Reproductive potential of individual male blue crabs, *Callinectes sapidus*, in a fished population: depletion and recovery of sperm number and seminal fluid

Matthew S. Kendall, Donna L. Wolcott, Thomas G. Wolcott, and Anson H. Hines

Abstract: We evaluated the depletion and recovery rates of sperm number and vas deferens weight following mating for male *Callinectes sapidus* both below (<127 mm carapace width) and well above (>140 mm) the fishery size limit for hard crabs in Chesapeake Bay (127 mm). Large males had low sperm count and vas deferens weight immediately after mating and required approximately 9–20 days to fully recover. After mating, small males had significant reduction in sperm number despite no significant change in vas deferens weight. Furthermore, small males with completely recovered seminal stores had significantly lower vas deferens weight than fully recovered large males but did not differ significantly from large males in number of sperm. The changes in vas deferens weight and sperm count following experimental mating suggest that large males delivered 21 times as much seminal fluid and 2.25 times as much sperm as small males. Field collections in a subestuary of Chesapeake Bay revealed that the majority (50–90%) of males had extremely low vas deferens weight relative to males with fully recovered sperm volume. Since the fishery targets males primarily, reducing both the number and average size of males in the population, many females may be mated with small or recently mated males that transfer less seminal material.

Résumé : Nous avons évalué les taux d'épuisement et de récupération du nombre de spermatozoïdes ainsi que la masse du vas deferens après l'accouplement chez des Crabes bleus, *Callinectes sapidus*, mâles de tailles inférieure (<127 mm de largeur de carapace) et bien supérieure (>140 mm) à la taille minimale de capture dans la baie de Chesapeake (127 mm). Chez les mâles de grande taille, le nombre de spermatozoïdes et la masse du vas deferens sont faibles immédiatement après l'accouplement; ils mettent environ 9–20 jours à revenir complètement à la normale. Après l'accouplement, les mâles de petite taille ont une réduction significative du nombre de leurs spermatozoïdes sans avoir de changement significatif dans la masse de leur vas deferens. De plus, après la récupération complète des réserves de spermatozoïdes, les petits mâles ont le vas deferens moins lourd que les grands mâles, sans qu'il y ait de différence significative entre les deux groupes dans le nombre des spermatozoïdes. Une étude du changement de masse du vas deferens et du nombre des spermatozoïdes après des accouplements expérimentaux laisse croire que les grands mâles libèrent 21 fois plus de liquide séminal et 2,25 fois plus de spermatozoïdes que les petits mâles. Des échantillons recueillis dans un sous-estuaire de la baie de Chesapeake indiquent que chez la majorité (50–90%) des mâles, la masse du vas deferens extrêmement faible par comparaison à des mâles ayant pleinement récupéré leurs réserves. Puisque la pêche cible surtout les mâles, ce qui réduit tant le nombre que la taille moyenne de ceux-ci dans la population, plusieurs femelles doivent s'accoupler avec de petits mâles ou avec des mâles qui viennent de se reproduire et qui libèrent moins de semence.

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Introduction

The reproductive contribution made by males is rarely examined when determining stock–recruit relationships. Sperm and seminal fluid are usually considered to be both energetically inexpensive for males to produce and easy to distribute to many females. The reproductive potential of a population is often estimated by counting mature females and, in managed species, spawning stocks are frequently maintained by allowing females to reach maturity prior to harvest, ignoring paternal influences altogether. Recent research suggests that this approach of assuming ample sperm and (or) seminal

M.S. Kendall.[1] National Oceanic and Atmospheric Administration / National Ocean Service, Center for Coastal Monitoring and Assessment, 1305 East-West Highway N/SCI1, Silver Spring, MD 20910, U.S.A.
D.L. Wolcott and T.G. Wolcott. North Carolina State University, Department of Marine Earth and Atmospheric Sciences, P.O. Box 8208, Raleigh, NC 27695-8208, U.S.A.
A.H. Hines. Smithsonian Environmental Research Center, P.O. Box 28, Edgwater, MD 21037, U.S.A.

[1]Corresponding author (e-mail: matt.kendall@noaa.gov).

fluid resources may be an oversimplification of the reproductive system in many decapod crustaceans (Ennis 1980; Smith and Jamieson 1991; Paul and Paul 1992). In many managed species in which only female spawning stock is protected, the consequence of overharvesting males can be a reduction in reproductive potential via sperm limitation or insufficient seminal fluid.

The male contribution to reproduction includes both sperm and seminal fluid. If sufficient numbers of spermatocytes are not available to fertilize a female's egg production, sperm limitation will result. Furthermore, insufficient availability of seminal fluid may also limit reproductive potential. Seminal fluid is not only the medium in which sperm is transferred but may also be necessary for successful sperm storage. For example, the seminal plasma of some brachyuran crabs contains a large amount of organic substances used for sperm metabolism (Subramoniam 1991). In addition, it has been shown that proteins found in seminal fluid play a critical role in effective sperm storage in *Drosophila melanogaster* (Tram and Wolfner 1999) and even act as antibacterial agents that may protect the reproductive tract of the mud crab, *Scylla serrata* (Jayasankar and Subramoniam 1999). Seminal fluid also plays a role in forming a "sperm plug" that may function to prevent sperm from leaking out of a female once deposited and (or) to block competing males from depositing additional sperm (Ryan 1964; Hartnoll 1969; Sainte-Marie and Sainte-Marie 1999).

Certain characteristics of a population's reproductive strategy suggest an inherent possibility of sperm and (or) seminal fluid limitation. For example, sperm limitation may occur in systems in which females fertilize multiple broods or large numbers of eggs using sperm stored from one mating event. Females may simply run out of sperm, as is the case for large American lobsters (*Homarus americanus*) that spawn twice in the same instar (Waddy and Aiken 1986), or female tanner crabs (*Chionocetes bairdi*) that do not receive sufficient sperm to fertilize multiple broods when inseminated only during their molt to maturity (Paul and Paul 1992).

