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Changing Fecundity and Reproductive Output in Females of a Chesapeake Bay Population of Blue Crab, *Callinectes sapidus*

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CHANGING FECUNDITY AND REPRODUCTIVE OUTPUT IN FEMALES OF A CHESAPEAKE BAY POPULATION OF BLUE CRAB, *CALLINECTES SAPIDUS*

by

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ABSTRACT

CHANGING FECUNDITY AND REPRODUCTIVE OUTPUT IN FEMALES OF A CHESAPEAKE BAY POPULATION OF BLUE CRAB, *CALLINECTES SAPIDUS*

Shannon L. Wells
Old Dominion University, 2009
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The Chesapeake Bay blue crab, *Callinectes sapidus*, is an important species both ecologically and as a fishery. Fishery dependent and independent data indicate that the population declined abruptly in the mid-1990s and has remained low. Previous work prior to the decline in population abundance found a significant relationship between size and fecundity for mature females. As with many heavily fished populations, this population has experienced a reduction in the size at maturity. The objectives of this study were to reassess the fecundity of the population, determine the lipid and protein energy allocated for reproduction, and to examine the concentrations of lipid fractions in eggs.

This study indicated that the size-fecundity relationship is absent or very weak. Fecundity estimates indicate that individual female fecundity has decreased by an order of magnitude from previously reported values. Egg production varied both inter- and intra-annually with a greater number of smaller eggs produced later in each season. This all suggests that allometric regulation is no longer the primary factor determining egg production in this population.

Negative changes in the allocation or availability of energetic resources may be having a significant impact on this population. Lipid and protein concentrations measured from the hepatopancreas, ovaries and eggs of mature female blue crabs demonstrated that first brood production is derived from stored resources in the

hepatopancreas. Later brood production is likely allocated for directly from ingested food. Egg lipid and protein concentrations showed considerable inter- and intra-annual variation, suggesting that there were tradeoffs between lipid and protein allocation. Concentrations of lipid fractions (triglyceride, phospholipid, and cholesterol) also showed variation within and between spawning seasons suggesting significant differences in egg quality.

Decreases in population fecundity in conjunction with observed variation in the biochemical content of eggs can have far reaching impacts on the population including larval survivability and recruitment. My results suggest that there are significant shifts in maternal effort, and potentially reproductive success, both inter-annually and seasonally. Fluctuations in energy available to an embryo, as well as the production of fewer embryos have significantly lowered the reproductive output of this population.

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CHAPTER I

INTRODUCTION

Understanding reproductive biology is important for the conservation of all species, but especially for those that are harvested. Various crustacean species are commercially harvested throughout the world leading to negative changes in population sizes and structures (Caputi 1993; Sarda 1993; Armstrong et al. 1998; Zheng and Kruse 2003; and others). Individuals must reproduce in order for a population or species to survive. Crustacean reproduction requires significant resources and investment primarily from mature females. Females must produce oocytes in the ovaries that will later become extruded eggs (Lee and Walker 1995; Spaargaren and Haefner 1994; Walker et al. 2003; Hasek and Felder 2006; Lee et al. 2006; Sanchez-Paz et al. 2006). Each of these oocytes must also be provided with necessary energy and structural components to allow the embryo to survive (Mourente and Rodriguez 1997; Garcia-Guererro et al. 2003; Pernet et al. 2003). To do this, quantities of food must be ingested well beyond what is needed for the growth and internal maintenance of the female. Most crustaceans can produce multiple broods within a spawning season, which requires that the processes of oocyte development and egg production be repeated (Wolin et al. 1973; Sibert et al. 2004). Changes in maternal investment and effort, as well as egg production, can dramatically affect any population and thus lead to further reduction in population size.

The Chesapeake Bay blue crab, *Callinectes sapidus*, is an important fishery and ecological species. In recent years this crustacean population has suffered from

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overfishing and habitat destruction within the Bay (Lipcius and Stockhausen 2002). Harvests began declining in the 1990's, along with the spawning stock, indicating a decrease in population size (Lipcius and Stockhausen 2002; Chesapeake Bay Commission Bi-State Blue Crab Technical Advisory Committee 2006). With a decline in the spawning stock, there is a subsequent decline in the number of larvae produced, and the number of post-larvae available for recruitment (Miller and Houde 1998; Lipcius and Stockhausen 2002). Generally, the focus of fishery management is to conserve egg production and recruitment. Declines in both parameters have led to further concern about the population and the fishery. To better understand the impact of these changes more knowledge was needed about population dynamics, especially female fecundity and reproductive output.

All crabs must molt their chitinous exoskeletons in order to grow (van Engel, 1958). Female blue crabs are believed to go through a final terminal molt at maturity before they are able to contribute to reproduction (van Engel 1958). Mating occurs before the female's exoskeleton hardens at the end of the terminal molt. As this is assumed to be the only occasion that a female will mate (van Engel 1958), enough sperm must be obtained in this coupling for the female to fertilize all of the broods she will produce throughout her two to three year lifetime (McConaugha 1992, 1999). Sperm stored in the spermathecae is used to fertilize the eggs as they are extruded from the body of the female. The fertilized eggs are expelled onto the female's abdomen, and subsequently attached to her pleopods (van Engel 1958; Millikin and Williams 1984).

The result is an egg mass, referred to as a sponge, visible on the abdomen of ovigerous females.

Over the past two decades there has been a marked shift in the size at maturity for the Chesapeake Bay blue crab population (VIMS Trawl Survey). There has also been an increase in the number of females that are less than 120 mm (VIMS Trawl Survey). This demonstrates that this is a shift in carapace width of the female population. This is not an artifact of overfishing because there are no limitations on size for harvesting mature females. For the purpose of this investigation, the definition of fecundity is the number of eggs produced per brood by a female blue crab. Previously reported data of blue crab fecundity from 1986-1987 showed an average of 3.2×10^6 eggs per brood, with a mean carapace width for mature females of 147 mm (Prager et al. 1990). This data also demonstrated that there was a positive allometric relationship between carapace width and fecundity in mature females (Prager et al. 1990). Given this relationship, the observed decline in the average carapace width of less than fifteen millimeters would result in a decline in population fecundity and fewer total larvae per brood.

Prager et al. (1990) indicated that this population experiences both interannual and interseasonal differences in reproductive output. The relationship between size and fecundity was not significant in this current study, and there was variation in egg diameter both within the season and inter-annually suggesting that there was another factor beyond allometry controlling reproductive output of this population. Because female size was not a controlling factor in these data, resource availability was considered a likely source. This could account for the interannual and interseasonal

variation previously seen, and could potentially lead to greater variation in a time when female size is not a determinant.

Body size is related to the amount of energy available to the female and how much of that energy is used for growth (Shertzer and Ellner 2002). Utilizing less energy for growth would result in smaller individuals, but other areas such as the reproductive organs could be enriched. Small females are assumed to belong to a younger year class than significantly larger ones. A chronologically earlier terminal molt would allow females to reproduce sooner, but at a reduced size. This could potentially lead to increased fecundity over the lifetime of an organism, as these females could produce more broods before death, with reduced fecundity per individual brood (Dickinson et al. 2006). Larger individuals may divert more resources into growth and would have more internal space for oocyte storage, but the reproductive period would be shorter than for an individual maturing earlier. Therefore crabs can grow at different rates leading to considerable variation in size and fecundity between different individuals.

All animals must ingest sufficient energy to maintain metabolism, grow, and reproduce. This is especially important for females that have reached maturity, and are developing oocytes within the ovaries. In brachyurans, lipids and proteins accumulate within the hepatopancreas, and are mobilized to the ovaries through the hemolymph during vitellogenesis (Spaargaren and Haefer 1994; Walker et al. 2003; Hasek and Felder 2006; Lee et al. 2006; Sanchez-Paz et al. 2006). It has been reported for other brachyuran species that during vitellogenesis there is an increase in ovarian lipid accumulation by up to 41% (Ying et al. 2006). Once lipids and proteins are in the ovaries they can be utilized to form oocytes that are later fertilized and extruded as eggs. The

quantity of lipids and proteins within each oocyte must be sufficient for the survival of the embryo and sustenance of the larvae at least to a feeding stage (Mourente and Rodriguez 1997; Garcia-Guererro et al. 2003; Pernet et al. 2003). Multiple forms of lipids are necessary in larvae for the development of cellular structures and energy storage (Garcia-Guererro et al. 2003; Graeve and Wehrtmann 2003; Calado et al. 2005). Larvae must have proteins for tissue growth, and metabolic energy (Garcia-Guererro et al. 2003; Graeve and Wehrtmann 2003). Blue crab larvae are able to feed upon hatching, but excess energy provided within the egg can be used after hatching to prolong the necessity for larval feeding. Larvae can also use the excess yolk for accelerated growth (Pernet et al. 2003; Lee et al. 2006). Eggs without sufficient yolk will result in embryonic or larval death and wasted reproductive effort. If food is in short supply, females can adjust the amount of lipids and proteins going into each egg with the lower limitation that larvae must have enough energy to reach the feeding stage. Alternatively, they can decrease the number of eggs that are produced (Calado et al. 2005). In times when food supplies are low, a reduction in individual fecundity may occur.

