Unnatural selection: Effects of a male-focused size-selective fishery on reproductive potential of a blue crab population

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Abstract

A male-focused size-selective fishery, like the one targeting the blue crab (*Callinectes sapidus* Rathbun) along the Atlantic and Gulf coasts of the U.S., has the potential to reduce the average size of the males in the population, reduce the density of males in the population, and/or raise the ratio of females to males. All of these may affect the mating dynamics of the population by reducing the amount of sperm that males provide to females and decreasing the number of males available for copulation. We investigated the effect of the fishery on a blue crab population in upper Chesapeake Bay by collecting crabs in areas of markedly different fishing pressure. Crabs were taken as individuals and as mating pairs, which permitted assessing the size of males currently mating in nature, and the seminal resources they possess and transfer to females. Average size of males in subpopulations that have been subjected to heavier fishing pressure is indeed smaller, and the smaller males pass less sperm and accessory fluid to females than would larger males if they were still present. Some males in pre-copulatory pairs are as sperm depleted as males that had just completed copulation, indicating that they are mating more frequently than they can replace their seminal resources. The most sperm-depleted males in the population are not even pairing or attempting to mate.
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1. Introduction

The blue crab (*Callinectes sapidus* Rathbun) has been subjected to intense fishing pressure in the Chesapeake Bay since 1880 (Van-Engel, 1958; Wenner, 1989; Abbe and Stagg, 1996). To protect the stock, fishing regulations were established to limit the take to hard crabs with a minimum carapace width (CW) of 127 mm and soft crabs of at least 76 mm. The protection of females to maintain the reproductive

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output of a population is a common focus of fisheries management. Since blue crab recruitment has been correlated with spawning stock, spawning sanctuaries have been established (Lipcius and Stockhausen, 2002) and regulations have been implemented to prohibit the catch of female blue crabs during egg-bearing stages of the reproductive cycle. The selective removal of large males from a population may significantly alter the mating dynamics of the population, and hence also reduce its overall reproductive success (Hines et al., 2003). Therefore it is crucial to understand the effect of a male-focused fishery on the mating dynamics, demography, and reproductive success of a population.

Disproportionate fishing pressure imposed on males could significantly change the population by: (1) reducing abundance of males, (2) increasing the operational sex ratio (OSR), and/or (3) decreasing the average size of the males (Sainte-Marie and Lovrich, 1994). Reducing the density of the males in the population may reduce the encounter rate, which could make it difficult for females to find a mate. Increasing the operational sex ratio, the "average ratio of fertilizable females to sexually active males at a given time" (as originally defined by Emlen and Oring, 1977), could leave insufficient males in the population to mate with all of the females, leaving some females unmated and without any reproductive contribution. Females also might have a reduced reproductive output if males, in attempting to mate all of the females they encounter, mate more frequently than they can recharge their seminal stores and hence transfer a reduced ejaculate to the female (Dewsbury, 1982; Kendall et al., 2002). Since an increased OSR has been shown to decrease duration of copulation in male-biased situations (e.g., the walnut fly Rhagoletis juglandis (Alonso-Pimentel and Papaj, 1996), water strider Gerris remigis Say (Clark, 1988), and blue crab (Jivoff, 1997a)), changes in the OSR are likely to be particularly important in species that pass more ejaculate with longer copulation (Jivoff, 1997a,b).

A decline in the average size of males may reduce the potential fecundity of females. In many species, such as the thysanuran Elaphrothrips tuberculatus and the water strider G. remigis, larger males have a competitive advantage (Crespi, 1989; Kruppa and Sih, 1993). This is true for the blue crab as well. Large males have larger chelipeds, which improves their success in aggressive interactions, increases their ability to hold ("cradle carry") females during pre- and post-copulatory mate guarding, and may aid in protecting the pair against predation (Jivoff, 1997b). Previous laboratory studies have also indicated that larger males pass larger ejaculates and more sperm to females (Jivoff, 1997a; Kendall et al., 2002). Removing the larger males from the population will increase the proportion of small males, provide them with unnatural opportunities to mate, and potentially result in delivery of smaller amounts of ejaculate, on average, to the females.

Reducing the number and size of males in the population may result in sperm limitation, in which the quantity or quality of sperm provided by the male is insufficient to fertilize the potential lifetime egg production of the female (Hines et al., 2003). The reproductive capacity of a female is related to the amount of sperm she receives (Tschinkel, 1987), and reducing the amount of sperm passed by males may reduce the reproductive potential of the population. Reducing the amount of fluid that accompanies the sperm could exacerbate this. Accessory fluid aids in storage and retention of sperm in female Drosophilia melanogaster (Neubaum and Wolfner, 1999; Tram and Wolfner, 1999) exhibits antibacterial activity in the mud crab Scylla serrata (Forskal) (Jayasankar and Subramoniam, 1991), and is a nutritive substance for sperm in both the male and female reproductive tracts of this species (Subramoniam, 1993). The accessory fluid in the blue crab is known to function as a sperm plug and may have other roles (Hines et al., 2003). The amount of accessory fluid given to a female blue crab is reduced in the absence of other males (Jivoff, 1997b), indicating that fluctuations in OSR may have consequences beyond those caused by changes in sperm delivery.