Mating systems in which females must store sperm for a long time between copulation and fertilization may also be sperm and (or) seminal fluid limited. Sperm viability may decline over time, especially if energy sources for metabolic requirements of sperm are insufficient. Seminal plasma has been shown to serve the nutritional requirement for metabolism of stored sperm in such species as the crab *S. serrata* (Subramoniam 1993). The transmission of adequate seminal fluid may be critical for maintaining sperm viability.

Populations that have a low density of males with high reproductive fitness may also be susceptible to sperm limitation. Reduced reproductive fitness in males may result when poor health or some other physiological constraint, such as molting or immaturity, results in decreased sperm and (or) seminal fluid production or transfer capability. High mating frequency of males also may reduce a population's reproductive potential. Females receive less sperm or seminal fluid if males mate more frequently than reproductive resources can be replenished. These males would deliver reduced quantities of sperm and (or) seminal fluid to their second and subsequent mates. For example, large male king crabs (*Paralithodes camtschatica*) show a reduction in fertilization success after nine successive matings (Powell et al. 1973).

Fertilization success may also be lower in small mature males that do not produce or transfer as much sperm and (or) seminal fluid as large mature males. In contrast with large male king crabs, small males show a reduction in fertilization success after only seven successive matings (Powell et al. 1973). In the crab *S. serrata*, there is an increase in spermatophore size as well as in the concentration of inorganic ions and organic substances in seminal fluid as males mature (Subramoniam 1993).

Populations with a low density of males (in any reproductive condition) may also be sperm limited. Under this condition, females may have to wait some period of time to find a mate, leaving their reproductive potential unfulfilled for that period. When densities of males are extremely low, females may not be able to find a mate at all. Large female American lobsters and Dungeness crabs (*Cancer magister*) probably experience difficulty finding suitable mates in areas where the fishery intensively exploits males (Ennis 1980; Smith and Jamieson 1991). This situation can be aggravated if the sexual receptivity of females is temporally limited. For example, female king crabs and snow crabs (*Chionocetes opilio*) are sexually receptive for only a few days each year (Sainte-Marie and Lovrich 1994).

Mating systems with one or more of these elements provide effective models with which to investigate sperm limitation. Blue crabs, *Callinectes sapidus*, have many of these attributes: males can mate repeatedly, females mate only once during a 2- to 3-year life span, females are sexually receptive only during their molt to maturity (~1 week during their entire lifespan), reproduction is highly seasonal at the population level (most mating in the Chesapeake Bay occurs during bursts in the spring and summer), and females use stored sperm and seminal fluid from an individual male's ejaculate over a period of several months to fertilize multiple broods of ca. $1–4 \times 10^6$ eggs each (Millikin and Williams 1980; Prager et al. 1990).

The likelihood that limiting conditions will arise in *C. sapidus* is aggravated by the effects of an intense fishery. In Chesapeake Bay (U.S.A.), the fishery has a minimum size limit (127 mm carapace width (CW)) as well as restrictions on the harvest of some ovigerous females and, hence, selectively removes large males from the population. A study during Maryland's summer fishery suggests that fishery pressure may be 3–6 times higher on males than on females (Casey et al. 1990). Remaining small males that escape the fishery are left to mate with the still relatively unfished female population (mature females are also fished from the population, but only after sperm resources are allocated). In addition, a monitoring program in mid Chesapeake Bay indicates that average male size has been declining over the past 30 years as pressure from the fishery has increased, whereas female size has shown no such decline (Abbe and Stagg 1996). Ejaculate size is positively correlated with male size (see Results; Jivoff 1995); therefore, although females in the population are presumably capable of maintaining normal levels of egg production (egg production is correlated with female size; Prager et al. 1990), they may actually be fertilizing fewer eggs with a reduced quantity of sperm and (or) seminal fluid received from fewer and smaller males.

It should be possible to determine if sperm limitation is occurring in this system by measuring ejaculate size

1170

Can. J. Fish. Aquat. Sci. Vol. 58, 2001

(amount of sperm and seminal material that females receive), estimating lifetime egg production for females, and determining if egg production exceeds the fertilization potential of sperm resources; however, researchers have had difficulty keeping mature females under suitable brooding conditions for long enough to obtain reliable estimates of lifetime egg production and fertilization rates (Sulkin et al. 1976; Darsono 1992).

Instead, we chose to explore the potential for sperm and (or) seminal fluid limitation in this system by identifying underlying mechanisms that could bring about the limitation. Operational sex ratios reveal whether male abundance is low, thus potentially inducing limiting conditions. Assessing this ratio involves not only counting the overall numbers of males and receptive females, but also identifying variables that affect competition for mates, such as the size, condition, and molt stage of males. It is also possible to determine if sperm limitation is likely to occur by examining the reproductive condition of males in areas where mating occurs. If most males have abundant seminal resources, sperm limitation is unlikely. Conversely, if many males in the population have low sperm and (or) seminal fluid volume, females mated with these males may experience reduced fertilization success. Because of the fishery's influence on the size distribution of males in the population, it is also important to determine the relationship between size and the reproductive potential of males, as well as to estimate the replenishment rates of sperm number and seminal fluid following mating for males of different sizes.

The purpose of this study was to determine the likelihood that sperm limitation occurs in a Chesapeake Bay population of *C. sapidus* by (*i*) determining the influence of male size and mating history on their reproductive potential, (*ii*) determining the temporal limitations for replenishment of sperm number and seminal fluid following mating for males larger and smaller than the fishery size limit, and (*iii*) determining the reproductive condition of males in an area where mating occurs.

