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Temporal and spatial variation in sperm stores in mature female blue crabs *Callinectes sapidus* and potential effects on brood production in Chesapeake Bay

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ABSTRACT: Fisheries that selectively harvest males have the potential to diminish the reproductive success of females due to reductions in the transfer of sperm and seminal fluid during mating. The purposes of this study were to investigate variation in sperm and seminal fluid quantities obtained during mating in mature female blue crabs *Callinectes sapidus* in Chesapeake Bay, USA, and to model potential effects of sperm reduction on lifetime brood production. We explored variation in sperm and seminal fluid quantity with respect to (1) season, (2) location, (3) operational sex ratio (OSR), (4) relative time since mating, and (5) fertilization, and used this information to model brood production. Mature female blue crabs were obtained from targeted or long-term collection efforts. Crabs were characterized by carapace condition, presence of a sperm plug (hardened seminal fluid), and presence of egg masses or egg remnants. They were dissected and processed to determine the quantity of stored sperm and spermathecae weight. Sperm quantity, but not spermathecae weight, of recently mated females varied seasonally and spatially, and was positively correlated with OSR. Females received as many as 3×10^9 sperm during mating, which declined to an average of 8×10^7 sperm before fertilization of the first brood. Both model simulations and estimates of sperm used for fertilization derived from sperm counts of females with and without evidence of spawning indicated that a reduction in lifetime brood production (sperm limitation) is likely in individual female blue crabs if they survive to a second spawning season.

KEY WORDS: Sperm limitation · Fisheries management · Sex ratio · *Callinectes sapidus* · Chesapeake Bay

INTRODUCTION

In fisheries for decapod crustaceans, selective removal of males often occurs when large males are most valuable to fishers (Millikin & Williams 1984, Sato 2012) and when fishery managers limit harvest of females to protect the spawning stock (Jamieson et al. 1998). Removing sexually mature males from a population has the potential to reduce population growth rate by reducing reproductive success, and removing only large males may lead to microevolution that opposes sexual selection for large size (Rowe & Hutchings 2003, Fenberg & Roy 2008, Lane et al. 2011). One way in which selective harvest of males may reduce reproductive success is through reductions in the operational sex ratio (OSR), the ratio of reproductively competent males to reproductively receptive females (Hines et al. 2003, Jivoff 2003a). When OSR declines, the remaining males may mate too frequently to regenerate a full supply

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of sperm and ejaculate between mating events, and thus transfer fewer sperm and less seminal fluid during each mating event (Kendall & Wolcott 1999, Kendall et al. 2002). This mechanism has been observed in harvested crabs (Smith & Jamieson 1991, Stevens et al. 1993, Lovrich et al. 1995, Carver et al. 2005, Sato 2011) and in experimental manipulations of un-harvested crabs (Sato & Goshima 2006). It may be exacerbated when females exhibit little mate choice and recently mated males remain dominant competitors for mating opportunities despite low sperm supplies (Kendall & Wolcott 1999). Reduced sperm stores have the potential to result in sperm limitation, defined herein as a decline in lifetime brood production due to insufficient sperm for successful fertilization. Sperm limitation is most likely to occur in species with brief mating seasons determined by life history, behavior, and seasonal events (Waddy & Aiken 1986, Sainte-Marie 1993), species with long-term sperm storage (Austin 1975), and species that produce multiple broods from a single mating event (Morgan et al. 1983, Paul & Paul 1992).

In this study, we evaluated potential effects of the selective harvest of males on reproduction of the blue crab *Callinectes sapidus* in Chesapeake Bay. Female blue crabs undergo a functional terminal molt to maturity after which they are receptive for up to several days and receive their full lifetime complement of sperm and ejaculate (Van Engel 1958). Most (>95%) mate successfully (Wenner 1989, Jivoff 1995, 1997a,b) and some mate with more than one male (Jivoff 1995, Wells 2009). Female maturation and mating takes place from spring through fall in Chesapeake Bay, with a peak in the number of females maturing in late summer (Hines et al. 1987). Mature females migrate (typically in fall) to high-salinity spawning areas in the lower bay (Van Engel 1958, Aguilar et al. 2005). Spawning takes place primarily from May to September in Chesapeake Bay (Van Engel 1958, McConaugha et al. 1983), during which time females are likely capable of producing up to 3–4 broods per year (Hines et al. 2003, Darnell et al. 2009). Lifetime brood production may reach up to 8 broods of eggs over the course of 2 spawning seasons in Chesapeake Bay (Hines et al. 2003, Dickinson et al. 2006, Darnell et al. 2009). The proportion of females surviving to reach their full reproductive potential in the wild may be low due to relatively high natural and fishing mortality (Miller et al. 2011).

Determining whether individual female blue crabs are sperm-limited is hindered by gaps in our understanding of decapod reproductive biology. *Chionocetes* spp. have been observed to use ca. 70 sperm per egg during extrusion of the first clutch and sperm:egg ratios of less than 7:1 prevented egg extrusion (Sainte-Marie & Lovrich 1994). For blue crabs, 'several hundred' sperm have been observed interacting with an individual egg under *in vitro* conditions (Brown 1966), but this may not reflect conditions required for fertilization in the wild. The sperm:egg ratio for wild blue crabs has been estimated at 100–400:1 immediately after mating and 20–30:1 at the time of fertilization, assuming that there is no loss of stored sperm over time (Hines et al. 2003). However, the quantity of viable stored sperm in female blue crabs declines by roughly half during the first 3 mo following mating (Wolcott et al. 2005), and the extent of further decline is unknown. This indicates that sperm:egg ratios at the time of mating may be lower for blue crabs than previously thought. Although the sperm:egg ratio needed for fertilization is unknown, observations of non-viable broods in Florida and declining brood volume with increasing brood number in North Carolina (Hines et al. 2003, Darnell et al. 2010) may be indicative of sperm limitation in later broods, but could also be due to other mechanisms such as declines in sperm viability.

Recent management changes in the Chesapeake Bay blue crab fishery have focused on increasing the proportion of females that reproduce during a first spawning season and survive to reproduce during a second spawning season (Miller et al. 2011). Survival of a significant number of females to a second spawning season has the potential to increase both total reproductive output of the spawning stock and population stability by buffering the impact of small year-classes (Miller et al. 2011). These benefits would not be realized if females are unable to fertilize broods in their second spawning season due to sperm limitation. The objectives of this study were to explore variation in sperm and seminal fluid quantity with respect to (1) season, (2) location, (3) OSR, (4) relative time since mating, and (5) fertilization. This information was used to model the effects of observed variation in sperm stores on an individual female's lifetime brood production.

MATERIALS AND METHODS

Biological data

Mature female blue crabs were collected and frozen during a variety of targeted sampling efforts (Table 1 and described below) during the period 1996–2012 to explore variability in the quantity of

sperm and seminal fluid transferred during mating and stored prior to spawning. Prior to dissection and processing, frozen crabs were placed in a bucket of cool, fresh water and allowed to thaw completely (approximately 1 h). Carapace width (CW) was measured as the distance between lateral spine tips. Carapace condition was determined to be clean, partially dirty, or dirty, and was used as a relative indicator of time since mating because mature female blue crabs will not mate again. Mature females classified as clean had clean carapaces with a bright white abdomen, indicating recent mating within the past several months. Crabs that were classified as partially dirty had some yellowing of the carapace and were assumed to be within their first year after mating. Crabs that were classified as dirty were brown in color with obvious darkening of the carapace, often had fouling organisms (barnacles, bryozoans, etc.) on the legs and carapace, and were assumed to be in their second year after mating.