The three main lipid types found in crustacean hepatopancreas and ovaries are triglycerides, phospholipids, and cholesterols (Mourente and Rodriguez 1997; Garcia-Guererro et al. 2003; Graeve and Wehrtmann 2003; Walker et al. 2003; Lee et al. 2006). It is these components that are believed to provide the greatest benefit to embryos and larvae by increasing survival and growth (Graeve and Wehrtmann 2003). The role of triglycerides primarily involves energy storage, whereas cholesterol and phospholipids are used in making structures such as membranes (Ravid et al. 1999; Graeve and Wehrtmann 2003; Sibert et al. 2004; Lee et al. 2006). Data from the larvae of lobsters

showed that triglycerides constituted as much as 68% of the total lipids, phospholipids constituted 30%, and cholesterol only 2% (Sibert et al. 2004). Lipids ingested by crustaceans are transformed within the body into other forms. Cholesterol is an exception to this, as crustaceans cannot synthesize de novo the cholesterol ring, and must receive it through their diets (Zandee 1967; Teshima and Kanazawa 1971; Ravid et al. 1999; Sanchez-Paz et al. 2006). Changes in any of these components within the hepatopancreas, ovaries, or eggs throughout the year or the spawning season may indicate significant changes in diet or maternal effort. This could have a great impact on larval survival. Direct correlations between the amount of maternal effort and the likelihood of survival have been found in other crustacean species (Calado et al. 2005).

The Chesapeake Bay blue crab has also experienced reductions in the availability of preferred prey species such as clams, oysters, and mussels (Hughes and Seed 1981; Blundon and Kennedy 1982; Clark et al. 1999). Harvest numbers for clams and oysters are at an all time low, and could have a severe impact even on a reduced crab population (VMRC Harvest Data). There are also seasonal differences in the amount and type of prey that are available within the Bay (Hines et al. 1990; Eggleston et al. 1992). Within the blue crab spawning season, females are often subjected to dwindling numbers of *Macoma balthica* and *Mya arenaria*, forcing them to find subpar alternative sources for food (Hines et al. 1990; Mansour and Lipcius 1991; Eggleston et al. 1992). Reduced prey availability can lead to necessary modifications in the way in which energy is utilized within the body, including diversion from reproduction. Fewer eggs may be extruded per brood when there is insufficient energy available to produce the maximum number based on body size. Alternatively, more energy than required for development

could be apportioned to each egg to enhance survival. This would result in further decreased egg numbers (Pernet et al. 2003; Calado et al. 2005; Lee et al. 2006). Decreased larval mortality could partially compensate for reduced egg numbers by increasing the number of larvae that survive from smaller broods.

Variations in fecundity, especially in crustaceans, suggest that the population has experienced some change or stress (Stearns 1992; Pinheiro and Terceiro 2000; Tuck et al. 2000; Ouellet and Plante 2004; Gimenez 2006). Examples of changes that have affected the fecundity of other crustacean species are food availability, predation pressure, and in some cases fishing pressure (Pampoulie et al. 2000; Pinheiro and Terceiro 2000; Gimenez 2006). The Chesapeake Bay blue crab has experienced changes in all of these parameters in recent years. This suggests that changes in current fecundity compared to previous estimates of fecundity for this population could be attributed in part to these external sources. While the Chesapeake Bay population of blue crabs was the focus of this study it is believed that the information gained from this research can be utilized in studies of the reproductive biology of other crustaceans and invertebrate species.

Concerns about the health of the Chesapeake Bay blue crab population have lead to increased conservation efforts. Fecundity has not been reassessed since the late 1980's, but those fecundity numbers are still being used in population estimates (Miller 2001). This current reassessment of the fecundity of mature female blue crabs was needed to provide an updated value for the Chesapeake Bay population. Examining the amount of energy apportioned to each egg provided information regarding the quality of the eggs being produced. Reduced egg quality can lead to wasted reproductive effort and embryonic death, but enhanced quality can increase the chances of survival (Pernet et al.

2003; Calado et al. 2005). This study has also provided insight into maternal reproductive effort within and between spawning seasons.

It was hypothesized that the reductions observed in mature female size would result in reduced overall fecundity of the population due to a reduction in the internal space available for oocyte storage. It was also hypothesized that female size would no longer be the primary regulating factor for egg production in this population due to extraneous environmental factors, such as food availability, altered reproductive patterns, and fishing mortality prior to a female reaching her full reproductive potential. Variation was also hypothesized for egg production and egg lipid, protein, and lipid fraction concentrations. Inter-annual and seasonal differences have been found in the number of eggs produced in previous studies of this population (Prager et al. 1990). This previous trend towards variability combined with the recent ecological changes seen in food availability and habitat destruction were expected to have a significant impact on reproduction in this population. The amount of effort put into reproduction by individual females was expected to be the same in spite of the inter-annual and inter-seasonal variability expected. This was based upon a previously reported trend in reproductive effort in a time when variability was also evident (Havens and McConaugha 1990). Fecundity of this population was hypothesized to be significantly less than expected based upon presently accepted data and models. This potential reduction in reproductive output could have a significant impact on the blue crab population, the state of the fishery, and the perceived ecological state of the Chesapeake Bay.

CHAPTER II

FECUNDITY OF AN EXPLOITED BLUE CRAB, *CALLINECTES SAPIDUS,* POPULATION

Introduction

In recent years mature female blue crabs in the Bay population have decreased in average size, indicating that females are maturing at smaller sizes (VIMS Trawl Survey). This suggests that they may be maturing at a younger age or that the allocation of energetic resources to growth has changed. Assuming that the previous allometric relationship between size and fecundity (Prager et al. 1990) is still valid for this population, a decrease in mean carapace width would reduce the number of eggs that a female could produce per brood. Smaller females produce fewer eggs per brood, but are able to produce more broods in a shorter amount of time than larger individuals (Dickinson et al. 2006). Females of various sizes had equivalent reproductive output within a season although individual brood sizes may have differed (Dickinson et al. 2006). This indicates that the smaller females observed in the Chesapeake Bay have the potential for producing the same number of eggs within a full reproductive season as large females, assuming that all are expending similar amounts of reproductive effort.

The contribution of the blue crab male to population fecundity has apparently also been affected in recent years. The average size of a mature male has declined due to intense fishing pressures on larger males. This has reduced the amount of sperm that can be stored within a male at any given time as male size is correlated with sperm production (Kendall et al. 2001; Kendall et al. 2002; Wolcott et al. 2005). The amount of

sperm stored in the spermathecae of the female must be sufficient to fertilize all of the broods that a female will produce in her lifetime, as females are assumed to only mate during the terminal molt (van Engel 1958). Smaller males may not be able to produce enough ejaculate to fertilize all of the oocytes for a single female, and the female would be unable to fulfill her reproductive potential as a result of sperm-limitation (Jivoff 1997; Kendall et al. 2001; Kendall et al. 2002).

Since females can be harvested in the Virginia portion of the Bay following the molt to maturity, smaller mature females may not survive to reach their full reproductive potential. Because of harvest pressure, early maturation may result in reduced total fecundity. Age is an important factor in regulating fecundity in many species, as younger females are generally less fecund than older females (Tuck et al. 2000). Declining fecundity of the population due to a higher percentage of younger and smaller mature females, in conjunction with intense harvest pressure, could further reduce the spawning stock. A diminished spawning stock combined with a further reduction in egg production could be catastrophic to the stability of the population.

Because of the decrease in average size of mature females in the Bay population since an earlier fecundity study was performed in the late 1980's (Prager et al. 1990), we thought it beneficial to reexamine fecundity. Little attention has been given to this important population parameter, and fishery managers are still utilizing the data from the earlier study to develop harvest targets (Miller and Houde 1998; Miller et al. 2005). Negative changes in the fecundity of this population, along with a declining spawning stock, could alter the fishery. Increased knowledge of fecundity and its variability is important for a greater understanding of the reproductive strategies of this population.

Materials and Methods

Mature gravid females with orange sponges were collected biweekly from a local crab processing house throughout five spawning seasons (May-September, 2002-2006), which corresponds with the potting season in VA. Collection from the local source allowed us to obtain a broad sample of females from the spawning ground of the lower Bay. Fishery dependent mature females were found to not differ significantly in carapace width from fishery independent sources (VIMS Trawl Survey). Their carapace widths were measured to the nearest mm, and the abdomens were removed, fixed in 10% buffered formalin and stored for later egg enumeration. The sponges were enumerated following the dry-weight method outlined in Prager et al. (1990). Eggs were removed from the pleopods, and three subsamples of two hundred eggs were counted from each sponge. Eggs that had fallen off the sponge during storage or processing were filtered and added to the remaining sponge. The sponge and three replicate samples were dried to a constant weight at 60^{o} C. The three subsamples were averaged to determine the mean egg weight and the average was used to calculate the total number of eggs in each sponge based on the total dry weight of the sponge. The relationship between carapace width and number of eggs per brood was modeled for each year using a linear regression.