Species that have a limited window for mating are especially susceptible to sperm limitation (Hines et al., 2003). Female blue crabs are subject to this; they are receptive for only hours to days, while still soft after their molt to maturity (the pubertal molt; Pyle and Cronin, 1950; Van-Engel, 1958), and only approximately 12% of them mate more than once during that time (Jivoff, 1997b). During that brief window for mating they receive all the sperm they will use to fertilize their lifetime's production of broods.

We examined impacts of the removal of large males on reproductive potential of the population. It is no longer possible to directly compare fished and unfished populations to examine the potential effects of the fishery because the fishery in the Chesapeake Bay, active since the 1880s, has left virtually no population unfished. Instead we field-collected paired and unpaired crabs from areas with differing fishing pressure, and compared the sizes of mating males and females, the ejaculate resources possessed by males available as mates, and the seminal resources they provided to females.

Because the experimental design was predicated upon blue crab reproductive biology, a brief review of mating behavior is presented here. Mating starts in late May in some locations in lower Chesapeake Bay with a synchronous molting of many prepubertal females (the "peeler run"), but in the upper Bay the onset is later (June–July) and more gradual. Mating continues Bay-wide throughout the summer (July through September). Prepubertal females approaching their terminal molt to maturity (distinguished by their purplish triangular abdomen) become attractive to mature males, with which they form a pre-copulatory pair ("doubler"). The male "cradle carries" the female for 2 or more days (precopulatory mate guarding) until she is ready to molt, assuring himself access to a female while she is receptive to mating (i.e., while she remains soft after her terminal molt). After the female molts, the male turns her over, inserts his two gonopods into her gonopores, and transfers sperm and accessory fluid over the next 5 to 12 h (Pyle and Cronin, 1950; Van-Engel, 1958). Upon completion of mating, the male turns the mature female (distinguished by a semicircular rather than triangular abdomen) upright and continues to cradle carry her. This post-copulatory mate guarding, which may last 2 or more days, helps assure the male's paternity by protecting the female from being preyed upon while she is still soft, and by denying access by other males until the introduced accessory fluid hardens to form a sperm plug (Van-Engel, 1958). This apparently is an effective means of preventing multiple inseminations, and blue crabs do not appear to fit the game theory models of sperm competition (e.g., Parker, 1990). Only a small proportion of females are found with two, or rarely three, sperm plugs (12.4% in our study area in recent years, Jivoff, 1997b), indicating that most females mate with a single male.

2. Methods and materials

2.1. Research location

This work was conducted in the summers of 1999 and 2000 in Chesapeake Bay waters near the Smithsonian Environmental Research Center (SERC). SERC is located on the Rhode River, a sub-estuary in the northwest Chesapeake Bay (38°51′N, 76°32′W) near Edgewater, Maryland, with predominantly silty-mud bottoms except some sandy areas near shore (Hines et al., 1987). Salinities fluctuate between 4–5 in spring and 12–15 in fall, and water temperatures range from 0 °C in January to 28–29 °C in July.

This site was chosen because it was the location of several previous studies of blue crab reproductive biology, and provided the opportunity to sample subpopulations of crabs subjected to differing degrees of fishing pressure. Fishing regulations prohibit the use of crab pots within the upper Chesapeake's tributary rivers. We regard the tributaries as areas with relatively low fishing pressure, limited to that from the commercial use of baited lines ("trotlines") and from recreational crabbers. We regarded bay waters outside the "pot line" at the mouth of the Rhode and West rivers (Fig. 1) as being subject to higher fishing pressure because commercial crab pots are permitted there. Just outside the "pot line" there often are hundreds of crab pots spaced as closely as 5–10 m.

The life history of the male blue crabs influences their size-specific susceptibility to the fishing pressure at the pot line. Postlarvae (megalopae) recruit into Chesapeake Bay from the Atlantic coastal waters, metamorphose into the first crab stage, and move up the estuary as they mature. While female blue crabs appear to molt to maturity throughout the system, male blue crabs do so preferentially in the tributary creeks (Pearson, 1948; Hines et al., 1987) and hence in the upper Bay (MD waters) are likely to reach the minimum legal size in areas where commercial potting is prohibited. However, when the mature males move out into Chesapeake Bay, they are exposed to a high risk of mortality as they pass
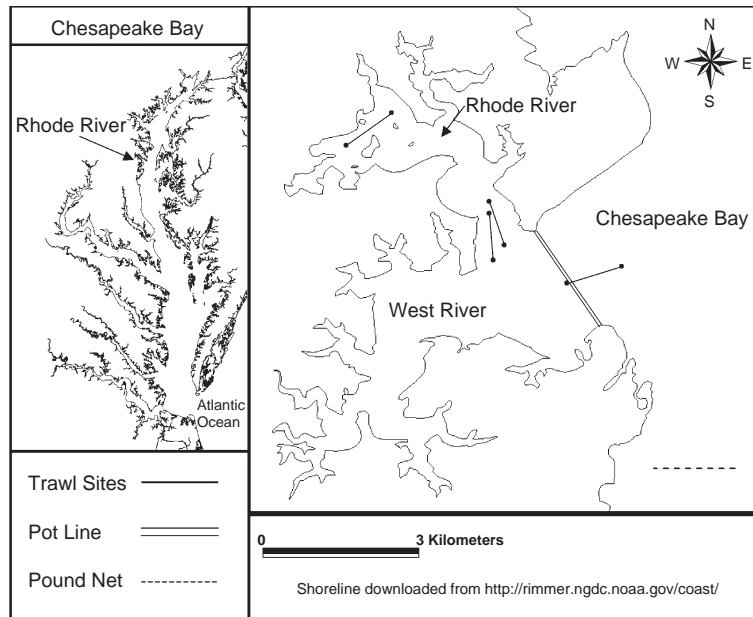
Fig. 1. Study site. Solid lines represent SERC trawl stations. Crab potting is permitted only bayward of the "pot line" (double line). Paired crabs were collected from the pound net south of the Rhode/West River mouth (dashed line).