Table 1. Collection sites, years, and number of mature female crabs processed for sperm enumeration. Collection sites are all located within Chesapeake Bay and are listed in order from north to south, with sites in Maryland above the dashed line and sites in Virginia below

Collection site	Year	No. crabs
Gunpowder River	2010	5
Magothy River	2010	5
Rhode River	1996	19
	1997	37
	1998	34
	2000	1
	2003	25
	2005	10
	2008	18
	2009	5
	2010	31
Choptank River	2010	5
Little Choptank River	2009	5
Patuxent River	2009	4
Manokin River	2009	5
Potomac River	2009	5
Rappahannock River	1996	113
	2000	32
York River	1996	1
	1997	27
	1998	4
	2000	18
	2010	3
James River	1996	29
	2010	3
Lower Chesapeake Bay	2000	6
	2010	15
	2011	31
	2012	75

Each crab was dissected by removing the abdomen and carapace. To determine whether spawning (and thus sperm use for fertilization) had occurred recently, the presence of external egg masses was noted and, if there was no visible egg mass, the pleopods were examined under a dissecting microscope for egg remnants. This method provided information on recent spawning but was likely not useful for determining whether females collected in May or June spawned the previous summer or fall because egg remnants could have been lost during the winter. Spermathecae were removed and weighed individually. The weight of spermathecae in recently mated females was indicative of the quantity of seminal fluid transferred during mating (Kendall & Wolcott 1999). Spermathecae were stored in 20 ml scintillation vials in 70% ethanol for sperm enumeration. We stored both spermathecae in the same vial for each crab dissected prior to October 2010 and stored each spermatheca separately after that time (e.g. 2012 lower bay samples).

To remove sperm from spermathecae, the entire contents of a vial (storage ethanol plus all spermathecae stored in that vial) were poured into a Petri dish. This ensured that loose spermatophores suspended in the storage ethanol were not lost. The spermathecal membranes were separated from the sperm plug (hardened seminal fluid plus spermatophores, indicative of recent mating) or from the spermatophores if the seminal fluid had already been resorbed. The spermathecal membrane was rinsed thoroughly with 70% ethanol to ensure that no spermatophores adhered to it and was discarded. Using a razor blade, the spermathecal contents were chopped into fine pieces (<1 mm). The spermathecal contents and liquid were then poured into a 16 ml glass PYREX Dounce homogenizer. The sample was homogenized to break apart the spermatophores into individual sperm cells. This process took approximately 10 min per sample when no seminal fluid was present and at least 1.5 h per sample when a hard sperm plug was present. After homogenization, the total volume of liquid plus spermathecal contents was recorded.

To enumerate the sperm, one drop of the sample was placed on a Petroff-Hausser Counting Chamber (with a 5 × 5 grid) and examined using a phase contrast microscope at 400× magnification. All 25 squares in the grid were counted and the number of sperm present was recorded. The total quantity of sperm per crab was calculated using the equation:

$$\text{Quantity of sperm} = \left(\frac{\text{Number of sperm cells counted}}{\text{Volume of counting chamber}}\right) \times \text{Dilution} \quad (1)$$

where the volume of the counting chamber was 1 × 1 × 0.02 mm, or 0.00002 ml, and the dilution was the total volume (ml) of homogenized liquid and spermathecal contents. This process was repeated 3 times per sample and the 3 calculations were averaged to produce the final number of sperm per crab. Beginning in 2010, only one spermatheca was counted for an individual crab and the number of sperm was doubled to calculate the sperm quantity. The change from processing 2 spermathecae to 1 spermatheca was made to minimize mechanical sample processing time in order to maximize sample sizes and because there is no theoretical or empirical reason for this subsampling to introduce bias (Duluc et al. 2005, Rodgers et al. 2011).

Seasonal variation and OSR

In 2010, blue crabs were collected from the Rhode River, Maryland, a sub-estuary of the middle Chesapeake Bay, in order to quantify seasonal variability of sperm stores and OSR. Crabs were purchased approximately every month from June to October from a commercial fisher working in the river. An initial determination of whether crabs were newly inseminated was made based on carapace condition and hardness. This was confirmed after dissection by the presence of seminal fluid or hardened sperm plugs within the spermathecae. A total of 5 to 7 individuals per sample date were later processed except for the July sample, when only 2 recently mated females were available. Differences in sperm quantities and spermathecae weight by month were evaluated using the nonparametric Kruskal-Wallis 1-way ANOVA by ranks due to low sample sizes. A post hoc Dunn's test was used to identify differences among groups. All statistical tests were performed using SigmaPlot version 12.3 and used $\alpha = 0.05$ to assess statistical significance.

To compare sperm quantity with OSR, we analyzed additional crabs collected from the Rhode River during a long-term otter trawl survey conducted by the Smithsonian Environmental Research Center. The trawl survey has been conducted yearly since 1982 from April to December with 3 replicate sample days per month. Each day, 3 stations within the river were sampled for 10 min (approximately 900 m tow distance) with a 4.88 m otter trawl (3.81 cm stretch mesh size). In many years, portions of the samples were placed in plastic zip-top bags and stored frozen. OSR within the Rhode River was calculated for each month in 2010 by dividing the total number of intermolt (stage C) males having CW >107 mm, a size at which >50% are sexually competent (Van Engel 1990), by the number of prepubertal females. Prepubertal females were identified by the triangular shape and dark color of the abdomen (Smith & Chang 2007). It should be noted that the trawl survey did not sample in very shallow water often occupied by blue crabs (Ramach et al. 2009), but it provided the best available data for calculating OSR. Visual inspection of the data indicated clustering of sample dates around OSR values of 2–3 (n = 8), 4–5 (n = 15), and 15 (n = 7). Because of this clustering, which made determination of the form of the relationship difficult, and low sample sizes, the 3 clusters of data were treated as groups and compared using Kruskal-Wallis 1-way ANOVA on ranks. A post hoc Dunn's test was used to identify differences among groups.

To determine whether seasonal patterns were consistent across years, recently mated females collected in 2005 and 2008 during August and September (the months with the lowest and highest sperm quantities in 2010) were processed for sperm quantity and spermathecae weight. Five individuals were processed for each sample period except September 2008, when only 4 recently mated females were available from frozen samples. Values for 2005 and 2008 were compared with 2010 values using Kruskal-Wallis 1-way ANOVA on ranks, again due to low sample sizes. A post hoc Dunn's test was used to identify differences among groups.

Spatial distribution

To examine spatial variation of sperm stores in Chesapeake Bay, mature female crabs were purchased in September 2010 from commercial fishers in the Gunpowder, York, and James Rivers for comparison with Rhode River crabs. Again, only crabs that were recently mated were selected for processing in order to control for differences in sperm quantity due to sperm loss over time (Wolcott et al. 2005) and sperm use during fertilization. Gunpowder River, Maryland, represented the upper bay (n = 5), Rhode River crabs from the long-term trawl survey described above represented middle bay (n = 6), and the York (n = 3) and James Rivers (n = 3) in Virginia represented the lower bay and were combined for analysis in order to have a comparable sample size (n = 6). Statistical differences were determined using

Kruskal-Wallis 1-way ANOVA on ranks due to low sample sizes. A post hoc Dunn's test was used to identify differences among groups.

Spawning area

To evaluate the sperm stores of mature female blue crabs that have reached the spawning area in the lower Chesapeake Bay, crabs were collected near the boundary of the blue crab spawning sanctuary where harvest of females is restricted during summer. The crabs were purchased from commercial fishers using crab pots at Thimble Shoals, Virginia, within approximately 5 km of the spawning sanctuary. Batches of 30 crabs were purchased on 6 June, 9 July, 21 August, 27 September and 13 November 2012, approximately every 6 wk during the mating and spawning season. All crabs in each collection were used to describe the composition of spawning area crabs with respect to carapace condition, evidence of spawning (presence of sponge or egg remnants), and presence of seminal fluid (sperm plug). A random subsample of 15 crabs from each collection date was later processed to quantify sperm quantity and spermathecae weight.