The mean carapace width and mean egg number for each year was compared to other years in the study using a one-way ANOVA with a post hoc Tukey's test. The number of eggs produced within each month of a spawning season was compared with the other months using a one-way ANOVA with a Tukey's test, to determine if there were within season differences in egg production.

The mean egg diameter for each sponge was determined to compare egg size within and between spawning seasons. Egg size changes may indicate differences in energy allocated to individual eggs, which may have an effect on egg and larval quality. Mean diameter was measured on an Olympus microscope equipped with an Optronics DEI-750 camera, and Image Pro imaging software. Images of ten randomly chosen eggs per sponge were digitized, which was sufficient based upon power analysis from preliminary data. The diameters of individual eggs were determined from the images using the linear measuring tool in the Image Pro software. The software was calibrated using an image of an ocular micrometer obtained at the same magnification used for taking the egg images. Intra- and inter-annual differences in mean egg diameter were examined using a one-way ANOVA with a post hoc Tukey's test.

These same images were analyzed to determine the percentage of eggs fertilized. Examining the eggs for fertilization involves the detection of the fertilization envelope. The fertilization envelope is clear, allowing for light penetration through part of the egg. An egg that was opaque was scored as not fertilized (Tabot and Goudeau 1988; Pongtippatee-Taweepreda et al. 2004).

Results

The mean number of eggs produced per brood for 2002-2006 was 8.7×10^5 ($\pm 5.3 \times 10^5$, n = 617), and the mean carapace width was 133.4 mm ($\pm$13.5 mm, n = 618) (Table 1.1). The mean number of eggs increased since the study was begun in 2002, but the number of eggs produced per brood for each year is less than the previously reported value of 3.2×10^6 (Prager et al. 1990). The mean egg diameter was 216.28 μm ($\pm$20.09 μm, n = 611). The mean and standard errors for each of these parameters for each year

sampled are given (Table 1.1). Superscripts after the mean of each parameter indicate groupings and significant differences between years.

There were inter-annual differences in all three parameters examined (Table 1.1). Mean carapace width ranged from 128.6 mm (± 13.1 mm, n = 121) in 2004 to 137.5 mm (± 14.3 mm, n = 128) in 2006. The mean carapace width for 2002 was significantly smaller than 2006 (Tukey-Kramer multiple pairwise comparison, n = 177, F = 7.587, $P \leq 0.001$), but was not significantly different from any other year. The mean carapace widths for 2003, 2005, and 2006 were not significantly different from each other, but the mean for 2004 was significantly smaller than 2003 (Tukey-Kramer multiple pairwise comparison, n = 241, F = 7.587, P = 0.012), 2005 (Tukey-Kramer multiple pairwise comparison, n = 322, F = 7.587, P = 0.006), and 2006 (Tukey-Kramer multiple pairwise comparison, n = 249, F = 7.587, $P \leq 0.001$).

Table 1.1. Inter-annual differences in reproductive output. Each column is the mean for the year. Different superscripts indicate significant differences and values with the same superscript are not significantly different. All differences are significant at the 0.05 significance level as determined by a Tukey-Kramer multiple pairwise comparison.

Year	Mean Carapace Width (mm)	Standard Error (SE)	Mean Number of Eggs ($x10^5$)	SE ($x10^5$)	Mean Egg Diameter (μm)	SE
2002	131.3 ^{a,b}	1.86	3.7 ^{d}	0.25	252.00 ^{g}	1.55
2003	134.1 ^{b,c}	1.27	4.3 ^{d}	0.19	224.84 ^{h}	1.58
2004	128.6 ^{a}	1.10	9.6 ^{e}	0.28	204.95 ^{i}	0.63
2005	133.8 ^{b,c}	0.97	9.1 ^{e}	0.24	205.63 ^{i}	1.10
2006	137.5 ^{c}	1.28	13.1 ^{f}	0.65	229.75 ^{j}	1.55

Analysis of egg abundance produced three significantly different groups. Egg abundance was lowest for 2002 and 2003. Subsequently, eggs/brood more than doubled for 2004 and 2005, which was significantly different from 2002 and 2003. There was a further, and highly significant increase in egg abundance in 2006, which was significantly different from all other years (Table 1.1). The mean egg diameter (ranging from 204.95 to 252 μm) was quite variable with the means for 2004 and 2005 being significantly smaller than all other years. The mean egg diameters for 2002, 2003, and 2006 were all significantly different from each other. Eggs from 2002 were the largest (252 μm $\pm$ 10.05 μm, n = 42) for all five seasons, followed by 2006 (229.75 μm $\pm$ 17.51 μm, n = 106) (Table 1.1). 2006 also had an extended reproductive period that began much earlier in May and lasted later into September than any other year. An extended reproductive period in conjunction with a greater average number of eggs per brood in 2006 suggests that there was likely greater egg production in this year than in other years.

Analysis of the aggregate data for the five-year study shows there were intra-annual differences in carapace width, egg number, and egg diameter (Table 1.2). As the season progressed female size declined but they produced a greater number of smaller eggs/brood. The mean carapace width of mature females in May was significantly larger than all other months (Tukey-Kramer multiple pairwise comparison, n = 618, F = 6.627, $P \leq 0.001$). The mean number of eggs produced peaked in July and August, which were significantly greater than all other months (Tukey-Kramer multiple pairwise comparison, n = 617, F = 19.985, $P \leq 0.001$) (Table 1.2). The opposite trend was true for the mean egg diameter. As the spawning season progressed mean egg diameter became smaller through August and then increased in September to values found in May and June.

Larger females at the start of the spawning season produced fewer but larger eggs. There was a negative correlation between egg number and diameter (Correlation, n = 618, r = -0.126, P = 0.002). When females produced a greater number of eggs the eggs were smaller in diameter, and vice versa.

Table 1.2. Intra-annual differences in reproductive output. Each column is the mean of 5 years. Different superscripts indicate significant differences and those with the same superscript indicate that there is not a significant difference. All differences are significant at the 0.05 significance level as determined by a Tukey-Kramer multiple pairwise comparison.

Month	Mean Carapace Width (mm)	Standard Error (SE)	Mean Number of Eggs ($x10^5$)	SE ($x10^5$)	Mean Egg Diameter (μm)	SE
May	139.8 [a]	1.81	6.5 [c]	0.52	226.95 [f]	1.71
June	134.9 [b]	0.88	6.7 [c]	0.31	222.62 [f]	1.32
July	130.1 [b]	1.19	10.2 [d]	0.50	205.49 [g]	1.11
August	130.7 [b]	1.04	10.6 [d]	0.41	213.39 [h]	1.83
September	133.7 [b]	1.68	8.1 [c]	0.51	223.54 [f]	2.78

The mean carapace width during this study was less than the 147 mm reported in the previous study (Prager et al. 1990). To ensure that the samples obtained for this study (fishery dependent) were representative of the Bay population, carapace widths were also compared to measurements taken of mature females during the VIMS trawl survey (fishery independent) during the same years. Three of the five years (2003 – ANOVA, n = 257, F = 1.803, P = 0.180; 2004 – ANOVA, n = 376, F = 3.039, P = 0.082; 2006 – ANOVA, n = 257, F = 2.365, P = 0.125) presented in this study were not significantly different from the trawl data, and the VIMS trawl survey females from the other two years were significantly larger (2002 - ANOVA, n = 175, F = 14.065, $P \leq 0.001$; 2005 – ANOVA, n = 469, F = 19.075, $P \leq 0.001$). This suggests that the crabs obtained in this

study are reflective of the Bay population. A previous study, during a period of high crab abundance, used crabs from the same fishery-dependent source and also determined that the carapace widths of mature females were not different from females obtained from a fishery-independent trawl survey (Havens and McConaugha 1990). The size distributions of carapace width have shifted to the left in recent years (Fig 1.1). The mean carapace width of mature females for 1980 was 148.1 mm, with a maximum size of 184.0 mm, and a minimum of 94.0 mm. The mean carapace width for 2005 was 133.8, with a maximum size of 167.1 mm, and a minimum of 102.5 mm. The mean carapace widths for these two years were significantly different, as determined by an ANOVA (P-value $\leq$ 0.001). The other years in this study also had a greater number of smaller female crabs similar to 2005 (Fig 1.1). The increase in the percentage of crabs in the smaller size ranges (<120mm) suggests that this is a shift in size at maturity and not attributed to over-fishing.

Previously reported data determined that there was a strong size-fecundity relationship for this population of blue crab (Prager et al. 1990). When carapace width was plotted against fecundity for each year of this study (Figs 1.2 a-e) there was at best a weak size-fecundity relationship. In 2002 and 2006 there was no significant relationship between size and fecundity. A weak but significant relationship was determined for 2003 (Regression, n = 119, R^2 = 0.032, P = 0.045), 2004 (Regression, n = 122, R^2 = 0.092, P = 0.001) and 2005 (Regression, n = 201, R^2 = 0.138, P $\leq$ 0.001).

