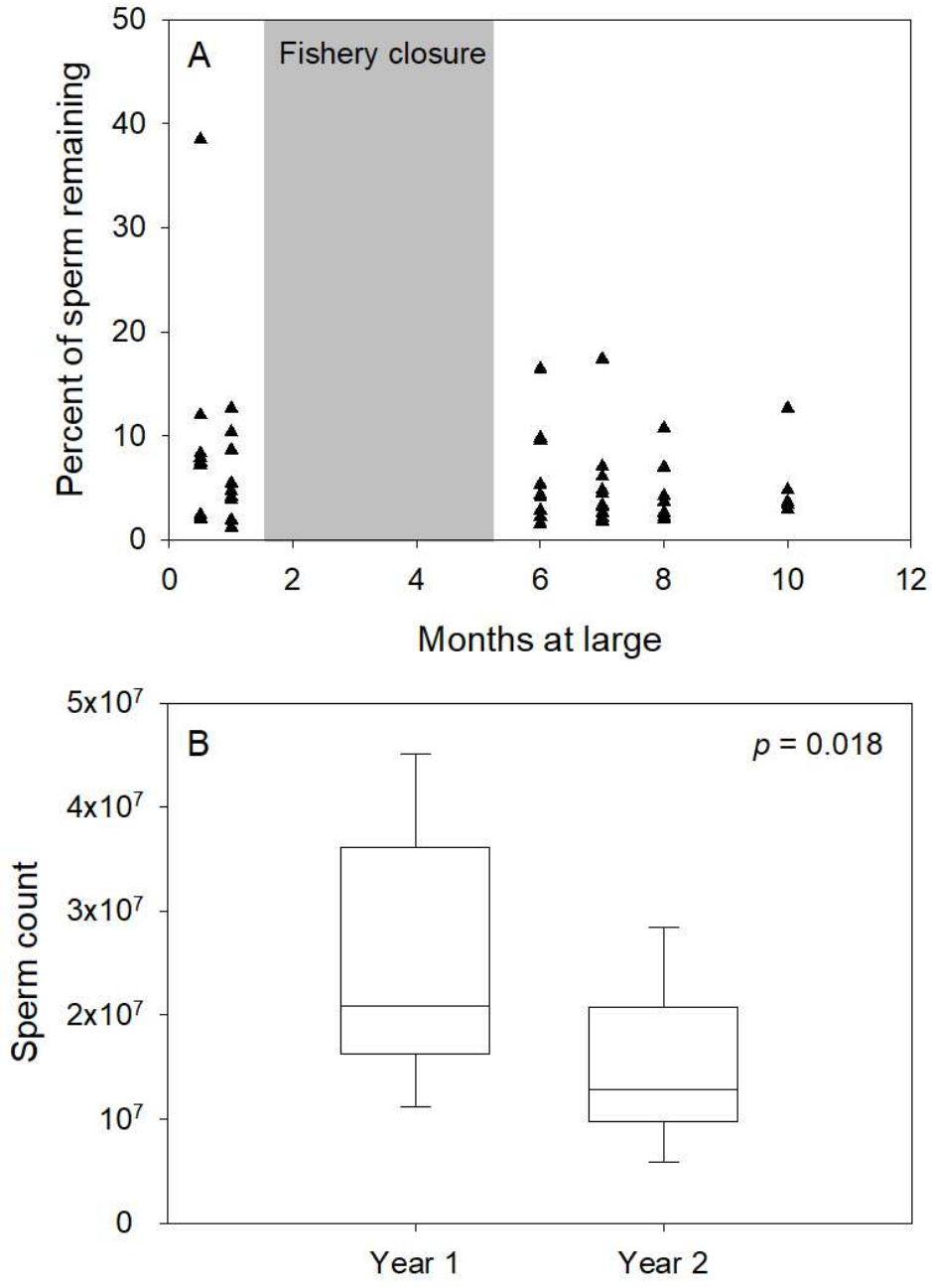
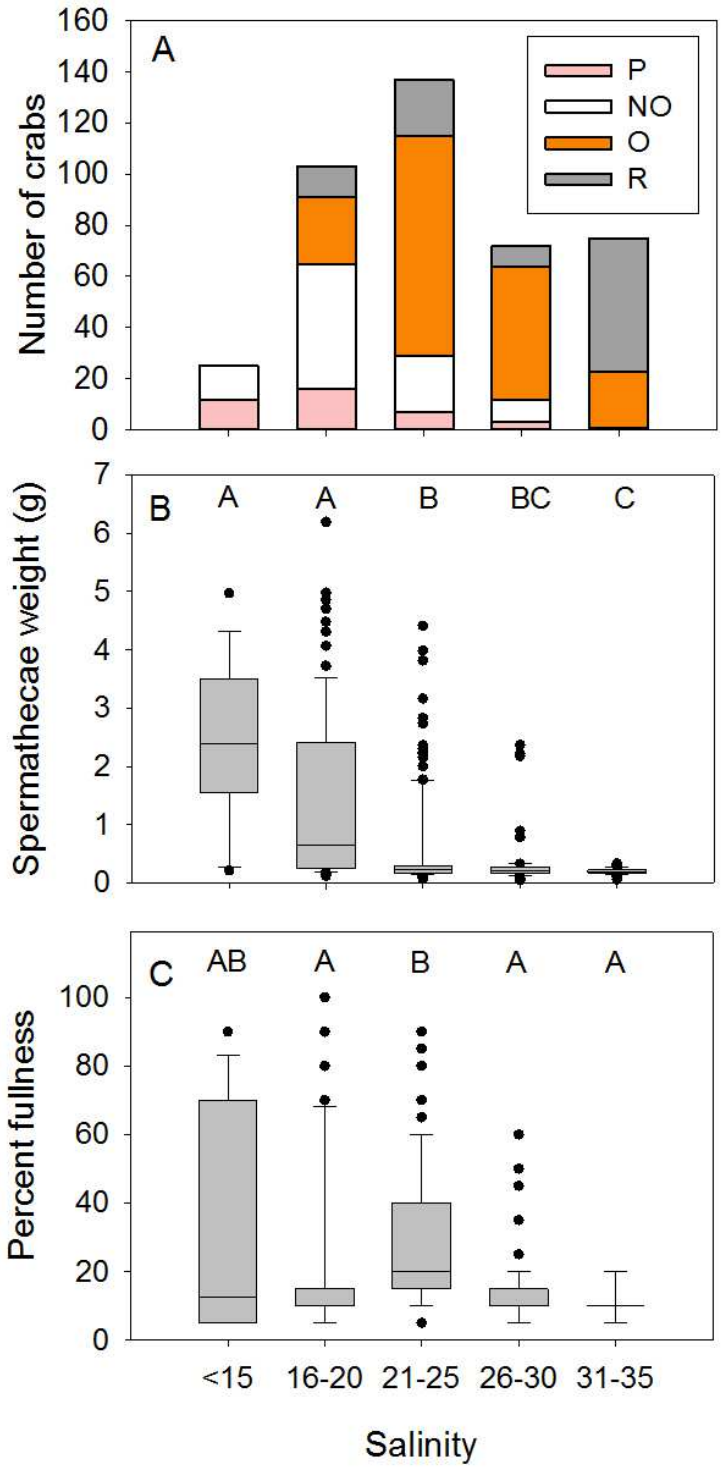
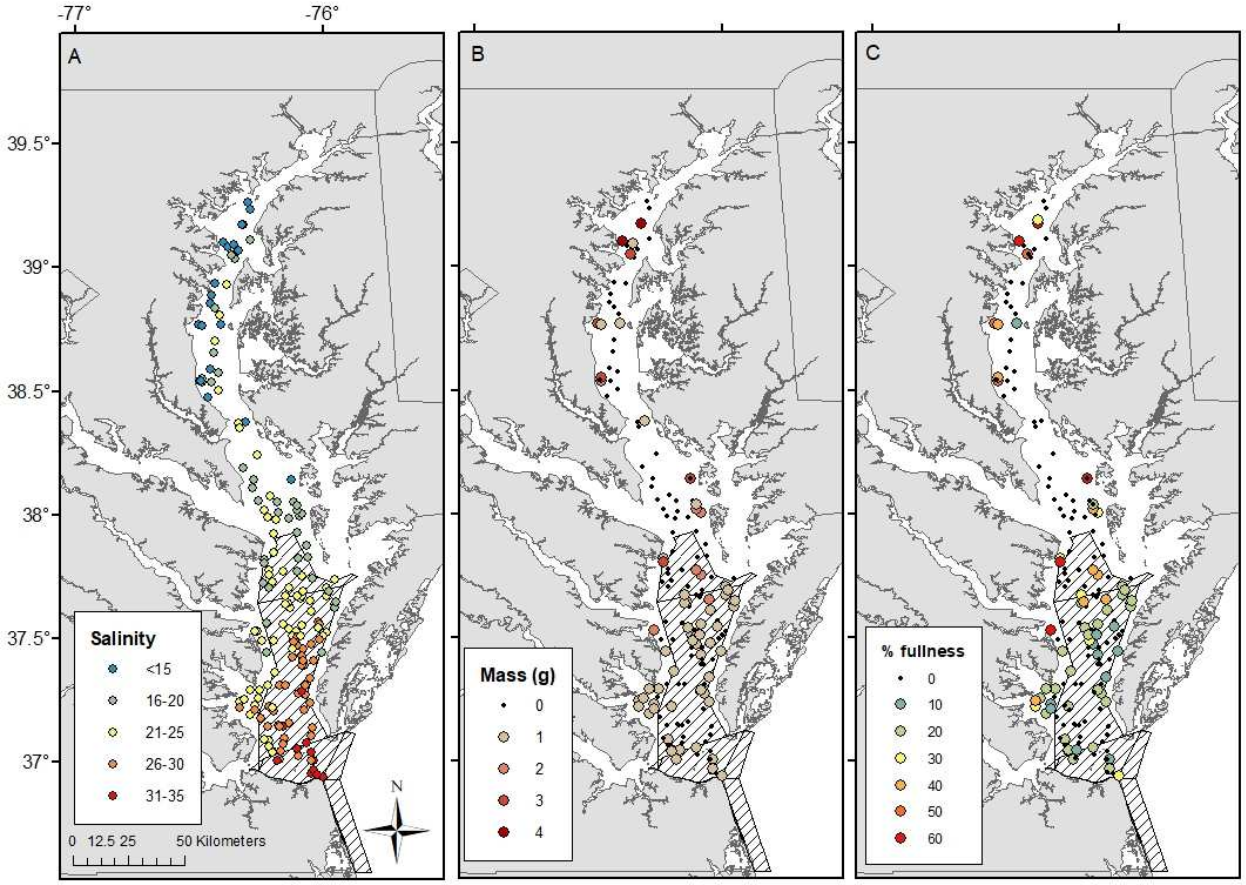

A
Fishery closure
Percent of sperm remaining
50
40
30
20
10
0
0
2
4
6
8
10
12
Months at large
B
p = 0.018
Sperm count
5x10^7
4x10^7
3x10^7
2x10^7
10^7
0
Year 1
Year 2

Figure 7.


A
P
NO
O
R
Number of crabs
160
140
120
100
80
60
40
20
0
B
A
A
B
BC
C
Spermathecae weight (g)
7
6
5
4
3
2
1
0
C
AB
A
B
A
A
Percent fullness
100
80
60
40
20
0
<15
16-20
21-25
26-30
31-35
Salinity

Appendix Figure A1. A) Salinity, B) mean spermathecae weight (g), and C) mean spermathecae percent fullness for mature female blue crabs *Callinectes sapidus* collected in the Chesapeake Multispecies Monitoring and Assessment Program in July 2015-2017. The hatched area indicates the spawning stock sanctuary that functions as a seasonal marine protected area.



Appendix Figure A2. Sperm quantity in recently-mated mature female blue crabs *Callinectes sapidus* collected at the same sites in fall 2014 and spring 2015. Asterisks indicate statistically significant differences based on Kruskal-Wallis Analysis of Variance on Ranks and post hoc tests. Error bars indicate standard error of the mean.