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EVIDENCE FOR SPERM LIMITATION IN THE BLUE CRAB, *CALLINECTES SAPIDUS*

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ABSTRACT

Reproductive success of female blue crabs may be limited by the amount of sperm received during the female's single, lifetime mating. Sperm must be stored in seminal receptacles until eggs are produced and fertilized months to years after mating. Further, intense fishing pressure impacts male abundance, male size and population sex ratio, which affect ejaculate quantity. We measured temporal variation in seminal receptacle contents in relation to brood production for two stocks differing in both fishing pressure on males and latitudinal effects on reproductive season: Chesapeake Bay, Maryland and Virginia, experienced intensive fishing and relatively short reproductive season; and the Indian River Lagoon, Florida, experienced lower exploitation and longer reproductive season. Nearly all (>98%) females were mated, and mating prevalence did not vary among sites during 1996. Seminal receptacle weight declined markedly for 2 mo following mating as seminal fluid disappeared to leave only spermatozoa for long-term storage, which suggests that seminal fluid serves as a short-term sperm plug. Seminal receptacle weight in upper Chesapeake Bay declined by 31% from 1992–1999, indicating that females received smaller ejaculates. In 1996, seminal receptacle contents were highest (3.75 g wet wt, $2.3 \times 10^3 \mu\text{g}$ DNA, 1.2×10^9 sperm) in Florida, but were significantly lower by: 25% for weight and 50% for sperm number at the upper Chesapeake Bay site; and 30% for weight and 65% for sperm number at lower Chesapeake Bay sites. Generally, females receive $2\text{--}3 \times 10^3$ spermatozoa and $10^8\text{--}10^9$ sperm cells for a full ejaculate, whereas females produce ca. 3×10^6 eggs per brood. Chesapeake Bay females appear to live about 3.5 yr, producing 1–3 broods (up to 9×10^6 eggs) per year and up to 6–7 broods (2.1×10^7 eggs) per lifetime. In contrast, Florida crabs produced up to 6–7 broods (2.1×10^7 eggs) per year, and up to 18 broods (5.4×10^7 eggs) per lifetime. In Florida, last broods produced by lab-held females were often infertile, indicating that females became sperm limited at the end of their lifetime. Experiments showed that male mating history affected female reproductive success, with females mated late in a sequence having only one third the brood hatching success of females mated early in the sequence. Sperm:egg ratios were estimated at 100:1 to 400:1 for the first brood but only about 20:1 or 30:1 for maximum lifetime broods over 2 seasons, suggesting that about 67×10^6 sperm are used per brood of 3×10^6 eggs. A model of brood production and sperm depletion in blue crabs indicates that sperm limitation may be common in Florida as females age, and in Chesapeake Bay as a result of fishery-induced reductions in initial quantities of sperm transferred at mating.

In many species, the ability of females to fertilize their eggs is influenced by the quantity and quality of sperm and other components of the ejaculate that are contributed by males (Gladstone, 1979; Ridley, 1989; Keller and Reeve, 1995; Reynolds, 1996). Reproduction becomes sperm limited when the number or quality of sperm received by females is not sufficient to fertilize the total potential egg production. The amount of ejaculate that males pass to females may decrease with the mating frequency of males, because males often require time to replenish their supply of sperm and seminal products (Ryan, 1967; Woodhead, 1985; Svard and Wiklund, 1986; Simmons, 1988; Pitnick and Markow, 1994; Birkhead and Fletcher, 1995; Birkhead et al., 1995; Cook and Gage, 1995). Females may receive insufficient sperm when mating with males before recovery of the sperm supply, especially if the male has previously mated with several females in succession (Woodhead, 1985; Svard and Wiklund, 1986; Pierce et al., 1990; Birkhead, 1991;

Simmons, 1988; Pitnick and Markow, 1994; Birkhead and Fletcher, 1995). The potential for sperm limitation is greatest when mating occurs during brief seasons requiring close coordination of life history, behavior and seasonal events. For example, mating in many crustaceans is closely coordinated with the molt cycle and seasonal maturation, which may be controlled by seasonal temperature and photoperiod cycles (Waddy and Aiken, 1986; Sainte-Marie, 1993). Sperm limitation may be aggravated by long periods of sperm storage in the female, which may reduce sperm quantity and quality (Austin, 1975; Nakatsuru and Kramer, 1982; Paul, 1984; Kirkendall, 1990; Paul and Paul, 1992; Saint-Marie, 1993). Similarly, sperm limitation may ensue as sperm are depleted by egg fertilization over a prolonged season or the lifetime of a female that has limited opportunities to replenish sperm stores (Morgan et al., 1983; Paul and Paul, 1992).

Intensive exploitation of males in several decapod crustacean fisheries may result in transfer of insufficient sperm to fertilize females' potential egg production (Dewsbury, 1982; Diesel, 1991) and thereby lead to sperm limitation of reproduction at the population level (Powell et al., 1973; Paul and Paul, 1992; Sainte-Marie et al., 1997). The potential limitation of sperm transfer results from fisheries' selectively removing males from the stock, because males are larger (e.g., *Chionoecetes opilio*) (Sainte-Marie and Carriere, 1995), and/or because males are preferred in the market place (e.g., *Callinectes sapidus*) (Millikin and Williams, 1984), making them more valuable than females. Also, males may be more vulnerable to fishing because of their habitat utilization and behavior, or because of fishing limitations designed to protect the female spawning stock (Jamieson, 1993).

Intense fishing pressure on males may lead to sperm limitation through at least three mechanisms. (1) Male : female ratios may be reduced to such low levels that remaining males have to mate repeatedly without sufficient time to recoup sperm stores, e.g., snow and tanner crabs (*Chionoecetes opilio*, *C. bairdi*) (Powell et al., 1973; Donaldson and Donaldson, 1992; Stevens et al., 1993; Lovrich et al., 1995) and dungeness crab (*Cancer magister*) (Smith and Jamieson, 1991). (2) Remaining males may be immature or too small to produce sufficient sperm, e.g., tanner crabs (*C. tanneri*) (Stevens et al., 1993) and snow crabs (*C. opilio*) (Lovrich et al., 1995; Sainte-Marie et al., 1997; Sainte-Marie and Sainte-Marie, 1999b). (3) Reductions in numbers of males may be so severe that females do not find mates, e.g., king crabs (*Paralithodes* spp.) (Gray and Powell, 1964; Powell et al., 1973).

Little is known about the quantities of sperm that may be "sufficient" to ensure reproductive success of a sustainable stock, or "insufficient" to achieve full fertility in brachyurans and other crustaceans. The amount of sperm required for successful fertilization probably requires ratios of spermatozoon to oocyte that are substantially higher than 1:1. In *Chionoecetes* spp., ratios for high fertilization success are reported to range from "several" sperm per egg (Adams and Paul, 1983) to actual expenditures of an estimated 70 stored sperm : oocyte for the first clutch extruded (Sainte-Marie and Lovrich, 1994). When sperm : egg ratios were below 7:1, females failed to extrude eggs (Sainte-Marie and Lovrich, 1994). Brown (1966) studied blue crab sperm structure and observed "several hundred" sperm interacting with eggs in laboratory culture, although this represented an in vitro condition that may not indicate natural sperm : egg ratios. Little is known for other species.

Blue crabs exhibit many life history features that make them vulnerable to sperm limitation. Generally, females mate only once at the time of their pubertal molt, when they receive their lifetime complement of sperm; whereas, males may mate repeatedly, although an unknown number of times (Millikin and Williams,

1984). The Chesapeake Bay blue crab stock is near the species northern limit distribution and has a very limited mating season. In upper Chesapeake Bay there is only a single mating period in late summer–early fall, while in the lower Bay there is both a spring (“peeler run”) and a late summer–fall peak of mating. The mating season in Chesapeake Bay coincides with the most intense period of fishing pressure, when primarily large males are removed. Females mated in the summer and fall must store sperm for 7–11 months over the winter before they fertilize broods the following summer (Millikin and Williams, 1984). Nothing is known about the fate and relative contribution to the total spawning stock for upper versus lower Chesapeake Bay females, nor for females mated in spring versus fall. The northern mating season contrasts with a much longer season at lower latitudes (Tagatz, 1968; Steele and Bert, 1994).