Relationships between spermathecae weight and sperm quantity

The loss of sperm between mating and fertilization of the first brood was evaluated by exploring relationships between sperm quantity and spermathecae weight for 2 sets of crabs. The first comparison was for crabs collected near the lower bay spawning area in 2012, as described above, that did not have evidence of spawning (n = 41). The second comparison was for crabs collected across a wide range of sampling locations (upper–lower bay), seasons, and years (1996–2012). For the second comparison, we pooled data for all 571 crabs analyzed for sperm quantity by our lab (Table 1). This included the sampling efforts described above as well as crabs sampled by trap or dredge in the Rappahannock, York, and James Rivers in Virginia and the Potomac River in Maryland, as well as by trotline in the Magothy, Patuxent, Manokin, and Little Choptank Rivers in Maryland. Crabs were processed following the methods described here and in Hines et al. (2003). The relationship between spermathecae weight and sperm quantity was determined by fitting a non-linear curve to the data using the Curve Fit tool (SigmaPlot v. 12.3). Goodness of fit was evaluated by examining residuals to ensure homoscedasticity.

Modeling

Lifetime brood production was modeled for mature females under different scenarios of initial numbers of sperm contributed at the time of mating followed by degradation or loss of sperm during storage. Initial contributions used were based on values observed in this study, including 3×10^9 (Rhode and Gunpowder Rivers in September), 1×10^9 (Rhode River in August), and 5×10^8 sperm (James and York Rivers in September). Three levels of sperm loss were used: (1) no loss, (2) 50% loss in the first 3 mo (Wolcott et al. 2005) with no additional loss, and (3) 20% monthly loss (the constant rate of loss necessary for a 50% decline in sperm in 3 mo). Brood production was also modeled using the average sperm quantity of females in the lower Chesapeake Bay spawning area that did not have evidence of spawning (8×10^7 sperm).

Females were assumed to mate in September and begin spawning in June (the 9th month after fertilization). Spawning/brood production was assumed to occur monthly during summer (June–September) for 2 yr after mating, for a maximum lifetime production of 8 broods. Some crabs follow different maturity and brood production schedules including mating in spring and producing the first brood the same summer, as has been observed for crabs in the lower bay (Aguilar et al. 2005) and in North Carolina (Darnell et al. 2009). These alternative schedules were not addressed here in order to simplify comparisons between scenarios. An average brood size of 3×10^6 eggs was used for all calculations (Hines 1982, Prager et al. 1990). The range of sperm:egg ratios needed to achieve successful fertilization included 1:1, 10:1, 25:1, 80:1, and 100:1, covering a range of possible values based on prior studies (Bressac et al. 1994, Hines et al. 2003, Sainte-Marie & Lovrich 1994). This resulted in values of the number of sperm required to fertilize a single brood that ranged from 3×10^6 to 3×10^8. The total number of complete broods that could be produced was calculated for each combination of sperm:egg ratio, initial contribution of sperm, and level of sperm loss. Although other factors such as mortality and nutrition likely affect an individual's lifetime brood production, we chose a relatively simple model to focus on the effects of variation in the sperm:egg ratio required for fertiliza-

tion, sperm stores, sperm use during fertilization, and other mechanisms of sperm loss.

RESULTS

Seasonal variation and OSR

The quantity of sperm stored in recently mated female blue crabs in the Rhode River in 2010 varied seasonally, with the highest values in September and October and the lowest values in August (n = 5–7 crabs per month except July, when n = 2). The mean sperm quantity for the entire season was $2.02 \times 10^9 \pm 1.88 \times 10^8$ (mean ± SEM reported here and for all subsequent values). Sperm numbers were moderate in June at $1.86 \times 10^9 \pm 2.63 \times 10^8$ and lowest in August ($1.02 \times 10^9 \pm 1.32 \times 10^8$; Fig. 1A), although the differences among months in June–August were not significant. Sperm quantity was significantly higher during September ($2.83 \times 10^9 \pm 5.06 \times 10^8$) and October ($2.85 \times 10^9 \pm 3.68 \times 10^8$) than in August ($p = 0.001$).

Spermathecae weight showed a seasonal pattern similar to that of sperm quantity, but there were no significant differences among months (Fig. 1B). The mean spermathecae weight for the season was 3.395 ± 0.227 g. Spermathecae weight was moderate in June (3.680 ± 0.589 g) and declined through August. In September, spermathecae weight increased to a high of 4.640 ± 0.700 g.

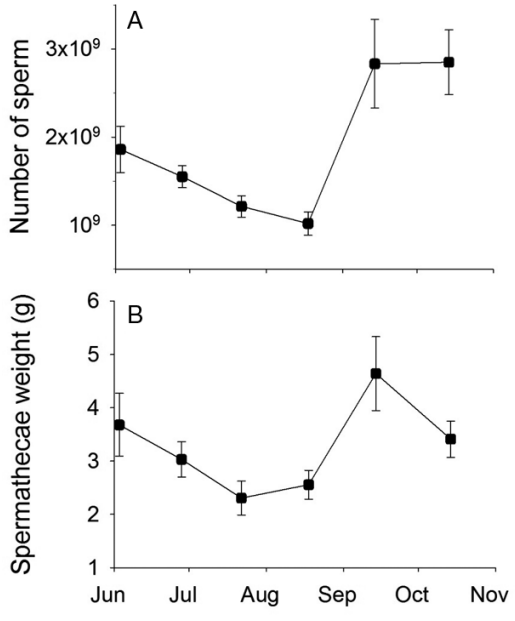

Fig. 1. Variation in (A) sperm quantity and (B) spermathecae weight in recently mated female blue crabs *Callinectes sapidus* during the mating season in the Rhode River, Maryland in 2010. Error bars indicate SEM

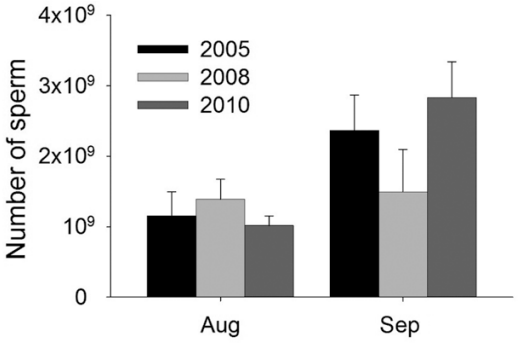

Fig. 2. Interannual variation in sperm quantity of recently mated female blue crabs *Callinectes sapidus* in August and September in the Rhode River, Maryland. Data shown are for 2005, 2008, and 2010. Error bars indicate SEM

The seasonal increase in sperm quantity from August to September 2010 persisted across multiple years. Sperm quantities in August were consistently lower than those in September in both 2005 and 2008, the 2 other years for which data were available (Fig. 2). However, the only significant difference between August and September was in 2010.

There was a positive, asymptotic relationship between OSR and sperm quantity (Fig. 3). OSR was lowest in August (2.38 reproductively active males per prepubertal female), corresponding to the minimum number of sperm ($1.02 \times 10^9 \pm 1.32 \times 10^8$) and near minimum for spermathecae weight (2.556 g). Sperm quantity was significantly lower ($p < 0.001$) when OSR was <3 (n = 8, $1.1 \times 10^9 \pm 1.0 \times 10^8$) than when it was between 4 and 5 (n = 16, $2.2 \times 10^9 \pm 2.4 \times 10^8$) or as high as 15 (n=7, $2.9 \times 10^9 \pm 3.7 \times 10^8$). OSR was unknown at the time of sampling and by chance no samples were obtained when OSR was between 5 and 15. Significant differences were also detected for spermathecae weight ($p = 0.019$), but these appeared to be due to 4 crabs with very high spermathecae weight (5–7 g) at moderate levels of OSR. There was no relationship between OSR and spermathecae weight when these data were removed.

