

ABSTRACT

DAVIS, JOHN HOWELL. Implications of a Decrease in the Mature Size of Female Blue Crab, *Callinectes sapidus*. (Under the direction of Donna L. Wolcott.)

The size-at-maturity of female blue crabs is declining and the proportion of very small mature females (<100mm carapace width (CW)) is increasing in North Carolina. Decreasing mature size of the female may have an effect on the mating success and mortality of size-disparate mating pairs. To test the effect of body size on mating behavior, I used controlled mating experiments comparing size-disparate mating pairs with similarly-sized mating pairs. Small males handled large females longer than any other mating pair combination; however, no increase in injury or mortality was found. Neither large nor small males transferred significantly different numbers of sperm to large and small females. Size-disparity appears to have no effect on mating success in blue crabs, suggesting that diversity of size-related genotypic traits can be preserved in the population. I also designed a mathematical model to estimate the lifetime spawning potential of different size-cohorts of female blue crabs subject to different mortality pressures. Cohorts of very small females (<100mm CW) that are not subject to fishery mortality produce 5.1 – 58.1% more eggs over two spawning seasons than larger, harvestable females (127-159mm CW), suggesting an evolutionary advantage to maturing at smaller sizes. I also used the model to estimate the efficacy of a proposed restriction on the commercial harvest of large females (>172mm CW), intended to increase the spawning potential of large females in order to increase population-wide recruitment, as well as the number of recruits that may possess a larger-size genotype. The proposed seasonal (Sept. – Apr.) commercial harvest of large females produced an estimated 23.2 – 35.1% increase in egg production over two spawning seasons. However,

since the large females compose less than 2% of the population, on average, the estimated increase in spawning potential for the entire population was negligible (0.46%).

Additionally, the 5% tolerance of the regulation allows for the harvest of the entire population of large females, suggesting that the proposed restriction will be ineffective.

Implications of a Decrease in the Mature Size of Female Blue Crab, *Callinectes sapidus*

by

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A thesis submitted to the Graduate Faculty of

North Carolina State University

In partial fulfillment of the

Requirements for the degree of

Master of Science

Marine, Earth and Atmospheric Sciences

Raleigh, NC

2006

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BIOGRAPHY

John Howell Davis was born in Spartanburg, SC, on August 1, 1976. Despite the derision of his older brother, John showed an interest in science from an early age. He excelled in biology and chemistry and attended a marine biology summer camp at Duke University at the age of 16. Following graduation from the Spartanburg Day School in South Carolina, John attended the University of South Carolina's Honors College as a Carolina Scholar. There, he majored in Biology and minored in Marine Science. He completed and defended an honors thesis regarding intron evolution in a closely-related family of fishes. John was a member of the Marine Science Undergraduate Society at USC, working with Loggerhead turtle restoration and beach erosion issues on the coast of South Carolina. John married Milli Wehr in 2000, with whom he suffered through Organic Chemistry at USC. After a brief stint in Oregon, John returned with his family to the Carolinas in 2002 to attend NCSU.

ACKNOWLEDGMENTS

I wish to thank my committee, Drs. Donna and Tom Wolcott, and Dr. David Eggleston, for their advice and help with building and polishing this thesis. Many reams of paper and red pens were sacrificed by them on my behalf in the editing process. I would like to especially think Donna and Tom for their patience in helping me develop these ideas into coherent scientific arguments. Thanks also go to Dr. Dan Rittschof and Gary Dickinson at Duke University for so graciously allowing me to use their unpublished data for my mathematical model. Special thanks to Dan for all of the help with my crab mating experiments. I would like to thank Duke University for allowing me to use their facilities at their laboratories near Beaufort, NC, free of charge. I wish to also thank my fellow graduate students that helped me with so many forgotten small things: Sean Ramach, Dave Medici, Geoff Sinclair, Linda Waters-Lindquist, Geoff Bell and Darren Parsons. Special thanks go to all the crabbers that contributed their time and crabs to my project, including Mark Hooper, Dell Newman, and especially Judy Cahoon and C&S Seafood.

I must also thank my family for their patience and moral support. My wife, Milli, had to put up with much strife and trauma during my graduate career, though she did get to live at the beach for one summer. Thanks to my boys, Rowan and Niall, for distraction from work. Also, thanks to my parents for their support throughout the years. Thank you all!

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Chapter 1: Implications of a reduced size-at-maturity to mating dynamics and reproductive potential of blue crabs in North Carolina

Introduction

The blue crab (*Callinectes sapidus*) is the most valuable commercial fishery in North Carolina in terms of total landings, processing, employment, and amount of gear used (Henry & McKenna 1998, NCDMF 2004). Over the past decade, North Carolina has been the top producer of blue crabs in the U.S. at over 24% of the total harvest, with an annual average of 50 million pounds valued at over \$37 million (NCDMF 2004). The economic impacts of the commercial fishery are far-reaching throughout eastern North Carolina. Many commercial crabbing operations are family businesses that have been passed down through many generations. In some of the more rural areas of eastern NC, especially the western side of the Pamlico and Albemarle Sounds where beach tourism is not a factor, the blue crab fishery often is the primary livelihood. In 2002, the commercial fishery contributed an estimated \$53 million in sales, \$32 million in total income, and more than 4000 full and part-time jobs statewide (NCDMF 2004).

Landings of blue crabs in North Carolina have decreased in recent years, possibly a consequence of declines in abundance of the blue crab stock indicated by most fishery-independent survey indices (Eggleston *et al.* 2004). Spawning stock biomass has decreased and fishing mortality has sharply increased since 1996, often to levels well above estimates of Maximum Sustainable Yield (MSY) (Eggleston *et al.* 2004). Concurrent with the generally increasing exploitation rate from 1992-2002 is a decreasing number of legal-sized crabs, a decreasing average size of mature females, and an increasing proportion of small

(<100 mm carapace width (CW)) mature female crabs (Eggleston *et al.* 2004). These observed changes in phenotype are consistent with selection for a genotype producing smaller size-at-maturity in female blue crabs. Small mature females are not susceptible to the fishery, because they can pass through the mesh of crab pots, the most common gear used. Additionally, cull rings in pots for hard crabs retain approximately 90% of females $\geq$ 122 mm CW, 10% < 117 mm, and 0% < 100mm (Guillory *et al.* 2004). A female's size is set at the molt-to-maturity, which is also her terminal molt (after which she can mate one or more times during a receptive period of ~1 week) (Van Engel 1958). Consequently, small females can spawn for the remainder of their natural lives while larger females are being removed by the fishery.

No change in the mature size of the male blue crab has been detected, due to the lack of survey data on the size-at-maturity of the male crab. Males attain sexual maturity over successive molts and retain the ability to continue growing and molting, unlike the female, in which size-at-maturity is easy to identify (for details of life history, see Appendix). Size-selective harvest of the blue crab (only males > 127 mm CW can be harvested) may alter the size structure of the males in the population simply by removing the majority of large males while leaving the small ones (Jivoff 2003). This size-selective harvest may change the mating dynamics of the population by allowing small males to mate repeatedly with reduced competition from large males (Carver *et al.* 2005).

If the effective size-at-maturity for blue crabs has any heritable genetic component, increased mating and spawning opportunities of small crabs relative to the larger, harvestable crabs could result in a further decrease in the average size-at-maturity of future spawning stocks due to evolution. A reduction in the mature size of both sexes could reduce the

average reproductive potential per crab in the population (leading to a declining abundance) (Prager *et al.* 1990, Jivoff 2003) and alter mating dynamics, which tend to favor larger crabs through male: male competition (Jivoff & Hines 1998, Jivoff 2003). Thus, it is important to investigate the mating behavior and mating success of small crabs in order to understand their potential genetic contribution to future generations.

It is well established that mortality selectively applied to one portion of the population can drive genetic change in that population (Policansky 1993). Mortality that differs according to size of the individuals imposes selection pressure on size-related life-history traits such as average adult size, size-at-maturity, and growth rate. Due to the difficulty of isolating genetic change from phenotypic plasticity, management of fisheries generally ignores the possibility of genetic change. Simply documenting changes in a population's life-history traits over time does not necessarily establish the occurrence of fishery-induced evolutionary change (Policansky 1993). For genetically-based changes in phenotype to occur in response to fishing pressure, changes must be heritable (genetic), and fishing pressure must result in different reproductive success for different genotypes (Policansky 1993).

Many phenotypic traits are influenced by heritable genotypic variation in fish species (Doyle 1983, Gall 1983, Wohlfarth 1983). Selective predation or mortality in the natural environment, as occurs in size-selective fisheries, results in the evolution of a variety of traits such as growth rate, color, morphology, and mating behaviors in freshwater crustaceans (Strong 1973), fishes (Gross 1978, Endler 1980, Reznick 1982), and many other organisms (Endler 1986). For example, genetically-controlled differences in growth rate among fishes in different latitudes (climates) are caused by size-dependent winter mortality (Conover &

Shultz 1995). Many studies of short-lived fishes have demonstrated genetic adaptation to different environments (Meffe & Snelson 1989, Taylor 1991, Bell & Foster 1994, Conover 1998). The rate of evolution can be quite rapid, occurring within a few generations (Reznick *et al.* 1997, Hendry *et al.* 1998, Thompson 1998). Local adaptive variation within populations can also be extensive, even in marine species that are often considered to have few barriers to gene flow (Conover 1998).

The common mechanism for fishery-induced differential reproduction of different genotypes is the size-selective nature of most fisheries, whether by gear selectivity or by management restrictions on harvestable size (Policansky 1993). Selective removal of the largest individuals in a population will reduce the potential genetic contribution of those individuals to future generations, especially if the fishery allows them little or no opportunity to mate and reproduce before harvest. Such fishery mortality will increase the reproductive potential of smaller mature individuals that are not harvested. Most fisheries cause high mortality, often 70% to 90% of the total population (Cushing 1968, Heard 1991, Rijnsdorp *et al.* 1991). Many exploited fish populations have been harvested for 50-100 generations, providing ample time for selection to produce observable genetic change.

Evolutionary change as a result of size-selective mortality has been documented in both experimental and natural fish populations. Rapid genotypic changes were reported in several life-history traits of an experimental population of Atlantic silverside, *Menidia menidia* (Conover & Munch 2002). Size-selective harvest produced significant changes in harvested biomass, mean weight, spawning stock biomass, egg size, and larval growth rate after only four generations. Significant life-history evolution within 30-60 generations has been reported in natural populations of guppies (*Poecilia reticulata*) by simply changing the

target of predation from adults to juveniles (Reznick *et al.* 1990). All of the guppies were reared in the same environment, eliminating environmental influences on life-history variation within cohorts (Reznick *et al.* 1990). Divergence in several traits has also been shown within 30 generations between adjacent populations of guppies in New Zealand, including growth rate and reproductive output (Quinn *et al.* 2001). Divergence of life-history traits between the two populations initially resulted from phenotypic plasticity (e.g. habitat-specific growth rates) but also had a genetic component, as evidenced by a lower survival rate of transplanted v. native populations.