Fig. 1.1. Size frequency distributions of carapace width for mature females from 1980 and 2005 to demonstrate the difference in sizes over a quarter century period (VIMS Trawl Survey). The size distribution has shifted to the left in all years of this study. Since all mature females are subjected to fishing pressure the increase in the percentage of crabs in the smaller size ranges (<120mm) suggests that this is a shift in size at maturity and not attributed to over-fishing.

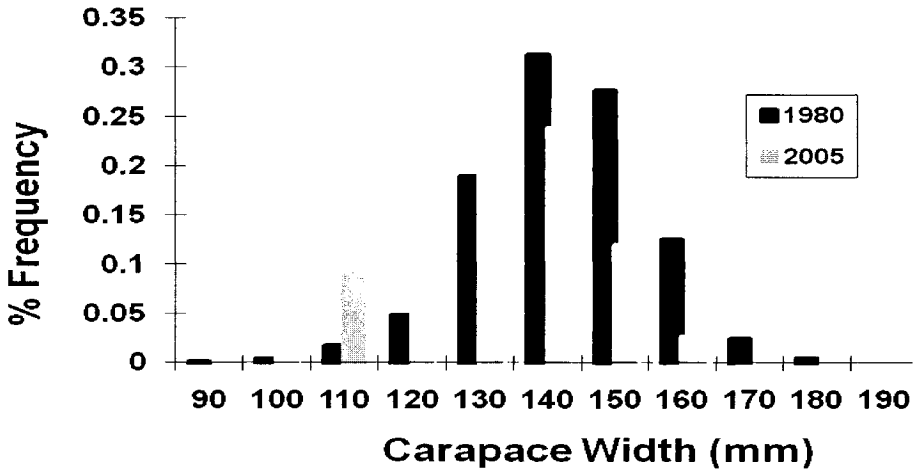


Fig 1.2 a-e. Scatter plots of carapace width vs. number of eggs from 2002-2006, a-2002, b-2003 (y = 3210.7x + 5243.5), c-2004 (y = 8105.8x – 57758), d-2005 (y = 9763.5x – 431682), and e-2006. There was no significant relationship between carapace width and the number of eggs for either 2002 or 2006. A weak but significant size-fecundity relationship was found for 2003, 2004, and 2005.

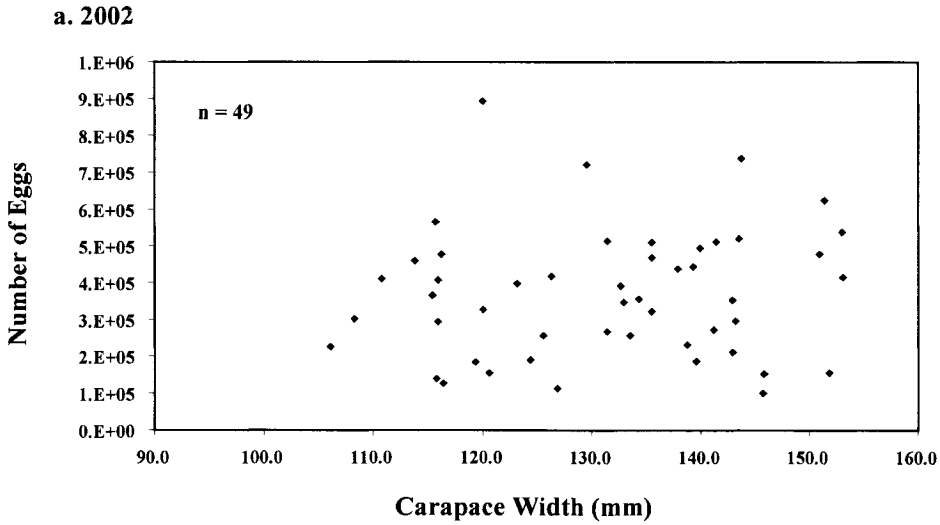
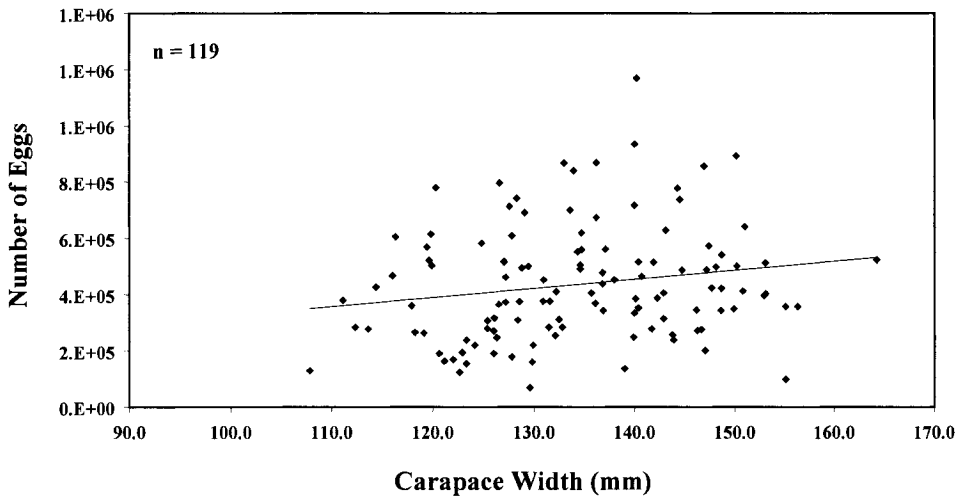


Fig 1.2 cont.

b. 2003



c. 2004

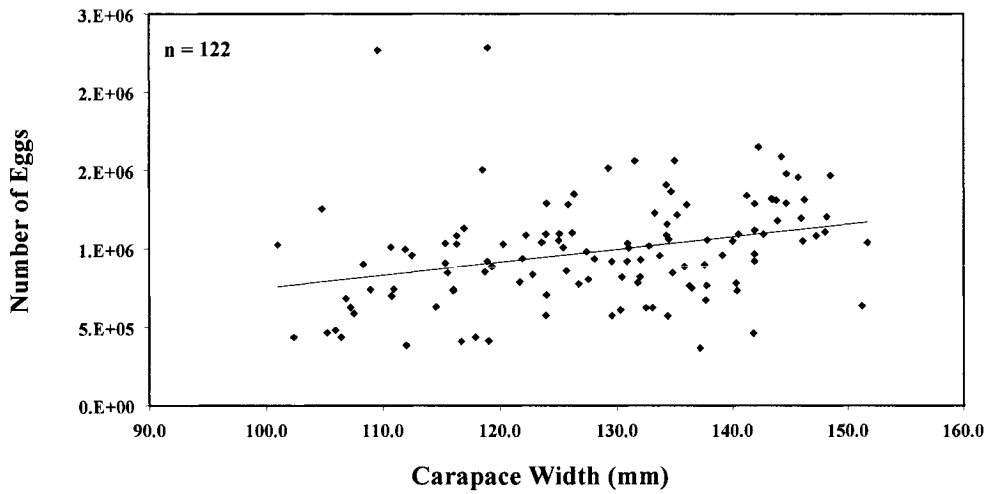
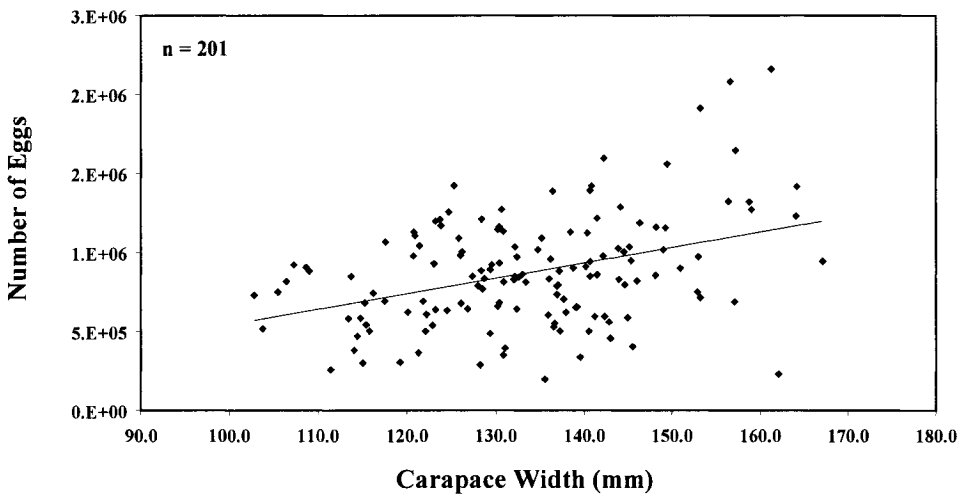
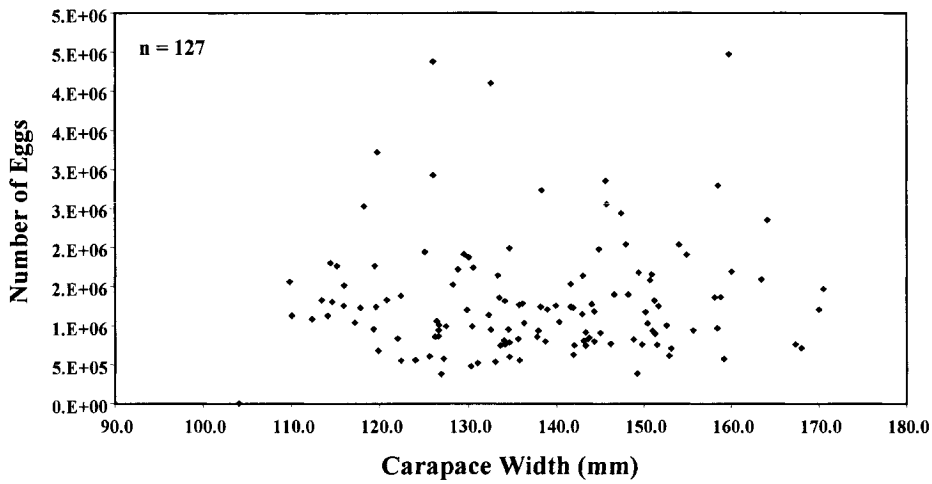