In two populations studied quantitatively, most (>95%) female blue crabs appear to mate successfully (South Carolina: Wenner, 1989; upper Chesapeake Bay: Jivoff, 1995, 1997a, 1997b). Jivoff (1995) noted that a small fraction (<10%) of females in upper Chesapeake Bay exhibited two ejaculates stored in their seminal receptacles, indicating that these females mated with two males. However, the amount of sperm transferred in these double matings has not been quantified. Our laboratory experiments and field sampling showed that the size of ejaculate transferred at mating depends on sex ratio (Jivoff and Hines, 1998b), male size (Jivoff, 1997a, 1997b) and their mating history (Jivoff and Hines, 1998a), such that low male:female ratios, small male size, and short recovery time before repeated mating each resulted in significantly reduced ejaculates. Both the male:female ratio and crab size have declined over the past 30 years in parts of Chesapeake Bay (Abbe and Stagg, 1996; Lipcius and Sockhausen, 2002), which would tend to reduce sperm transferred at mating. Experimental analysis of males showed that vas deferens weight dropped significantly after mating compared to unmated males; and the majority of male crabs in a subestuary of upper Chesapeake Bay had vas deferens weights that were significantly lower than the level characteristic of “fully charged” males and even of those that had just mated once (Kendall and Wolcott, 1999).

The purpose of this study was to explore the potential for sperm limitation in blue crabs by considering six components of evidence. First, we compared annual patterns of ejaculate storage by females among locations in upper and lower Chesapeake Bay (Maryland and Virginia) and in the Indian River Lagoon (Florida). Chesapeake Bay was selected to represent a population with an intensive fishery, with relatively short mating and brooding seasons separated by cold winters requiring long periods of sperm storage. The Indian River Lagoon represents a population with a less intensive fishery and much longer mating and brooding seasons, such that mating is followed by egg production in the same season. Second, to assess whether some females receive disproportionately low levels of sperm in nature, we measured variation in ejaculate quantity among individual females and among years for the population at one location in upper Chesapeake Bay. Third, to test for spatial variation in quantity of sperm provided to females, we compared quantities of sperm stored in the seminal receptacles of newly mated females from upper and lower Chesapeake Bay and the Indian River Lagoon. Fourth, to consider whether females may run out of sperm, we estimated sperm:egg ratios for the number of eggs produced per brood, per season and per lifetime for females in Chesapeake Bay and the Indian River Lagoon. Fifth, to test whether reduction in sperm transferred to females may result in lower fertilization success, we conducted an experiment measuring brood production and hatching success as a function of male mating history. Finally, to integrate our observations of the

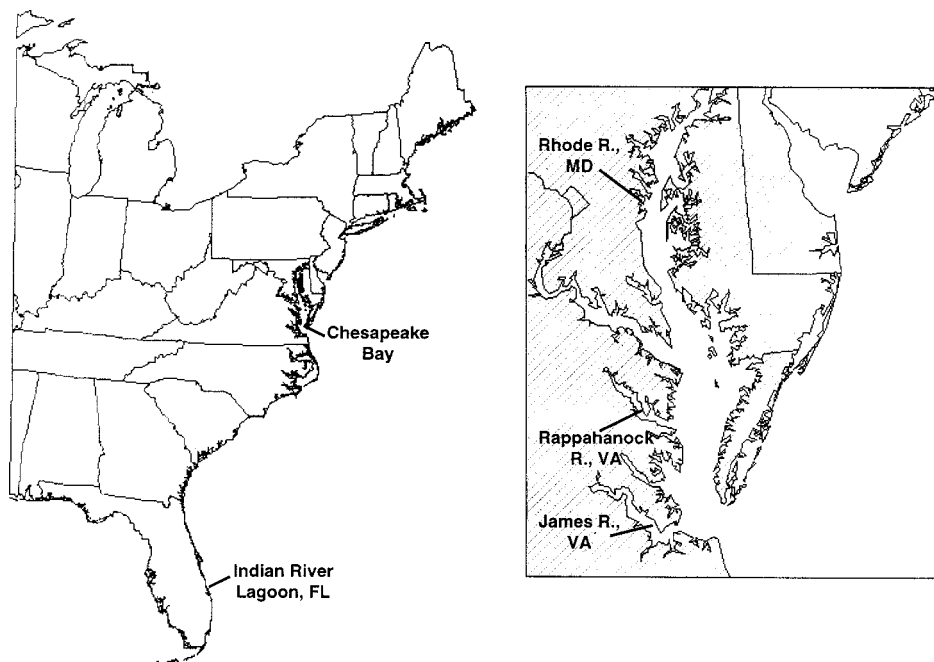


Figure 1. Map showing collecting sites for mature female blue crabs in Chesapeake Bay, Maryland and Virginia, and in the Indian River Lagoon, Florida. Inset shows sampling sites in upper and lower Chesapeake Bay.

sequence of reproductive events and to assess the potential for sperm limitation in blue crabs, we developed a simple conceptual model of mating, brood production and levels of sperm stores. Rather than providing definitive determination of sperm limitation, our intent is to analyze variables that affect sperm storage and brood production in blue crabs, so that we may identify the bounds of variables, or combinations of variables, leading to the potential for sperm limitation.

MATERIALS AND METHODS

SAMPLING METHODS AND LOCATIONS.—Mature female blue crabs were collected from one location in upper Chesapeake Bay (Rhode River), and from three locations in lower Chesapeake Bay (Rappahannock and James Rivers), and one site (Sebastian Inlet in the Indian River Lagoon, Florida) (Fig. 1). Crabs from the Rhode River were collected by trawl during the months of April to December during 1992–1999 (except not in 1995). Crabs from the lower Chesapeake Bay sites were collected in 1996 by trap during April to November and by dredge during December. Crabs from Florida were collected by trawl and trap throughout the year of 1996. The following variables were recorded for each crab: carapace width (including lateral spines), molt stage, shell condition (clean or dirty), ovarian development (5 stages, including no development), and brood development (5 stages, including no brood). In addition, the seminal receptacles were dissected from each female and weighed (wet weight to nearest mg), scored for presence or absence of ejaculate material, and assigned a percent fullness by visual estimation of volume of the ejaculate. Seminal receptacles were then stored frozen or in 70% ethanol. Seminal receptacles from a subset of females collected from each site were processed to estimate sperm quantity, either by nucleic acid analysis or direct sperm counting. Filled seminal receptacles contain non-cellular seminal fluid and sperm as the only cellular material. Nucleic acid of sperm cells is virtually only DNA. Thus, calculations of total nucleic acid from seminal receptacle contents estimate total sperm DNA and therefore are indirect estimates of sperm quantity. The subset consisted only of post-molt females to assure that these contained the full complement of the ejaculate before potential attrition by degradation or use of seminal fluid and sperm.

DNA ANALYSIS.—Seminal receptacles stored in 70% ethanol were first rehydrated by soaking for

48 h in aqueous buffer (0.1 M NaCl, 0.05 M EDTA, 0.1 M Tris-HCl, pH 7.4) with fluid changes twice daily; frozen samples were processed directly. Nucleic acid, consisting primarily of sperm DNA, was extracted from all samples following a protocol modified from Strassman et al. (1996). For each crab, contents of both seminal receptacles were removed, pooled and homogenized in a Dounce homogenizer with 15 ml aqueous buffer (0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl, 0.05 M EDTA, 0.05% SDS, pH 9.1). The homogenate was incubated 45 min in a 65°C water bath, followed by 45 min on ice after the addition of 4.3 ml of 8 M potassium acetate. After incubations, samples were centrifuged at 10,000 rpm for 20 min at 4°C. To each supernatant was added 12.5 ml of 100% EtOH at 20°C and allowed to incubate overnight at 20°C. Samples were then centrifuged at 10,000 rpm for 20 min at 4°C, and the pellets were washed twice with 10 ml ice-cold 70% EtOH. The washed pellets containing DNA were allowed to dry without heat and then resuspended in 4 ml deionized water. Optical density (OD) of each sample was calculated from absorbance readings on a UV spectrophotometer at 260 nm. DNA concentration was estimated using the formula $OD_{260} = 50 \mu\text{g DNA/ml}$ with a 1 cm light path (Sambrook et al., 1989). Protein contamination was estimated by the ratio of OD_{260} to OD_{280} . Averaged ratios from each sampling site ranged from 1.3 to 1.5, indicating some protein contamination; however, ANCOVA showed no significant differences among sampling sites ($F = 1.21$, $P = 0.317$), so comparisons among sites were not influenced by protein contamination.

SPERM COUNTS.—For direct sperm counts, seminal receptacle contents of each crab were removed and pooled. Samples stored in 70% EtOH were processed in 70% EtOH, while frozen samples were processed in full-strength artificial seawater (ASW). Receptacle contents were minced in 20–30 ml 70% EtOH or ASW until pieces were 2–5 mm in size. This tissue was stained with 100 μl 1% aqueous crystal violet to aid in identification of sperm cells. Stained tissue was ground in a Dounce homogenizer and filtered through 25 μm mesh Nitex cloth. Retained solid particles were examined microscopically for intact spermatophores. If any remained, the grinding was repeated. Sperm cell number in the filtrate was estimated by 3 replicate counts in a hemacytometer under a microscope.