Evolutionary change as a response to heavy exploitation by the fishery has been reported for a number of important fishery species. Divergence has been reported between fished grayling (*Thymallus thymallus*) populations due to different degrees and types of exploitation over 44-88 years (9-22 generations) (Haugen & Vøllestad 2001). The major cause of phenotypic differences found between populations was adaptive evolution caused by different mortalities under different fishing intensities. DNA comparisons support the conclusion that genetic drift occurred between the populations (Haugen & Vøllestad 2001). Age and length at maturation has shifted toward younger age and smaller length in many exploited fish species such as North Sea plaice (*Pleuronectes platessa*) and Atlantic cod (*Gadus morhua*) due to evolution (Ernande *et al.* 2003, Grift *et al.* 2003, Barot *et al.* 2004). Short-term fluctuations from plastic responses are superimposed on a persistent, long-term trend resulting from genetic selection.

Demonstrations of genetic selection in crustaceans have been limited primarily to short-lived planktonic species, and are less common for large marine ones such as lobsters and crabs (Jamieson 1999). One of the few comprehensive discussions of phenotypic

plasticity v/s genetic change in the blue crab showed marked concurrent decreases in spawning stock abundance and recruitment, larval abundance, and adult female size (and thus, size-at-maturity) in the Chesapeake Bay (Lipcius & Stockhausen 2002). Many possible genetic mechanisms were discussed for why female crabs were maturing at younger ages and smaller sizes: optimization of mating opportunities when larger potential mates were sparse (Smith & Jamieson 1991, Jamieson *et al.* 1998); maximizing lifetime fecundity in the absence of mate-competition with other females (Lipcius 1995); size-selective fishery exploitation of large females resulting in selection for smaller females (Policansky 1993, Jamieson 1999); and compensatory responses of the population to low densities such as decreases in mean size, age or instar at maturity (Charnov *et al.* 1978, Charnov 1979, Hannah & Jones 1991, Wellbourn 1994, Sparkes 1996). Distinct genotypic influences on population change were not identified, however; a combination of genetic mechanisms, phenotypic plasticity and size-selective exploitation may have acted in concert to produce covariation in female size and spawning stock abundance (Lipcius & Stockhausen 2002).

Differences in mating behavior and success attributable to differences in mature size have been studied in the context of competition between individuals. The mating success and seminal contribution of male crabs depend primarily on male: male interactions, which favor large males (Jivoff 2003); however, at the low male: female sex ratios found in many fished populations, competition between males is decreased (Jivoff & Hines 1998, Jivoff 2003). Since the size of a male crab does not affect its ability to subdue the female during the pre-molt mating interactions (Jivoff 2003), in the absence of male: male competition, mating opportunities of small and large males will be similar. The mating success and semen

transfer for mating pairs of disparately-sized mates has not been well studied for heavily fished blue crab populations that are likely to have little male: male competition.

The potential consequence of reduced sperm transfer is a lower maximum reproductive potential of the female, hence a reduced genetic contribution to subsequent generations by both mates. Limitations to the amount of sperm available to females in the population and the reproductive contribution of the male are rarely considered when determining stock-recruit relationships (Kendall *et al.* 2001). The assumption that sperm and seminal fluid are in ample supply may be an oversimplification for many decapod crustaceans (Ennis 1980, Smith & Jamieson 1991, Paul & Paul 1992). Recent research suggests that female crabs may become sperm limited when males are overharvested, making the number of sperm received during mating a potential limiting factor for their reproductive potential (Kendall *et al.* 2001, Kendall *et al.* 2002, Hines *et al.* 2003).

The potential consequence of decreased amounts of seminal fluid (in contradistinction to sperm) transferred to the female is a reduction in the reproductive potential of the male. The seminal fluid, which makes up the bulk of the ejaculate, is thought to function primarily as a “sperm plug,” which increases the chances that the first male’s sperm will fertilize the eggs by limiting the amount of semen that can be transferred to the female by any subsequent mates (Jivoff 1997, Wolcott *et al.* 2005). Multiple sperm plugs, indicating multiple matings, have been detected in field populations (Jivoff 1997). Reduced transfer of seminal fluid in size-disparate mating pairs would enhance mating opportunities between that female and subsequent males, thereby increasing sperm competition.

If pairings between crabs that do not differ greatly in size are more successful, then the relative genetic contribution of same-sized pairs (either small or large) will increase. The

actual genetic contribution of crabs in large: large mating pairs, however, is likely to be retarded by size-selective fishery mortality on the large females (see Chap. 2). Conversely, an increased mating success for a small: small mating pair vs. a small: large mating pair could accelerate the decrease in size of mature female crabs that has been observed in the blue crab population of North Carolina, because the small crabs are not subject to fishery mortality (Eggleston *et al.* 2004).

Since the proportion of very small mature females in the population is increasing, and larger males are selectively removed by the commercial harvest, leaving all of the smaller mature males, I examined the ability of small females and males to mate with “normal-sized” (much larger) crabs. A large disparity in size of the crabs in a mating pair may make it difficult to complete the various cradle-carry and mating positions (see Appendix for details of blue crab mating biology). Increased handling of a female that is much larger or smaller than the male crab could increase stress, injury, or mortality for the female when she is soft and vulnerable. I hypothesized that, when compared to pairings between crabs of similar size, pairings of a small crab with a large crab would exhibit the following differences:

- More time needed for male to manipulate and position the female
- Decreased duration of mating
- Increased frequency of disengagement from cradle-carry or mating position
- Increased frequency of limb loss by the female due to handling during mating
- Increased mortality of females
- Transfer of fewer sperm and less seminal material

Materials & Methods

Study Site

Blue crab mating experiments were conducted during the summer and fall of 2004 at the Duke Univ. Marine Laboratory on Piver's Island, Beaufort, NC. Healthy, whole crabs were purchased from local fishermen. All crabs were harvested using crab pots in the Jarrett Bay portion of Core Sound, in Mill Creek and other tributaries of the Newport River north of Morehead City, and in the White Oak River near Swansboro and Bogue Inlet, NC.

Experimental Protocol

Male crabs were grouped into two size classes based on their carapace width (CW) (measured between the tips of the large lateral spines): “small” (< 120mm CW; mean size = 112.5mm) and “large” (> 150mm CW; mean size = 160.3mm). The small group represented the smallest sexually mature males in the population; they were below the minimum legal size of harvest (<127mm CW). I used only small males that met the observable criteria for sexual maturity (abdomen completely free from the sternum, and the penes and second pleopods inserted into the first pleopods; Van Engel 1990). The large group represents a subset of the largest mating males in the local population.

Males were collected from mid-April through June and during September of 2004. Males selected for experimentation had fully hardened shells; none were recently molted “white-bellies.” To assure replete sperm stores, males were held and fed for 9-14 days (Kendall *et al.* 2001) prior to the commencement of mating.

Female pre-pubertal (pp) crabs were collected from mid-April through mid-July and from mid-September through mid-October of 2004. “Small” pp females were defined as those < 95mm CW (mean size = 85.6mm). I would have preferred to use an 80mm cut-off to

assure a mature post-molt size of $< 100\text{mm}$ CW, but insufficient numbers of such small individuals were obtainable. “Large” pp females were defined as those $> 115\text{mm}$ CW (mean size = 124.4mm). This cutoff value was chosen to achieve a difference of approximately 30mm in CW between the two groups after their molt to maturity, similar to the difference between the small and large males used in this experiment.

All crabs were held in large, round tanks (diameter = 122 cm ; volume = 1098 liters) with filtered circulating seawater pumped from Bulkhead Channel near Bogue Sound, NC. Salinity ranged from $27 - 33\text{ppt}$ and temperature from 18°C to 28°C . Fluorescent lighting was kept on 24 hours a day to facilitate video taping. Male crabs were fed daily with fish (primarily menhaden, *Brevoortia tyrannus*, and croaker, *Micropogonias undulatus*) for the duration of the 9-14 day holding period. Densities in holding tanks were ≤ 12 crabs per tank for the large male crabs, or ≤ 20 crabs per tank for the small male crabs or pp females. Dead crabs and uneaten fish were removed twice daily. All female crabs were held for at least 24 hours to acclimate them to the laboratory environment before the commencement of mating experiments. Female crabs were chosen for mating once they developed a distinct red line along the edge of the swimming paddle, indicating imminent molt.

Crabs selected for mating experiments were placed together in mating tanks (glass aquarium tanks holding approximately 70 liters of seawater, with aeration). Blinds were placed between the tanks to prevent visual interaction between mating pairs. A male crab was placed into the tank first and allowed at least 15 minutes to adjust. The female crab was not introduced until the male crab ceased all aggressive behavior or movement induced by human handling. Each of the following treatments was assigned randomly to tanks:

Treatment #1 – small male with small female (N=37 mating trials; 22 completed matings)

Treatment #2 – small male with large female (N=22 mating trials; 13 completed matings)

Treatment #3 – large male with small female (N=25 mating trials; 20 completed matings)

Treatment #4 – large male with large female (N=22 mating trials; 15 completed matings)

Every 24 to 36 hours, as needed, dirty water and feces were slowly (5-10 min.) siphoned out of the mating tanks while fresh seawater was added to keep the water level well above the crabs. The water was changed only when the mating crabs were quiescent in a cradle-carry or mating position, and never during or right after a period of active movement or molting. To minimize possible stress to the crabs while water was being changed, the hoses were kept as far from the crabs as possible and visual stimuli were minimized either by dimming the lights or by putting an additional blind in front of the tank.

Behavior was either observed in real time or recorded with a video camera for all mating pairs until mating (or attempts) ended. Video cameras were positioned beside each mating tank so that all behaviors could be observed. Recordings included a time-stamp. The following behavioral response variables were quantified: (1) the handling time required for the male to position the female into the (a) initial cradle-carry before the female's molt, (b) cradle-carry after the female had molted and completely freed herself from her old shell, (c) mating position, and (d) post-mating cradle-carry; and (2) duration of mating. Individual handling times were defined as seconds required for the male to achieve a cradle-carry or mating position (see Appendix), from the first attempts until the female was secured under the male for at least 30 seconds. The duration of mating was recorded for each pair, starting when the male achieved a mating position and ending when the male withdrew his gonopods and allowed the female to close her telson ("apron").

I also quantified the (3) frequency of disengagements from the cradle-carry or mating position, (4) frequency of limb loss by the female, (5) mortality of the female, and (6) sperm and seminal fluid transferred to the female. After all mating activities or mating attempts had ceased for at least 6 hours, crabs were placed in plastic storage bags in a freezer for later dissection.