Fig 1.2 cont.

d. 2005



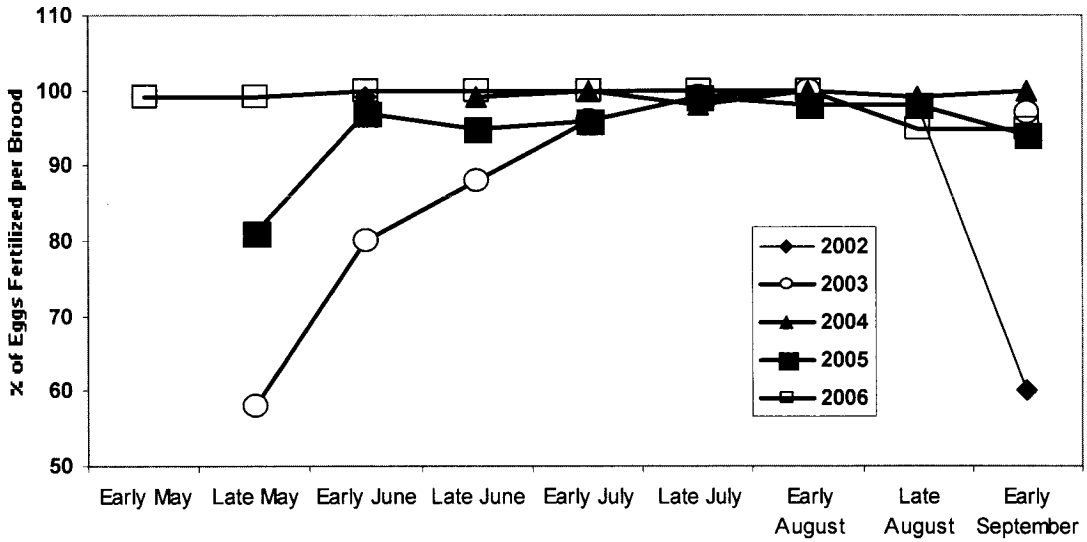
e. 2006



The annual percentage of fertilized eggs/brood was not significantly different from 100% for years 2002, 2004, 2005, and 2006 (Fig 1.3). In 2003 egg fertilization

averaged 88% per brood for the season, which was significantly lower than 100% (Tukey-Kramer multiple pairwise comparison, n = 612, F = 16.067, $P \leq 0.001$). The low percentage of fertilization in May of 2003 (58%) depressed the yearly average. Comparison of intra-annual differences found that the average percent fertilization was significantly lower for May (82%, Tukey-Kramer multiple pairwise comparison, n = 612, F = 21.212, $P \leq 0.001$) than for all other months. This can primarily be attributed to the 2003 and 2005 data. The lowest percentage fertilization in most years was at the beginning of the season. Fertilization increased significantly as the season peaked (June, July).

Fig 1.3: Intra-annual differences in the percentage of eggs fertilized per brood for 2002-2006. The average percent fertilization for 2003 was significantly lower than all other years. Considering all years, May had the lowest average percent fertilization.



Discussion

In decapod crustaceans, egg production associated with size at maturity has generally been described by an allometric relationship between carapace width and egg number (Hines 1982; Prager et al. 1990). This relationship assumes that females are producing the maximum number of eggs possible based on this allometric relationship (Hines 1982; Prager et al. 1990). Our current work suggests that this assumption is no longer valid for the blue crab in Chesapeake Bay. Data on egg production per brood for the last 5 years indicates that there is at best a weak size-fecundity relationship in this population. There is also strong inter-annual variation in this relationship with two (2002, 2006) of the five years having no significant size-fecundity relationship. The absence of a strong size-fecundity relationship coupled with an order of magnitude decrease in fecundity per brood suggests that allometric regulation is no longer the primary factor determining egg production in this population.

The 2006 spawning season was an anomaly compared to the other 4 years. An increase in egg numbers, egg size, and reproductive season duration in 2006 indicates that reproduction of the population was greater for the season. While there was no significant size-fecundity relationship for 2006, mature females were larger in 2006 than in any other year of the study. There was also a significant increase in both mean egg number per female, and mean egg diameter, indicating that individual females put more effort into reproduction during the 2006 season compared to other years. Increased egg lipid and protein concentration throughout the 2006 reproductive season (see Chapter 3) supports the hypothesis that there are factors other than allometry affecting the fecundity of this population.

In this study there were intra- and inter-annual differences in egg diameter and egg number suggesting tradeoffs between the two. The greatest numbers of eggs/brood were produced in July and August of each year. Eggs were smallest in diameter at this time in the annual reproductive cycle. This demonstrates that seasonal changes in reproductive allocation occur in this population. A higher number of eggs produced per brood in conjunction with smaller individual eggs during this period indicate that the females were allocating the energy available for reproduction differently as the spawning season progressed.

One potential factor driving this is the availability of resources to produce eggs. Blue crab preferred prey sources have also exhibited population declines in recent years (Hughes and Seed 1981; Blundon and Kennedy 1982; Clark et al. 1999). Declines in the quantity and/or quality of resources necessary for egg production could affect the number of eggs produced and the amount of yolk available to be apportioned. Further research is needed to ascertain the full importance of the role of resource availability in reproduction of this population.

Populations experiencing stress may express phenotypic plasticity to compensate for and alleviate stress (Stearns 1992). Stress in the forms of positive or negative changes in population density, as well as changes in natural and harvest mortality, habitat, and food availability can affect the reproductive strategies of a population (Stearns 1992; Xu et al. 1993; Trippel 1995; Marteinsdottir and Begg 2002). The Chesapeake Bay blue crab population has experienced a decline in the spawning stock in recent years that may have affected the number of larvae produced and subsequent recruitment (Lipcius and Stockhausen 2002). Harvest mortality has increased in this population to the point of

overfishing (Miller and Houde 1998). Juvenile blue crab habitat in the form of submerged aquatic vegetation has also dramatically declined in the Bay (Lipcius et al. 2005). These stresses may be affecting reproductive strategies of the Chesapeake Bay blue crab population.

The patterns of egg fertilization seen over the course of this study show that significantly fewer eggs are fertilized in the broods produced at the beginning of each season with the exception of 2006. As each season progressed the percentage of fertilized eggs within a brood was not significantly different from 100%. Fertilization in 2006 was not significantly different from 100% at any time in the season. The increased reproductive output in the 2006 season suggests that more fertilized eggs were produced than in other observed seasons. The low rates of fertilization at the beginning of the reproductive season in May suggest that overwintering mature females with low or deteriorated sperm reserves are the primary contributors. Females that have already produced broods prior to overwintering will have lower sperm reserves than newly mated females leading to lower fertilization success (Wolcott et al. 2005). Over time some of the sperm left in the spermathecae can degrade and lose viability, or leak out of the crab due to the degradation of the sperm plug, which will further decrease fertilization (Wolcott et al. 2005). This is evidence for possible sperm limited female reproduction within the Bay. The slight decrease in September fertilization rates may be attributed to a small portion of the mature females in the population becoming sperm limited at the end of the season. Smaller males are capable of transferring less sperm than their larger counterparts (Carver 2001). In this population larger males experience intense fishing pressure due to size harvest regulations that protect smaller males. This skews the

population to smaller males that are able to produce and transfer less sperm per mating (Carver 2001). The North Carolina blue crab population has also shown a shift to smaller males due to fishing pressure, and these males are transferring reduced amounts of sperm (Wolcott et al. 2005).

Although there is evidence for sperm limitation among some individuals in the Chesapeake Bay population, a high percentage of individuals from the same time period have broods that are 100% fertilized. Sperm limitation does not appear to affect the majority of the population. Those individuals that have fully fertilized broods in September likely had greater mating success, or have not produced enough broods to deplete their sperm reserves. The other possible explanation for increased fertilization among individuals is that there is continued migration of mature females into the reproductive population (Jones et al. 1990). Immature females may be maturing early enough to contribute a late season sponge, and would not have low sperm reserves.