In some seminal receptacles, the seminal fluid was broken down sufficiently for the collection of loose, intact spermatophores. For these samples, spermatophores were removed from the two receptacles, pooled, strained through 25 μm Nitex mesh, and spread in a single layer in a 2,500 mm^2 counting dish. The number of spermatophores per pair of receptacles was estimated by 3 replicate counts of 25 mm^2 subsamples under a dissecting microscope. Sperm cells per spermatophore were also estimated by removing a single spermatophore, grinding it in 10 μl 70% EtOH or ASW in a prepared mini-mortar and pestle (Strassman et al., 1996) and counting sperm cells microscopically in a hemacytometer for 3 replicate subsamples.

BROOD PRODUCTION ESTIMATES.—We conducted experiments in the Indian River Lagoon, Florida, to estimate the number of broods produced per season by female blue crabs. We collected mature females by trap at Sebastian Inlet and transferred them to large (1 m \times 2 m \times 1 m) tanks with flowing estuarine water. The tanks were shaded to minimize algal growth. Salinity in the holding tanks varied between 26–30 ppt, while temperatures varied between 22–27°C, which was consistent with ambient water conditions in the Lagoon. Crabs were fed pieces of fish and squid daily. Each crab was marked with a unique number and examined daily for condition and brooding status (brood absent or present; if present, the brood was categorized into 4 developmental stages—early no-eyed embryos, late no-eyed embryos, early eyed embryos, late eyed embryos), which allowed us to record the sequence of brood production and hatching. We collected 3 separate cohorts of crabs to follow brooding sequence: one cohort was collected on 20 August and held until 15 November 1994; a second was collected on 4 April and held until 1 June 1995; and third was collected on 4 April and held until 30 September 1995.

MATING EXPERIMENTS.—We conducted a mating experiment in the Indian River Lagoon, Florida, during April to August 1998 to test the effect of male mating history on brood hatching success of females. We obtained crabs from fishermen working traps at Sebastian Inlet. Mature intermolt males were collected by trap, and prepubertal females in late premolt stage were obtained from a soft-shell crab shedding operation. Maturity was distinguished by morphology and coloration of pleopods and abdomen, while molt stage was determined by carapace hardness and coloration along the edge of the fifth pereopod (Millikin and Williams, 1984; Van Engel, 1990). Males were held and fed in tanks with flowing estuarine water for 12 days prior to use in experiments, thereby assuring that they had fully recovered stores of sperm and seminal fluid (Jivoff, 1995). Prepubertal females were held in the tanks for 2 hours before use in experiments, to be sure they appeared healthy. For mating, each female was placed in a small (25 cm \times 25 cm \times 25 cm) basket with a male from one of four randomly assigned mating sequences: 0 prior matings (i.e., the first mating in the laboratory for that male); 1 prior mating (i.e., the second mating in the laboratory for that male); 2 prior matings; and 3 prior matings. Females usually molted within 12–24 h and copulation usually ensued immediately. The mated pair was separated once males ended copulation and returned to a cradle-carry position (Jivoff, 1995). Mated males were moved immediately to the next mating, so that they would copulate with the next female in the sequence within 2 days, minimizing time for recovery of their stores of sperm

and seminal fluid (Jivoff, 1995). Mated females were held for 4 days in separate cages until their carapace hardened. Then females were transferred to a large floating tank system that pumped flowing estuarine water through large (200 liters) containers supplied with aeration and 5 cm of sand on the bottom. Each container held 1–2 mature females, which were fed frozen fish and squid daily. Each female was uniquely marked and her brooding status was recorded every other day.

SPERM:EGG RATIO.—Numbers of sperm supplied to females were estimated from our counts (above). We estimated egg fecundity per brood, per season and per lifetime of females using a combination of our estimates of brood production and information from the literature (Hines, 1982; Millikin and Williams, 1984; Prager et al., 1990).

STATISTICAL TREATMENTS.—We used ANCOVA to test for spatial variation in quantity of ejaculate weight, DNA quantity, and sperm count, using collecting location as class variable and female carapace width as a covariate (SAS, 1990). When ANCOVA indicated significant effects, SNK comparisons were used to determine differences among multiple means. Independence of brood hatching success among mating treatments was tested using *G*-tests (Sokal and Rohlf, 1995).

RESULTS

MATING SUCCESS.—A gross estimate of mating success of female blue crabs in the field was determined as the frequency of mature females with ejaculate stores present in their seminal receptacles. Mature females from all three collecting locations had very high mating success, with ejaculate stores present in 100% of females collected at the Rhode River ($n = 619$), 98% ($n = 1,241$) in lower Chesapeake Bay tributaries, and 100% ($n = 396$) in the Indian River Lagoon, Florida. Ejaculate stores were absent in one side of the paired seminal receptacles in rare instances ($\ll 1\%$ of females) at each location: 3 females at the Rhode River; 3 females at lower Bay tributaries; and 2 females in the Indian River Lagoon, Florida. Similarly, double inseminations (2 ejaculates in the same seminal receptacles) occurred occasionally at each location: 10 females at the Rhode River; 3 females at lower Chesapeake Bay tributaries; and 4 females in the Indian River Lagoon, Florida.

TEMPORAL AND SPATIAL VARIATION IN EJACULATE STORES.—The seasonal pattern of variation in mean wet weight of seminal receptacles (pair combined) differed among collecting locations (Fig. 2). In the Rhode River, mature female blue crabs were not present in the system until late June–early July, when maturation and mating began. Mating activity peaked during July and August, and continued through much of September. Average seminal receptacle weight increased during the summer from about 2 g in July to about 3 g in October, when mature females left the Rhode River to migrate down to the lower Chesapeake Bay (Turner et al., 2003; Hines et al., unpubl.). In the lower Chesapeake Bay tributaries, female maturation and mating began in May. Seminal receptacle weight was maximal at levels of about 1.75 g during the spring period of synchronized maturation and mating (the “spring peeler run”), and remained high during the summer, except for a dip weight during July. Seminal receptacle weights declined to minimal levels of about 0.5 g by December for the winter period. In Florida, maturation and mating occurred over a longer season beginning in March with peaks occurring during July and October. Average seminal receptacle weight started at a low level of about 0.5 g in March and increased gradually during the mating season to about 1.5 g by October.

Seminal receptacle weight as a function of ovarian developmental stage exhibited a nearly consistent pattern among the three main collecting sites (Fig. 3). Seminal receptacles were heaviest immediately after maturation and mating when ovaries had not developed at all, except in lower Chesapeake Bay, where initial weight of seminal receptacles was aberrantly low, (but the sample size, $n = 13$, was small for this category). Seminal receptacles at this stage were typically large, thick-walled organs full of pink seminal fluid and white spermatophores; the re-

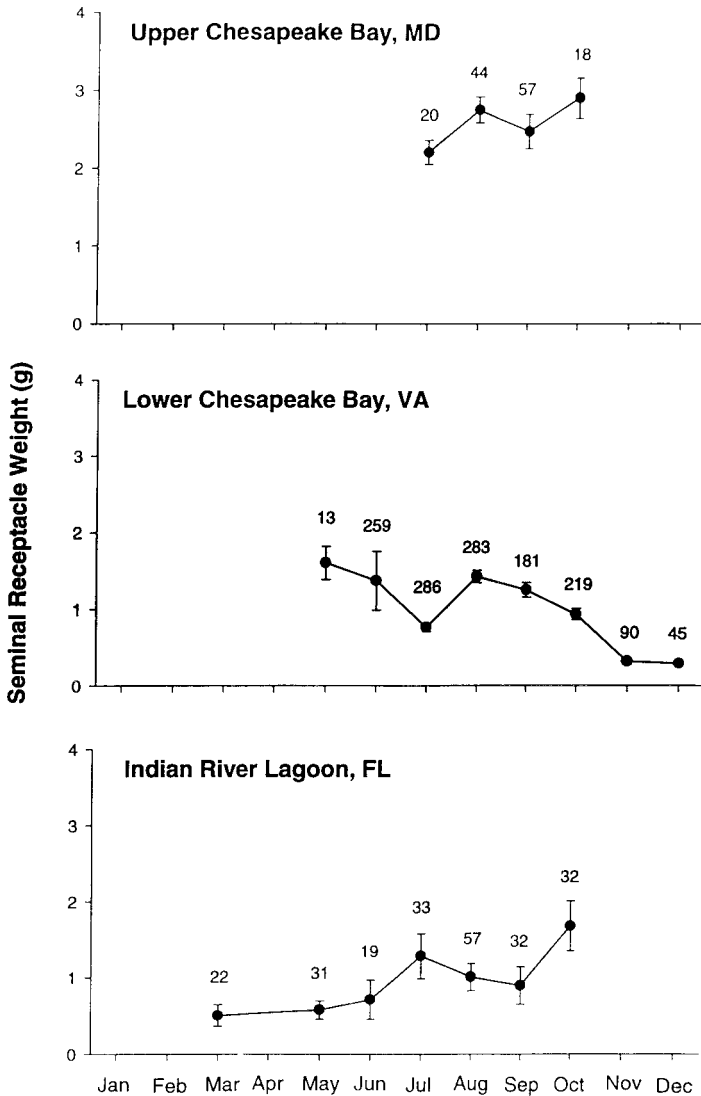


Figure 2. Seasonal variation in weight of seminal receptacles of female blue crabs from upper and lower Chesapeake Bay and from Indian River Lagoon, Florida during 1996. Means \pm SE of mean and number of mature females are indicated for each female.

ceptacles filled much of the ventral volume of the body cavity. As ovaries developed, seminal receptacles became thin-walled, more flaccid, and declined in average weight until reaching a constant level of about 0.5 g by ovarian stage 3. In Florida seminal receptacle weight declined more quickly and reached a constant low level by ovarian stage 2.