through the phalanx of pots just outside the "pot line". This spatially differentiated fishing pressure allowed us to compare crabs inside and outside of the pot line to estimate its effects on crab size, ejaculate resources possessed by males, and ejaculate stores provided to females by their mates.

2.2. Field collections

Unpaired and paired crabs were collected and analyzed to assess the number of sperm (by direct counts) and seminal fluid (weight) in each sex. Size of crabs (carapace width "CW" measured point to point) and limb autotomy were noted. Sex of crabs, and maturity of females, were determined by abdominal morphology; maturity of unpaired males was assessed by whether the telson could be retracted.

Unpaired crabs from inside the "pot line" were haphazardly selected from catches of the SERC trawling program (trawl sites shown in Fig. 1). (It was not possible to know if any of these had been paired because they would have been separated by the trawl.) Paired crabs were collected by going 2–3 times/week in August of 1999 and August/September of 2000, to several commercial pound nets 2–3

km south of the Rhode River (approximate location shown in Fig. 1). These are rows of poles set perpendicular to the tidal currents in 2–3 m depth. When in use they are hung with lengths of netting. Paired blue crabs in nature apparently are attracted to such structures, and intact pairs could be dip-netted from them with relative ease if the netting was in place, the tidal height was appropriate, water was clear and weather was calm. (Unpaired crabs were rarely seen on, and never captured from, pound nets.) Favorable conditions allowed capture of pairs on August 4 and 5 of 1999, and on August 8, 9, 17, 24, 28 and September 14 of 2000. We collected paired crabs in three stages of the mating process: (1) pre-copulatory mate guarding (38 pairs with pubertal female in premolt), (2) copulation (9 pairs), and (3) post-copulatory mate guarding (13 pairs with female in postmolt adult instar). All crabs were measured. Seminal resources were measured in males from pre-copulatory pairs, and in both members of post-copulatory pairs. Pre-copulatory females were released, since they had not received any sperm. In pairs collected during copulation, it could not be known how far sperm transfer had progressed, so crabs were released and measurements

of seminal resources in each member were omitted as irrelevant.

2.3. Estimation of ejaculate contents

To measure the amount of seminal resources or ejaculate they contained, the males' vasa deferentia and females' spermathecae were removed and weighed. A factor, based on a correlation with CW of the mature female, was used to correct for the weight of the spermathecae themselves: wt. of spermathecal walls (g)=0.01*CW (mm)−0.1224 (D. L. Wolcott, unpubl. data). Since the weight of the vas deferens wall is insignificant, a correction factor was not required for males.

Direct counts of sperm in the spermathecae and vasa deferentia were used to determine the number of sperm present in female and male crabs respectively. After the organ was weighed it was preserved in 70% alcohol. To prepare for counting, the organ was homogenized in artificial seawater (salinity=30), passed through a 35 μm filter to remove tissue debris, and stained with 1% Gentian Violet (1 drop/10 ml filtrate). A sub-sample was pipetted onto a Petroff-Hauser spermacytometer and counted under 1000×. Replicate counts were averaged and used to extrapolate to the total number of sperm.

The amount of sperm and accessory fluid was measured in field-collected unpaired (trawl samples) and paired crabs (from pound nets). Data from unpaired male crabs gave an indication of the available seminal stores in the general population. Those from paired crabs revealed the seminal stores in males before they mated, the amount retained by males immediately after mating, and the amount transferred to the females during mating. Data from paired crabs were also used to correlate ejaculate volume with size of the male. The measurements of seminal stores from males in the field were compared with previous field measurements and with laboratory data (Kendall et al., 2001, 2002) for "fully recharged" or recently mated males in the laboratory.

2.4. Statistical analysis

Two-way ANOVAs were used to test for size (CW) differences among crabs attributable to sampling method (pound nets in/out of river, trawls) or stage

of mating (pre- or post-copulation). Two-way ANOVAs were also used to test for differences in the ejaculate components in pre- and post-copulatory crabs. Normality and homogeneity of variance were tested using residuals and the data were log or square root transformed when necessary. When it was difficult to determine which transformation would be most appropriate based on the residuals, the Box-Cox test was employed. Correlations between male CW and female CW (in pre-copulatory pairs), and the amount or percent of the male's sperm and/or fluid that had been transferred to a female (in post-copulatory pairs) were examined through a series of models. The first model used main (CW and year) and interaction (CW by year) terms. If the interaction was not significant it was removed from the model, and a second model was run with main effects for CW and year. If year was significant in this main effects model, significance of the effect of carapace width was determined from this model. If year was not significant in the main effects model, year was removed and a third model with CW as the only predictor was run in order to test for the effect of male carapace width.