The fecundity of the Chesapeake Bay blue crab population has dramatically declined in the past two decades. The shift in size frequencies and the increase in absolute number of mature females at smaller sizes support the hypothesis that females are maturing at smaller sizes. A decline in fecundity due to declining carapace width would be expected in this population based on the previously determined relationship between size and fecundity (Prager et al. 1990). The further decline in fecundity per brood as evidenced by the lack of a strong size-fecundity relationship in this study suggests that population fecundity has declined further than expected. Changes in size at maturity might suggest a diversion of resources from growth into other systems, or that maturity is occurring sooner in the life cycle (Stearns 1992; Shertzer and Ellner 2002).

Other preliminary evidence based on lipofuscin aging techniques suggests that the shift seen in this population can be attributed in part to a reduction in age at maturity (Wells and McConaugha unpublished).

Early maturation is often viewed as increasing fitness in a population by contributing new offspring to the population more quickly (Stearns 1992). Changes experienced by the population can cause a phenotypically plastic reaction allowing females to reach maturity earlier and therefore contribute to reproduction sooner. The driving forces for this are increased mortality and decreased density within the population (Stearns 1992). The Chesapeake Bay blue crab population has experienced increased natural and harvest mortality, as well as a decrease in density, likely leading to reproductive phenotypic plasticity in mature females. Other heavily exploited populations have been shown to shift the size and age at maturity, increasing the probability that individual females will contribute to the reproductive effort of the population (Gadgill and Bossert 1970; Ermande et al. 2004). The trade-off is reduced individual fecundity per brood. In the case of the blue crab, a female maturing earlier will contribute to population reproduction earlier. It is then possible for a female to produce more broods within her lifetime, offsetting some of the loss in egg number in individual broods (Dickinson et al. 2006). Larger individuals may divert more resources into growth and would have more internal space for oocyte storage, but the lifetime reproductive period would be shorter than it would for an individual maturing earlier. In the Chesapeake Bay fishery, females can be harvested once they reach maturity, regardless of size, making it less likely that a female will survive to produce multiple broods. By maturing sooner, fewer eggs are produced per brood, thereby lowering brood

fecundity. Harvesting females before they can produce multiple broods eliminates the potential to counteract lowered brood fecundity leading to a substantial decrease in population fecundity as well as a decrease in the number of larvae available for recruitment.

This data suggests that the overall fecundity of this population has declined further than would be expected based upon estimates of spawning stock abundance and previous size-fecundity relationships. The lack of a significant relationship between size and fecundity is indicative of a population experiencing stress. Further investigation is necessary to ascertain what stressors and factors are contributing to the variability seen in reproduction both inter- and intra-annually. It is obvious that females are capable of reproductive phenotypic plasticity. Reproductive allocation can vary, leading to changes in egg numbers and sizes throughout the reproductive season as was seen in this study and in the Beaufort, NC blue crab population (Dickinson et al. 2006). It is also likely that females are maturing sooner as exhibited by their smaller average size at maturity, but they should theoretically be able to produce a greater number of broods throughout their lifetime (Dickinson et al. 2006). By maturing earlier, females in the Chesapeake Bay may actually be reducing population fecundity further because they are producing smaller broods and their reproductive period may be truncated due to harvest regulations in the Bay. This change in fecundity in conjunction with the recent decreases observed in population numbers and harvests are indicative of a population in crisis. The magnitude of variation in fecundity demonstrated in this study could lead to the collapse of the fishery if current estimates are not incorporated into fisheries management models.

CHAPTER III

VARIATION IN BLUE CRAB, *CALLINECTES SAPIDUS*, LIPID AND PROTEIN CONCENTRATIONS RELATING TO REPRODUCTION

Introduction

Reproduction is a very energy expensive process. Reduced prey availability or increased energy costs can result in energy reallocation within the body, including diversion from reproduction. This can result in decreased fecundity within a population, as observed for the Chesapeake Bay blue crab population (see Chapter 2). Fewer eggs may be extruded per brood when there is insufficient energy available to produce the maximum number of eggs based on allometry. Alternatively, extra energy could be apportioned to individual eggs which would result in a further decrease in egg numbers, but a greater likelihood of larval survival (Pernet et al. 2003; Calado et al. 2005; Lee et al. 2006). Increased numbers of larvae surviving to metamorphosis could partially compensate for reduced egg numbers per brood. Blue crab females also have the potential to produce multiple broods within a lifetime. If there are fewer eggs per brood, but the female produces multiple broods with higher quality eggs, the number of larvae available for recruitment could be greater than expected (Dickinson et al. 2006).

The dramatic changes observed in the fecundity of the Chesapeake Bay blue crab demand further investigation into the reproductive output of this population. The gonadal index (GI) allows us to determine if the crabs were allocating a consistent amount of energy to ovarian tissue relative to their body size as compared to previous data. Protein

and lipid concentrations in the hepatopancreas, ovaries, and eggs of mature females can provide insight into the utilization and mobilization of energy in the blue crab within and between years and spawning seasons. Changes in reproductive effort, both inter- and intra-annually can also be ascertained from this information. Altogether, this will provide more concise information about the reproductive strategies of the population, which can be used to understand population dynamics and for fishery management.

Materials and Methods

Mature female blue crabs were collected for two years (2005, 2006) from a local crab-processing house. During the spawning season they were collected bi-weekly, and monthly at other times. Collection from the local source allowed us to obtain a broad sample of females from the spawning ground. Maturity was determined by the presence of an egg mass on the abdomen, or by the shape of the abdomen. The entire crab was weighed to the nearest mg, and the hepatopancreas and ovaries were dissected and also weighed to the nearest mg. The ratio of ovarian weight to the total weight was used to calculate the GI. A subsample of the hepatopancreas, ovaries, and eggs from females were taken for lipid and protein analysis. These samples were stored frozen (-80^{o} C) and under nitrogen to prevent the oxidation of tissues, until analysis was completed.

Hepatopancreas, ovaries, and eggs were analyzed for total lipids using the Bligh and Dyer (1959) method of extraction and gravimetric analysis. For the hepatopancreas and ovaries 1 mg of tissue was preweighed. Egg analysis was performed with 100 eggs. All samples were replicated three times to determine an average concentration. Egg number was used as opposed to egg weight so that the data could be extrapolated to a per egg basis. A chloroform, methanol, and water extraction (2:2:1) was used in conjunction

with sonication for complete lysis of cells and the greatest amount of lipid recovery. Each sample was extracted and sonicated three times, and at the end of the third round the chloroform layer was removed and placed into a pre-weighed test tube. The chloroform layer was evaporated to dryness under a steady stream of nitrogen and the test tube was reweighed. The total amount of lipid in each sample was the difference in the tube weight. An average lipid concentration was determined from the three sample replicates. Significant differences in lipid concentration between years and within a reproductive season were determined using a one-way ANOVA with a Tukey's post hoc test.

Protein concentration was determined by the fluorescamine technique (Undenfriend et al. 1972; Lorenzen and Kennedy 1993) on a Biotek FLX800 fluorescent microplate reader equipped with a PC running KC Junior software. A milligram of ovarian and hepatopancreas tissue, as well as 100 eggs were again utilized in replicates of three for this analysis. Bovine serum albumin was used to generate a standard curve for each plate. Each sample was sonicated in MilliQ for ten minutes for the greatest amount of protein recovery. Duplicate 200 μL samples along with blanks and standards were pipetted into a 96-well microplate. Fluorescamine, at a concentration of 3 mg/mL, was added to each well. The plates were read with an excitation of 400 nm and an emission of 460 nm. Average protein concentration for each sample was calculated from the replicates. Significant differences in protein concentration between years and within a reproductive season were determined using a Tukey-Kramer multiple pairwise comparison.

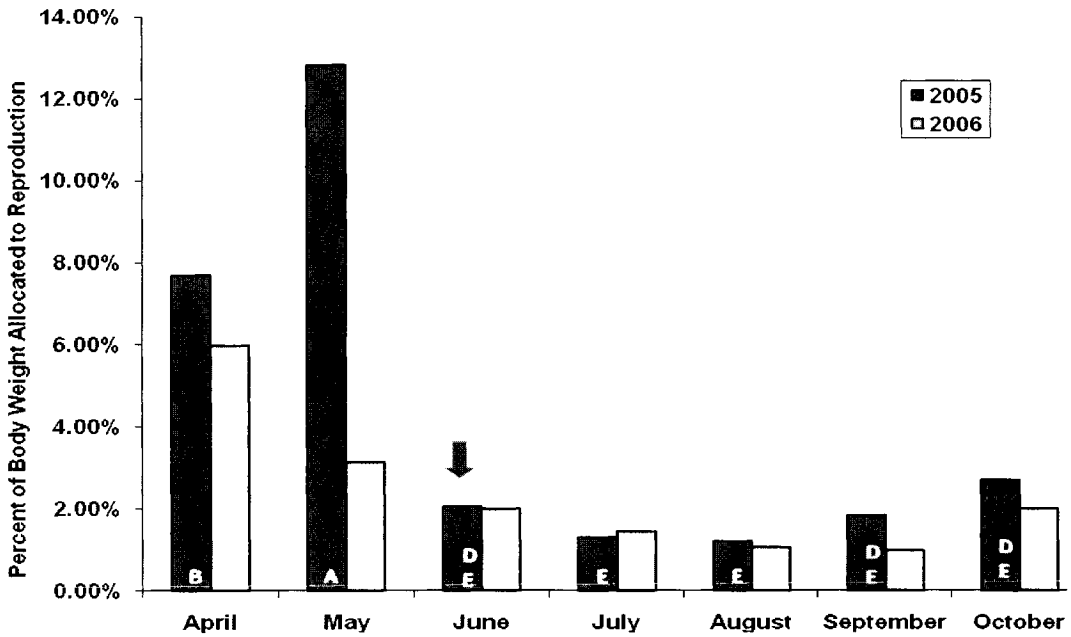
The mean egg diameter for each sponge was measured to determine if there was a relationship between egg size and lipid or protein content within the egg. Mean diameter was measured on an Olympus microscope equipped with an Optronics DEI-750 camera, and Image Pro imaging software. Images of ten randomly chosen eggs per sponge were digitized, and the diameters of individual eggs were determined from the images using the linear measuring tool in the Image Pro software. The software was calibrated using an image of an ocular micrometer obtained at the same magnification used for taking the egg images.