Regression analysis (Fig. 4) revealed that seminal receptacle weight of female blue crabs collected near the mouth of the Rhode River declined significantly from 1992–1999 (SemReceptacle Wt = -0.166 Year + 19.0) ($R^2 = 0.511$; $P < 0.001$), and declined significantly by 31% from 3.70 g per pair in 1992 to 2.54

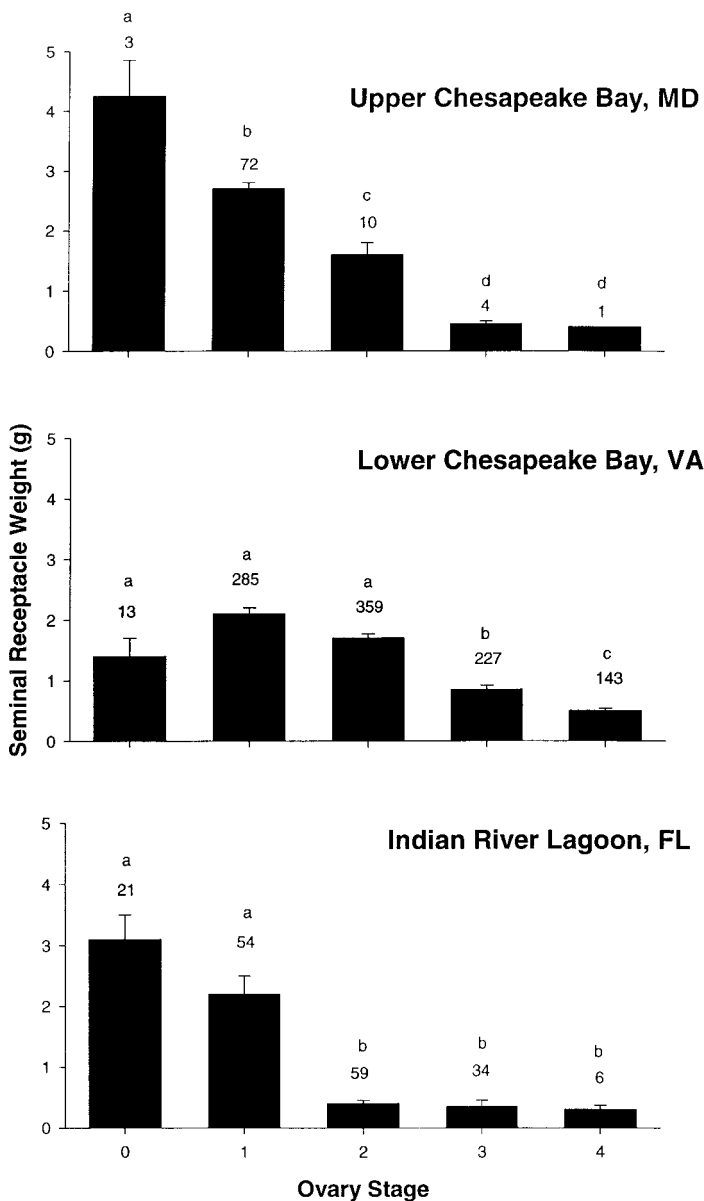


Figure 3. Change in seminal receptacle weight as a function of ovarian developmental stage in female blue crabs from upper and lower Chesapeake Bay and Indian River Lagoon, Florida. Stage 0 indicates no yolk visibly developing in ovaries, while stage 4 indicates fully ripe ovaries. Letters above histograms indicate samples that did not differ in SNK multiple comparison of means.

g in 1999 (ANOVA, $F_{(6,219)} = 5.773$, $P < 0.001$). The lowest annual mean of 2.50 g occurred in 1996.

In 1996 seminal receptacle weight, DNA quantity, and sperm count all exhibited significant spatial variation in newly mated, postmolt female crabs (Fig. 5). All three variables were significantly lower in Chesapeake Bay than in Florida (ANCOVA with female size as covariate, $P < 0.001$). Within Chesapeake Bay,

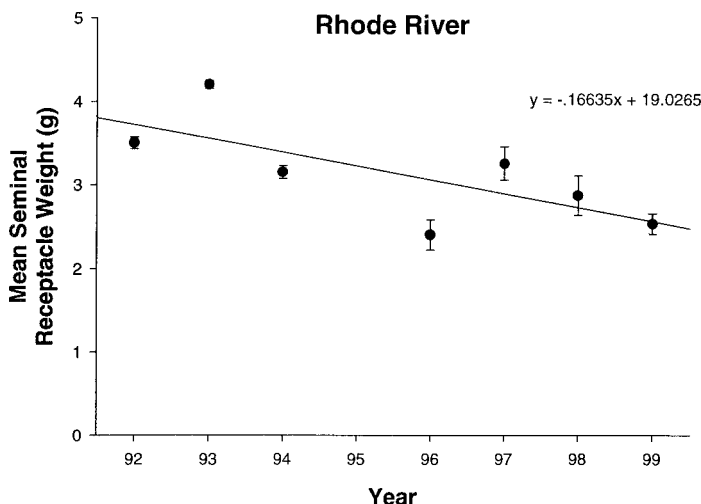


Figure 4. Decline of seminal receptacle weight of female blue crabs from upper Chesapeake Bay from 1992–1999. Mean \pm SE of mean and regression line are plotted for mature females collected in the Rhode River, MD. No samples were collected in 1995.

however, seminal receptacle weight in the James River did not differ significantly from that in the Rhode River. Apart from this exception, seminal receptacle weight, DNA quantity and sperm count were lowest in lower Chesapeake Bay (1.9 g wet wt, $0.55 \times 10^3 \mu\text{g}$ DNA, and 4.1×10^8 cells, respectively) and highest in the Indian River Lagoon (3.4 g wet wt, $2.4 \times 10^3 \mu\text{g}$ DNA, and 12×10^8 cells, respectively). Importantly, the seminal receptacles of Florida females uniformly appeared “bulging full” of ejaculate, whereas those of females at the Chesapeake Bay sites ranged from 10–100% full. As a group, all the sites in Chesapeake Bay had lower mean levels of receptacle weight, DNA quantity and sperm counts than those in Florida. Although our sample sizes are relatively small for these site comparisons, they are consistent with the temporal pattern of seminal receptacle weights involving a much larger number of crabs.

For 6 females with freshly deposited ejaculates in the Rhode River sample, there was a mean of 2.32×10^4 spermatophores per ejaculate, with a mean of 2.25×10^4 sperm per spermatophore and 5.21×10^8 sperm per ejaculate.

BROOD PRODUCTION.—In Chesapeake Bay, females are believed to produce 1–3 broods during summer, with most females in most years producing one brood and producing a second brood in some years, with possible production of a third brood late in the summer season (Van Engel, 1958; Millikin and Williams, 1984; Prager et al., 1990). In contrast, females that we held in tanks in Florida were capable of producing many more broods, sometimes at intervals of 2–3 weeks with only a few days between hatching and extrusion of the next brood (Fig. 6). Of the 16 females held during August to November 1994, most crabs produced 3 broods during a 9-week period from late August through October. During the spring of 1995, a cohort of 10 females produced either 2 broods (80%) or 3 broods (20%) over an 8-week period from April through May. A third cohort of females held over spring and summer 1995 produced as many as 6 broods over a 24-week period from April through September: 23% produced 6 broods; 31% produced 5 broods; 15% produced 4 broods; 15% produced 3 broods; and 15% produced two broods. If these females continued to produce broods during the late summer and