Materials and methods

All experiments were conducted at the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland, U.S.A. (38°51′N, 76°32′W) (Fig. 1). SERC is located on the Rhode River, a subestuary draining into northwest Chesapeake Bay. Salinity in the Rhode River ranges from 5 to 15 psu (practical salinity units) from spring to fall and the temperature ranges from 0 to 29°C from winter to summer.

Reproductive resources of males: the role of size and mating history

We compared the reproductive condition of small and large male crabs, as well as the recovery rates of sperm stores for crabs above and below the fishery size limit (127 mm CW). Male blue crabs were collected by crab pot in the Rhode River and separated into large (>140 mm CW) and small (115–127 mm CW) size-classes. Size-class limits were selected to ensure that all individuals in the small size-class were mature (males reach maturity at ~110 mm CW; Van Engel 1990) but below the current harvestable size limit for the blue crab fishery in Maryland. The large size-class limit (>140 mm CW) was selected to provide a contrast group well above the legal limit of the fishery. Only intact intermolt (hard-shelled; molt stage C) animals were used in these experiments.

To permit the recovery of sperm resources, males were held in large submerged cages near the SERC dock for 20 days prior to experimentation. Preliminary research indicated that this provided sufficient time for seminal resources that might have been spent prior to collection to be replenished. They were fed a mixed diet of fish and molluscs placed in the enclosures each day. The amount of food offered was based on estimated daily feeding rates for crabs of this size (Nye 1989). At the conclusion of the recovery period, crabs were assessed for molt stage and general condition (e.g., missing limbs, parasites, etc.). We assumed that the enclosures provided adequate habitat for growth and behavior, because mortality was low (<10% during recovery periods) and most crabs were found to be in good condition (active and intact).

Following the initial holding period, we measured the recovery rate of vas deferens weight and sperm number for both size-classes of males following copulation. Animals with fully replenished sperm resources were split into five groups. The first group consisted of control (unmated) males. Individuals in the remaining groups were mated twice in close succession to deplete their sperm levels to nearly zero. Receptive females in the advanced premolt stage (D_3) of the prematurity instar, termed "pubertal," were obtained from commercial shedding operations near the study site. In most cases, males mated twice in 2–4 days if offered a D_3 molt stage pubertal female after their first mate was removed (D_3 females molt or are receptive to mating in 0–2 days). For large males (>140 mm), groups of mated male crabs were then sacrificed 0, 3, 9, and 20 days after their last mating and assessed for reproductive condition by weighing their vasa deferentia and performing sperm counts. Food was supplied ad libitum during recovery periods. Crabs below the legal size limit of the fishery (127 mm) were assessed for reproductive condition prior to mating and at 0 and 3 days after they had mated twice in close succession (but not at 9 and 20 days).

The total number and concentration of sperm were determined for each male. Following determination of wet weight, the entire vasa deferentia and their contents were homogenized in 2 mL of artificial seawater (30 psu) and stained using 7–8 drops of 1% gentian violet. Samples were then passed through a 35-μm filter, to separate large bits of ruptured spermatophores from sperm cells. Both the filter and filtrate were examined to ensure that all spermatophores were ruptured and all sperm were passing through the filter. Filtrate volume was raised to 100 mL/g of vas deferens with 30 psu artificial seawater, to dilute sperm sufficiently to avoid their overlapping each other in the counting chamber. Ten microlitres of the evenly mixed suspension was placed on each of two Petroff-Hausser spermacytometers and allowed to settle for 5 min. Sperm were counted under phase contrast at 400× magnification and low–medium light. If the two cell counts differed by >10%, the original sample was resuspended and the procedure performed again. This approach minimized variability in the sperm count data due to sampling technique. The total number of sperm for each male was then calculated by dividing the number of cells counted by the volume counted and multiplying this quotient by the total dilution volume ((number of cells counted/volume counted) × total dilution volume). Sperm concentration was determined for each individual by dividing the total number of sperm by the weight of the vasa deferentia.

Reproductive condition of male blue crabs in the Rhode River

Crabs were sampled by trawl from a variety of habitats and depths throughout the Rhode River to determine the reproductive condition of the population during the mating season (June–October) in 1996 and 1997. Otter trawls (7 mm cod end) were conducted three times per month in each of four sites (Fig. 1): a shallow (~2 m) mud-bottom site at the head of the subestuary, a sand-bottom site (~2 m) at the mouth of the subestuary, a ~4 m deep mud-bottom site at the mouth