Results

Mean ovarian weights were significantly greater in the months prior to spawning, decreased once eggs were extruded, and increased slightly once the spawning season was completed. Fig. 2.1 shows the trends in gonadal index for 2005 and 2006. Resources in the ovaries were used for the production of oocytes, and later extruded as eggs. Spawning season is defined in this study as the time when extruded eggs were present. The 2005 spawning season occurred over a three-month period from June-August. Spawning for 2006 began in early May and lasted three weeks into September. The annual gonadal index for 2005 was significantly higher than 2006 (ANOVA, $n = 318$, $F = 4.575$, $P = 0.033$), but this is primarily due to the large gonadal index detected in May of 2005. Ovarian weight for the months measured in 2006 never reached the highest levels observed in May of 2005. The percentage of body weight allocated to gonads during the spawning months of June, July, and August was not significantly different between years with means at or below 2.0%. The only month that was significantly different between

years was May, which was significantly higher in 2005 (Tukey-Kramer multiple pairwise comparison, n = 318, F = 73.496, P < 0.001).

Fig. 2.1. Gonadal index demonstrating the average percentage of body weight that mature females allocated to ovaries. Consistently, a greater portion of the body weight was attributed to the ovaries prior to the onset of spawning. The arrows indicate when spawning commenced for each year. Different letters within the bars of each figure indicate significant differences. Bars that have the same letter(s) are not significantly different.



The lipid concentrations of the hepatopancreas peaked prior to the onset of spawning each year (Fig 2.2a). There was a decrease in lipid concentration during the spawning season, and a slight increase once spawning was completed, suggesting a recovery of resources in the hepatopancreas. ANOVA indicated significant differences in both inter- and intra-annual lipid concentrations. Inter-annual lipid concentrations in the hepatopancreas were significantly higher in 2005 compared to 2006 (ANOVA, n = 123,

F = 25.390, P $\leq$ 0.001). The highest lipid concentrations for 2005 occurred in May and June, which were significantly higher than the lower concentrations found in August (Tukey-Kramer multiple pairwise comparison, n = 60, F = 5.961, P = 0.003), and September (Tukey-Kramer multiple pairwise comparison, n = 60, F = 5.961, P = 0.00p). In 2006, May also had the highest lipid concentration, which was significantly higher than the lower concentrations found in September (Tukey-Kramer multiple pairwise comparison, n = 63, F = 3.149, P = 0.017) and October (Tukey-Kramer multiple pairwise comparison, n = 63, F = 3.149, P = 0.031).

Total lipid concentrations in the ovaries were highest prior to spawning, decreased during the spawning season, and increased after the season was complete (Fig 2.2b). The average concentration for 2005 was not significantly higher than 2006 (ANOVA, n = 121, F = 2.305, P = 0.132). The highest average lipid concentration for 2005 occurred in May, just prior to spawning. The ovarian concentration decreased in 2005 at the onset of spawning and continued to decrease through the end of spawning in August. September and October showed an increase in lipid content in the ovaries after spawning. Changes in ovarian lipid concentration were slightly different in 2006, because of the extended reproductive season. Lipid concentrations for 2006 varied during the period from April to August but there were no significant differences. A significant decrease in gonadal lipid content was observed in September (Tukey-Kramer multiple pairwise comparison, n = 61, F = 4.508, P = 0.008), the final month of the 2006 spawning season. October showed a slight recovery in ovarian lipid concentration after the prolonged 2006 spawning season.

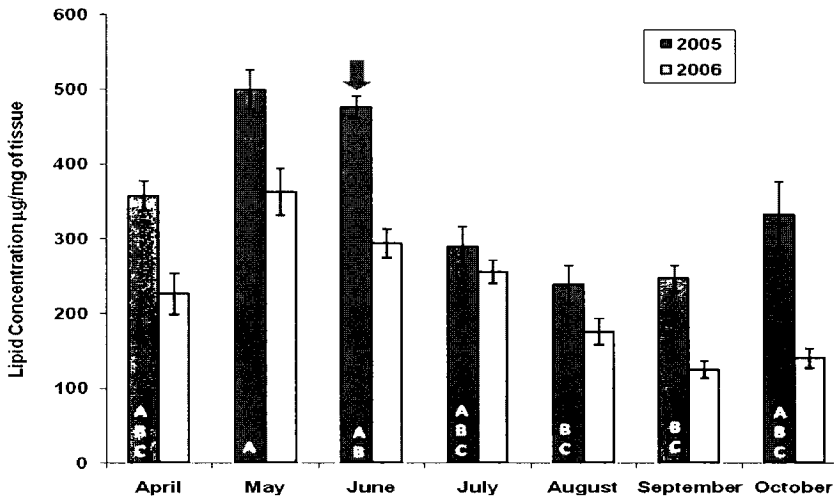
The protein concentrations in the hepatopancreas peaked prior to the onset of spawning and another peak was observed in the last month of spawning each year (Fig 2.2c). Decreases in protein concentration were detected in the middle of the spawning season and again after spawning was complete. As with the hepatopancreas lipid concentrations, protein concentrations were significantly higher in 2005 than in 2006 (ANOVA, $n = 119$, $F = 14.417$, $P \leq 0.001$). The concentrations were highest in April of 2005, which was significantly higher then the lowest concentration for that year which occurred in September (Tukey-Kramer multiple pairwise comparison, $n = 59$, $F = 3.420$, $P = 0.027$). In 2006 the highest protein concentration occurred in April followed by significantly lower concentrations in May (Tukey-Kramer multiple pairwise comparison, $n = 60$, $F = 5.267$, $P \leq 0.001$), June (Tukey-Kramer multiple pairwise comparison, $n = 60$, $F = 5.267$, $P = 0.001$), July (Tukey-Kramer multiple pairwise comparison, $n = 60$, $F = 5.267$, $P = 0.008$), and October (Tukey-Kramer multiple pairwise comparison, $n = 60$, $F = 5.267$, $P = 0.050$).

The ovarian protein concentration was significantly higher in 2005 than in 2006 (ANOVA, $n = 117$, $F = 11.565$, $P = 0.001$) for all months. Proteins were highest prior to spawning and decreased once eggs were extruded, followed by a recovery after spawning each year. In 2005 the highest concentrations were in April, May and October, just prior to and following spawning, but these were not significantly different from other months. Significantly lower concentrations occurred in 2005 in September (Tukey-Kramer multiple pairwise comparison, $n = 59$, $F = 3.324$, $P = 0.040$), immediately following spawning. 2006 was different from 2005 again likely due to the extended spawning period. The highest concentration in 2006 occurred in April, but significantly decreased

in June (Tukey-Kramer multiple pairwise comparison, n = 61, F = 4.508, P = 0.030). The lowest protein concentrations were during spawning.