Spatial distribution

Sperm quantity, but not spermathecae weight, differed by sampling location within Chesapeake Bay (n = 5–6 crabs per location) in September 2010. Sperm quantity in the lower bay was significantly lower than that in both the middle and upper bay ($p = 0.003$); however, the middle and upper bay were not

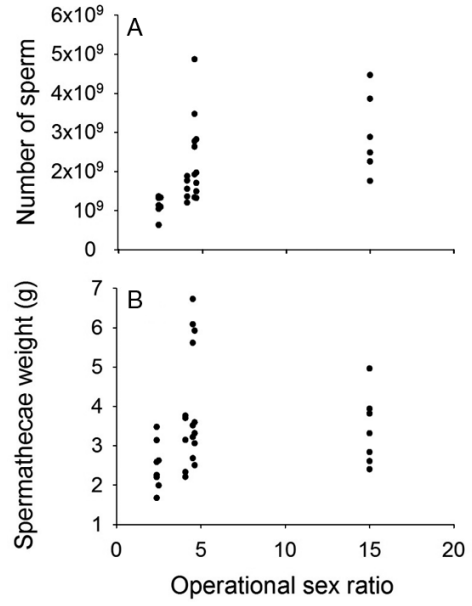

Fig. 3. Relationships between operational sex ratio (OSR) and (A) sperm quantity and (B) spermathecae weight of recently mated female blue crabs *Callinectes sapidus* collected in the Rhode River, Maryland, in 2010. Each data point represents an individual crab. Data for OSR were calculated for the month in which each crab was collected, such that all crabs collected in the same month have the same value for OSR

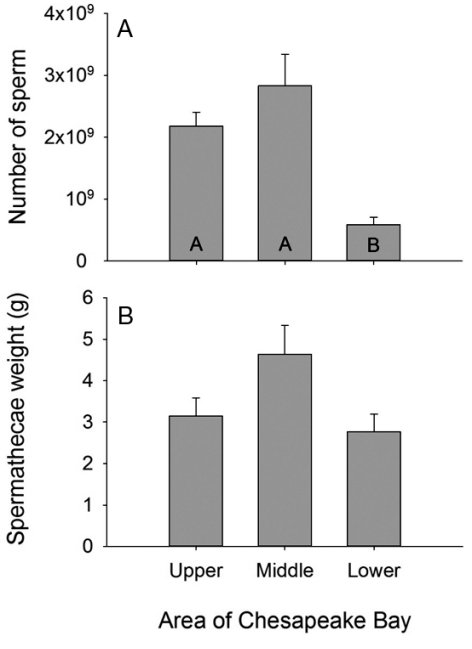

Fig. 4. Spatial variation in (A) quantity of sperm and (B) spermathecae weight for recently mated mature female blue crabs *Callinectes sapidus* collected in 2010 from the upper, middle, and lower Chesapeake Bay. Error bars indicate SEM. Different letters at the bottom of vertical bars indicate statistical differences ($\alpha = 0.05$)

different from each other (Fig. 4A). Spermathecae weight was highest in the middle bay and lower in both the upper and lower bay, but there was no significant difference among the sites (Fig. 4B).

Spawning area

There were strong seasonal differences in the composition of females in the spawning area of the lower Chesapeake Bay in 2012 (n = 30 crabs per month). The proportion of female crabs with evidence of prior spawning in the lower bay was highest in June (80%) and August (100%) (Fig. 5A). In September and November, the samples were dominated by crabs without evidence of prior spawning (100% and 93%, respectively). Samples in July were fairly evenly split between those with and without evidence of spawning. Nearly all crabs without evidence of spawning had clean or partially dirty carapaces in June and July, whereas a majority of crabs without evidence of spawning in September and November had partially

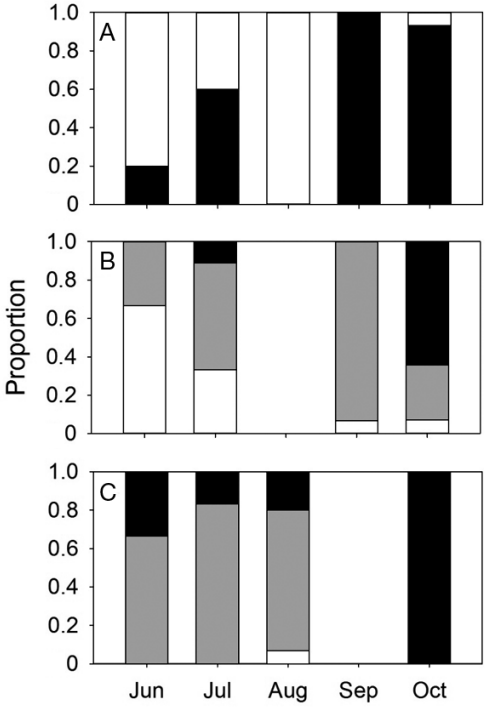

Fig. 5. Proportion of samples of mature female blue crabs *Callinectes sapidus* collected in the spawning area of the lower Chesapeake Bay in 2012 with respect to (A) proportion of crabs with (white) or without (black) evidence of spawning (n = 30 crabs each month), (B) carapace condition of crabs without evidence of spawning, and (C) carapace condition of crabs with evidence of spawning. Carapace conditions are clean (white), partially dirty (gray), and dirty (black)

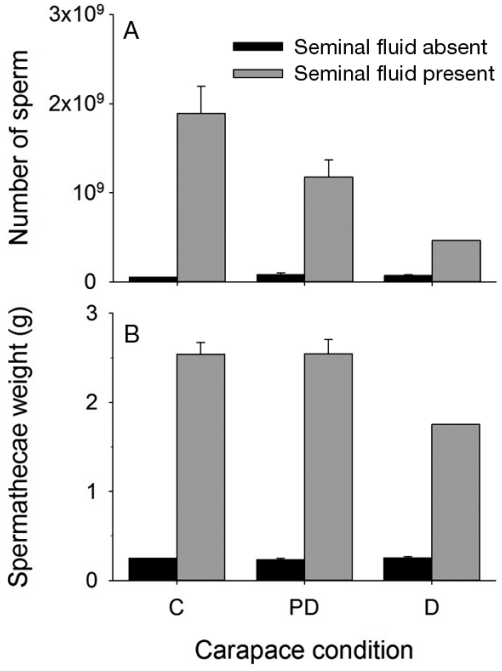

Fig. 6. Variation in (A) sperm quantity and (B) spermathecae weight for mature female blue crabs *Callinectes sapidus* collected in the spawning area of the lower Chesapeake Bay in 2012 with respect to carapace condition (clean [C], partially dirty [PD], and dirty [D]) and presence/absence of seminal fluid (hardened sperm plug). Error bars indicate SEM

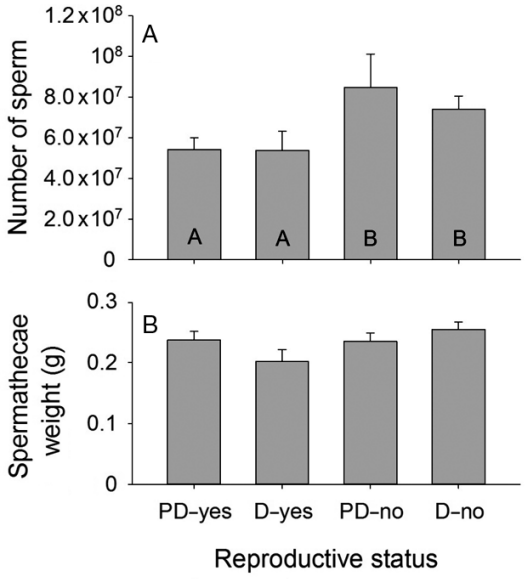

Fig. 7. Variation in (A) sperm quantity and (B) spermathecae weight for mature female blue crabs *Callinectes sapidus* collected in the spawning area of the lower Chesapeake Bay in 2012 for which no seminal fluid was present (i.e. the sperm plug was resorbed). Crabs were classified by carapace condition (partially dirty [PD] or dirty [D]) and presence (yes) or absence (no) of evidence of spawning (egg remnants on pleopods). Different letters at the bottom of vertical bars indicate statistical differences (α= 0.05). Error bars indicate SEM

dirty and dirty carapaces (Fig. 5B). Crabs with evidence of spawning were typically partially dirty through September, shifting to 100% dirty in November (Fig. 5C).

The number of sperm was highest for clean, recently mated crabs with seminal fluid and decreased with increasing carapace dirtiness (Fig. 6A). Crabs without seminal fluid had low sperm numbers regardless of carapace condition. The weight of the spermathecae for crabs with seminal fluid was similar for clean and partially dirty crabs and decreased for dirty crabs (Fig. 6B). However, the latter difference could not be tested because only a single individual had both a dirty carapace and seminal fluid. Crabs without seminal fluid had low spermathecae weights regardless of carapace condition, as seminal fluid accounts for a large proportion of spermathecae weight.