Laboratory Dissection

In the laboratory, the carapace width of each crab was measured to the nearest millimeter using dial calipers. The two spermathecae (sperm storage organs) were removed from female crabs and weighed. The percent fullness of the organs was visually estimated. The spermathecae were cut open and the contents removed. The spermatophores and the seminal fluid (often hardened into a “sperm plug”) were extracted by scraping the spermathecal wall and rinsing it with 30 psu seawater. The mass of the extracted seminal fluid and sperm (if any) was calculated by subtracting the mass of the empty spermathecal wall from the total mass. If only one spermatheca contained sperm and seminal fluid, its data were doubled to estimate the contents had ejaculate been transferred to both. Lack of ejaculate on one side was usually caused by a physical problem such as a broken gonopod on the corresponding side of the male. All such males possessed a seminal vesicle replete with sperm and seminal fluid on the side where no ejaculate was transferred, compared to a much smaller, depleted vesicle on the side corresponding to the successful ejaculate transfer. It is assumed that failure to transfer ejaculate on one side is not affected by size-disparity, as all such males were able to achieve the mating position and appeared to be mating normally. The fertilization of the female crab on one side occurred in only 5 of 70 pairs and in three of the four treatments, too infrequent for statistical analysis.

The spermathecal contents were mixed in a Dounce homogenizer with 30 psu seawater until all spermatophores were disintegrated into the supernatant. If the seminal fluid had hardened into a sperm plug, it was minced with a scalpel or razor blade into pieces < 2 mm before homogenization. Material adhering to the spermathecal wall was scraped off with a scalpel and rinsed into the homogenate with 30 psu seawater. The spermathecal wall was examined under the microscope to ensure that no sperm remained. The homogenized supernatant was filtered through 35 μ m Nitex cloth supported by a funnel, directly into a graduated cylinder. The filter was rinsed with 30 psu seawater to flush through any loose sperm. The material remaining on the filter was examined under a microscope; if any sperm were detected, it was rehomogenized and filtered again. This process was repeated as necessary.

The filtrate was stained with eosin Y red dye (one drop of 1% eosin Y in 30 psu seawater per 5 ml of sample volume). The total volume of the dyed supernatant was recorded. The sample was poured into a dish and mixed with a magnetic stir bar for approximately five minutes. The sperm cells in 10 μ l aliquots were counted in a Petroff-Hausser spermacytometer under an oil-immersion microscope at 1000X. Two to three slides were counted for each sample and averaged. The total number of sperm in the sample was calculated by extrapolating from the spermacytometer volume counted to the total sample volume.

For any mating pair that failed to mate or to transfer sperm to the female, the male crab was examined to determine sexual maturity of the gonads as indicated by the presence of mature spermatophores in the anterior *vas deferens* (Van Engel 1990). Any mating pair in which the male was determined to be sexually immature was excluded from the dataset.

Statistics

One-Way Analysis of Variance (ANOVA) ($\alpha=0.05$) was used to detect significant differences between treatments for the following factors: the handling time required to achieve the pre-molt cradle carry, the post-molt cradle carry, the flip of the female into the mating position, and the flip of the female into the post-mating cradle carry; the combined handling time for the previous four factors; the duration of mating; the weight of spermathecal contents (amount of semen transferred to the female); and the number of sperm transferred to the female. Data for handling times were log-transformed, and data for number of sperm square-root-transformed, to satisfy assumptions of normality. Homogeneity of variance was tested using Brown & Forsythe's test (Brown & Forsythe 1974) as well as by visually comparing between treatments the distribution and range of residuals. Tukey's Studentized Range Test, which controls Type I error rate, was used for contrast of means between treatment levels in cases where there was a significant treatment effect.

Results

Both the time it took male crabs to "flip" females from a cradle carry to a mating position, and the total handling time during mating, varied significantly with size disparity between mates (one-way ANOVA; mating position, $F = 5.02$, $df = 3, 63$, $p = 0.0035$; total handling; $F = 9.97$, $df = 3, 53$, $p < 0.0001$). Small males took significantly longer (mean = 74s) to flip large females from a cradle carry to a mating position than males in the other treatments (means = 27-37s; Fig. 1). Similarly, total handling time over the entire experiment was significantly longer for small males with large females (mean = 208s) than in

the other treatments (means = 89-94s; Fig. 2). No differences were found between treatments for the handling time required to achieve the pre-molt cradle carry, the post-molt cradle carry, or the flip of the female into the post-mating cradle carry (one-way ANOVA, all $p > 0.05$).

Large males mated for a significantly longer time than did small males (one-way ANOVA; $F = 8.29$, $df = 3, 54$, $p = 0.0001$; Fig. 3). Large males mated for an average of 902-923 minutes, whereas small males mated for an average of 658-754 minutes (Fig. 3). For males in a given size class, female size had no significant effect on mating duration (one-way ANOVA, all $p > 0.05$).

Volume of semen transferred to a female crab's spermathecae was positively correlated with the duration (min.) of mating (Fig. 4), as was the number of sperm transferred (Fig. 5). The large male with small female treatment (#3) was excluded from the regression analyses because the amount of semen and sperm that could be transferred was apparently limited by the size of the females' spermathecae (females were 100% full after mating).

Instances of male crabs disengaging from or "dropping" the female from the cradle-carry position more than twice (3-4 times per treatment) did not occur often enough for statistical analyses. Males that dropped the female only once or twice were not considered due to the small amount of handling required for the male to re-cradle the female. These disengagements almost always occurred before the female molted when she was still protected by her shell. Even in pairs with 3+ drops, there was no injury or mortality.

Most mortality during the mating experiments occurred shortly after the mating pair was combined and before the female had molted. Mortality later in the mating experiment was too low (~5%) for statistical analysis (i.e., 4 deaths in 70 mating pairs). The same was

true for limb loss, which only occurred in ~5% of females (5 females with limb loss out of 70).

Large males mated with large females transferred significantly more sperm than did small males with small females or small males with large females (one-way ANOVA; $F=5.23$, $df = 3$, 66 , $p= 0.0027$; Fig. 6). Large males mated to small females did not transfer significantly more sperm than did all small males (Fig. 6), probably due to volume limitations in the size of the spermathecae in small v. large females. Female size had no effect on the number of sperm received. Small males transferred statistically similar numbers of sperm to large and to small females (Fig. 6), as did large males.

Large females received significantly more semen from large males (mean = 5.8g) than did small females (one-way ANOVA; $F = 39.39$, $df = 3$, 66 , $p < 0.0001$; Fig. 7). No difference was found in the amount of semen received from small males by large or small females (Fig. 7). Large males mated to small females transferred significantly more semen than did small males with small females, but not more than did small males mated to large females (Fig. 7).

All females mated with large males had 100% full spermathecae after mating, except one large female that received 80% and another 90%. Large females mated with small males received ejaculate volumes ranging from 25% to 90% of their spermathecal capacity, with an average of 49% fullness. Small females mated with small males received ejaculate volumes ranging from 20% to 100% of their spermathecal capacity, with an average of 76%.

Discussion

Differences in size between mates in paired blue crabs apparently do not reduce reproductive potential, based on numbers of sperm transferred, below that expected when the paired crabs are of similar size (Fig. 6). Female body size had no significant effect on the number of sperm transferred by males within size groups; both large males well above the legal fishery limit of 127mm CW and small males below the legal limit (< 120 mm CW) transferred similar numbers of sperm to large and small females, suggesting that sperm transfer does not limit or affect the reproductive potential of males in size-disparate mating pairs. Large males did transfer more sperm to large females than did small males, but this result is probably due to the larger sperm stores possessed by larger males. A large male is thought to have a higher reproductive potential when mating with a larger female, since her fecundity per brood increases with body size (Prager *et al.* 1990); however, recent evidence suggests that this increased fecundity with size may be offset by a lower brood frequency compared to relatively small females, resulting in statistically similar potential larval production per unit time for all female crabs > 100 mm CW (Dickinson *et al.* in press).

Female body size had no significant effect on the amount of seminal fluid received from small males (Fig. 7). Large males did transfer larger ejaculates to large females than to small females; however, those large males mated to small females were apparently limited by the smaller capacity of the small females' spermathecae. Seminal fluid transferred by blue crabs hardens into a sperm plug shortly after mating, preventing subsequent copulation with other males (Jivoff 1997, Wolcott *et al.* 2005). All of the females paired with large males were 100% full (except 2 large females, 80% and 90% full); the amount of seminal fluid transferred is inconsequential, as similar prevention of subsequent copulation by other males

is achieved. Females paired with small males may retain the ability to mate again, due to the significantly smaller amount of semen transferred by small males, resulting in a smaller sperm plug that does not fill the females' spermathecae. Additional mating opportunities could increase the female's potential lifetime fecundity, if it is likely to be limited by the availability of sperm (Hines *et al.* 2003). Such additional matings could increase the total number of sperm received by these females to an amount similar to that transferred by a single large male. Large male crabs in the field today, however, are at low male: female ratios, mating more frequently, and providing less sperm and seminal fluid (Jivoff 2003), unlike the seminally replete males used in my experiment. If male abundance is too low, additional mating may not occur, increasing the possibility of sperm limitation (Hines *et al.* 2003).

I expected that size-disparate mating pairs would experience increased physical stress as a consequence of increased handling and limb-loss, leading to increased mortality for the female, which is soft and vulnerable during this time. Small males took longer to achieve the post-molt flip of the female to a mating position when she was large; nevertheless, there was no increase in injury or mortality of large females mated with small males. Unless the (assumed) stress of prolonged handling led to delayed mortality, there apparently is no evolutionary penalty for small: large pairings.

Pairwise Reproductive Benefits

There was no evidence that a small female benefits by mating with a small male. I expected that a large male was more likely to injure or kill a small female, or that a large male would be unable to manipulate a crab so much smaller than himself and fail to successfully transfer an adequate amount of sperm and semen. Neither occurred; there was

no increase in injury or mortality, nor any difference in sperm contribution between large and small males paired with small females, suggesting that small females can mate successfully with any male in the population. The small females might benefit from the longer guarding time provided by larger males (Jivoff & Hines 1998, Jivoff 2003). Longer guarding would allow females more time to harden their shells under the protection of the male crab, but this advantage may diminish under high fishing pressure; guarding time decreases at low male: female sex ratios (Jivoff 2003).

Large males provided more sperm than did smaller males when paired with large females, providing reproductive benefit to the large female, consistent with other reports of greater sperm transfer by larger males (Jivoff & Hines 1998, Jivoff 2003). However, large males in the field today may have depleted sperm stores because male: female sex ratios in the population are low and the remaining males apparently are mating frequently, with short inter-mating intervals (Jivoff 2003). Under these circumstances, a depleted large male may also mate for a shorter duration. The amount of ejaculate the male crab has to offer appears to be correlated with mating duration. The intense, size-selective harvest of males by the commercial fishery has been implicated in altering the size-structure and sex ratio of the blue crab mating population (Jivoff 2003, Eggleston *et al.* 2004, Carver *et al.* 2005). Such conditions negate most of the advantages to any female that preferentially mates with large males.