Fig. 1. Trawl locations within the Rhode River subestuary. Inset shows the location of SERC on Chesapeake Bay (38°51′N, 76°32′W).
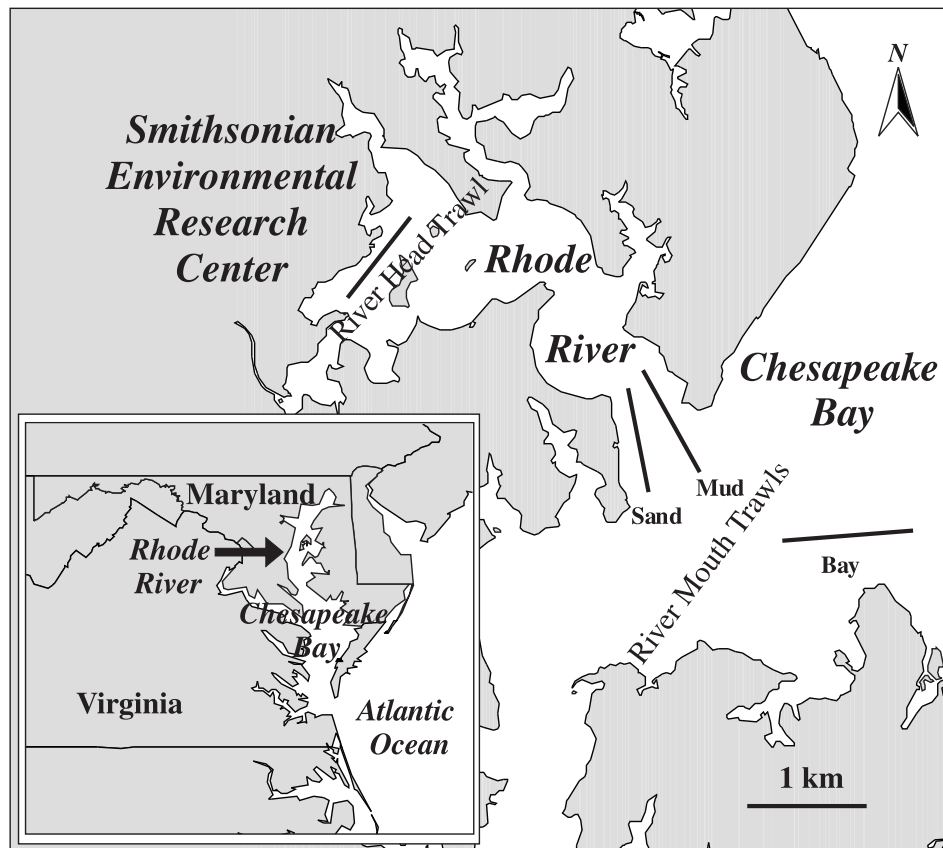
of the subestuary, and a deep (~4 m) site in Chesapeake Bay off the mouth of the Rhode River. While no sampling strategy is perfectly representative of a population, our sampling design captured a suitable cross section of blue crabs in the Rhode River for our study, since it incorporated multiple habitat types used by crabs and locations from the head of the subestuary to its opening into Chesapeake Bay (Hines et al. 1987). This sampling strategy enabled us to (*i*) estimate the overall reproductive condition of male blue crabs in the Rhode River, (*ii*) determine patterns in reproductive condition based on location in the Rhode River, (*iii*) assess temporal differences in reproductive condition between and within reproductive seasons, and (*iv*) estimate the operational sex ratio in the Rhode River.

Size, sex, molt stage, and autotomy status (intact vs. missing or regenerating limbs) were recorded for each crab. The reproductive condition of male crabs was determined by weighing the vasa deferentia. Scatter plots of vas deferens weight versus CW were generated from the data for each month. Data for all locations were pooled in each month, because there were no location effects (see Results). Sperm counts could not be conducted on field samples, owing to time constraints.

The average weight of vasa deferentia for crabs mated a single time following complete recovery of sperm resources was determined and was used as a reference point with which to compare the reproductive condition of field-collected males. Mean vas deferens weight was obtained by holding a group of 15 males, whose sizes covered a large range (115–160 mm CW), for 20 days (resulting in complete recovery of sperm resources as described above), allowing them to mate a single time, and then weighing the vasa deferentia. The proportions of trawl-caught crabs that lay above and below this value were classified as being either "recovered" or "depleted," respectively. The reproductive condition of a control group of males held under identical conditions but prevented from mating was also determined, to provide a reference level for a "fully recovered" male.

Statistical analyses

Vas deferens weight, sperm-count, and sperm-concentration data from recovery-rate experiments were log-transformed to meet assumptions of normality and homoscedasticity during tests of significance. Vas deferens weight, sperm count, and sperm concentration were compared among groups, using *t* tests for pairwise comparisons (e.g., large unmated vs. small unmated) and the differences of least squares means (LSD) procedure for comparisons involving multiple means (Zar 1996). Field data were analyzed to identify temporal and spatial differences in male reproductive condition, by comparing mean vas deferens weights for each month and location using a mixed-model analysis of covariance ($\alpha = 0.05$) (year was analyzed as a random variable, while location, autotomy status, and molt stage were considered fixed) with a LSD mean comparison procedure using crab size as a covariate (preliminary analysis revealed that crab size had a significant influence on vas deferens weight). Differences in vas deferens weight based on autotomy status (intact vs. missing or regenerating limbs) and molt stage (A, early post molt; B, late post molt; C, intermolt; D, premolt; E, ecdysis) were examined in the same way.

Results

Reproductive resources of males: role of size and mating history

Small unmated males in the experimental group had a significantly lower mean vas deferens weight than large unmated males (1.41 vs. 4.23 g, $t = 3.60$, $p < 0.001$; Fig. 2).

Fig. 2. Vas deferens weight of small males (with carapace width (CW) below the legal limit of the fishery (<127 mm); denoted by open bars) and large males (with CW > 140 mm, well above the legal limit of the fishery; denoted by shaded bars) prevented from mating (unmated) and sacrificed 0, 3, 9, and 20 days after two consecutive matings (small males not tested at 9 and 20 days). Means ± standard error of the mean are plotted.