Crabs from the lower bay without seminal fluid had significantly lower sperm quantities if they had spawned compared with crabs without evidence of spawning, irrespective of carapace condition ($p = 0.010$; Fig. 7A). The range of sperm quantities was large for partially dirty and dirty crabs without seminal fluid and with evidence of spawning, with a minimum value of 4.27×10^6 and maximum of 1.07×10^8 (n = 33). For comparison, crabs without evidence of spawning had sperm quantities ranging from 4.02×10^7 to 1.32×10^8 (n = 14). Spermathecae weight for crabs without seminal fluid was very low and not significantly different among carapace conditions or spawning evidence (Fig. 7B).

Relationships between spermathecae weight and sperm quantity

There was a positive relationship between spermathecae weight and sperm quantity. Spermathecae weight varied from 0.05 to 6.99 g and sperm quantity varied from 1.2×10^6 to 2.2×10^{10} (Fig. 8). For crabs collected in the lower bay in 2012, the relationship was bimodal (Fig. 9A). Most individuals had either high sperm quantities ($1.31 \times 10^9 \pm 1.69 \times 10^8$) and spermathecae weights (2.516 ± 0.123 g), or low quantities ($7.61 \times 10^7 \pm 6.70 \times 10^6$) and weights ($0.248 \pm 0.033$ g). When data for all 571 crabs collected between 1996 and 2012 were included, the relationship was asymptotic ($\log_{10}y = 7.590 + 1.609[1 - e^{-0.9659x}]$, $r^2 = 0.536$; Fig. 9B). Sperm quantity reached an as-

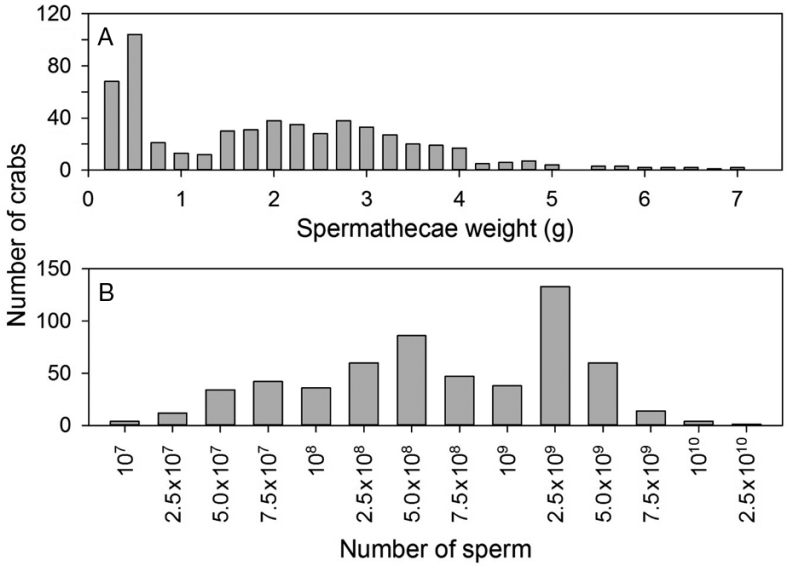

Fig. 8. Histograms for (A) spermathecae weight and (B) sperm quantity for all female blue crabs *Callinectes sapidus* processed for sperm quantity by our lab during 1996–2012 (n = 571). Spermathecae weight and sperm number classes indicate the number of crabs with values less than or equal to each bar's position on the *x*-axis

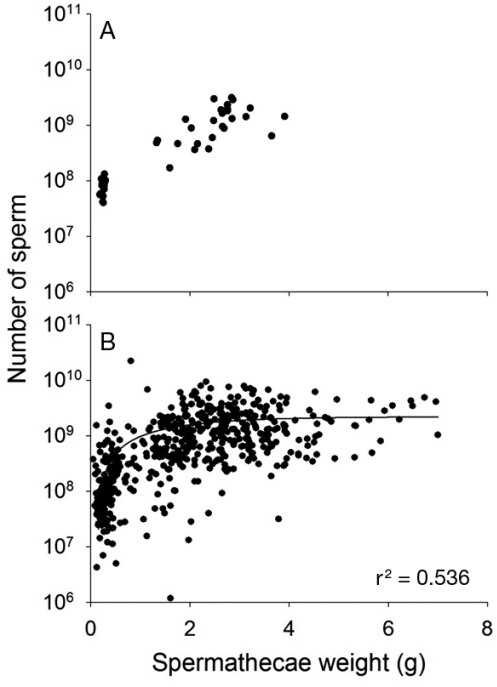

Fig. 9. Relationship between spermathecae weight and sperm quantity for (A) mature female blue crabs *Callinectes sapidus* collected in the spawning area of the lower Chesapeake Bay in 2012 that did not have evidence of prior spawning (n = 41) and (B) all mature female blue crabs collected throughout Chesapeake Bay and analyzed for sperm quantity by our lab (n = 571). A regression line and squared correlation coefficient are shown for the sigmoid relationship in B

ymptote of 1.58×10^9, although substantial variability in sperm quantity remained among individuals with high spermathecae weight.

Modeling

Modeled brood production for individual female blue crabs varied from a minimum of zero to a maximum of 8 broods over a simulated 2-yr adult lifespan (Table 2). In simulations with no sperm loss except by fertilization, 8 full broods were produced except at sperm:egg ratios of 80 or 100. In simulations with 50% reduction in sperm prior to spawning, declines in initial sperm load from 3×10^9 to 5×10^8 resulted in a dramatic decrease from 8 to 3 broods at a sperm:egg ratio of 25. Smaller decreases in brood number were observed at other combinations of sperm:egg ratio and initial sperm load. When sperm load declined at a monthly rate of 20% throughout the adult lifespan, reductions in initial sperm load had the greatest effect at a sperm:egg ratio of 10, resulting in a reduction from 4 broods to 1.

When the observed value of sperm quantity for females in lower Chesapeake Bay without evidence of spawning was used (8×10^7 sperm), predicted lifetime brood production of an individual female was lower than for the scenarios described above. Only 2 full broods were predicted at a sperm:egg ratio of 10:1 without additional sperm loss and one full brood with 20% loss per month (Table 2). At a sperm:egg ratio of 1:1, 5 to 8 full broods could be produced depending on whether additional sperm were lost. No more than a single full brood would be possible at a sperm:egg ratio of 25:1 or higher.

DISCUSSION

Sperm stores in recently mated female blue crabs were lower during summer in the Rhode River sub-estuary of Chesapeake Bay compared with other seasons, corresponding to reductions in the OSR. Sperm quantities were highest in fall, nearly 3×10^9, when there were as many as 15 mature males available to mate with each pre-pubertal female. These high sperm quantities, likely obtained from males with suf-

Table 2. Model simulations of brood production under various levels of sperm:egg ratio, initial sperm quantity, and sperm loss. Data reported are number of full broods produced assuming a brood size of 3×10^6 eggs. The initial sperm quantity (**bold**) is either for different contributions of sperm at the time of mating (Scenarios 1–3) or for females that have already migrated to the spawning area (Scenario 4). In Scenario 4, sperm loss prior to brood 1 is not applicable (N/A) as this loss would be accounted for in the initial sperm quantity