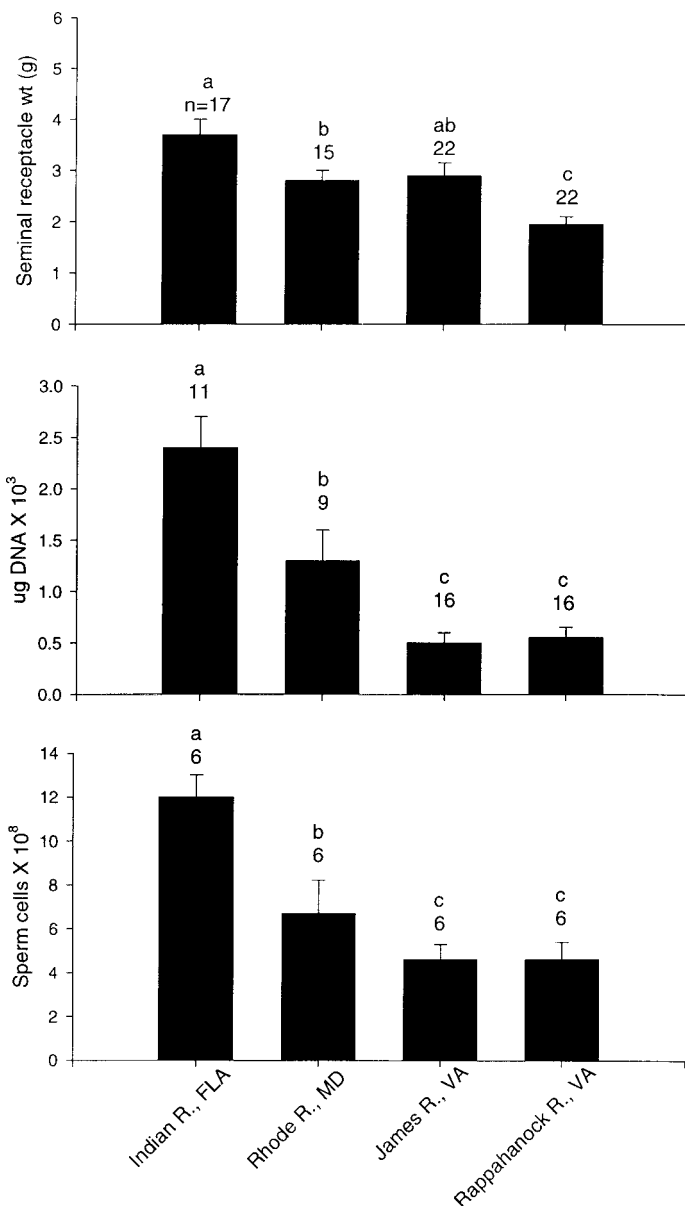


Figure 5. Geographic variation in seminal receptacle weight, weight of DNA, and number of sperm cells in seminal receptacles of female blue crabs. Means \pm SE of mean and sample size are plotted for samples from: Indian River Lagoon, FL; Rhode River, upper Chesapeake Bay, MD; James River and Rappahannock River, VA in lower Chesapeake Bay. Letters above histograms indicate samples that did not differ in SNK multiple comparison of means.

fall like the previous summer's cohort, then we infer that these Indian River Lagoon females might have produced as many as 7 broods over an entire brooding season from April to October.

Several of the females in the 1995 cohort produced fifth or sixth broods that were not fertile (the eggs turned clearish white, soon turned light tan, did not

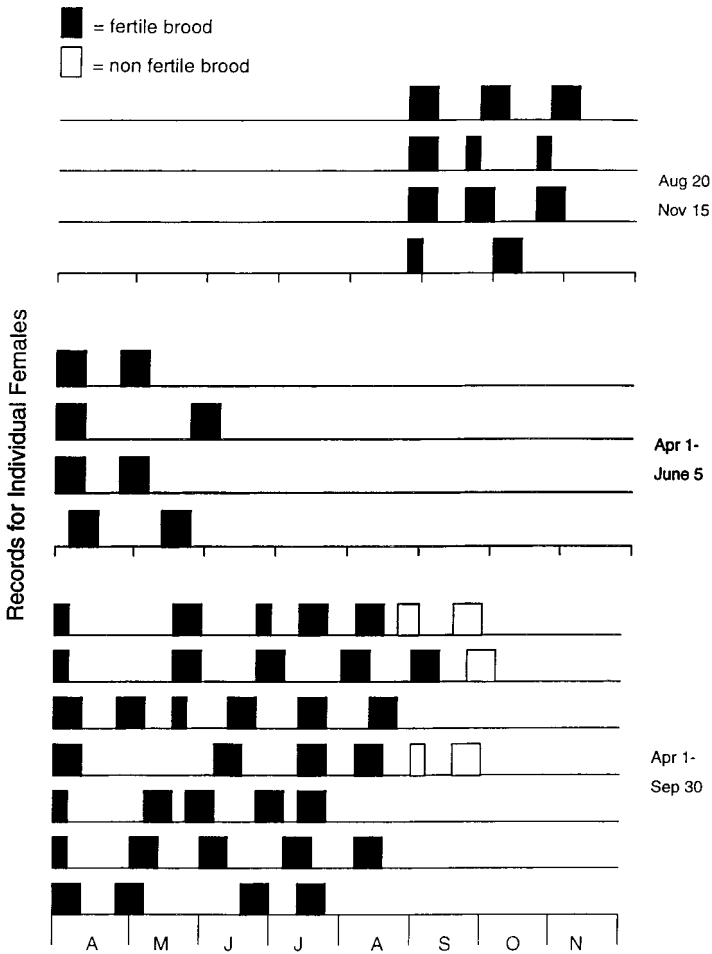


Figure 6. Sequences of brood production in female blue crabs held in laboratory tanks for the Indian River Lagoon, Florida. Representative examples of records are shown for individual females from 3 cohorts of crabs held from August–November 1994, April–May 1995, and April–September 1995. Blocks indicate the observed periods of brood incubation; solid blocks indicate fertile broods; open blocks indicate unfertile broods.

develop to hatching and deteriorated; see Dorsono, 1992): 25% of the fifth broods, and 50% of the sixth broods were not fertile.

If we assume that females live a maximum of about 2 years after the season of their molt to maturity (Millikin and Williams, 1984), then we estimate that females may produce a maximum number of broods per lifetime that varies among sites. Because females from upper Chesapeake Bay apparently do not begin to brood until the summer season after mating (due to migration and over-wintering), they may produce up to 3 broods in each of 2 seasons, for a maximum total of 6 broods during their lifetime. Since females from lower Chesapeake Bay often mature and mate earlier and do not migrate far, they may produce at least one brood in the same year as mating, and then up to 3 broods in each of 2 subsequent seasons, for a maximum total of 7 broods per lifetime. Females mating early in the season (April) in the Indian River Lagoon, Florida, can begin brooding as

soon as 2 months later (see mating experiments below) and might produce as many as 4 broods in the same year they mated (see also below), with as many as 7 broods during each of 2 subsequent seasons, for a maximum total of 18 broods per lifetime.

SPERM: EGG RATIOS.—Estimates for sperm:egg ratios were similar for female blue crabs from upper Chesapeake Bay, lower Chesapeake Bay, and Florida (Table 1). Egg production estimates were derived from published estimates of average blue crab fecundity at about 3×10^6 eggs per brood (Hines, 1982; Prager et al., 1990), and our estimates of maximum brood production per season and lifetime (above). Due to the spatial variation in sperm stores, we estimate that sperm:egg ratios for the “first brood produced” ranged about 2.5 fold from 150 sperm per egg in the lower Chesapeake Bay to 400 sperm per egg in Florida. However, the spatial variation in sperm stores in combination with variation in brood production resulted in similar lifetime sperm:egg ratios. For females producing the maximum number of broods, lifetime ratios may be as low as about 20 or 30 sperm per egg for both Chesapeake Bay and Florida—levels that do not consider loss of sperm during storage.

EFFECT OF MALE MATING HISTORY ON FERTILIZATION SUCCESS.—Sequential mating experiments in Florida showed that male mating history has significant effects on brood fertility (Fig. 7). Females began to produce broods 2 months following experimental mating. The number of broods produced per female ranged from 0 to 7, but the mean number of 2 broods produced per female did not vary among mating treatments (G -test, $df = 3, 95; \chi^2 = 1.79, P > 0.5$). However, hatching success of broods declined significantly with male mating history (G -test, $df = 3, 95; \chi^2 = 12.79, P < 0.01$). Females mated to males with fully charged sperm stores (no access to females in previous 12 days) had significantly higher hatching success (75% of broods) than females mated to males that had mated several times with less than 3 days between mating either 2–3 times (ca. 40% hatched broods), or 4 times (20% fertile broods).