3. Results

3.1. Evidence for fishing pressure: size differences inside and outside of pot line

Mature males that were trawled in the river (mean CW 137 mm, S.E.=1.27) were significantly larger (p=0.0006, MS=135.27, df=2) than those collected from the pound nets in the Chesapeake Bay (mean CW 128 mm, S.E.=2.35). Conversely, mature females trawled from the river (mean CW 156 mm, S.E.=2.97) did not differ significantly in size (p=0.47, MS=48.18, df=2) from those that were collected from the pound net in the Chesapeake Bay (mean CW=158 mm, S.E.=0.92).

To determine if differences in male size were due to sampling method, the carapace widths of mature males collected from pound nets within the Rhode River during a previous study were compared with those of mature males that were trawled in the Rhode River during those same months and years (1991–1994) (Jivoff, 1997a). In that study the male crabs collected at pound nets within the Rhode River were
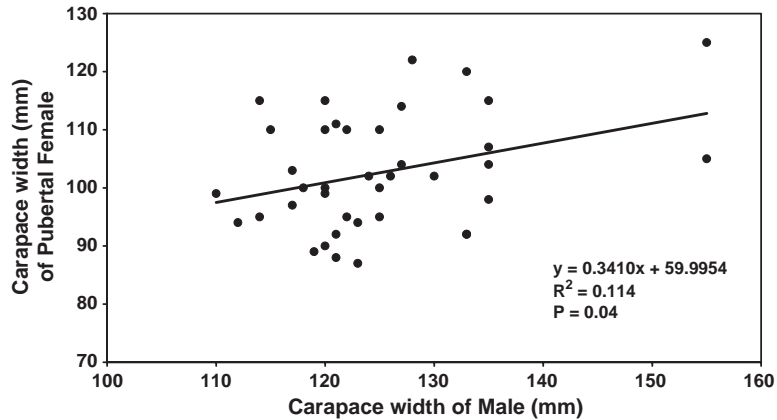
Fig. 2. Relative sizes of males and females in pre-copulatory pairs, showing weakly size-assortative mating.

significantly ($p=0.003$, MS=489.82, $df=14$) larger (mean CW 136 mm, S.E.=0.80) than those collected nearby in the trawls (mean CW 133 mm, S.E.=0.94, $p=0.003$). This indicated that the smaller average size of males collected from the pound nets out in the Chesapeake Bay was not an artifact of sampling method.

3.2. Size of males in mating pairs

Unless otherwise indicated, size of males did not differ significantly between years ($p>0.05$) and the data were pooled over the 2 years. There was no difference ($p=0.3$, MS=136.1, $df=2$, $F=1.34$) in the average CW of the paired male crabs in any of the mating stages: pre-copulatory (125 mm), mating (124 mm), or post-copulatory (130 mm). About half of the males engaged in mating were below the minimum legal size for the fishery. There was a significant correlation between male CW and pre-pubertal female CW ($p=0.04$, MS=416.65, $df=1$) indicative of size-assortative mating, but the regression explains only 11% of the variance (Fig. 2). All of the comparisons from pound net mating pairs were affected by the nearly complete absence of large males from the population. Because the upper end of the size range was severely under-represented (Figs. 2–5), the two fairly large males in the samples may have had a disproportionate effect on the statistics.
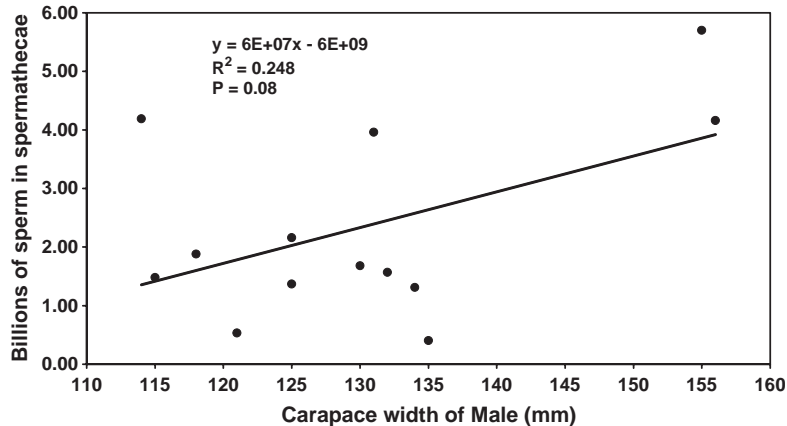