Fig. 3. Sperm counts of small males (with carapace width (CW) below the legal limit of the fishery (<127 mm); denoted by open bars) and large males (with CW > 140 mm, well above the legal limit of the fishery; denoted by shaded bars) prevented from mating (unmated) and sacrificed 0, 3, 9, and 20 days after two consecutive matings (small males not tested at 9 and 20 days). Means ± standard error of the mean are plotted.
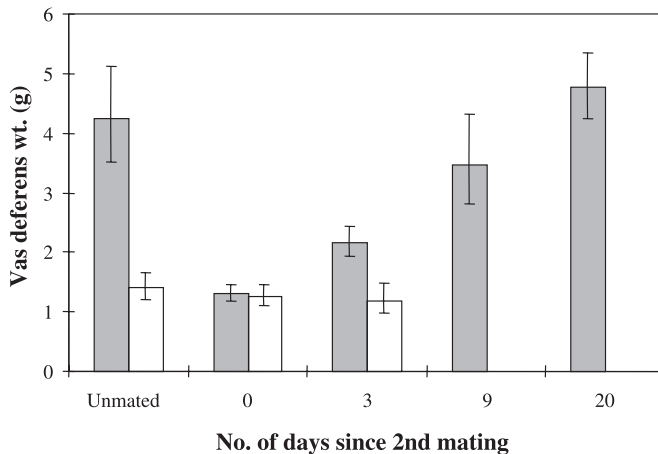
Immediately after mating, large males had a very low mean vas deferens weight (1.31 g) that was significantly lower than that of large males allowed even a 3-day recovery (2.16 g) ($t = 2.07$, $p < 0.04$). The mean vas deferens weight of large males allowed to recover for 3 days was significantly lower than that of large unmated males ($t = 2.52$, $p < 0.02$) or large males allowed to recover for 20 days ($t = 3.14$, $p < 0.004$; Fig. 2). Nine days following mating, large males had a mean vas deferens weight significantly higher than that of males sacrificed immediately after mating ($t = 3.39$, $p < 0.002$) but not significanly different from that of large unmated males and those allowed a 20-day recovery.

Regardless of recovery time, small males consistently had low vas deferens weight that was significantly below that of large males (except large males allowed a 0- or 3-day recovery time following mating). There were no differences in mean vas deferens weight among small unmated males (1.41 g), small males that had just mated (1.27 g), and small males allowed a 3-day recovery (1.19 g).

Sperm counts revealed no significant differences between small unmated males and large unmated males (Fig. 3), despite the weight of the vasa deferentia of small males being only a fraction of that of a large male (Fig. 2). Large males sacrificed immediately after mating and those allowed a 3-day recovery had very low sperm counts (7.2×10^8 and 8.4×10^8 sperm, respectively) that were significantly less than those of large unmated males (2.5×10^9 sperm) ($t = 2.74$, $p < 0.01$ and $t = 2.32$, $p < 0.03$, respectively) and large males allowed a 20-day recovery (2.1×10^9 sperm) ($t = 2.64$, $p < 0.01$ and $t = 2.22$, $p < 0.04$, respectively). Nine days following mating, the sperm counts of large males were intermediate between (and statistically indistinguishable from) those of unmated males and those that had just mated.

Small males allowed a 0- or 3-day recovery time had the lowest sperm counts of all groups (4.1×10^8 and 4×10^8
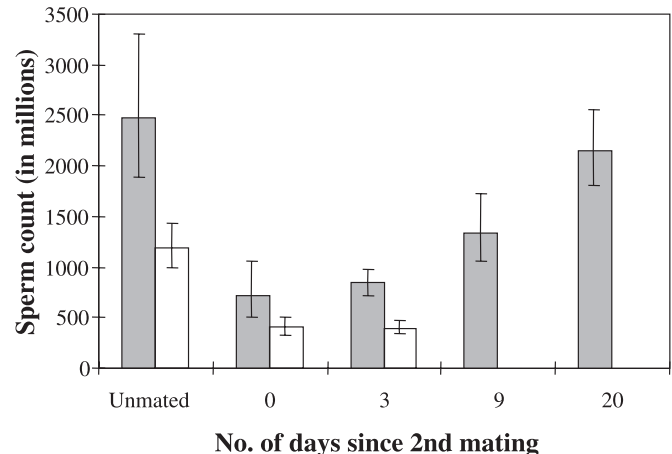
sperm, respectively); these values were significantly lower than those of small unmated males (1.2×10^9 sperm) ($t = 2.84$, $p < 0.008$ and $t = 2.89$, $p < 0.007$, respectively), despite no significant difference in vas deferens weight (Fig. 3).

Mating history also had an influence on sperm concentration in small males. Small unmated males had a higher mean sperm concentration (1.01×10^9 sperm cells × (vas deferens weight (g))$^{-1}$) than small males evaluated just after mating (2.97×10^8 sperm cells × (vas deferens weight (g))$^{-1}$) ($t = 3.99$, $p < 0.0004$) or those allowed a 3-day recovery (4.25×10^8 cells × (vas deferens weight (g))$^{-1}$) ($t = 3.33$, $p < 0.002$; Fig. 4). No significant differences in sperm concentration were found among large males or large and small males with similar mating histories.

The quantity of reproductive resources delivered by males was estimated by measuring differences in vas deferens weight and sperm counts before and after the experimental matings. Large males experienced an average drop of 2.92 g (70%) in seminal material and 1.78×10^9 (70%) sperm as a result of the two mating events, whereas for small males, seminal fluid decreased by only 0.14 g (10%) and sperm by 7.9×10^8 (70%) as a result of the two mating events.

Reproductive condition of males in the Rhode River

The sex ratio for crabs caught in trawls was highly variable over the course of the reproductive season in both years. The mean ratio of males with a CW over 110 mm (the minimum size at which males were observed paired with females in the field) to pubertal females was ~2:1 in 1996 and ~7:1 in 1997.