If the average size of female crabs in North Carolina continues to decrease and the proportion of very small “pygmy” females continues to increase, understanding the mating dynamics and potential fecundity of these members of the population will become increasingly important. Mature mated female crabs < 100 mm CW have been observed in

trawl surveys in NC waters in increasing numbers (Eggleston *et al.* 2004) and anecdotal evidence from crab fisherman indicates that large males mate or attempt to mate with these very small females. Recent evidence suggests that the spawning potential per unit time for females < 100 mm CW is significantly lower than that of larger ones (127-159mm CW; Dickinson *et al.* in press), which could contribute to further declines in recruitment and spawning stock abundance, if the proportion of pygmy females in the population continues to increase.

A more important question may be whether the lifetime spawning potential of a large female, or the reproductive benefit to a male by mating with a large female, will ever be realized under intense fishery pressure (see Chap. 2). Reproductive potential of males ultimately depends on the spawning potential of their mate(s). Females that spawn for two full seasons are likely to become sperm limited (Hines *et al.* 2003); however, few large females will live long enough to realize their full reproductive potential before being harvested. Chapter 2 examines the effect of fishery mortality on the reproductive potential of female crabs.

Evolution of size-related life history traits is dependent on differences in the reproduction of genotypes for size (Policansky 1993), and limited evidence for the blue crab (Lipcius & Stockhausen 2002) suggests that there is at least some genetic influence to size-at-maturity. The genotypic influence on the size-at-maturity of the crabs used in this experiment is unknown. The growth rate and mature size of the blue crab can be affected by physical environmental variables such as salinity and temperature (Fisher 1999). However, the effective size-at-maturity of female crabs in North Carolina appears to have decreased

over time independent of salinity differences (Eggleston *et al.* 2004), suggesting that the mature size of the females in my experiments is at least partially influenced by genotype.

Historically, fisheries theory has assumed that harvested fishery populations can recover from harvesting and quickly return to a pre-fished equilibrium, and that over the long term, harvest will not change the inherent dynamics of the population (Conover 2000). It has become increasingly apparent, however, that rapid life-history evolution is occurring in exploited wild fish species (Reznik *et al.* 1990, Haugen & Vøllestad 2001, Quinn *et al.* 2001) and that recovery of overexploited fish populations is unexpectedly slow (Hutchings 2000, Hutchings & Reynolds 2004). If fisheries managers continue to ignore these population-level changes and fail to investigate the underlying causes, their management strategies will become increasingly ineffective, even potentially detrimental to the population of the harvested species as well as to the viability of the fishery. My experiments suggest that crabs representing all but the smallest sizes in the population are able to mate with each other without any substantial differences in mortality or reproductive potential, indicating that the diversity of size-related genotypic traits can be preserved in the population if fishery pressure is reduced or changed to increase the mating opportunities and spawning potential of larger crabs. However, as long as the commercial fishery preferentially harvests large individuals while leaving smaller ones, genotypes for larger size are likely to decrease in the population, making recovery of the size-structure of the population to a more “natural” state increasingly unlikely.

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Appendix

Blue crab life history & mating dynamics

The average life span of the blue crab is three years with a maximum of five to eight years (Eggleston *et al.* 2004, NCDMF 2004). Blue crabs progress through an oceanic, planktonic larval (zoeal) stage and a postlarval (megalopal) stage before entering the estuaries, at which point the juvenile crab assumes a heavier, hard shell and a benthic lifestyle (NCDMF 2004). They reach maturity at approximately 12 to 18 months of age. The exact age and growth rate of the blue crab is difficult to assess, since periodic molting of the hard exoskeleton produces a discontinuous growth pattern, and salinity and water temperature affects growth rate (Fisher 1990). Year-class strength of the blue crab population is determined by the annual larval recruitment into the estuaries, which is affected by the abundance of the spawning stock (Eggleston *et al.* 2004).

Female blue crabs attain sexual maturity in a single pubertal or terminal molt. The transition to sexual maturity is easily identifiable in the female blue crab by the marked change in the shape of the abdomen from triangular to rounded. The male blue crab, however, often reaches sexual maturity over successive molts and exhibits few external signs that can reliably indicate sexual maturity (Van Engel 1990). The male crab also possesses the ability to continue growing and molting after sexual maturity has been achieved. For these reasons, it is difficult to identify changes in size-at-maturity for the male crabs within the population.

As a pre-pubertal (pp) female approaches the molt to maturity, she will pair with a male crab. The male places the female in a pre-copulatory embrace or “cradle-carry” position under his own body and guards the female against predators and male competitors

(Van Engel 1958, Jivoff 1997). Females mate only immediately after the molt to maturity, though the male can mate repeatedly throughout its lifetime (Van Engel 1958, Hines *et al.* 2003). Female crabs can mate with multiple males if she does not receive a full complement of ejaculate from a single male. After the molt, the male flips the female onto her back and mates for up to 12 hours before returning the female to an upright cradle-carry position. The male will continue guarding the female for a variable amount of time, usually until her shell is sufficiently hardened. The female crab stores the seminal contribution in two storage organs or spermathecae and uses the sperm to fertilize multiple broods of eggs throughout its lifetime.

Spawning usually occurs in the vicinity of inlets where the larvae exit the estuaries and move offshore (NCDMF 2004). The female extrudes the eggs and broods them in an egg “sponge” under her tail (telson) for approximately 15 days until they hatch. The number of eggs per spawn ranges from 700,000 to 8,000,000, depending on the size of the female crab (Prager *et al.* 1990, NCDMF 2004).

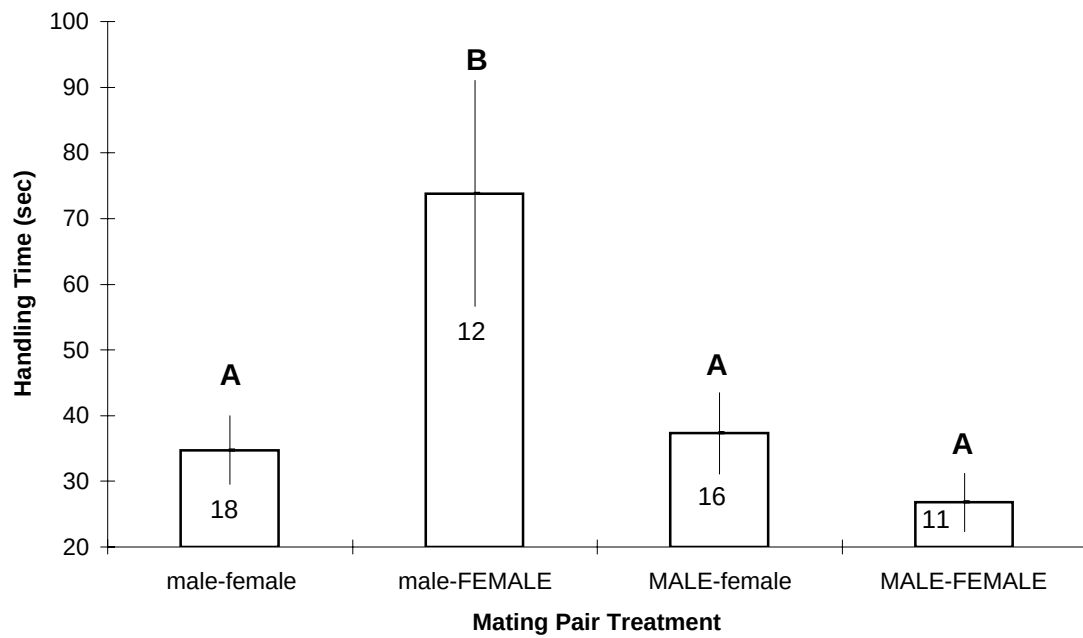


Figure 1: Mean (+/- SE) handling time for male blue crabs to flip the female from the cradle-carry to the mating position as a function of crab sex by size treatments.

Different letters denote treatment means that varied significantly from one another as identified with a Tukey's multiple comparisons test. Large crabs in capital letters; small crabs lower-case.

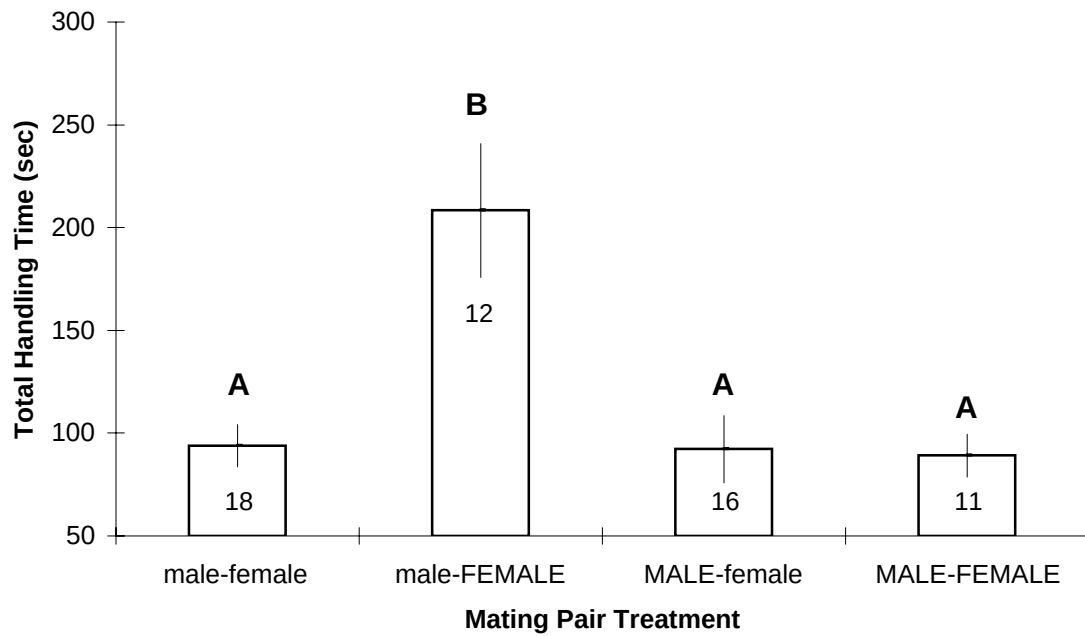


Figure 2: Mean (+/- SE) sum handling time for the male crab to achieve all cradle-carry and mating positions as a function of crab sex by size treatments.

Different letters denote treatment means that varied significantly from one another as identified with a Tukey's multiple comparisons test. Large crabs in capital letters; small crabs lower-case.