Scenario	Sperm:egg ratio				
	1	10	25	80	100
(1) 3×10^9 at the time of mating					
No loss	8	8	8	8	8
50% loss prior to brood 1	8	8	8	6	5
20% monthly loss	8	4	3	1	0
(2) 1×10^9 at the time of mating					
No loss	8	8	8	4	3
50% loss prior to brood 1	8	8	6	2	1
20% monthly loss	6	3	1	0	0
(3) 5×10^8 at the time of mating					
No loss	8	8	6	2	1
50% loss prior to brood 1	8	8	3	1	0
20% monthly loss	5	1	0	0	0
(4) 8×10^7 in spawning area					
No loss	8	2	1	0	0
50% loss prior to brood 1	N/A	N/A	N/A	N/A	N/A
20% monthly loss	5	1	0	0	0

ficient time between mating events to regenerate a full complement of sperm, were similar to the 3.4×10^9 sperm observed for Chesapeake Bay females mated with 'fully recovered' males in laboratory experiments (Kendall et al. 2002, Carver et al. 2005). These sperm stores were greater than the sperm stores of 1.2×10^9 in laboratory experiments conducted in North Carolina (Wolcott et al. 2005) and in crabs collected from the field in Florida (Hines et al. 2003). The estimate from Florida appears to have been a substantial underestimate because sperm quantities decline with time after mating (Wolcott et al. 2005) and sampling was not restricted to recently mated females as in the present study. It is unclear why Wolcott et al. (2005) observed lower sperm stores in recently mated females from North Carolina. In August, when the OSR was only 3 mature males for each pre-pubertal female, sperm stores in recently mated females declined to just 36% of the full complement observed in fall. The sperm stores of females in August were similar to the level of sperm transferred by recently mated males in laboratory experiments (Kendall et al. 2002), indicating that these females likely mated with males with too short an interval between mating events to regenerate a full complement of sperm. The OSR may have been lowest in August because of intense fishing for mature males during summer and a late summer increase in the number of females molting to maturity (Hines et al. 1987), although we did not investigate the factors affecting OSR in this study. The seasonal pattern of sperm limitation was consistent across 3 yr of sampling, indicating that it occurs annually in Chesapeake Bay.

The seasonal reduction in sperm stores described here is consistent with males encountering pre-pubertal females too frequently to regenerate a full supply of sperm and ejaculate between mating events (Kendall & Wolcott 1999, Kendall et al. 2002). In experimental manipulations with the unharvested crab *Hapalogaster dentata*, Sato & Goshima (2006) observed significant declines in the number of sperm ejaculated with increased male mating frequency. In heavily fished areas of Chesapeake Bay, male blue crabs in pre-copulatory embrace have been observed with depleted sperm stores equivalent to males that have just mated, indicating reduction in sperm stores due to frequent mating (Carver et al. 2005). Similar evidence for reduction in sperm stores in fished decapod populations has been observed for Dungeness crab *Cancer magister* (Smith & Jamieson 1991), snow crab *Chionocetes opilio* (Lovrich et al. 1995), Tanner crab *Chionocetes bairdi* (Stevens et al. 1993), and coconut crab *Birgus latro* (Sato 2011), suggesting that reduced sperm stores may be a common consequence of male-selective fishing for decapod crabs. All females in the present study had mated, suggesting that the Chesapeake Bay blue crab population does not experience reproductive impairment due to an inability to find mates.

Spatial variation in sperm quantities in newly mated females was indicative of reduced sperm stores received by females molting to maturity in Virginia tributaries of Chesapeake Bay during September 2010. Female crabs from the middle and upper bay had stores of $>2 \times 10^9$ sperm on average in September, indicating that they received nearly a full load of sperm. In contrast, females from the lower bay had <25% of the sperm load of middle bay crabs. Such a pattern might be due to differences in fishery management between Maryland in the middle and upper bay and Virginia in the lower bay, although there were no data available to investigate this hypothesis directly. Alternatively, the spatial pattern could be due to random sampling variability considering the small sample sizes (5–6 individuals per location) and inclusion of only 3 locations.

Unlike sperm quantity, the spermathecae weight of recently mated females did not vary with season,

OSR, or collection location. Variation in spermathecae weight is driven primarily by the quantity of seminal fluid transferred during mating, which is affected both by male size and male mating frequency (Jivoff 1997a,b, 2003a,b). Although small males transfer much less seminal fluid than large males during mating, the contribution of sperm is independent of male size (Kendall et al. 2001). Taken together, these results suggest that the variation we observed in sperm quantities was due to male mating frequency (which varies with OSR) and that male size, which was an unknown in our study, may have obscured the effect of mating frequency on spermathecae weight.

The quantity of stored sperm declined significantly with time since mating. Although there is not a precise method of determining time since mating, the hardened seminal fluid (sperm plug) present after mating is absorbed approximately 1 mo later (Wolcott et al. 2005). In our study, females that no longer had a hardened sperm plug had sperm quantities that were 1 to 2 orders of magnitude less than the full complement of a recently mated female. This decline was observed in females with and without evidence of spawning, suggesting that it was due to sperm loss and not simply the use of sperm for fertilization, which occurs during egg extrusion onto the abdomen. In North Carolina, Wolcott et al. (2005) observed 50% declines in sperm stores of captive females in 3 mo prior to fertilization, consistent with our results from field collections. It remains unclear whether sperm loss occurs primarily during breakdown of the sperm plug or continuously following mating. Nor is it clear whether this attrition results from a process of leakage and sloughing or from deterioration—all independent of use during fertilization.

In the lower Chesapeake Bay spawning area, the composition of females shifted from primarily older, reproductively active females in summer to younger, recently mated females in fall. In summer, most females were either ovigerous, with external sponges of fertilized eggs, or had remnants of egg cases from previous broods. These reproductive females had significantly lower sperm stores (5.4×10^7 sperm) than females without evidence of spawning (7.8×10^7 sperm), as would be expected due to the use of sperm during fertilization. It should be noted that the females collected from the biologically relevant spawning area in lower Chesapeake Bay were collected by commercial fishers working 5 km outside the border of the spawning sanctuary, where fishery harvest of mature females is prohibited during the spawning season (Lipcius et al. 2003). The presence of spawning females outside of the sanctuary in this area was expected given that the sanctuary was designed to protect approximately 75% of the spawning grounds (Lipcius et al. 2003) and has been shown to be an effective management tool (Lambert et al. 2006a). This was not indicative of the sometimes substantial difference between biologically relevant spawning areas for blue crabs and spawning sanctuaries designated by fishery managers, as observed in more southern estuaries (Rittschof et al. 2009).

The high prevalence of recently mated females in the spawning area in fall, and to a lesser extent in early summer, is consistent with our understanding of the seasonal timing of mating and the spawning migration (Hines et al. 1987, Aguilar et al. 2005). Interestingly, we did not observe consistent differences in the quantity of stored sperm with respect to carapace condition except for recently mated females with seminal fluid (a sperm plug) present (Fig. 6A). This result is indicative of substantial uncertainty in the value of carapace condition as a relative measure of time since mating in mature female blue crabs, as the mechanisms underlying changes in carapace condition have not been evaluated rigorously. A validated technique for determining time since mating will be needed to determine the pattern and rate of decline in sperm stores due to causes other than fertilization. Potential indicators of blue crab age include lipofuscin concentration in eye stalks (Ju et al. 1999) and growth rings in the eye stalk or gastric mill (Kilada et al. 2012), but additional validation is needed to determine whether these techniques are useful for determining time since mating.

Although reductions in sperm stores in recently mated females were substantial in some locations and seasons, determining whether the blue crab spawning stock in Chesapeake Bay is sperm-limited at a level to affect population dynamics is much more challenging. We addressed this problem by modeling the lifetime brood production of females with various initial sperm quantities, patterns of sperm loss, and sperm:egg ratios required for fertilization. As in Hines et al. (2003), we assumed an average brood size of 3×10^6 eggs and a reproductive season typical for Chesapeake Bay. In model results, the effect of different initial sperm quantities was difficult to assess due to the strong influence of (1) the timing of sperm loss, (2) the rate of sperm loss, and (3) the sperm:egg ratio. For example, the lifetime brood production of females in the spawning area without evidence of spawning (initial sperm quantity of 8×10^7) was 8 broods at a sperm:egg ratio of 1:1 and only 2 broods at 10:1, assuming fertilization was the only mechanism of sperm loss. When additional sperm loss occurred at 20% per month (based

on observations in Wolcott et al. 2005), females with no evidence of spawning were capable of producing 5 full broods at 1:1 and only one full brood at 10:1. The sperm:egg ratio needed for successful fertilization of a full brood in blue crabs has previously been estimated at 20:1 or 30:1 (Hines et al. 2003), although this estimate was likely too high because it did not take into account any sperm loss after mating. Our modeling results suggest that this number must be between 1:1 and 10:1 in order for females to fertilize the 2 to 7 broods observed in brood production studies in North Carolina and Florida (Hines et al. 2003, Darnell et al. 2009).