There were no differences in mean vas deferens weight among locations in the Rhode River subestuary ($F_{(3,3)} = 2.29$, $p < 0.25$) or among months ($F_{(4,2)} = 3.01$, $p < 0.26$), during the reproductive season. Intact crabs had a higher mean vas deferens weight than crabs missing or regenerating limbs (pooled mean, 1.57 vs. 1.24 g; $F_{(1,244)} = 4.85$, $p < 0.028$). Molt stage B crabs had significantly lower mean

Fig. 4. Sperm concentration (number of sperm/vas deferens weight) for small males (with carapace width (CW) below the legal limit of the fishery (<127 mm); denoted by open bars) and large males (with CW > 140 mm, well above the legal limit of the fishery; denoted by shaded bars) prevented from mating (unmated) and sacrificed 0, 3, 9, and 20 days after two consecutive matings (small males not tested at 9 and 20 days). Means ± standard error of the mean are plotted.
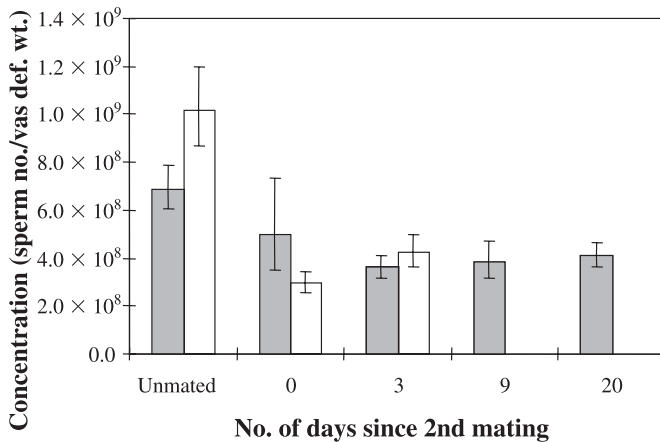
vas deferens weight than molt stage C crabs (0.52 vs. 1.46 g; $t_{244} = 5.05$, $p < 0.0001$). No other differences in vas deferens weight among molt stages were found, although a larger sample size for molt stage A, D, and E crabs would allow the effects of molt stage to be interpreted with more confidence.

At all times during the 1996–1997 mating seasons, at least 50% of the males in the population were scored as "depleted" (had vas deferens weight below that expected had they mated a single time following complete recovery of sperm volume; Figs. 5 and 6). In both years, during July when the mating season begins in earnest, depleted males comprised as much as 64–74% of the male population (Figs. 5a and 6b). Furthermore, there was a large number of males that even had vas deferens weights much lower than the average value expected had they mated once after fully recovering their sperm volume (i.e., below 1.38 g).

Discussion

A large proportion of the males across all size-classes in the Rhode River had low vas deferens weight. A variety of causes may be responsible. We demonstrated that small males simply have low vas deferens weight. Other males may have gametogenesis and production of seminal fluid precluded by the energetic requirements of molting or limb regeneration. At any given time, 20–30% of crabs in the Rhode River basin are either premolt or post molt (Hines et al. 1987) and 17–25% of crabs are missing and (or) regenerating at least one limb (Smith and Hines 1991). Males with significant limb loss are less competitive for mates than intact males (Smith 1992). For molting or injured males, energetic expenditure on sperm production may not contribute to their reproductive success as much as energy put toward regeneration of chelae or limbs or calcification of their exoskeleton. Another contributing factor may be simply that

a high incidence of mating in the population leaves many males depleted.

At first consideration, the absolute sex ratios of 2:1 and 7:1 for the 2 years of this study suggest that there are plenty of males to mate with the female population (without regard to the amount of sperm females receive). However, these are probably very liberal estimates of the "operational sex ratio," a value that provides a more accurate depiction of which males in the population are likely to mate. To estimate this value, we should eliminate or devalue the males that are not likely to be mating (e.g., males that are molt stages A, B, D, and E (which are never observed paired in the field) and those that have lost even one cheliped and are significantly less able to defend females from intact competitors; Smith 1992). Competitive interactions between males can also influence the operational sex ratio. Laboratory experiments revealed that large males have better pairing and mate-stealing success than small males (Jivoff 1997a, 1997b). The mating history of males is also a determinant of pairing success (Jivoff 1997a, 1997b). Males that have recently mated (and therefore have low vas deferens weight and sperm count; see Results) are, in fact, better competitors for mates than males without recent experience (Kendall and Wolcott 1999). Given these considerations, the operational sex ratio will always be less than the absolute ratio of adult males to pubertal females, and an estimate of 1:1 may be more appropriate for the Rhode River system.

There were no significant differences in mean vas deferens weight among locations or months over the reproductive season in the Rhode River. However, these are not the most informative comparisons for examining spatial and temporal trends in male reproductive condition. Females do not mate with the "*average*" male crab; females receive sperm from *individual* males in the population. Examining mean reproductive condition instead of reproductive condition of individuals can be misleading. For example, in a population, the average amount of stored sperm per male may be high but, if individual males that have very low sperm volume mate with some percentage of the females, those females may be sperm limited. Conversely, the average level of stored sperm per male in a population may be very low but, if the few males in the population with a large volume of stored sperm are competitive dominants, they may do most of the mating and provide their mates with ample sperm. Therefore, rather than examining mean vas deferens weight over some time interval or location, we suggest that scatter plots of vas deferens weight versus crab size yield a more meaningful biological perspective on the reproductive condition of the male population.

We observed a large number of males in the population with a vas deferens weight much lower than that expected had they just mated a single time after fully recovering their sperm volume. This suggests that these males lack a full complement of sperm and (or) seminal fluid between consecutive mating events. In fact, many males in the field were observed to have vas deferens weights much lower than even the most depleted groups observed in the laboratory study. Females mated with these males would receive particularly low quantities of sperm and seminal fluid.

Following mating, large males recover most of their seminal resources (sperm and seminal fluid) in less than 9 days.