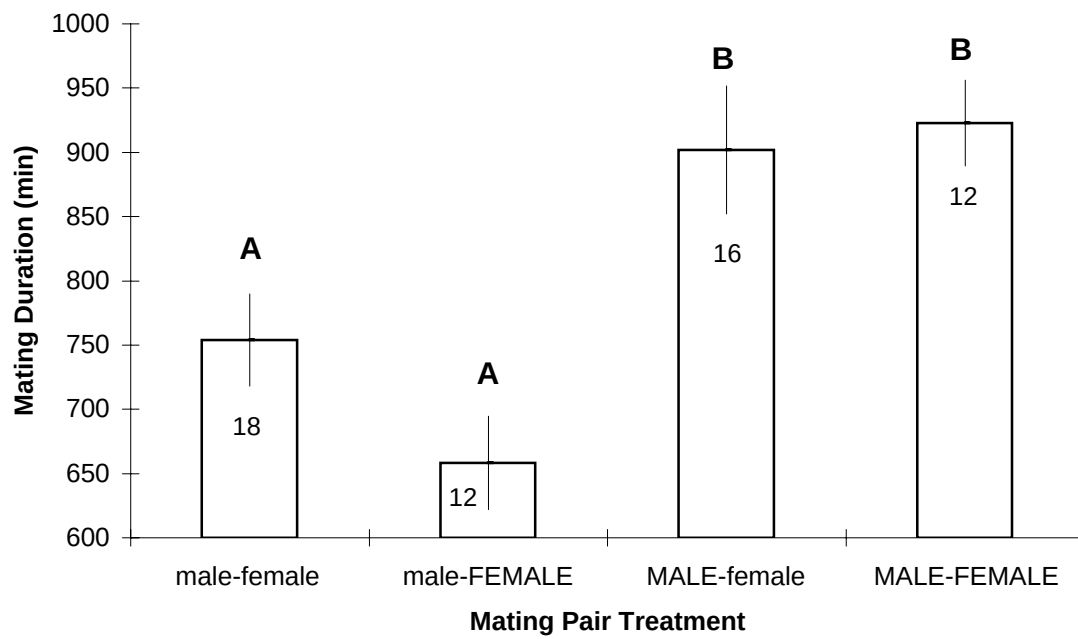


Figure 3: Mean (+/- SE) duration of mating as a function of crab sex by size treatment: Different letters denote treatment means that varied significantly from one another as identified with a Tukey's multiple comparisons test. Large crabs in capital letters; small crabs lower-case.

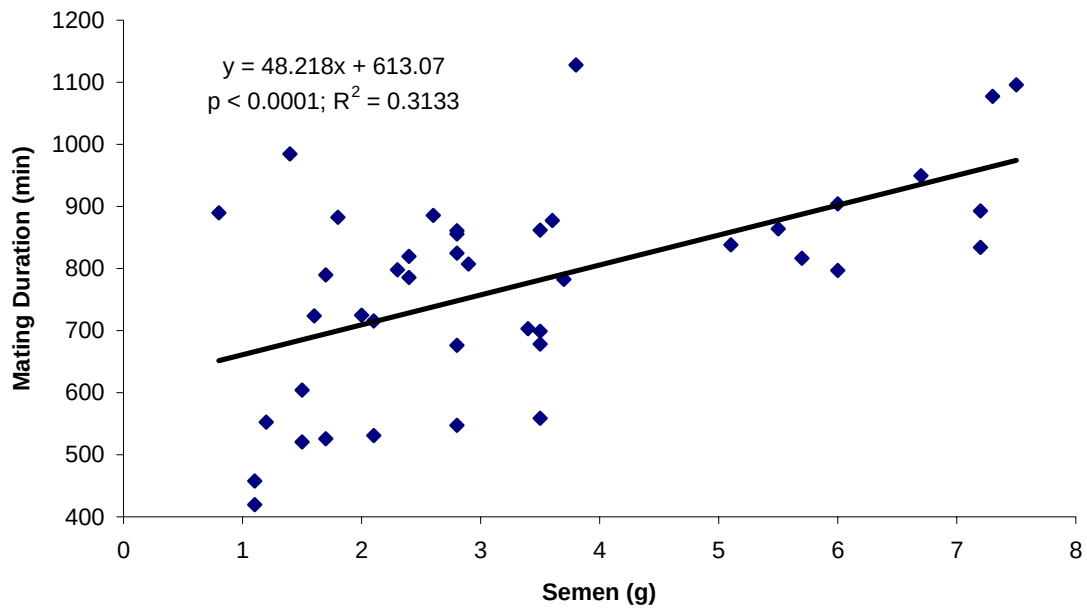


Figure 4: Relationship between semen transferred to a female blue crab by a male and the duration of mating. N=42.

Large males paired with small females were excluded from the regression analysis, since the quantity of semen is likely affected by the limited size of the female crab's spermathecae.

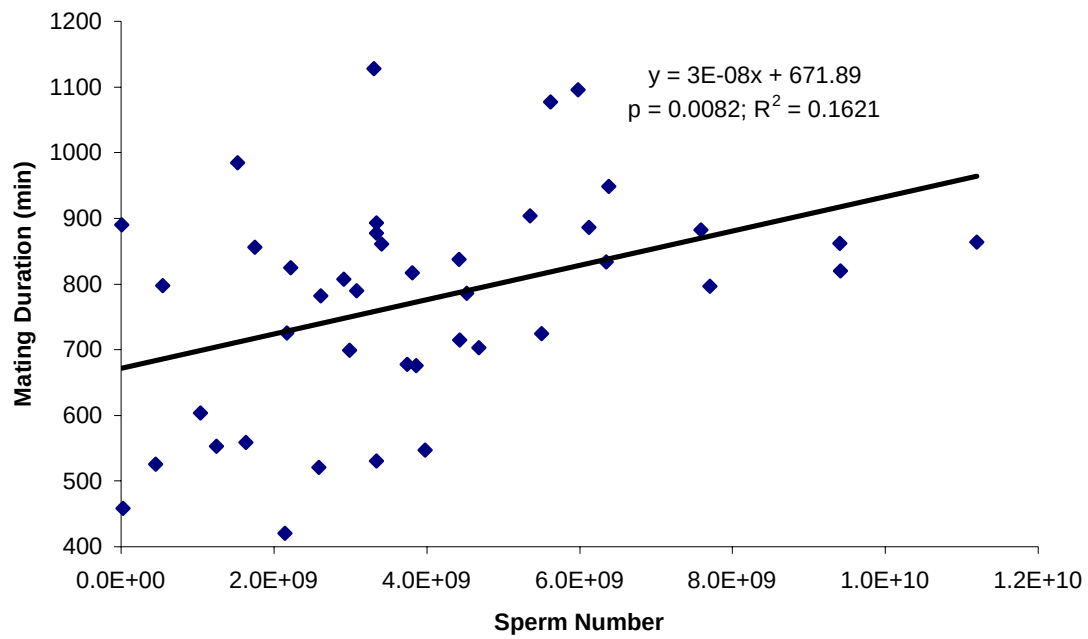


Figure 5: Relationship between sperm transferred to a female blue crab by a male and the duration of mating. N=42.

Large males paired with small females were excluded from the regression analysis, since the quantity of sperm is likely affected by the limited size of the female crab's spermatecae.

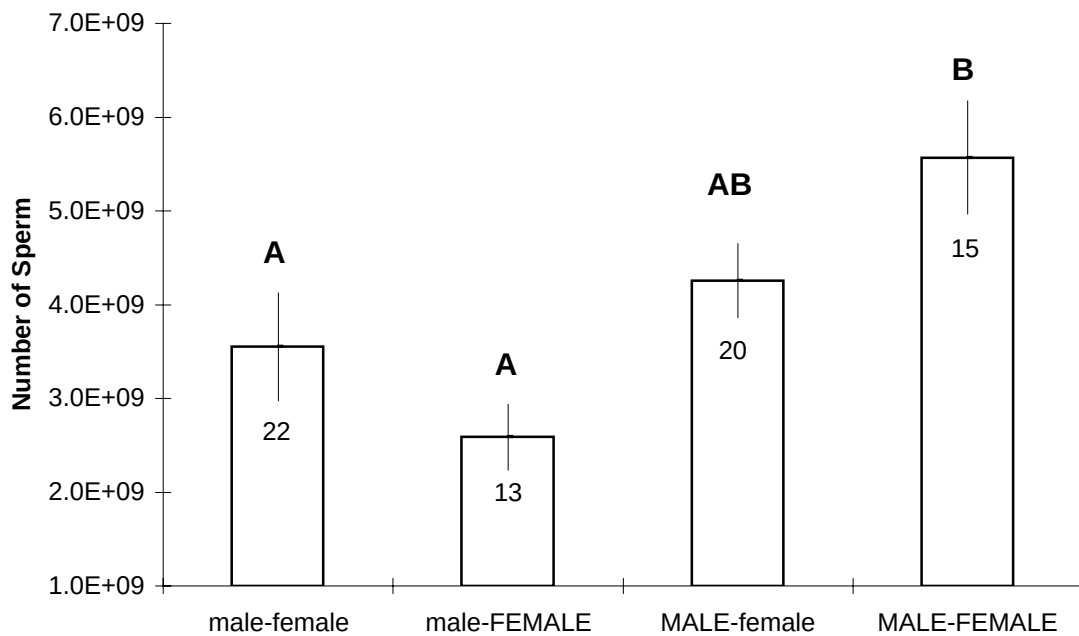


Figure 6: Mean (+/- SE) number of sperm transferred by the male crab as a function of crab sex by size treatments.

Different letters denote treatment means that varied significantly from one another as identified with a Tukey's multiple comparisons test. Large crabs in capital letters; small crabs lower-case.

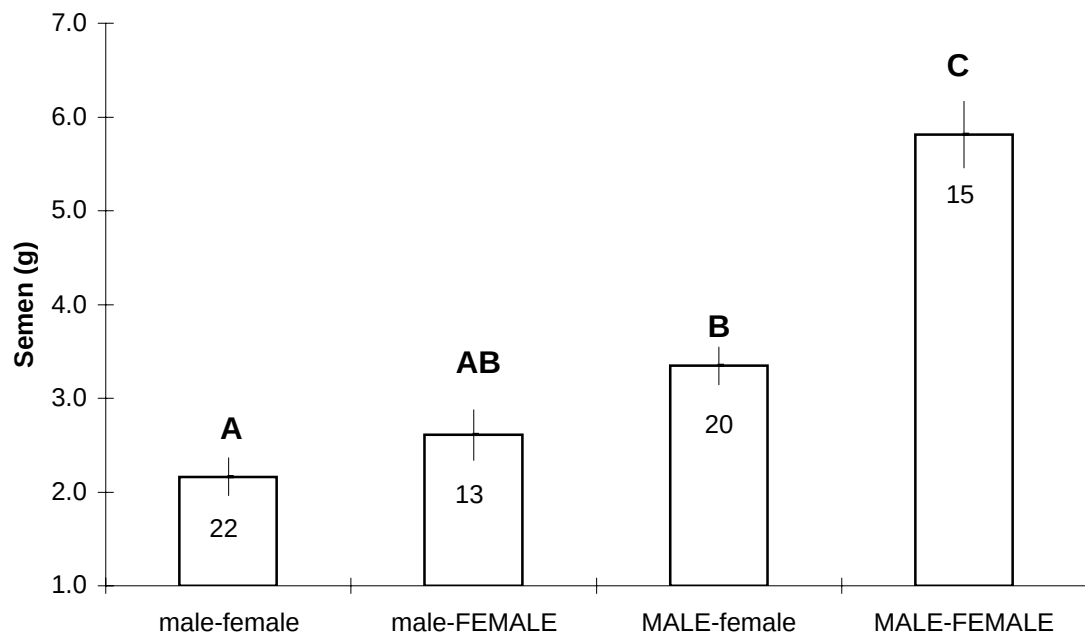


Figure 7: Mean (+/- SE) volume of semen transferred by the male crab as a function of crab size by size treatments.