Fig. 3. Total number of sperm transferred to (post-copulatory) females' spermathecae as a function of their mate's size (carapace width).
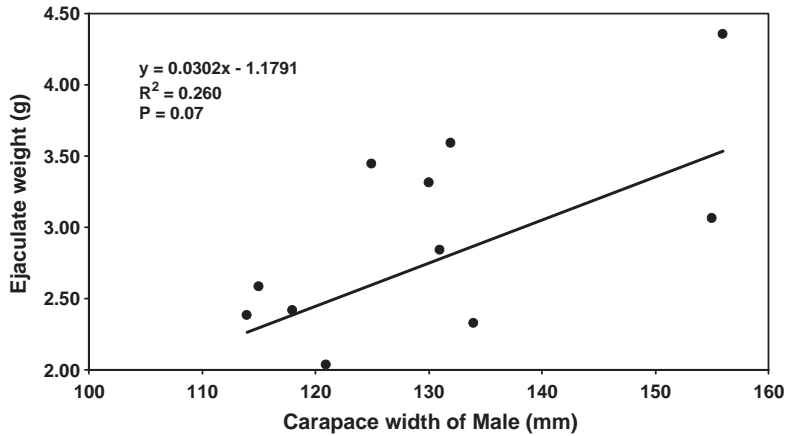
Fig. 4. Weight of ejaculate (principally seminal fluid) in post-copulatory females' spermathecae as a function of their mate's size (carapace width).

3.3. Transfer of sperm and accessory fluid as function of males' size

Essentially all of the mature females collected from this population, whether taken unpaired or as members of post-copulatory pairs, had mated (evidenced by ejaculate in their spermathecae). The number of sperm they received ranged from 0.04×10^9 to 5.7×10^9. Those that mated with the larger males may have received larger amounts of both sperm and fluid but the relationship is not strong. The regressions of sperm/fluid transfer vs. CW of the male are only weakly suggestive of a relationship; they are significant only at alpha levels of 0.08 (MS=7.84, df=1) and 0.07 (MS=1.91, df=1) respectively with the number of samples available, and explain only about 25% of the variation (Figs. 3 and 4). In both 1999 and 2000 there was a strong correlation between a male's size and the percentage of his sperm given to his mate during copulation (p=0.02, R^2=0.799). Because the relationship differed between the years, they were analyzed separately. In 1999 the average proportion of the
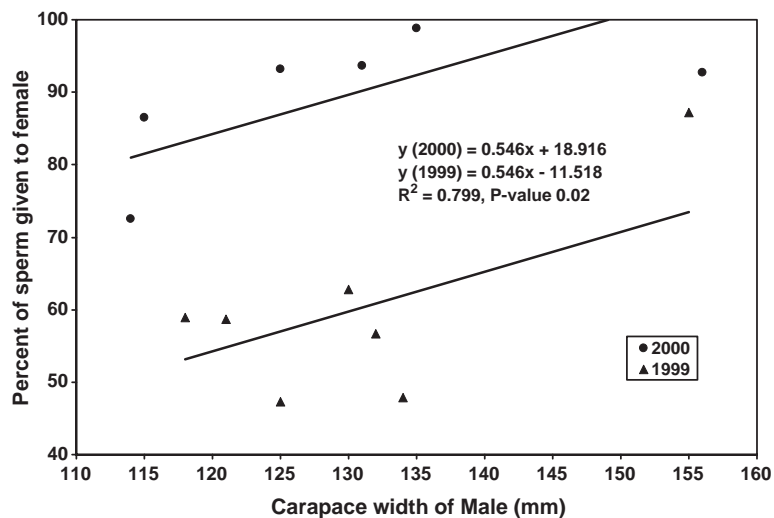

Fig. 5. Proportion of males' sperm that was transferred to their (post-copulatory) mates, as a function of the males' size (carapace width). Triangles and lower regression line, 1999; circles and upper regression line, 2000.

male's sperm given to a female was 60% (range 47–87%); in 2000, it was 90% (range 72–99%) (Fig. 5).

The percentage of accessory fluid that was transferred to a female averaged 58% (range 49–70%), and was not significantly related to the male's size ($p=0.14$). Year was not significant in this analysis ($p>0.88$) and the data were pooled over the 2 years.

3.4. Seminal stores of males

Pre-copulatory males, on average, possessed a significantly greater number of sperm (30.89×10^8, S.E.$=3.20 \times 10^8$), than did post-copulatory males (7.638×10^8, S.E.$=5.55 \times 10^8$, $p=0.0001$); they also had more accessory fluid (3.312 g, S.E.$=0.14$ g) than did post-copulatory males (1.928 g, S.E.$=0.25$ g, $p=0.001$) (Fig. 6). The number of sperm possessed by either pre- or post-copulatory males bore essentially no relationship to their carapace width (Fig. 7).