1174

Can. J. Fish. Aquat. Sci. Vol. 58, 2001

Fig. 5. Scatter plots of vas deferens weight (g) versus carapace width (mm) for pooled trawls during July–September (*a–c*) of the 1996 reproductive season. The horizontal line in each plot denotes the vas deferens weight of a crab allowed full recovery of sperm resources and then mated a single time.
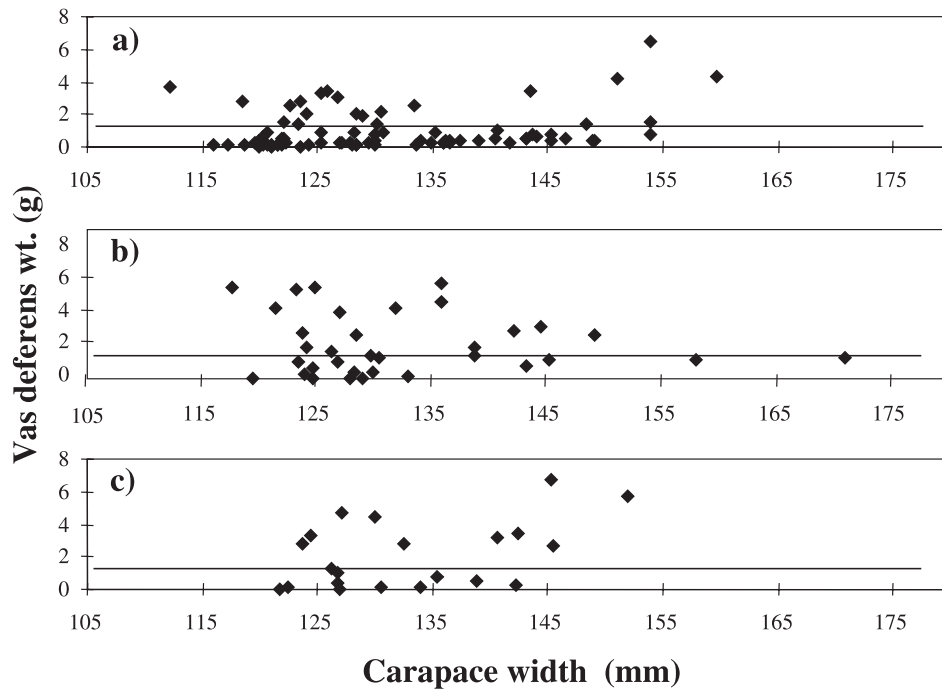

Vas deferens weight is recovered very quickly; as few as 9 days were required for large males to have a vas deferens weight significantly higher than that of males which had just mated and statistically indistinguishable from the vas deferens weight of fully recovered males. However, the recovery of sperm number probably takes longer and is apparently still underway at 9 days following copulation; at this point, large males have levels of sperm that are intermediate between just-mated males and fully recovered males and overlap statistically with both. Despite these small differences in the recovery rate of sperm number and seminal fluid for large males, the experimental samples demonstrate that, for large males, complete recovery of all seminal material is attained between 9 and 20 days following mating. Small males require more than 3 days to recover sperm number (vas deferens weight does not vary), although the time required for complete recovery cannot be determined from this study (we did not follow a group of small males for a longer recovery period, because vas deferens weight levels had not dropped much following mating). Since males typically guard females for 2–5 days after mating and for ~5 days prior to mating (these values are estimated from field observations and laboratory experiments, respectively; Jivoff 1995), the sum of these times suggests that large males that mate repeatedly would have sufficient time to replenish most of their number of spermatozoa and at least some of their seminal fluid (vas deferens weight) between consecutive matings. However, large males are being selectively removed from the population by the fishery. The fisheries mortality (*F*) on age 1+ is estimated to be as high as 0.8–0.9 in some years (Sauls et al. 1995). Those males that remain will spend less time guarding mates as the sex ratio

declines (Jivoff 1997*a*), which will reduce the time available for recovery of reproductive resources between consecutive matings. Furthermore, small males that escape the fishery are left to service a large portion of the female population. Field observations in the Rhode River revealed that sublegal males are paired with approximately one-third of receptive females (Jivoff 1995, 1997*b*). Males below the legal size limit of the fishery generally have available only a fraction of the seminal material of large males. Females mated with males of below 127 mm CW probably received smaller quantities of seminal fluid and, possibly, fewer sperm than if they had mated with a large recovered male.

We were also able to infer the amount of seminal material that males delivered to females by comparing vas deferens weight and sperm number observed in males before and after mating. In our experiments, both large and small males delivered approximately 70% of their stored sperm, although the difference in absolute numbers was bigger for large males. In fact, large males delivered 2.25 times as much sperm and 21 times as much seminal fluid as small males. Interestingly, small males experienced essentially no change in vas deferens weight following mating, whereas large males lost nearly 70% of their premating vas deferens weight, indicating that small males deliver scant amounts of accessory seminal fluid with their sperm.

The delivery of male reproductive resources is affected by the interaction of male size and mating history. Additionally, levels of reproductive resources are characterized by high variability, both in the experimental crabs and in the field population. While large fully recovered males appear to be capable of delivering much greater quantities of seminal fluid and sperm than small males, the levels of reproductive