Different letters denote treatment means that varied significantly from one another as identified with a Tukey's multiple comparisons test. Large crabs in capital letters; small crabs lower-case.

Chapter 2: Size-selection in the North Carolina blue crab population based on differential spawning potential

Introduction

The blue crab, *Callinectes sapidus*, has an important ecological role as a predator and scavenger in the coastal waters and estuaries of North Carolina. It also supports the most important commercial fishery in the state, with average (1993-2002) annual landings of approximately 50 million pounds valued at almost \$35 million dollars (NCDMF 2004). Recent data suggest that the population of NC blue crabs may be declining as a result of heavy exploitation by the fishery (Eggleston *et al.* 2004). Steadily decreasing biomass and an increasing trend in fishery harvest, often above the estimated Maximum Sustainable Yield (MSY) for the species, are of particular concern for the future viability of the commercial fishery (Eggleston *et al.* 2004).

A declining trend in the average size of mature female crabs from 1987-2002 and an increase in the proportion of extremely small ($< 100\text{mm}$ carapace width (CW) as measured from spine to spine) mature female crabs has been reported (Eggleston *et al.* 2004). Selective removal of large individuals by the fishery may be contributing to the reduction in size, possibly as a compensatory response of the population favoring earlier maturity under intense exploitation (Bertelsen & Cox 2001). The mature size of the female is positively correlated with fecundity, as measured by brood size (Prager *et al.* 1990, Dickinson *et al.* in press). However, smaller mature females may have artificially elevated fecundity because they can escape through cull rings in commercial crab pots, allowing them to continue

producing broods while larger females have been harvested. If the decline in the average size of mature females in the population continues, the concurrent size-dependent reduction in fecundity will likely contribute to a decline in recruitment. A significant spawning stock-recruitment relationship has been identified in the Chesapeake and Delaware Bays (Tang 1985, Lipcuis & Van Engel 1990, Helser & Kahn 1999) and in North Carolina (Eggleson *et al.* 2004). Reduced larval output per crab will have the same result as the lower larval output and recruitment related to reduced spawning stock abundance. Additionally, if the reduced size-at-maturity of the small females has any genetic component, the potential increase in their proportional contribution to the spawning stock, due to selective removal of larger females, is likely to result in a further decline in size-at-maturity in future generations.

Observed changes in life-history traits could indicate either a shift in genotype or a manifestation of phenotypic plasticity. Simply documenting changes over time does not establish evolutionary change (Policansky 1993). There are many confounding factors that make detection and measurement of evolutionary change difficult. Growth rate and size-at-maturity can be significantly affected by environmental variables such as temperature and salinity (Fisher 1999). Little is known about how much genetic influence there is on these traits. Determining whether evolution occurs in the natural environment would require observation of large numbers of individuals over multiple generations. Laboratory studies would be impractical for this species, which inhabits many different environments during its complex life cycle.

There is no direct evidence that distinguishes the genetic influences on life-history traits from environmental and phenotypic influences in blue crabs; however, evolutionary theory and many examples from other more easily studied species under intense exploitation

suggest that genetic change in the heavily fished North Carolina blue crab population is likely. For example, differences in growth rate among fishes in different latitudes (climates) can be affected by size-dependent winter mortality (Conover & Shultz 1995). Atlantic silverside (*Menidia menidia*) subjected to size-selective harvest in laboratory populations showed significant changes in biomass, mean weight, and size-at-maturity in only four generations (Conover & Munch 2002). Selection on the adult size of this species resulted in the rapid evolution of multiple traits that influence population growth rate and productivity (Conover & Munch 2002, Roff 2002). There are many reports of a decline in the size-at-age of natural populations in response to fishing pressures. Examples include grayling (*Thymallus thymallus*) (Haugen & Vollstad 2001), cod (*Gadus morhua*) (Chen & Mello 1999, Sinclair *et al.* 2002), Pacific salmon (*Oncorhynchus spp.*) (Ricker 1981), plaice (*Pleuronectes platessa*) (Grift *et al.* 2003), and red porgy (*Pagrus pagrus*) (Harris & McGovern 1997).

Environmentally-induced genetic change among crustaceans has been reported almost exclusively in short-lived planktonic species, but rarely for large marine invertebrates such as lobsters and crabs (Jamieson 1999). There has been a marked and concurrent decrease in spawning stock abundance and recruitment, larval abundance, and adult female size (and thus, size-at-maturity) of blue crabs in the Chesapeake Bay (Lipcius & Stockhausen 2002). Many possible genetic mechanisms are discussed in Lipcius & Stockhausen (2002) for why female blue crabs are maturing at younger ages and smaller sizes, including: optimizing mating opportunities when larger potential mates were sparse (Smith & Jamieson 1991, Jamieson *et al.* 1998); maximizing lifetime fecundity in the absence of mate-competition with other females (Lipcius 1995); size-selective fishery exploitation of large females

resulting in selection for smaller females (Policansky 1993, Jamieson 1999); and compensatory responses of the population to low densities, such as decreases in mean size, age, or instar to maturity (Charnov *et al.* 1978, Charnov 1979, Hannah & Jones 1991, Wellbourn 1994, Sparkes 1996). Although a combination of genetic mechanisms, phenotypic plasticity, and size-selective exploitation may have acted in concert to produce concurrent declines in female size and abundance, a rapid decrease in larval recruitment over a short 1 to 2 year time span was the primary cause of the significant reduction in stock abundance. A progressive change over evolutionary time spans has yet to be demonstrated for the blue crab (Lipcius & Stockhausen 2002).

I designed a mathematical model to find the extent to which disproportionate fishing pressure on large females increases the relative reproductive output of small females that are able to escape through cull rings in crab pots, thus avoiding harvest by the fishery (Guillory *et al.* 2004). If variation in size-at-maturity in female blue crabs has any genetic basis, differential survival based on size, and the resultant changes in lifetime fecundity (spawning potential), could be driving genetically-based reduction in size of mature female blue crabs. The model was also used to estimate the fishing pressure at which females maturing at smaller sizes will have a selective advantage (greater reproductive potential) relative to large females.

To estimate the reproductive or spawning potential of female crabs, I needed to estimate the number of eggs/brood as a function of female body size (CW), the total number of lifetime broods expected, and natural and fishery-induced mortality rates. Model input data were obtained from the 2004 North Carolina Blue Crab Fishery Management Plan (NCDMF 2004), including stock assessment data by Eggleston *et al.* (2004), as well as a

series of field and laboratory experiments by Dickinson, Rittschof and Latanich (Dickinson *et al.* in press, G.H. Dickinson & D. Rittschof, Duke University, pers. comm.) which addressed the spawning biology of North Carolina blue crabs. The results of the latter study incorporate size-at-maturity, brood size, and brood frequency into a single measure of size-specific spawning potential, per unit time. Using this information, I calculated the lifetime spawning potential of different-sized females. I also determined the point at which the fishery mortality rate (F) brings about equal lifetime spawning potential in relatively small v. large mature females, which was then compared to fishery mortality estimates from a stock assessment of the blue crab in North Carolina (Eggleston *et al.* 2004) to assess the potential for evolution of size-at-maturity of female blue crabs in the NC population. I hypothesized that 1) under high fishing pressure, smaller females that are not subject to fishery mortality will have a greater lifetime spawning potential than larger, harvestable females, and 2) the fishery mortality rate (F) at which small and large females have an equal spawning potential will be lower than current estimates of fishery mortality for the North Carolina population of blue crabs.

The model was also used to explore how a proposed upper size limit for commercial harvest of mature female blue crabs in North Carolina would affect reproductive potential (NCDMF 2004, Eggleston *et al.* 2004). The stock assessment (Eggleston *et al.* 2004) proposed that harvest of females over 6.75 inches CW (172mm) be prohibited all year as one of several recommended strategies to increase stock abundance and the average size-at-maturity of female blue crabs in North Carolina. Since fecundity is positively correlated with carapace width (Prager *et al.* 1990), protecting the largest females presumably would increase larval production per crab in the population and allow the most fecund individuals to

produce multiple broods over their lifetime (Eggleston *et al.* 2004). The larvae from the protected large females would also presumably possess a genotype for larger size, thus preserving a more “natural” mature size in the North Carolina blue crab population.

The NC Marine Fisheries Committee is considering the implementation of this upper size limit for females in the hard crab fishery from September through April of each year (NC MFC meeting, New Bern, NC 12 May 2004). The model in this study was used to estimate the increase in spawning potential of the large females affected by the proposed upper size limit, both for year-round closures as initially recommended by Eggleston *et al.* (2004), and seasonal (September through April) closures, which was the subsequent recommendation in the most recent blue crab fishery management plan (NCDMF 2004). The efficacy of the upper size limit is then evaluated based on the number of eggs those large females are likely to produce.

Materials & Methods

Using a mathematical model, I applied different mortality rates to daily spawning potential (no. of eggs/crab/day) of hypothetical size-cohorts of mature female blue crabs to estimate the average daily spawning potential. Over time, members of the hypothetical cohorts died and the average daily spawning potential (per crab) of the initial cohort decreased. The mathematical function describing this process was then integrated for specific periods of time when spawning is presumably occurring to estimate the total, lifetime spawning potential of each size-cohort (no. of eggs/crab). Natural mortality (M) was applied to all size-cohorts and fishery mortality (F) to size-cohorts that were subject to fishery harvest.

Model Parameters & Assumptions

The model in this study used values of the average daily spawning potential, adapted from Dickinson *et al.* (in press). Spawning potentials from their experiments were calculated by multiplying the total number of broods produced by each female during the observation period by the average brood volume of that female, divided by the length of the observation period (described as ml eggs/week; Dickinson *et al.* in press). Spawning potentials of individual crabs were then averaged by four size groups: (1)78-100mm CW, (2)101-126mm CW, (3)127-159mm CW, and (4)162-176mm CW. A significant difference in this measure of spawning potential was found only between the smallest size group (78-100 mm. CW; 12.67ml eggs/week) and a larger size group (127-159 mm CW; 28.93 ml eggs/week), so I focused on these two size groups when comparing the reproductive output of “small” v. “large” females. The largest size group (162-176mm CW) had a relative small N and did not have a spawning potential significantly different than any other size group. The model estimated the relative lifetime spawning potential of these two groups under different levels of F. The largest size group from these experiments (162-176mm CW), approximating the size group that would be protected by a proposed upper size limit on the harvest of females > 172mm CW, was used for model runs examining the effect of a proposed restriction on the harvest of large female crabs. The spawning potential reported for this size group was 27.94 ml eggs/week (Dickinson *et al.* in press).