Even though the pre-copulatory males generally had greater seminal stores than did post-copulatory males, many of them had less than predicted if they had been "fully recharged" (4–5 g vas deferens weight, 2 to 2.5×10^9 sperm, Kendall et al., 2001) and the ranges overlapped broadly. The sperm count ranged from 7.28×10^8 to 1.02×10^{10} (average 3.08×10^9) in pre-copulatory males and 4.65×10^6 to 1.59×10^9 (average 7.9×10^8) in post-copulatory males. Twenty-five percent (10 of 39) of the pre-copulatory males had sperm counts that fell into the same low range ($\leq 1.59 \times 10^9$) observed in the post-copulatory males, and three of them had sperm counts even lower than the average of the post-copulatory males. Similarly, the range of accessory fluid in pre-copulatory males was 1.469 g to 5.862 g (average 3.312 g) and in post-copulatory males was 1.349 g to 2.649 g (average 1.94 g). Twenty eight percent (11 of 39) pre-copulatory males had vas deferens weights falling in the range observed in post-copulatory males,
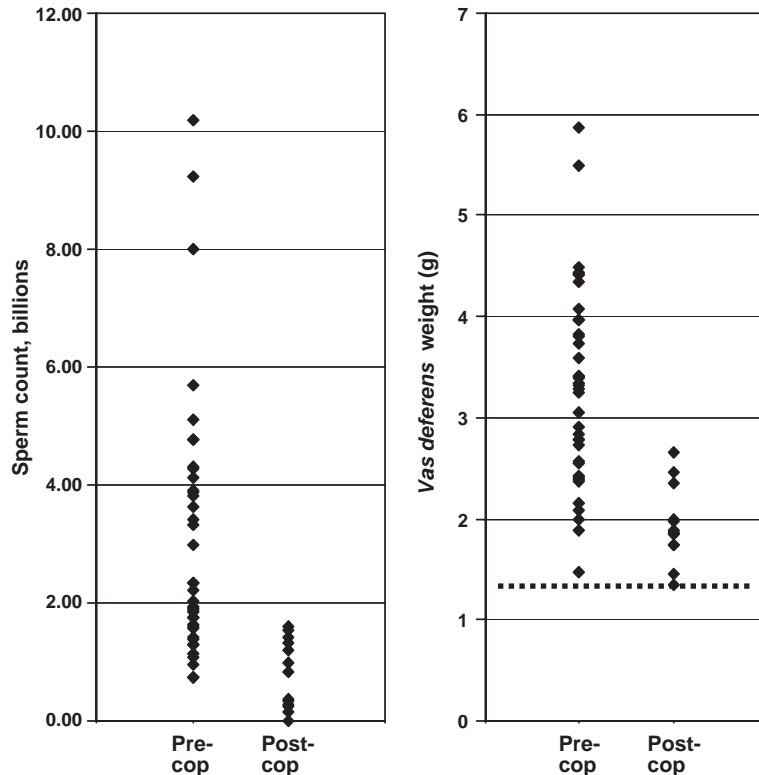

Fig. 6. Numbers of sperm in vasa deferentia (left panel) and total weight of vasa deferentia (right panel) of pre- and post-copulatory paired males. Dotted line in right panel is 1.38 g threshold for "sperm depleted".
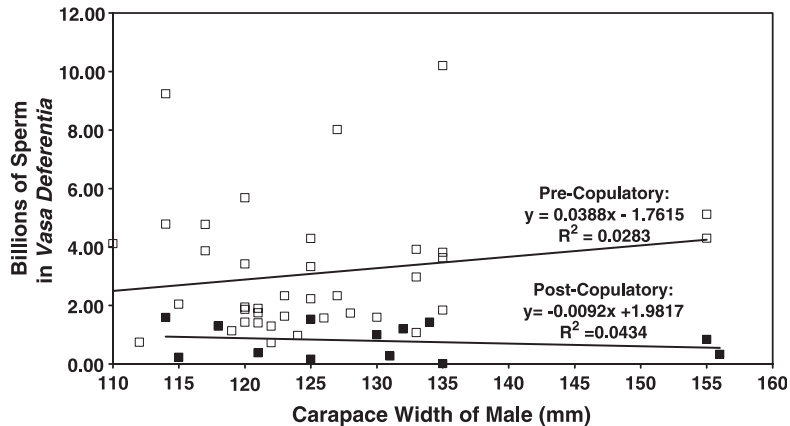
Fig. 7. Number of sperm in vasa deferentia of paired males as a function of carapace width. Open squares and upper regression line, pre-copulatory males; solid squares and lower regression line, post-copulatory males.

with two even below the average for post-copulatory males.

Kendall et al. (2001) demonstrated that more than 50% of unpaired intermolt male crabs collected from the Rhode River in 1996 and 1997 were sperm-depleted, with vas deferens weights below the 1.38 g observed in males immediately after laboratory matings. In our study, 26% of the unpaired crabs (n=15) collected from the Rhode River were depleted below the 1.38 g threshold. Although our sample size was small, it demonstrated that seminal resources of our unpaired males exhibited the same range of depletion as observed earlier by Kendall et al. (2001). None of our pre-copulatory males were "sperm-depleted" according to the 1.38 g criterion.

The number of sperm in vasa deferentia of pre-copulatory males ranged from 0.73×10^9 up to 10.2×10^9. In this population of actively mating males, with unknown recent mating histories, there was no relationship between sperm stores and carapace width; a linear regression explained less than 3% of the variance (Fig. 7).