CONCEPTUAL MODEL OF SPATIAL VARIATION IN MATING, BROOD PRODUCTION AND SPERM STORES.—By synthesizing the data on seasonal timing of mating, rates of brood production, and the levels of sperm transferred, we developed a simple schematic model that summarizes these activities, and that allows inferences about sperm storage levels (Fig. 8). Females in upper Chesapeake Bay mate from late June through September and migrate down Bay to join the females in the lower Bay, so there is essentially no brooding activity in the upper Bay (except occasionally in high salinity years (Hines, pers. obs.) (Fig. 8A). Females mated in the upper Bay thus store sperm over winter and produce up to 3 broods per summer for up to 2 seasons in the lower Bay, totaling up to 6 broods per lifetime (Fig. 8B). Females in lower Chesapeake Bay mate from late May to September, with those mating early in the season producing broods by late summer. These lower Bay females also store sperm over winter and then produce up to 3 broods per season for up to 2 seasons, totaling 7 broods (Fig. 8B). Females in the Indian River Lagoon, Florida, mate from March to September, and brood eggs from April to October (Fig. 8C). Whereas females mated early in the season begin producing up to 4 broods in the first season after about 2 months following mating, those mated late in the season do not begin brooding until the following spring. Early- and late-mated females store sperm over the short winter and then produce up to 7 broods per year for up to 2 seasons. Thus, early mated females could produce as many as 18 broods over 2.5 seasons ($4 + 7 + 7$) while late mated females might produce as many as 12 broods over 2 seasons.

Based on our measurements of seminal receptacles in wild-caught and exper-

Table 1. Geographic variation in estimates of sperm : egg ratios for three sampling sites. Calculations per brood, per season, and per lifetime are based on average number of eggs and maximum number of broods and lifespan for each site.

Site	Sperm per ejaculate	Per brood			Per season			Per lifetime		
		No. eggs	Sperm : egg ratio	No. broods	No. eggs	Sperm : egg ratio	No. broods	No. eggs	Sperm : egg ratio	No. broods
Upper Chesapeake Bay, Maryland	6×10^8	3×10^6	200:1	3	9×10^6	66:1	6	1.8×10^7	36:1	6
Lower Chesapeake Bay, Virginia	4×10^8	3×10^6	133:1	3	9×10^6	44:1	7	2.1×10^7	21:1	7
Indian River Lagoon, Florida	1.2×10^9	3×10^6	400:1	7	2.1×10^7	57:1	18	5.4×10^7	22:1	18
				6	1.8×10^7	68:1	12	3.6×10^7	33:1	12

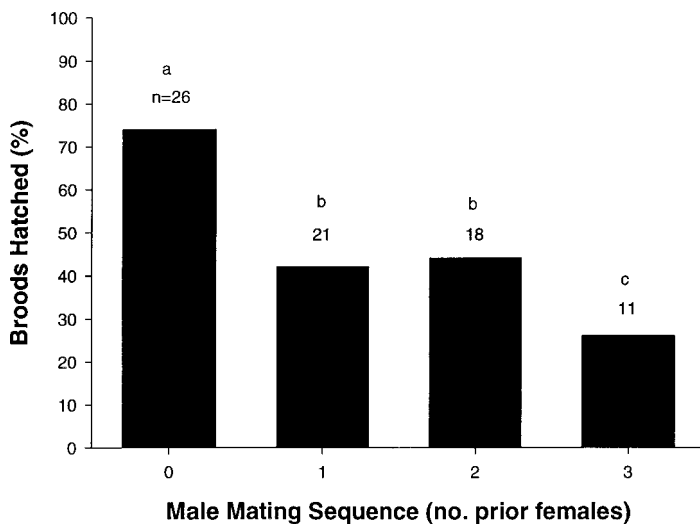


Figure 7. Relationship between brood hatching success and male mating history for female blue crabs in laboratory mating experiments in Indian River Lagoon, Florida. Histogram bars indicate percent of first broods hatching as a function of the number of prior females mated in sequence to males. Sample sizes indicate number of females in each group. Letters above histograms indicate treatments that did not differ in SNK multiple comparison of means.

imental females, our observations of brood production, and our mating experiments in the Indian River Lagoon, we hypothesize that females in the Florida population receive the full (maximum) complement of sperm, but that they also exhaust their supply of sperm late in their sequence of brood production (Fig. 8C). This level is presumed to be the maximum amount because: (1) it is the highest mean level recorded in the field for several years and locations (Hines et al., unpubl. data); (2) it equivalent to seminal receptacle weights and sperm counts delivered in experimental matings by males that have been held for a period of 20 days without access to females (Jivoff, 1995; Kendall et al., in press); and (3) ejaculate levels reach asymptotic levels after this time period of experimental abstinence (Kendall and Wolcott, 1999). If our estimate of the number of broods per lifetime is correct, then we can partition the full allocation of sperm among the 12–18 broods, yielding a sperm : egg ratio of about 22:1 or 33:1. This assumes parsimoniously that the sperm are partitioned equally among broods during the sequence, and it neglects any attrition of sperm during periods of prolonged storage. Thus, we estimate that each brood of 3×10^6 eggs uses about 68×10^6 to 100×10^6 sperm (including any attrition during storage).

Based on similar assumptions and these sperm : egg ratios, we also estimated the “fertilization capacity” of the sperm stores that we recorded for each location (Table 2) and diagrammed the incremental decline in sperm stores (Fig. 8D). Florida females, as defined in the model, receive just enough sperm to fertilize their potential egg production capacity. Females in the upper Chesapeake Bay receive about 50% of the sperm found in Florida crabs, which would be enough to fertilize about 9 broods—more than the expected production of 6 broods over a 2-year lifetime total. However, females in the lower Chesapeake receive about 35% of the sperm found in Florida crabs, which would fertilize only about 6 of the 7 broods that we estimate as their potential lifetime capacity. Thus, sperm stores may become exhausted at the end of the females’ reproductive life in the Indian

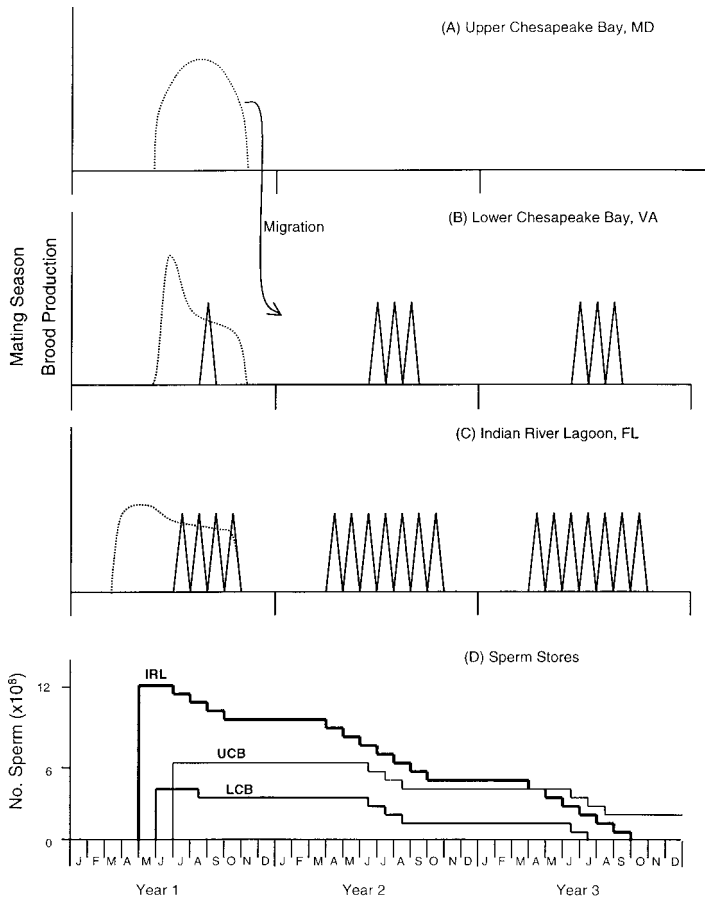


Figure 8. Conceptual model of geographic variation in mating season and brood production, and for sperm stores of female blue crabs from: (A) upper and (B) lower Chesapeake Bay and (C) from Indian River Lagoon, Florida. Curved lines indicate mating season. Arrow from upper Chesapeake Bay panel indicates that females migrate to join the lower Chesapeake Bay population at that time. Spikes indicate timing of estimated maximum number of broods produced over the lifespan of hypothetical females. (D) Sperm stores reflect use of sperm for egg fertilization (and attrition during storage), corresponding to the broods produced.

River Lagoon, and sperm stores may be limiting for females mated in the lower Chesapeake Bay.

DISCUSSION

These initial findings all indicate that ejaculate size and sperm quantities received by female blue crabs vary significantly in space and time, with females in the Chesapeake population having received lower levels than the Indian River Lagoon, Florida, population during at least one year of comparison (1996). Ejaculate weight and sperm number appeared near maximum levels (3.6 g, 1.2×10^9 sperm, respectively) in females at the Florida site, and these levels correspond well with ejaculates of fully charged experimental males that had no access to females for 2–3 weeks in both Chesapeake Bay and the Indian River Lagoon (Hines et al., unpubl. data; Kendall and Wolcott, 1999, Kendall et al., 2001).

Table 2. Geographic variation in estimated fertilization capacity of sperm stores. Maximum number of broods are estimated for 2 rates of sperm use per brood.