I converted spawning potential values in ml eggs/crab/week to number of eggs/crab/day for use in the model by dividing ml eggs/crab/week by 7 days and multiplying by a ratio of 122,761 eggs/ml of brood volume. The ratio of 122,761 eggs to 1 ml of brood volume was calculated by first counting the average eggs/ml in four brood stages of North

Carolina ovigers, from early (yellow and orange) to late (brown and black) (D. Wolcott, unpubl. data). The four ratios were combined into a single conversion factor by weighting each according to the proportion of that stage in the sample population from Dickinson *et al.* (in press)(G.H. Dickinson & D. Rittschoff, Duke University, pers. comm.). The resulting daily spawning potential of the small (78-100mm CW) and large (127-159mm CW) size groups under comparison in the model were 2.22×10^5 eggs/day and 5.05×10^5 eggs/day, respectively. The daily spawning potential of the largest (162-176mm CW) size group was 4.90×10^5 eggs/day (though smaller than the spawning potential of the 127-159mm CW size group, the spawning potential of the 162-176mm CW size group was not significantly different).

To keep the model manageable, I assumed no differences in growth rates between the size classes. The crabs used (Dickinson *et al.* in press) were all mature females collected from the population at large. It was not known whether the relatively small ones had reached maturity at an earlier age than had larger ones, or at the same age after slower growth. The model's estimated spawning potential for a cohort of small females would not be affected by differences in growth rate.

A 184-day spawning period from 1 May to 31 Oct. for NC blue crabs is assumed in this model based on two criteria: 1) mature sponges (egg masses) are found from late April to early May in North Carolina (Ballance & Ballance 2002), and 2) the majority (91%) of crabs sacrificed in mid-October had mature ovaries and were therefore able to continue clutch production to the end of October and into early November (Dickinson *et al.* in press). The remaining 181 days of the year are defined as the overwintering period, during which no spawning occurs. Fishery mortality (F) was not applied to any size-cohort during the

overwintering period when crabs are buried in the sediment, because there is no winter dredge fishery in North Carolina.

The instantaneous annual natural mortality rates used in the model were obtained from a North Carolina blue crab stock assessment (Eggleston *et al.* 2004), which argues that the most likely estimates of natural mortality are $M=0.87$ and $M=0.55$, based on a maximum lifespan ($t_{\max}$) of 5 years and 8 years, respectively. The fishery mortality rate (F) at which the two size groups of blue crabs reach an equal spawning potential was calculated by the model and compared to the current estimates of F reported in the stock assessment: $F=0.9$ and $F=1.28$ (corresponding to $t_{\max}=5$ and $t_{\max}=8$, respectively; Eggleston *et al.* 2004).

Natural mortality (M) is commonly reported as an instantaneous annual mortality and assumed to be applied evenly throughout the year (Eggleston *et al.* 2004). Due to a lack of information for the blue crab regarding seasonal variation in natural mortality, the model in this study assumes the same. Fishery mortality (F) is also commonly reported as an instantaneous annual rate. Estimates of temporal variation in fishery mortality are available for the blue crab, due to the comprehensive collection of commercial fishery data since 1994. Fishery mortality from the hard crab fishery using crab pots is concentrated in the summer months, with more than 88% of the harvest occurring from May through October (NCDMF 2004). The hard crab fishery, including all harvest of mature female crabs, accounts for 97% of the total blue crab harvest in North Carolina, 95% of which is attributable to the use of crab pots (NCDMF 2004). The model in this study, therefore, treats all fishery mortality as harvest of hard crabs by pots (ignores the effects of the much smaller peeler harvest, which is not size-selective). Since the majority of the fishery mortality is occurring at the same approximate time as the spawning season defined in the model, the model assumes that the

annual fishery mortality (F) will all occur during the 184 days defined as the spawning season. For simplicity, the fishery mortality is also assumed to be constant over the course of a spawning season, rather than increasing during the middle of summer and decreasing in the fall and spring, as actually occurs (NCDMF 2004).

The cull rings in hard crab pots are the likely mechanism by which size selection may be occurring for the female crab (i.e., gear selectivity; Policansky 1993), since there is no restriction in North Carolina on the harvest of mature female blue crabs. Mature female crabs from the larger size group (127-159mm CW) cannot escape through the 2-5/16" cull rings in crab pots and thus are susceptible to fishery mortality. More than 90% of females ranging in size from 127-131 mm CW, and 100% of those > 137mm CW, are retained in crab pots (Guillory *et al.* 2004). Mature female crabs < 100mm CW (the smaller size group in the model) all can escape (Guillory *et al.* 2004).

Female crabs that are able to escape through cull rings (as measured by Guillory *et al.* 2004) do not necessarily do so. Behavioral studies of actual cull ring escapement by blue crabs are currently being conducted in NC by Paul Rudershausen (North Carolina State University Department of Zoology, pers. comm.). The model in this study assumes that small females experience no fishery mortality, based on personal observations of commercial crab harvesting in North Carolina, where the occurrence of small mature females (<100 mm CW) in the crab pots was very low.

The model assumes that there is a delay of approximately 12 weeks (84 days) between mating and the production of the first brood (Hopkins 2002), unless mating occurs in the fall, which is too late for any spawning before the season ends. All females are then assumed to produce broods continuously for the remaining portion of the defined spawning

seasons. Though brood production by a single crab is a series of discrete events, average brood production per crab of a cohort of female crabs is assumed to be a continuous function in this model. The model is discontinued at the end of the second spawning season, as two seasons of spawning are probably the maximum (D. Wolcott, pers. comm.), and females that have produced broods for two full seasons are likely to become sperm limited (Hines *et al.* 2003, Dickinson *et al.* in press).

Two model runs were performed for each size-group, representing 1) a cohort of female crabs maturing and mating at the beginning of the spawning season during the spring “peeler run,” and 2) a cohort of female crabs of both size groups maturing and mating toward the end of the spawning season (1 Sep) during the fall “peeler run.” The latter group would begin spawning the following spring on 1 May. Model runs were also completed for two estimates of current mortality rates: $F = 0.9$ when $M = 0.87$, and $F = 1.28$ when $M = 0.55$ (Eggleston, *et al.* 2004).

The basic function used in this study was:

$$DSP_t = (DSP_0)(Z^*)^t \quad \text{Eq. 1.}$$

where DSP_t = daily spawning potential of a cohort on day t , DSP_0 = initial daily spawning potential of a cohort (eggs/crab/day), Z^* = daily survivorship probability, and t = time in days. DSP_0 for small females (78-100mm CW) was 2.22×10^5 eggs/crab/day, DSP_0 for large females (127-159mm CW) was 5.05×10^5 eggs/crab/day, and DSP_0 for the largest females (162-176mm CW), approximating those females that would be protected by an upper size limit (>172mm CW) on commercial harvest was 4.90×10^5 eggs/crab/day.

As crabs died, the expected spawning potential per crab in the initial cohort declined. The daily survivorship probability (Z^*) varied depending on whether fishery mortality was being applied. Natural mortality (M) and fishery mortality (F) are typically reported as instantaneous annual rates. These mortalities were combined and converted to a daily survivorship probability by:

$$Z^* = e^{-(M/365 + F/184)} \quad \text{Eq. 2.}$$

I divided M by 365 days/year to produce a daily natural mortality rate. F was divided by 184 to produce the daily fishing mortality rate during the defined 184-day fishing season. The $F/184$ term was removed from Eq. 2 for the 181-day overwintering period, during which no harvest occurs. The F term was removed entirely from Eq. 2 when modeling cohorts of females that are not subject to harvest, such as the small size-group, and the largest size-group when under a year-round harvest restriction. For the model runs estimating lifetime spawning potential of the largest size-group of females under a seasonal harvest restriction, the $F/184$ term in Eq. 2 was removed for the 181-day overwintering period and for the 123 days between 1 May and 31 August each year. Fishery mortality was applied only for the last 61 days of the season from 1 Sep to 31 October.

The model function (Eq. 1) for each size-group was integrated over the time intervals when spawning is presumably occurring to estimate the total (lifetime) expected spawning potential per crab of each size-cohort. Newly mature and mated female crabs experienced mortality, but produced no eggs for approximately 12 weeks (84 days) after mating (Hopkins 2002). The spring model runs started during the spring “peeler run,” at the beginning of a 184 day spawning season on 1 May. Expected egg production per crab for the first season

was the integral over days 84-184, representing the spawning that occurs until the end of the defined spawning season. Expected egg production per crab for the second season was the integral over days 365-549 of the spawning season, representing an entire second season of spawning (1 May – 31 Oct). The two integrations were added to obtain the estimations of lifetime spawning potential (eggs/crab) for spring cohorts.

The fall model runs began during the fall “peeler run,” on 1 Sept., and estimated the spawning potential of cohorts of female crabs molting to maturity and mating during this latter part of the spawning and harvest season. Newly mature and mated female crabs experienced mortality, but produced no eggs for approximately 12 weeks (84 days) after mating (Hopkins 2002). Since the spawning season ends before 12 weeks pass, spawning is assumed to begin at the start of the next season (on 1 May). Expected egg production per crab for the first spawning season was the integral over days 242-426 (242 = 61 days remaining in the initial season plus 181 days overwintering; 426 = 242 days plus 184 days of spawning). Expected egg production per crab for the second season was the integral over days 607-791 of the spawning season, representing an entire second season of spawning (1 May – 31 Oct). The two integrations were added to estimate lifetime spawning potential (eggs/crab) for fall cohorts.

Results

The model estimated that current fishery mortality is sufficient to give small (78-100 mm CW) females an advantage in reproductive output over large (127-159 mm CW) females. The estimated egg production during two spawning seasons was equal between the small- and large-size groups at fishery mortality rates ranging from $F = 0.835$ (when $M =$

0.87) to $F = 0.81$ (when $M = 0.55$) for spring-mating females, and $F = 0.845$ (when $M = 0.87$) to $F = 0.78$ (when $M = 0.55$) for fall-mating females. The estimated average fishery mortalities for the years 1995 to 2001 (from a stock assessment report by Eggleston *et al.* 2004) were $F = 0.9$ (when $M = 0.87$) and $F = 1.28$ (when $M = 0.55$). Both of these F -values from Eggleston *et al.* (2004) exceed the values of F at which the model predicted that small females would reach a reproductive potential equal to that of large ones, suggesting that the fishery is capable of selecting for smaller size. At these F -values from Eggleston *et al.* (2004), the model estimated that the small-size group will have spawning potentials 6.1% and 58.1% higher than the large-size group for spring-mating crabs, and 5.1% and 56.8% higher for fall-mating crabs, for $F = 0.9$ (when $M = 0.87$) and $F = 1.28$ (when $M = 0.55$), respectively (Table 1).

The estimated egg production per crab in completely protected (year-round closure) cohorts of the largest female blue crabs (162-176mm CW) increased by 142% to 261% over those under current fishing practices for the spring cohort at mortality rates of $F = 0.9$ (when $M = 0.87$) and $F = 1.28$ (when $M = 0.55$), respectively. For the fall cohort, the increases in estimated egg production per crab were 140% and 258% at mortality rates of $F = 0.9$ (when $M = 0.87$) and $F = 1.28$ (when $M = 0.55$), respectively (Table 2).