In our field study, a difference of 2.4×10^7 sperm was observed between females with and without evidence of spawning. Assuming (1) an average brood size of 3×10^6, (2) that females with evidence of spawning had only produced one brood, and (3) that there was no sperm loss to mechanisms other than fertilization, this difference is indicative of 8 sperm used per egg during fertilization of the first brood. If an equal number of sperm are used for each subsequent brood, only 2 additional full broods could be produced. This estimate is consistent with our modeling results, which suggested that only 2 full broods could be produced at a somewhat higher sperm:egg ratio of 10, assuming no sperm loss except during fertilization. However, it is inconsistent with observed brood production of up to 7 fertilized broods (Hines et al. 2003, Dickinson et al. 2006, Darnell et al. 2009), suggesting that fertilization may be possible at even lower sperm:egg ratios. Snow crab *Chionocetes opilio* have been observed to cease egg extrusion at ratios below 7:1 (Sainte-Marie & Lovrich 1994). It is possible that decapod species such as blue crabs that store sperm from a single mating may be capable of fertilization at lower sperm:egg ratios than species such as snow crabs that mate multiple times. Very low sperm:egg ratios are not unheard of in the Arthropoda, as some *Drosophila* species have ratios as low as 1:1 (Bressac et al. 1994). Direct quantification of the number of sperm used during fertilization in the blue crab is needed.

Two important limitations of our model were the assumptions that brood size and fertilization success are equal for all broods. In a study of captive crabs in North Carolina, brood size declined by 50% from brood 1 to 5, and the percentage of extruded embryos developing normally decreased 40% from brood 1 to 4 (Darnell et al. 2009). In Florida, Hines et al. (2003) occasionally observed that later broods (5th to 7th observed brood) were non-fertile. These combined findings provide strong evidence that both brood size and fertilization success decline over time in blue crabs, but these factors were left out of the model because mechanisms are unknown. Possible mechanisms include insufficient sperm stores, as indicated in our modeling and field-based estimates of brood production, or loss of sperm viability, declining female condition due to fouling or disease, or a combination of mechanisms. Understanding the extent to which insufficient sperm stores contribute to declines in the size and fertilization success of later broods is important for determining whether increasing the number of females surviving to spawn in a second spawning season is an effective management strategy for enhancing the reproductive output of the spawning stock. If sperm limitation is an important factor affecting brood production in an individual's second spawning season as indicated in this study, fishery managers could potentially increase the reproductive output and stability of the blue crab population in Chesapeake Bay through actions aimed at maintaining OSR in nursery habitats at the time of mating at sufficient levels to prevent sperm limitation.

Although this study refines our understanding of some aspects of blue crab reproductive biology, it also highlights data gaps critical to assessing the potential for population-level sperm limitation. First, the pattern and rate of sperm loss following mating is important to determining whether the low sperm quantities we observed in spawning areas of the lower Chesapeake Bay are indicative of natural loss of sperm following mating or sperm reduction at the time of mating. Second, the sperm:egg ratio required for successful fertilization is unknown, yet it is a key factor in determining whether an individual female is sperm-limited. Third, our model was focused on lifetime brood production and thus did not consider mortality. And fourth, experiments are needed to determine whether reductions in the quantity of sperm acquired during mating result in actual reductions in the number or size of fertilized broods that can be produced. Management strategies in Chesapeake Bay are focused in part on increasing the reproductive output and stability of the spawning stock by increasing the number of females spawning for a second year (Lambert et al. 2006b), yet these second-year females are most likely to be affected by sperm limitation. Mark-recapture experiments indicate that the annual survival rate of mature female blue crabs in Chesapeake Bay is 0.15 ± 0.01 (mean ± SE) (Lambert et al. 2006b); however, these experiments were conducted in 2002–2006 prior to closure of Virginia's winter dredge fishery and the extent of any subsequent increase in survival rate is unknown. In the

present study, carapace condition did not appear to be a conclusive indicator of whether females were in their first or second spawning season. Therefore, developing a reliable method for distinguishing between first- and second-year spawners should be a priority.

In summary, we observed reduced sperm stores in recently mated female blue crabs in Chesapeake Bay when OSR was lowest (August) and in lower bay tributaries in September. Sperm stores declined by at least an order of magnitude from the time of mating to fertilization of the first brood. The composition of the lower bay spawning stock matched expectations, with reproductively active females dominating in spring and summer and newly arrived, recently mated females dominating in fall. Reproductively active females in the lower bay had significantly lower sperm quantities than females without evidence of prior spawning, indicating that we were able to detect the use of sperm for fertilization. Modeling of lifetime brood production and estimates based on apparent sperm use during fertilization indicated that sperm limitation is likely for females that survive to a second spawning season. Information on the proportion of crabs surviving to a second spawning season and sperm:egg ratios required for successful fertilization is needed in order to better understand the degree to which the reductions in sperm observed in recently mated females in this study may be affecting the total reproductive output of the blue crab population in Chesapeake Bay.

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LITERATURE CITED

Aguilar R, Hines AH, Kramer MA, Wolcott TG, Lipcius RN (2005) Timing and route of movement and migration of post-copulatory female blue crabs, *Callinectes sapidus* Rathbun, from the upper Chesapeake Bay. J Exp Mar Biol Ecol 319:117–128

Austin CR (1975) Sperm fertility, viability and persistence in the female tract. J Reprod Fertil Suppl 22:75–89

Bressac C, Fleury A, Lachaise D (1994) Another way of being anisogamous in *Drosophila* subgenus species: giant sperm, one-to-one gamete ratio, and high zygote provisioning. Proc Natl Acad Sci USA 91:10399–10402

Brown GG (1966) Ultrastructural studies of sperm morphology and sperm–egg interaction in the decapod *Callinectes sapidus.* J Ultrastruct Res 14:425–440

Carver AM, Wolcott TG, Wolcott DL, Hines AH (2005) Unnatural selection: effects of a male-focused size-selective fishery on reproductive potential of a blue crab population. J Exp Mar Biol Ecol 319:29–41

Darnell MZ, Rittschof D, Darnell KM, McDowell RE (2009) Lifetime reproductive potential of female blue crabs *Callinectes sapidus* in North Carolina, USA. Mar Ecol Prog Ser 394:153–163

Darnell MZ, Darnell KM, McDowell RE, Rittschof D (2010) Postcapture survival and future reproductive potential of ovigerous blue crabs *Callinectes sapidus* caught in the central North Carolina pot fishery. Trans Am Fish Soc 139:1677–1687

Dickinson GH, Rittschof D, Latanich C (2006) Spawning biology of the blue crab, *Callinectes sapidus*, in North Carolina. Bull Mar Sci 79:273–285

Duluc C, Sainte-Marie B, Brethes JC (2005) Value of indicators for assessing recent mating in brachyuran crabs. J Mar Biol Assoc UK 85:129–135

Fenberg PB, Roy K (2008) Ecological and evolutionary consequences of size-selective harvesting: How much do we know? Mol Ecol 17:209–220

Hines AH (1982) Allometric constraints and variables of reproductive effort in brachyuran crabs. Mar Biol 69: 309–320

Hines AH, Lipcius RN, Haddon AM (1987) Population dynamics and habitat partitioning by size, sex, and molt stage of blue crabs *Callinectes sapidus* in a subestuary of central Chesapeake Bay. Mar Ecol Prog Ser 36:55–64

Hines AH, Jivoff PR, Bushman PJ, van Montfrans J, Reed SA, Wolcott DL, Wolcott TG (2003) Evidence for sperm limitation in the blue crab, *Callinectes sapidus.* Bull Mar Sci 72:287–310

Jamieson GS, Phillips A, Smith BD (1998) Implications of selective harvests in Dungeness crab (*Cancer magister*) fisheries. Can J Fish Aquat Sci 125:309–321