Site	Sperm stores	Fertilization capacity (no. of broods)	
		6.7×10^7 sperm per brood	1×10^8 sperm per brood
Upper Chesapeake Bay, Maryland	6×10^8	9	6
Lower Chesapeake Bay, Virginia	4×10^8	6	4
Indian River Lagoon, Florida	1.2×10^9	18	12

Ejaculates in Chesapeake Bay were lower than those in Florida by about: 25% weight and 50% in sperm number at the upper Chesapeake Bay site; and 50% in weight and 65% in sperm number at lower Chesapeake Bay sites. The Chesapeake and Florida sites differ both in fishing pressure and in factors related to latitude that affect the timing and duration of the reproductive season. Because these effects are potentially confounded without sampling replication of more sites, we proceeded to explore the interaction of the factors affecting sperm storage and depletion as a function of brood production at each site. This analysis allows consideration of the potential for sperm limitation in blue crab reproduction.

The quantity of ejaculate, and possibly sperm, transferred to females in upper Chesapeake Bay appears to have declined significantly during the 7-year period 1992–1999, while fishing pressure increased and the stock declined significantly (Miller and Houde, 1999; Lipcius and Stockhausen, in press). Mating success (percent of females mated) during this period, however, remained high in both the Chesapeake Bay and Indian River Lagoon populations, as indicated by the presence of ejaculate in >98% of post-molt females. Concurrently during past decades male:female ratio and male size have declined in parts of Chesapeake Bay (Abbe and Stagg, 1996), and these factors affect mating duration and ejaculate quantity (Jivoff and Hines, 1998a, 1998b). Thus, most females appear to be mating successfully, but with smaller males that mate more frequently to deliver smaller ejaculates than large, fully charged males (Kendall and Wolcott, 1999; Kendall et al., in press). During this period of decline, lowest levels occurred in 1999 and 1996, which was the only year that we compared levels between Chesapeake Bay and Florida. However, 1992 and 1993 at the start of the decline, seminal receptacle weight in the Rhode River crabs (3.5–4.1 g) was similar to the high level in Florida in 1996 (3.75 g), indicating that sperm levels in the upper Chesapeake Bay were at near full levels in the early 1990s. Due to declines in the spawning stock over this same period, reproductive output and larval recruitment declined significantly in Chesapeake Bay (Lipcius and Stockhausen, 2002). The potential interaction of declining reproductive stock and declining sperm stores is unknown, because we understand so little about variation in egg production in blue crabs.

The fate of the stored ejaculate is poorly understood, but the dynamics of seminal receptacle weight provide clues to the function of the seminal fluid, which forms the major portion of the ejaculate (Fig. 9). Mating success can be assessed shortly after mating, because of the large mass of the seminal receptacles filled with pink seminal fluid. The seminal fluid disappears from the seminal receptacles during a period of two to three months following copulation, leaving behind only a relatively small mass of sperm, which are difficult to detect visually without careful microscopic examination. Seminal fluid may serve in sperm storage to sustain sperm metabolism or to function as antibacterial agents that may protect

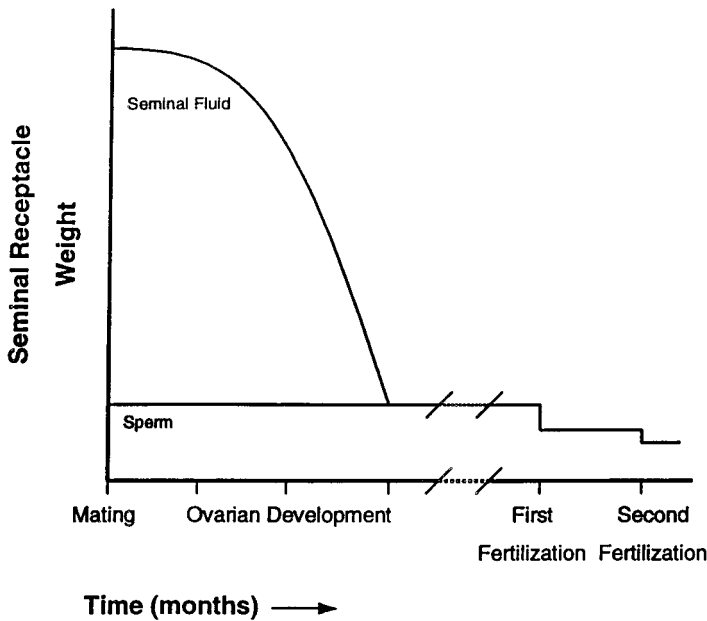


Figure 9. Schematic of changes in seminal receptacle contents following mating and brood fertilization.

the reproductive tract (e.g., the mud crab *Scylla serrata*, Subramoniam, 1991, 1993; Jayasankar and Subramoniam, 1999; the insect *Drosophila melanogaster*, Neubaum and Wolfner, 1999; Tram and Wolfner, 1999). However, the seminal fluid stored by Chesapeake blue crab females mated in summer/fall disappears by December, several months before fertilization and brooding begins in early summer. Similarly in Florida, seminal fluid disappeared within two months after mating in the spring, leaving sperm alone in the seminal receptacles for months to years as brood production proceeded during the first summer and potentially the following two years. Thus, the seminal fluid is not serving a role of sustaining sperm during most of the storage period in *Callinectes sapidus*. The fluid may serve as a “nuptial gift” of nutritional value when absorbed by the female, although a single prey item is often larger in biomass (Hines et al., 1990), and the chemical composition of the fluid appears to be of doubtful nutritional value (Johnson, 1980; Diesel, 1991). While its nutritional value remains unknown, the seminal fluid quickly hardens (within a few days) after insemination to a waxy consistency, and it seems more likely to serve as a sperm plug to prevent sperm from leaking out of the female or to block competing males from depositing additional sperm (Ryan, 1967; Hartnoll, 1969; Jivoff, 1997a; Sainte-Marie and Sainte-Marie, 1999). Sperm plugs also occur in several other groups of crabs, including cancrids and majids (Hartnoll, 1969; Diesel, 1991; Jensen et al., 1996; Sainte-Marie and Sainte-Marie, 1999a, 1999b; Hines, pers. obs.).

The seasonal variation in seminal receptacle weight reflected the seasonal periods of mating activity at each sampling site. Seminal receptacle weight showed a dip in July in the lower Chesapeake Bay (Fig. 2). This dip may reflect a bimodal peak of mating (May and August) for lower Bay blue crabs. It probably does not reflect the influx of females migrating down from the upper Bay, since they

migrate rapidly to the lower Bay in October and do not move down the Bay earlier in the season (Turner et al., this volume; Hines et al., unpubl. data).

To assess sperm limitation, we considered sperm stores relative to annual and lifetime fecundity, which may be estimated as a product of the number of eggs per brood and brood production capacity, and the number of reproductive years over the lifespan. Published estimates of fecundity per brood are similar at about 3×10^6 eggs for both Chesapeake Bay and the Indian River Lagoon, although variance is high and fecundity is significantly positively correlated with female size (Hines, 1982; Prager et al., 1990; Hines, unpubl. data). Our observations of brooding sequences by female blue crabs in the Indian River Lagoon, Florida, indicate that yearly brood production at lower latitudes is much greater than previously thought. Previous work in Chesapeake Bay indicated that blue crabs in the field produce 1–3 broods per season (Millikin and Williams, 1984), and a similar rate of brood production was inferred for the Indian River Lagoon (Tagatz, 1968). More recently, Prager et al. (1990) estimated from population brooding frequencies that Chesapeake blue crabs produce one brood in most years and two broods in other years. Our laboratory study in Florida is the first to track sequences of brood production by individual females over periods of months, indicating that these females produced up to 7 broods in rapid, although variable rate of succession over a prolonged season. Since sustained records of individual females are not available for Chesapeake Bay, it is not clear whether females at higher latitudes may also produce numerous broods. However, the brooding season is considerably shorter at higher latitude, and anecdotal observations indicate that there is a delay between broods produced by females in the Chesapeake and Delaware Bays (Hines, pers. obs.). Accordingly, the present consensus is that Chesapeake blue crabs produce 1–3 broods per year.