If an upper size limit is seasonally imposed (September through April), as proposed by the NCDMF (2004), estimated egg production per crab increases over that predicted for present, unrestricted harvesting practices by 17.5% and 26.2% for the spring cohort at mortality rates of $F = 0.9$ (when $M = 0.87$) and $F = 1.28$ (when $M = 0.55$), respectively (Table 3). For the fall cohort, the increase was 45.9% and 70.6% at mortality rates of $F = 0.9$ (when $M = 0.87$) and $F = 1.28$ (when $M = 0.55$), respectively (Table 3).

The proportions of female crabs that spawn during the spring and fall are not equal. Approximately 60% of the annual harvest of soft and peeler crabs falls within one month of the model's 1 May spring season starting date (landings by month; NCDMF 2004). Approximately 15% of the annual harvest of soft and peeler crabs occurs within one month of the model's 1 September fall starting date (landings by month; NCDMF 2004). These landings data were used as a proxy for the relative abundance of maturing female crabs during the two defined "peeler runs"; about 80% are in the spring cohort and 20% in the fall. The percent increases in spawning potential from the model were weighted by these proportions and combined to produce an estimate for the entire population. Compared with year-round harvest, seasonal closure increases spawning potential for all cohorts by 23.2% at mortality rates of $F=0.9/M=0.87$ and by 35.1% at mortality rates of $F=1.28/M=0.55$. This scaling was not performed for the comparison between the small- and large-size females (Table 1) or for the comparison between year-round harvest and complete closure of the harvest on large females (Table 2), as increases in spawning potential were very similar in spring and fall cohorts.

Discussion

The model shows that, under current levels of fishery mortality, smaller female crabs in the population have a reproductive advantage over larger females. Depending on the mortality estimates used, small females produce up to 58% more eggs over two spawning seasons than do larger females. Additionally, estimated rates of annual fishery mortality (F) in 1995-2001, for rates of natural mortality (M) of 0.55 and 0.87, ranged from ~ 1 to 1.5 (Eggleston *et al.* 2004), which exceeds the values of F at which the model predicted equal

reproductive potential for small and large females. This evidence for differential reproduction of the two size classes suggests that the observed decline in female size may be a result of an evolutionary response to intense selection for size in the blue crab, since differential reproduction of genotypes is one of the two factors that must be present to drive genetic change (Policansky 1993). The reproductive contribution of the very small (<100 mm CW) females to future generations is becoming increasingly important to stock abundance and size-at-age structure; presently mature females (<100mm CW) constitute 12% of the population and the proportion is rising (Eggleston *et al.* 2004).

It has often been assumed that fishery populations are able to recover from harvesting and reach a pre-fished equilibrium in a relatively short time, and that long-term harvest will not change the inherent dynamics of the population (Conover 2000). It has become increasingly apparent, however, that significant changes in heavily fished populations of marine species are occurring. For the blue crab, continued reductions in the size-at-maturity of the females, which the model suggests could be driven by intense size-selection by the fishery, would result in decreasing revenue for the commercial fishery. Additionally, the lower spawning potential of very small female crabs (< 100 mm CW) compared to that of larger females (if not removed by the fishery) (Dickinson *et al.* in press) will likely result in lower potential recruitment in subsequent generations if the proportion of very small females in the population continues to increase. The potential effect of lower larval output per crab on recruitment is likely similar to that of lower spawning stock abundance. A significant spawning stock-recruitment relationship has been identified for the North Carolina population of blue crabs, such that low levels of spawning stock abundance produce low

subsequent recruitment and high levels of spawning stock abundance produce high subsequent recruitment (Eggleston *et al.* 2004).

To keep the model manageable, I assumed no differences in growth rates between the size classes. It was not known whether the relatively small females had reached maturity at an earlier age than had larger ones, or at the same age after slower growth. A female crab that reaches maturity at a younger age can begin spawning sooner, reducing generation time (potentially increasing “*r*”). The model’s estimated spawning potential for a cohort of small females would not be affected by differences in growth rate; however, over long periods the shorter generation time would result in an even greater reproductive advantage for the small females.

The evolutionary inferences from the results of this modeling exercise depend on the assumption that there is some genetic influence on the size-at-maturity of female blue crabs. Although environmental factors such as salinity and temperature have a marked effect on growth rate and size-at-maturity (Fisher 1999), a statistically significant decline over time in the mean size of mature females in NC, after the effects of salinity were removed (Eggleston *et al.* 2004), suggests a genetic basis. It must also be assumed that there is some degree of heritability to size-at-maturity. There is no reported evidence for such heritability in blue crabs, but heritability of life history traits affecting size-at-age and mature size has been observed in so many other species that it seems likely in blue crabs as well. If this is the case, the proportionally higher reproductive output shown in this model for small female crabs is likely to result in a tendency toward smaller size-at-maturity in future generations of blue crabs.

Evidence for a reduction in the mature size of male crabs is lacking, primarily because males do not have an obvious molt to maturity or terminal molt. Male blue crabs are under size-selective pressures as well, due to regulations prohibiting the harvest of males <127 mm CW. If there is genotypic variation in and heritability of size-at-maturity, and size-selective evolutionary pressures in both sexes of the blue crab, the potential for significant changes in size in the population is compounded and could produce further declines in the blue crab stock and average size (see Chap 1).

The potential effects of the proposed maximum legal size restriction for female crabs also are estimated by the model. One of the expected benefits of this upper size limit is that larger females, allowed to reach their full reproductive potential, would contribute significantly more recruits to subsequent generations than would smaller harvestable females, thus increasing recruitment and future stock abundance. These recruits presumably also would possess the genotype for large size-at-maturity. Their contribution to future generations would preserve a more “natural” size structure in the population (Eggleston *et al.* 2004). These benefits are questionable, however. The higher larval production predicted by Eggleston *et al.* (2004) is based solely on brood size and does not take into account brood frequency, which is negatively correlated with the CW of female blue crabs. When brood frequency is factored in, the spawning potential per unit time of all female crabs > 100 mm CW was found to be statistically similar (Dickinson *et al.* in press).

The proportion of the large females (> 172 mm CW) in the NC commercial hard crab harvest is, on average, less than 2% (Eggleston *et al.* 2004). The spawning potential found by the model for these large individuals was up to 35% above that of smaller females if an upper size limit was imposed seasonally, or 261% higher if they were protected year-round.

Since the large females compose only 2% of the population, the increase in spawning potential, and thus larval production, for the entire population is a maximum of 0.46% with the seasonal upper size limit, and 5.2% for the year-round limit. A seasonal upper size limit will have a negligible (<1%) impact on larval production. The original proposal of a year-round upper size limit, which should produce about a 5.2% increase in larval production, might increase recruitment over successive generations, especially if the proportion of larger females (> 172 mm CW) increased due to the predicted upward shift in the size-structure of the population. However, like the 127mm lower limit on male crabs, the proposed upper size limit regulation on female crabs allows up to 5% of harvested crabs to be of “protected” sizes (NCDMF 2004). Such a regulation, if intended to protect < 2% of the population, would be meaningless.

If the primary goal of Fisheries Management is to increase larval output and subsequent recruitment, protection of a larger proportion of the female population, either by lowering the upper size cutoff or by protecting a different size group altogether, would be necessary to produce significant increases in spawning potential and larval output (Eggleston *et al.* 2004, Dickinson *et al.* in press). Unlike males, harvested female hard crabs are graded by weight and not by individual size. Since all female crabs > 100 mm CW have similar spawning potentials per unit time (Dickinson *et al.* in press), a restriction on smaller (less valuable) harvestable females is likely to produce as much of an increase in larval production per crab as would a restriction on larger females. A lower size limit, such as that imposed on the harvest of male crabs <127 mm CW, or an increase in cull ring size, would also be much easier to implement.

If the primary goal of the Fisheries Management is to increase larval production by, and thus the genetic contribution of, large females in an attempt to stem the decreasing trend in the mature size of the female blue crab, the proposed upper size limit will not accomplish that goal unless the 5% tolerance is removed or the maximum legal size cutoff is lowered to protect a group larger than 5% of the population. Increasing the genetic contribution of these large females is directly dependent on increasing their spawning potential and larval output, which cannot be achieved by the proposed seasonal upper size limit. If the tolerance level was reduced to zero or the upper size cut-off value was reduced, enough large females could escape fishery mortality and contribute the putative large size-at-maturity genotype to future generations. In addition to preserving a larger size-at-age structure, an effective large size refuge could eventually increase the proportion of large females > 172 mm CW in the population, increasing the potential benefits of the regulation.

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Table 1: Spawning potential (per crab in the initial cohort) of large (127-159 mm CW) and small female blue crabs (72-100 mm CW) for spring and fall “peeler runs.” Two estimates of natural (M) and fishery (F) mortality were used.

	Spring Cohort			Fall Cohort		
	Size Group	Eggs/crab	% Difference	Size Group	Eggs/crab	% Difference
M=0.87, F=0.9	Large	2.83×10^7	6.1%	Large	2.51×10^7	5.1%
	Small	3.01×10^7		Small	2.64×10^7	
M=0.55, F=1.28	Large	2.44×10^7	58.1%	Large	2.49×10^7	56.8%
	Small	3.86×10^7		Small	3.91×10^7	

Table 2: Spawning potential (per crab in the initial cohort) of the largest female blue crabs (162-176 mm CW) are compared for cohorts subjected to the current, year-round harvest regime and to no harvest. Two estimates of natural (M) and fishery (F) mortality were modeled.

	Spring Cohort			Fall Cohort		
	Harvest Type	Eggs/crab	% Difference	Harvest Type	Eggs/crab	% Difference
M=0.87, F=0.9	Current	2.74×10^7	142%	Current	2.42×10^7	140%
	No Harvest	6.63×10^7		No Harvest	5.82×10^7	
M=0.55, F=1.28	Current	2.36×10^7	261%	Current	2.41×10^7	258%
	No Harvest	8.51×10^7		No Harvest	8.62×10^7	

Table 3: Spawning potential (per crab in the initial cohort) of the largest female blue crabs (162-176 mm CW) are compared for cohorts subjected to the current, year-round harvest regime and to a proposed seasonal harvest (Sept.-Apr.). Two estimates of natural (M) and fishery (F) mortality were modeled.

	Spring Cohort			Fall Cohort		
	Harvest Type	Eggs/crab	% Difference	Harvest Type	Eggs/crab	% Difference
M=0.87, F=0.9	Current	2.74×10^7	17.5%	Current	2.42×10^7	45.9%
	Seasonal	3.22×10^7		Seasonal	3.54×10^7	
M=0.55, F=1.28	Current	2.36×10^7	26.2%	Current	2.41×10^7	70.6%
	Seasonal	2.97×10^7		Seasonal	4.11×10^7	