4. Discussion

Our observations indicate that the size-selective male-focused fishery has affected the size of male blue crabs in the population, and probably thereby the delivery of seminal resources to females, in the Rhode River area. Males collected from pound nets

where crab pots are abundant were smaller (average CW 128 mm) than those collected by trawl inside the pot line where potting is prohibited (average CW 137 mm). This was not due to differences in collection methods (trawling vs. dip-netting from pound nets); control measurements of males from pound nets inside the pot line confirmed that they were larger than males collected nearby in trawls. Neither could the size difference be attributed to environmental factors that differed between river and Bay, since females collected from outside the pot line were the same size as those in the river. It appears that in MD waters the larger males are being "filtered out" of the population as they move from tributaries and must run the gauntlet of pots just outside the pot line on their way out into the Bay. Sexually mature males grow by small (25–33%) size increments at each molt (Pyle and Cronin, 1950; Van-Engel, 1958), and in the years of this study became susceptible to the fishery as soon as they reached a CW of 127 mm. Females, on the other hand, typically remain below the legal size until their final molt to maturity, at which they may increase in size (width) by up to 56% (Darsono, 1992). They go from an average prepubertal size of 115 mm to an average mature size of 155 mm (Abbe and Stagg, 1996). Consequently, the size selectivity imposed by fishing pressure presumably has little impact on females: prepubertals are almost all sublegal (except those taken as "peelers") and matures are almost all legal (Abbe and Stagg, 1996).

Since larger females are capable of producing larger broods (Hines, 1982) and larger males may give more seminal stores to females, selection should favor individuals of both genders pairing with as large a mate as possible. Models of optimal sperm allocation suggest that serially mating males with finite sperm resources should allocate sperm preferentially to the highest quality females, and husband their sperm early in the breeding season while assessing the quality available in the population of females (Galvani and Johnstone, 1998). The applicability of such models to blue crabs probably is limited; the species evolved in (unfished) populations, in which there often is a pulse of females molting to maturity at the beginning of the season. Because the females' receptivity is so abbreviated, and because the natural sex ratio was not biased toward females, males probably would gain little advantage by postponing mating. There is limited evidence for mate choice; size-assortative mating has been observed in the laboratory, as larger male blue crabs competitively displace smaller ones from large prepubertal females (Jivoff, 1997a). Progressive displacement of smaller males in nature would lead to an increasing association between large males and large females during the course of the mating period (pre-copulatory embrace, mating, post-copulatory embrace). There was no such increase in the relative size of males through the mating sequence in our paired crabs; the size-assortative mating we observed (Fig. 2), which accounted for only 11% of the variance, seems to have been established at initial pair formation.

Whether this competitive displacement would have occurred if the largest size class of male blue crabs had not been almost eliminated from the population cannot be tested (note the size distribution of males in Fig. 2). Males as large as 200 mm still occur within the Rhode River (pers. obs., SERC trawls), but at the pound nets in the Bay they ranged from 110 to only 156 mm. Females that mate in the Bay may be at a reproductive disadvantage compared to those that mate in the river, where the absence of crab pots allows a few large males to persist, or to females in the Bay population of several decades ago, before the fishery eliminated almost all of the large males.

Whether large males provide a larger ejaculate to females is exceedingly important for fisheries management issues. The logical assumption is that as

males molt to larger sizes they increase the size of their reproductive tracts and seminal stores, and hence their reproductive potential. If this is true, the preferential removal of large males could be decreasing the reproductive potential of the population. Prior studies involving sperm limitation and blue crab reproduction have varied in their conclusions regarding the amount of sperm that different size classes of males provide to females (Jivoff, 1997a; Hopkins, 2002; Kendall et al., 2002). These studies were conducted in controlled laboratory environments where the mating status of the male prior to copulation, the number of males, and the number of females present during mating were predetermined. Our results are from males that were field-collected from natural situations and undisturbed by experimentation, but could be collected only in modest numbers that limit the power of statistical tests (six and seven pairs in the 2 years). The data nevertheless indicate that larger males may give a larger number of sperm and greater absolute amount of accessory fluid to females (weak correlation, Figs. 3 and 4), and that larger males give a significantly greater proportion of their sperm resources to the female during mating (Fig. 5). However, in our population with mixed mating histories, the number of sperm males possess is essentially independent of their size (Fig. 7), perhaps because the male's recent mating history has a far greater effect than his size (Kendall et al., 2002). What is clear is that females molting to maturity in areas with heavy fishing pressure are likely to mate with smaller males than would females from lightly fished areas. What we do not know is if the amount of sperm they receive is less than they would have received from the largest male size classes that presumably were dominant competitors for mates, but now have become so rare.

Although larger males provide females with greater absolute amounts of accessory fluid than do smaller males, they do not give a greater percentage of their seminal fluid stores. In blue crabs, accessory fluid is known to act as a sperm plug that may aid in sperm retention and presumably protects the male's paternity by hindering additional mating (Jivoff, 1997b). Since the production of accessory fluid involves an energetic cost (Turner et al., 2003), it may be beneficial to give the female only enough to fulfill these roles. Sperm production also entails an energetic cost

(Dewsbury, 1982), but since delivering an increased number of sperm increases the potential number of eggs fertilized, that energy expenditure may have been favored by selection. These considerations might lead to the prediction that small males, unable to defend mates for long against larger competitors, would invest principally in sperm that might win them some paternity, rather than wasting energy on fluid that would be insufficient to form an adequate sperm plug. This does not appear to be the case; small and large males contribute similar proportions (about 58%) of their seminal fluid during mating.