Estimation of lifetime brood production and fecundity depends on the lifespan of female blue crabs. Although it is not possible to age blue crabs directly in the field, analysis of population dynamics, size structure and tagging studies indicated clearly that females live for an average of 1–2 years after attaining sexual maturity (2.5–3.5 years total lifespan) (Millikin and Williams, 1984; Rugulo et al., 1997; Miller and Houde, 1998). Thus, while our estimate of lifetime brood production and fecundity for Florida blue crabs is much higher than previously thought (up to 18 broods and 5.4×10^7 eggs), our estimates for the Chesapeake population remain consistent with long established estimates (6–7 broods and 1.8×10^7 to 2.1×10^7 eggs). More limited data from tagging studies indicate that females may live considerably longer than 3 years. In North Carolina, Fischler (1965) estimated that a few crabs live as long as 5 years. Limited data from recent tagging studies (McConaughy, pers. comm.) indicate that females might live as long as 8 years. If this greater longevity is true, and if females remain reproductively active throughout this period, then maximum lifetime brood production and fecundity could be at least twice the 3 year average. Little comparative data is available for populations at lower latitudes, but Tagatz (1968) indicated maturation at age 1–1.5 years and a lifespan of about 3 years in northeast Florida, similar to Chesapeake Bay. For females at lower latitudes of warmer climate to have brood production similar to the estimate for Chesapeake Bay, Florida crabs would have to live only about 1.5 years. Conversely, if females experience greater longevity in lower fishing intensity at lower latitude, then they may have even greater potential brood production.

Our estimates of maximum egg production and our counts of sperm stored by females allow one of the few quantitative estimates of sperm:egg ratios for marine organisms. Estimated sperm:egg ratios of about 20:1 or 30:1 for blue crabs

appear low in comparison to mating systems of mammals ($10^9:1$) (Smith, 1984; Cummins and Woodall, 1985) or free-spawning fish ($10^4:1$ to $10^5:1$) (Shapiro et al., 1994; Fauvel et al., 1999; Ciereszko et al., 2000). Insect sperm:egg ratios range from $10^4:1$ in some lepidoptera to 1:1 in some *Drosophila* spp. (Bressac et al., 1994). However, there are few other quantitative estimates of sperm:egg ratios for comparison in crabs. For snow crabs (*Chionoecetes* spp.), ratios of 70:1 appear to be in a range similar to blue crabs, and as ratios drop below about 10:1 egg extrusion may stop (Sainte-Marie and Lovrich, 1994). For blue crabs, sperm:egg ratios are initially an order of magnitude higher (100:1 or 400:1) but inevitably decline as sperm are used for successive fertilizations. Our estimates of sperm:egg ratios do not consider unknown potential effects of attrition or loss of sperm viability over long periods (months to years) of sperm storage. In addition to blue crabs, several other crab species can store sperm for long periods, including cold-water species that may miss annual remating, such as cancrids (Jensen et al., 1996) and some majids (Sainte-Marie, 1993), which may go 2.5 years or more without re-mating when they "skip-molt." Thus sperm:egg ratios over the longer period likely become critical as sperm are used, lost or diminished in quality.

Although we have not determined empirically the minimum amount of sperm needed for fertilization, we believe that our model of brood production and sperm utilization sets reasonable bounds to the quantity. The validity of the model can be evaluated by assessing its assumptions. Central to the model are our observations that females in the Indian River Lagoon, Florida, which we presume to receive a full complement of sperm (about 1.2×10^9 sperm), eventually appear to run out of sperm, as evidenced by the broods that did not hatch at the end of the reproductive sequence. If the estimated maximum number of broods produced in Florida (18) is too high (perhaps enhanced by laboratory feeding, or by over-estimating longevity), then the quantity of sperm used per brood would be even greater than our estimate of 68×10^6 sperm per brood of 3×10^6 eggs. But if this is true, then the Chesapeake females are even more likely to be sperm limited. If the Florida females produced infertile broods as an artifact of captivity affecting egg quality rather than sperm availability, and could actually fertilize more broods, then the sperm:egg ratio would be substantially lower than 20:1. Sperm:egg ratios that are substantially lower than 20:1 seem likely to be sperm limited; but if this lower ratio is adequate, then the lower sperm stores in Chesapeake females may be sufficient to fertilize the lower number of broods they produce. Attrition of sperm is appreciable during storage by the female in some species (Paul, 1948). If this were also the case for blue crabs, then the operational sperm:egg ratio would be even lower than 20:1. Thus, our model's prediction (that blue crabs in both Florida and Chesapeake Bay run out of sperm) would only be in substantial error (i.e., they need far less sperm than we estimated) if the sperm:egg ratio used per brood is well below 25:1 or the potential number of broods produced is much lower than our estimates for both latitudes. Our direct observations of female brooding sequences in Florida, and the estimate of brood production in Chesapeake Bay, suggest that these parameters in the model are in fact reasonable upper limits. Lower limits of brood production over 2 years may be 2 broods in the Chesapeake Bay and 8–10 broods in Florida, which would not approach sperm limitation but would indicate that reproductive output is low even if not sperm limited.

Our model includes a parsimonious assumption that sperm are allocated in equal increments among broods (Fig. 8D). While clues to sperm use may be found in the pattern of brood infertility in Florida females held in the lab and in our mating experiments, our methods simplify the inferences that we can draw. Our

data indicate that entire broods of some females went unfertilized after relatively few broods (wild caught females) or after few male matings (in lab experiments). However, our methods only quantified broods that developed through to hatching as a categorical variable, because it was beyond our technical resources to quantify the numbers of eggs produced and the partial fertility of broods as continuous variables without sacrificing females and broods. We observed high variability in fecundity per brood, as noted by others for blue crabs (Hines, 1982; Prager et al., 1990; Hines, unpubl. data), and we did not quantify unfertilized eggs within a brood, which may be sloughed off during brooding (Dorson, 1992; Hines, unpubl.). While much more difficult to assay, these factors could provide evidence for partial fertility rather than "all-or-none" fertility of broods. We tracked in brood sequences in wild caught females that undoubtedly differed greatly in both age and previous brooding history, with some near the beginning, and others near the end of their life span. The quantity of sperm delivered to females may decline precipitously after only one or two matings (Kendall and Wolcott, 1999; Kendall et al., 2001), so females late in a mating sequence can receive a much reduced ejaculate. Thus, there may be high variation in both the number of eggs produced and the quantity of sperm transferred. Our data provide a conservative assay that male mating history affects reproductive success. Our model of brood production and sperm storage focuses on the average amounts of sperm allocated equally to maximum numbers of broods. Obviously, other combinations could be considered, but we do not feel that we have any basis to assume unequal allocation of sperm at this time.

Although management of crab fisheries often focuses on protection of females, our analysis of blue crabs indicates that removal of males may have significant impacts on reproductive success. Fisheries that target males appear to be altering the operational sex ratio, size composition, and male mating history of several species of decapods, including king crabs *Paralithodes* spp. (Powell et al., 1973) and several brachyuran crab, *Chionoecetes* spp. (Powell et al., 1973; Ennis et al., 1990; Paul and Paul, 1992; Sainte-Marie et al., 1997), *Cancer magister* (Smith and Jamieson, 1991; Elner and Beniger, 1995; Hankin et al., 1997), and *Callinectes sapidus* (Abbe and Stagg, 1996; Jivoff, 1997a; Jivoff and Hines, 1998). Recently, analysis of various stock models and long-term data for Chesapeake Bay blue crabs indicate that the stock is suffering significant over-fishing (Miller and Houde, 1998). Fishery-independent winter dredge survey data indicate that the abundance and size of over-wintering population of females has decreased (Lipcius and Stockhausen, in press). However, intense fishing on male blue crabs in some parts of Chesapeake Bay is also imposing long-term reductions of male size and male:female sex ratio (Abbe and Stagg, 1996). At this point, our sampling indicates that the reduction in males apparently has not resulted in significant proportions of females going unmated. However, small male size negatively affects ejaculate quantity, and reduced male:female sex ratio reduces mating time, and presumably sperm transfer (Jivoff, 1995, 1997, 1998; Jivoff and Hines, 1998a, 1998b). Thus, in addition to the problem of stock depletion of females leading to the more traditional view of stock reproductive limitation, we argue that the Chesapeake blue crab stock may suffer from excessive removal of large males, which can affect population reproductive success in complex and profound ways. We hypothesize that significant reduction in male size and the "operational" male:female sex ratios by over-fishing of males in the population could lead to sperm limitation, whereby females receive insufficient quantities of sperm to fertilize the full potential of their egg production. Just as importantly, our data indicate that the Florida population at lower latitude and longer brooding season may also run

out of sperm after producing many more broods than previously thought by the end of their reproductive lifetime. This suggests that intense fishing pressure at such lower latitudes could have an even greater impact on reproductive potential than it is having in Chesapeake Bay.

ACKNOWLEDGMENTS

We thank Midge Kramer, Laura Nye, and Karen Metcalf for assistance in processing samples and managing the data. Many students at VIMS and SERC assisted with crab collections and dissections. Rob Andrews assisted with preparing the figures. Funded by: NSF OCE-9711843 to A.H.H. and P.R.J.; NSF OCE-97155 to T.G.W. and D.L.W.; Smithsonian Environmental Sciences Program to A.H.H.; Disney Wildlife Conservation Fund to A.H.H. and T.G.W.; and a Smithsonian Postdoctoral Fellowship to P.J.B.

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