Jivoff PR (1995) The role of mate guarding, male size and male investment on individual reproductive success in the blue crab, *Callinectes sapidus*. PhD dissertation, University of Maryland, College Park

Jivoff PR (1997a) The advantages of large body size in sexual competition among males in the blue crab, *Callinectes sapidus.* Biol Bull 193:368–380

Jivoff PR (1997b) The relative roles of predation and sperm competition on the duration of post copulatory mate guarding in the blue crab, *Callinectes sapidus*. Behav Ecol Sociobiol 40:175–185

Jivoff PR (2003a) A review of male mating success in the blue crab, *Callinectes sapidus,* in reference to the potential for fisheries-induced sperm limitation. Bull Mar Sci 72:273–286

Jivoff PR (2003b) Summary session paper, Blue Crab Symposium 2000, reproduction and embryonic development. Bull Mar Sci 72:265–272

Ju SJ, Secor DH, Harvey HR (1999) Use of extractable lipofuscin for age determination of blue crab *Callinectes sapidus*. Mar Ecol Prog Ser 185:171–179

Kendall MS, Wolcott TG (1999) The influence of male mat-

ing history on male-male competition and female choice in mating associations in the blue crab, *Callinectes sapidus* Rathbun. J Exp Mar Biol Ecol 239:23–32

Kendall MS, Wolcott DL, Wolcott TG, Hines AH (2001) Reproductive potential of individual male blue crabs, *Callinectes sapidus*, in a fished population: depletion and recovery of sperm number and seminar fluid. Can J Fish Aquat Sci 58:1168–1177

Kendall MS, Wolcott DL, Wolcott TG, Hines AH (2002) Influence of male size and mating history on sperm content of ejaculates of the blue crab *Callinectes sapidus.* Mar Ecol Prog Ser 230:235–240

Kilada R, Sainte-Marie B, Rochette R, Davis N, Vanier C, Campana S (2012) Direct determination of age in shrimps, crabs, and lobsters. Can J Fish Aquat Sci 69: 1728–1733

Lambert DM, Lipcius RM, Hoenig JM (2006a) Assessing effectiveness of the blue crab spawning stock sanctuary in Chesapeake Bay using tag-return methodology. Mar Ecol Prog Ser 321:215–225

Lambert DM, Hoenig JM, Lipcius RM (2006b) Tag-return estimation of annual and semi-annual survival rates of adult female blue crabs. Trans Am Fish Soc 135: 1592–1603

Lane JE, Forrest MNK, Willis CKR (2011) Anthropogenic influences on natural animal mating systems. Anim Behav 81:909–917

Lipcius RN, Stockhausen WT, Seitz RD, Geer PJ (2003) Spatial dynamics and value of a marine protected area and corridor for the blue crab spawning stock in Chesapeake Bay. Bull Mar Sci 72:453–470

Lovrich GA, Sainte-Marie B, Smith BD (1995) Depth distribution and seasonal movement of *Chionoecetes opilio* (Brachyura: Majidae) in Baie Sainte-Marguerite, Gulf of Saint Lawrence. Can J Fish Aquat Sci 73:1712–1726

McConaugha JR, Johnson DF, Provenzano AJ, Maris RC (1983) Seasonal distribution of larvae of *Callinectes sapidus* (Crustacea: Decapoda) in the waters adjacent to Chesapeake Bay. J Crustac Biol 3:582–591

Miller TJ, Wilberg MJ, Colton AR, Davis GR and others (2011) Stock assessment of blue crab in Chesapeake Bay 2011. Tech Rep TS 614 11, University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, Solomons, MD

Millikin MR, Williams AB (1984) Synopsis of biological data on the blue crab, *Callinectes sapidus* Rathbun. NOAA Technical Report NMFS 1, FAO Fisheries Synopsis 138

Morgan SG, Goy JW, Costlow JD (1983) Multiple ovipositions from single matings in the mud crab *Rhithropanopeus harrisii*. J Crustac Biol 3:542–547

Paul AJ, Paul JM (1992) Second clutch viability of *Chionoecetes bairdi* Rathbun (Decapoda, Majidae) inseminated only at the maturity molt. J Crustac Biol 12:438–441

Prager MH, McConaugha JR, Jones CM, Geer PJ (1990) Fecundity of blue crab, *Callinectes sapidus*, in Chesapeake Bay: biological statistical, and management considerations. Bull Mar Sci 46:170–179

Ramach SM, Darnell MZ, Avissar NG, Rittschof D (2009) Habitat use and population dynamics of blue crabs, *Callinectes sapidus*, in a high-salinity embayment. J Shellfish Res 28:635–640

Rittschof D, Darnell MZ, Darnell KM, Goldman M, Ogburn MB, McDowell R (2009) Informing managers: blue crab spawning biology and stock assessment. In: Kruse GH, Eckert GL, Foy RJ, Lipcius RN, Sainte-Marie B, Stram DL, Woodby D (eds) Biology and management of exploited crab populations under climate change. Proc 25th Lowell Wakefield Fish Symp. Alaska Sea Grant College Program, Anchorage, AK, p 91–108

Rodgers PJ, Reaka ML, Hines AH (2011) A comparative analysis of sperm storage and mating strategies in five species of brachyuran crabs. Mar Biol 158:1733–1742

Rowe S, Hutchings JA (2003) Mating systems and the conservation of commercially exploited marine fish. Trends Ecol Evol 18:567–572

Sainte-Marie B (1993) Reproductive cycle and fecundity of primiparus and multiparus female snow crab, *Chionoecetes opilio*, in the northwest Gulf of Saint Lawrence. Can J Fish Aquat Sci 50:2147–2156

Sainte-Marie B, Lovrich GA (1994) Delivery and storage of sperm at first mating of female *Chionoecetes opilio* (Brachyura: Majidae) in relation to size and morphometric maturity of male parent. J Crustac Biol 14:508–521

Sato T (2011) Plausible causes for sperm-store variations in the coconut crab *Birgus latro* under large selective harvesting. Aquat Biol 13:11–19

Sato T (2012) Impacts of large male-selective harvesting on reproduction: illustration with large decapod crustacean resources. Aqua-BioSci Monogr 5:67–102

Sato T, Goshima S (2006) Impacts of male-only fishing and sperm limitation in manipulated populations of an unfished crab, *Hapalogaster dentata.* Mar Ecol Prog Ser 313:193–204

Smith SG, Chang ES (2007) Molting and growth. In: Kennedy VS, Cronin LE (eds) The blue crab: *Callinectes sapidus*. Maryland Sea Grant College, College Park, MD, p 197–254

Smith BD, Jamieson GS (1991) Possible consequences of intensive fishing for males on the mating opportunities of Dungeness crab. Trans Am Fish Soc 120:650–653

Stevens BG, Donaldson WE, Jagga JA, Munk JE (1993) Morphometry and maturity of paired Tanner crabs, *Chionoecetes bairdi*, from shallow- and deepwater environments. Can J Fish Aquat Sci 50:1504–1516

Van Engel WA (1958) The blue crab fishery in Chesapeake Bay: reproduction, early development, growth, and migration. Commer Fish Rev 20:6–17

Van Engel WA (1990) Development of the reproductively functional form in the male blue crab, *Callinectes sapidus.* Bull Mar Sci 46:13–22

Waddy SL, Aiken DE (1986) Multiple fertilization and consecutive spawning in large American lobsters, *Homarus americanus*. Can J Fish Aquat Sci 43:2291–2294

Wells SL (2009) Changing fecundity and reproductive output in females of a Chesapeake Bay population of blue crab, *Callinectes sapidus*. PhD dissertation, Old Dominion University, Norfolk, VA

Wenner EL (1989) Incidence of insemination in female blue crabs, *Callinectes sapidus.* J Crustac Biol 9:587–594

Wolcott DL, Hopkins CWB, Wolcott TG (2005) Early events in seminal fluid and sperm storage in female blue crab *Callinectes sapidus* Rathbun: effects of male mating history, male size, and season. J Exp Mar Biol Ecol 319: 43–55

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