Fig. 6. Scatter plots of vas deferens weight (g) versus carapace width (mm) for pooled trawls during June–September (*a–d*) of the 1997 reproductive season. The horizontal line in each plot denotes the vas deferens weight of a crab allowed full recovery of sperm resources and then mated a single time.
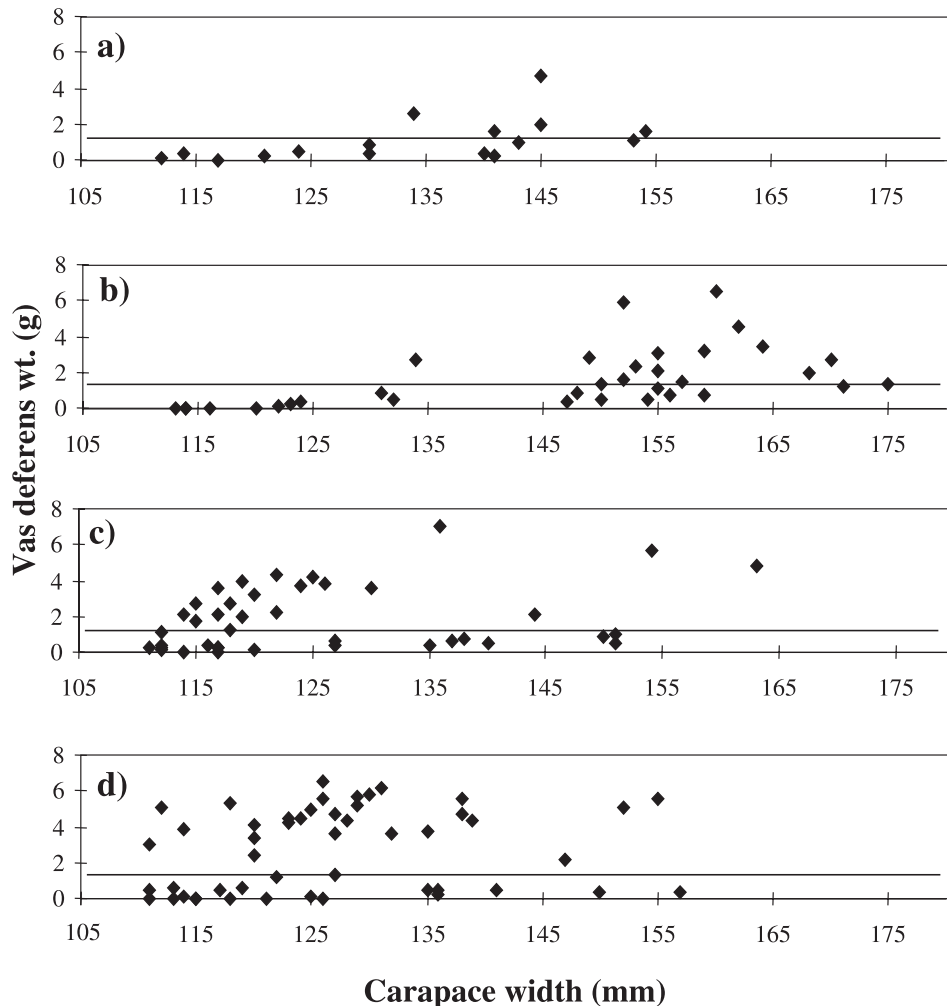
resources for many large males in nature may be similar to those of small males, especially if the large male has been mating recently.

Females mating with large depleted males or small males of any reproductive history receive small quantities of seminal fluid. The long-term consequences of receiving less seminal fluid are unclear. Seminal fluid is not only the medium in which sperm are transferred but also has been suggested to influence reproductive success by serving as a source of sperm nutrition during storage (Subramoniam 1993), as an antibacterial agent protecting the female reproductive tract (Jayasaker and Subramoniam 1999), and as a "sperm plug" that may both prevent deposited sperm from leaking out of a female's seminal receptacles and deny access to a female's reproductive tract to subsequent males (Ryan 1964; Hartnoll 1969). Males delivering reduced quantities of seminal fluid but normal numbers of spermatocytes may experience reduced reproductive success due to sperm loss from inadequate nutrition, bacterial infection, or simple leakage from a female's reproductive tract.

It should be noted that the dual role of the "sperm plug" might have both positive and negative influences on the pos-

sibility of sperm limitation. Since smaller males have less seminal fluid, their sperm plugs may be less effective. While this may result in some sperm leakage (making sperm limitation more likely), it could increase the potential for a female to receive sperm from two males because of an ineffective plug; this would reduce her likelihood of being sperm limited.

We have documented high incidences of males with very reduced seminal resources in a natural population and demonstrated that stored sperm and seminal fluid become depleted as a result of mating and remain so for some days. These results confirm the importance of two potential mechanisms by which the reproductive potential of the blue crab mating system could be limited by reproductive resources contributed by males (sperm and (or) seminal fluid): (1) a reduction in the male:female operational sex ratio and (2) a high incidence of mating by small or frequently mating males with low vas deferens weight (for large males, frequent mating is probably not a source of reduced fertilization success, if mate-guarding times observed in the laboratory accurately reflect those in the field). To understand the impact of these mechanisms on the reproductive output of females and definitively determine if sperm limita-

1176

Can. J. Fish. Aquat. Sci. Vol. 58, 2001

tion is occurring, it remains to (*i*) determine the amount of sperm and seminal fluid received by females in the field, (*ii*) examine the influences of male size and mating history on the amount of sperm and seminal fluid actually transferred to females, (*iii*) quantify the relationship between sperm number, seminal fluid volume, and fertilization success, (*iv*) identify additional influences on operational sex ratio and their effects on the reproductive potential of the population, and (*v*) identify the role(s) of seminal fluid in the blue crab mating system.

Proper management of the fishery must incorporate all aspects of the stock–recruit relationship. Often, management strategies consider only mature females in this regard (Jones et al. 1990; Sauls et al. 1995; Prager 1996). Blue crabs and other crustacean species appear to be susceptible to fishery-induced sperm limitation (Smith and Jamieson 1991; Paul and Paul 1992; Sainte-Marie and Lovrich 1994). Our study suggests that we must consider the attributes of the male contribution to reproductive success (i.e., sperm and accessory seminal fluid), to obtain an accurate assessment of reproductive potential at the population level in the blue crab.

Acknowledgements

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