The average percent of a male's stored sperm given was 60% in 1999 and 90% in 2000. In all instances the males were transferring more than half of their seminal stores to their mates. The higher percentage of sperm transferred to a female during mating in 2000 may have been an effect of low population density on mating dynamics. If a male is encountering very few potential mates, there may be selective value to investing heavily in the ones he does find. The numbers of crabs during the 2000 fishing season were particularly low; routine trawl sampling in the Rhode River by SERC showed that the average number of crabs was less than half of the 1999 abundance (Carver, 2001), and many of the commercial crabbers abandoned potting because it became unprofitable.

All of the unpaired and paired adult postmolt females examined during this study had mated. Other studies of mating success in blue crabs indicate that more than 97% of the females in several populations successfully mate (South Carolina, Wenner, 1989; Chesapeake Bay, Jivoff, 1997a; Jivoff and Hines, 1998; D.L. Wolcott, unpubl. data). If essentially all of the female crabs mate, then the population will be sperm limited only if the number of sperm supplied is less than that needed to fully fertilize a female's lifetime potential brood production. A large proportion of the unpaired male intermolt crabs sampled from the Rhode River was sperm depleted, with vas deferens weights below the average of 1.38 g for recently mated male crabs (Kendall et al., 2001). However, all males collected from pre-copulatory paired males in our Chesapeake Bay site had ejaculate stores above the 1.38 g threshold (Fig. 6). This indicates that the most-depleted males in the general population were not actively mating.

Because the most-depleted males are not mating, the Operational Sex Ratio is actually higher than would be calculated from the ratio of adult females to adult males. One consequence is that males are mating more frequently than they can recharge their seminal stores; this apparently causes a greater reduction in sperm delivery than does the reduction in the average size of males (Kendall et al., 2002). Although pre-copulatory males had heavier vasa deferentia than males in the general population, and had, on average, more seminal stores than post-copulatory males, some of the pre-copulatory males that were about to mate were as sperm depleted as those males who had just completed copulation. Clearly, many females are mating with males having seminal resources reduced by prior matings, and are receiving less ejaculate than if they had mated with a more fully recharged male. The probability of mating with a depleted male is particularly high during a "peeler run", when large numbers of prepubertal females synchronously molt to adulthood and present males with receptive mates at intervals too short to permit regeneration of seminal resources. These females receive smaller ejaculates than do those that mate at a comparable location during other seasons (D.L. Wolcott, unpubl. data).

It is difficult to assess the degree to which size-selective fishing pressure, by modulating the average size of males and the OSR, is affecting population reproductive output. Sperm is considered limiting if a female receives an insufficient amount to fertilize her full reproductive potential (Hines et al., 2003), but how much is "sufficient" is poorly understood. If sperm delivery is reduced but the amount that the female receives is still enough to fertilize her lifetime reproductive output, then the reduction is inconsequential. There is by now considerable evidence, albeit circumstantial, that females in the Chesapeake Bay are becoming sperm limited prior to reaching their full brood potential (Hines et al., 2003).

How many sperm are "enough"? This is a function of how many broods a female may be expected to produce in her lifetime, the size of each brood, the number of sperm required to fertilize each egg, and the viability of the sperm during storage in the spermathecae. In the Chesapeake Bay population we sampled, all but one of the females mated with a male smaller than 140 mm CW. Those females received an average of 2.34×10^9 sperm. A sperm/

egg ratio of about 83:1 is used during fertilization (P. Jivoff, pers. comm.), allowing us to calculate that this amount of sperm is sufficient to fertilize about nine average-sized broods of 3×10^6 eggs each. This calculation assumes that all sperm remain viable from mating until production of the first brood, but Hopkins (2002) showed that nearly half of the sperm disappear during that period in crabs that produce the first brood in the same summer they mated. This brings the estimated potential number of fertile broods down to about 4.5, even assuming that there is no further mortality of sperm between broods. A large proportion of female blue crabs, at least from upper Chesapeake Bay, mates in one summer but produces no broods until the next spring (Turner et al., 2003). The proportion of sperm surviving such long storage is unclear. Although under laboratory conditions 50% of the sperm disappear within 12 weeks after mating, sperm stores of North Carolina females collected from the field in April (before mating begins) are not markedly different from those of females collected the previous November (Hopkins, 2002). If females initially receive small ejaculates from small or previously mated males, or if a large proportion of the sperm are lost over the winter, females that have begun brooding may possess insufficient sperm to fertilize the maximum potential number of broods (6–7 in Chesapeake Bay) postulated by Hines et al. (2003). Females that receive small ejaculates may not be able to produce even one fully fertile brood. Given increasing evidence of excessive pressure from the fishery (e.g., Lipcius and Stockhausen, 2002), it will be imperative to obtain direct measures of how reduced sperm delivery by small or depleted males is affecting reproductive output of females in nature.

Acknowledgements

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