Fig 2.2 a – d. – Hepatopancreas and ovarian lipid and protein concentrations. Lipid and protein concentrations were generally higher prior to and after spawning in both the hepatopancreas and ovaries. The concentrations decreased as spawning commenced and resources were mobilized to the eggs. Arrows in each figure indicate the onset of spawning for each year. Months with different letters indicate significant differences, and those with the same letter are not significantly different.

a: Hepatopancreas Lipid Concentrations
n = 10-18 per month



b. Ovarian Lipid Concentration
n = 10-18 per month

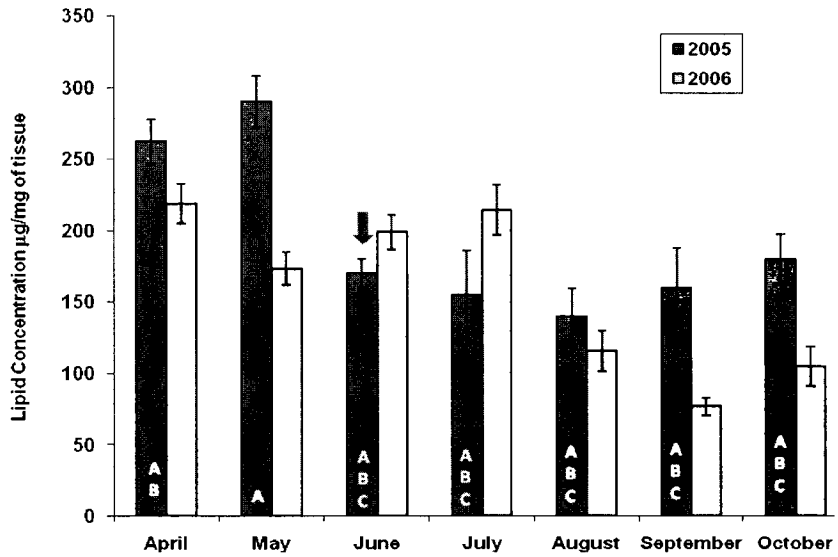
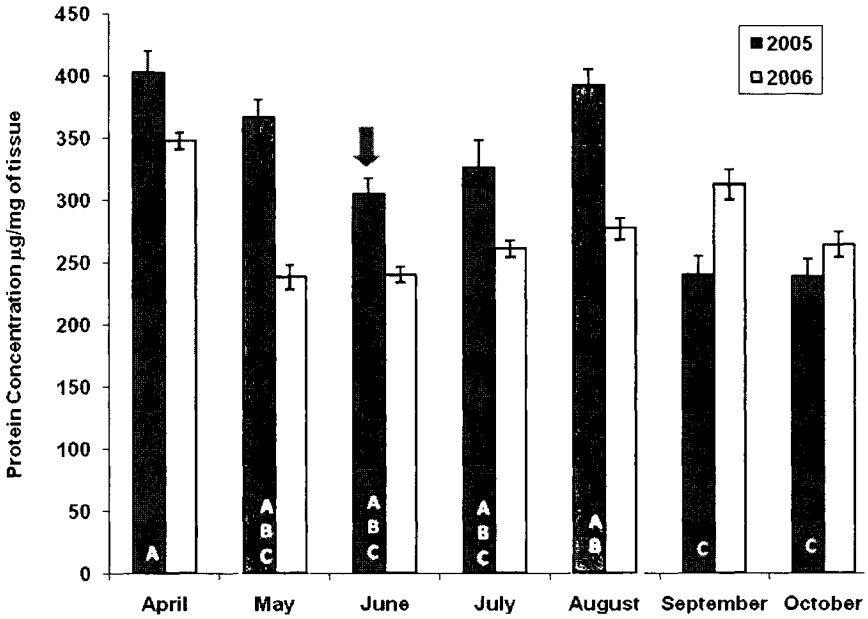
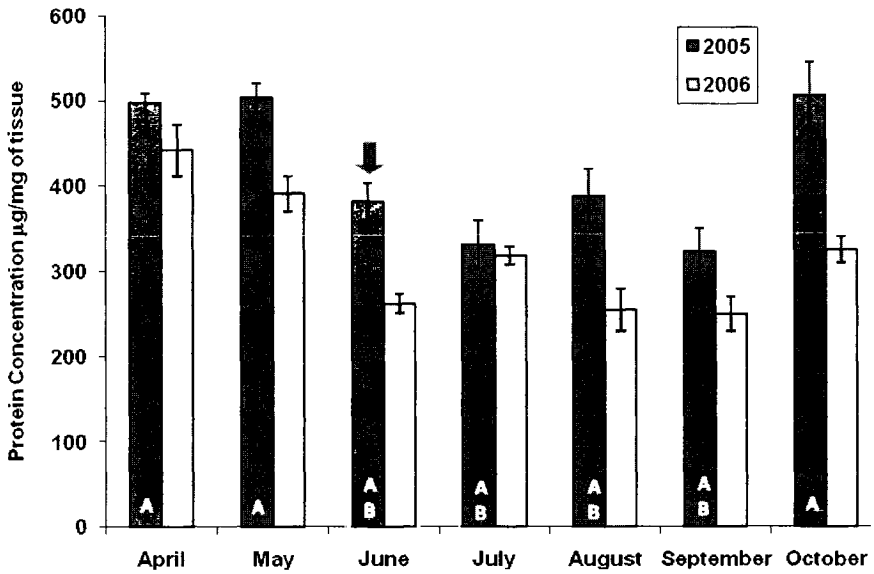


Fig 2.2 cont.

c. Hepatopancreas Protein Concentration
n = 10-18 per month



d. Ovarian Protein Concentration.
n = 10-18 per month



The trend in lipid concentrations within the eggs was quite different between 2005 and 2006 (Fig 2.3a). There was a significant difference in the overall egg lipid concentration between the two years (ANOVA, n = 96, F = 9.769, P = 0.002). 2006 egg lipid concentrations were higher than 2005 in all months of spawning, which was the opposite of what was observed in the hepatopancreas and ovaries. In 2005 the highest average concentration was found in July, but it was not significantly higher than the average for June. The average concentration August was significantly lower than the July lipid concentration in 2005 (Tukey-Kramer multiple pairwise comparison, n = 45, F = 4.254, P = 0.022). The highest average concentration for 2006 came in the month of May, decreased throughout the spawning season to the lowest concentration detected in August. The August lipid concentration was significantly lower than May (Tukey-Kramer multiple pairwise comparison, n = 51, F = 3.469, P = 0.013). There was a slight increase in lipid concentration in September of the 2006 reproductive season.

The protein concentration in eggs for both the 2005 and 2006 spawning seasons showed opposing trends as compared to the lipid concentrations measured in the same months (Fig 2.3b). No significant difference was detected for overall average protein concentration within the eggs between the two years (ANOVA, n = 93, F = 0.758, P = 0.386). The highest concentrations were at the beginning and end of spawning in 2005. In 2006 the highest concentrations were seen at the end of spawning in August and September, when lower concentrations of lipids were observed. A significantly lower concentration of protein in eggs was observed in June (Tukey-Kramer multiple pairwise comparison, n = 48, F = 3.331, P = 0.018). May, the month of highest lipid concentration, had lower protein content than August or September.

Lipid and protein concentrations within the eggs were also positively correlated with the average diameter of the eggs from each brood sample (Correlation, $n = 93$, $r = 0.283$, $P = 0.003$ and $r = 0.407$, $P \leq 0.001$, respectively). This demonstrates that larger eggs contain a greater amount of bulk lipid and protein than smaller eggs, and suggesting that larger eggs are better quality eggs.

Fig 2.3: a & b. Egg lipid and protein concentrations showed varying trends for both years. The months that the lipids are the highest are the months when proteins were the lowest and vice versa for both years. Also, 2006 lipid and protein concentrations were higher than in 2005, which is the opposite of that observed in the hepatopancreas and the ovaries. Different letters within the bars of each figure indicate significant differences. Bars that have the same letter(s) are not significantly different.

a. Egg Lipid Concentration
n = 11-16 per month

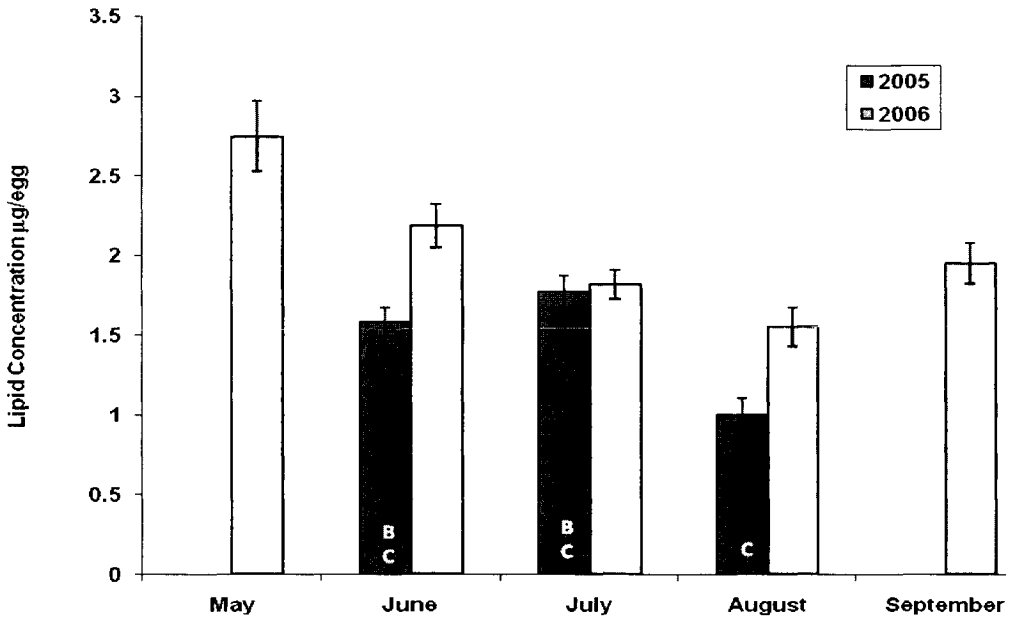


Fig 2.3 cont.

b. Egg Protein Concentration
n = 11-16 per month

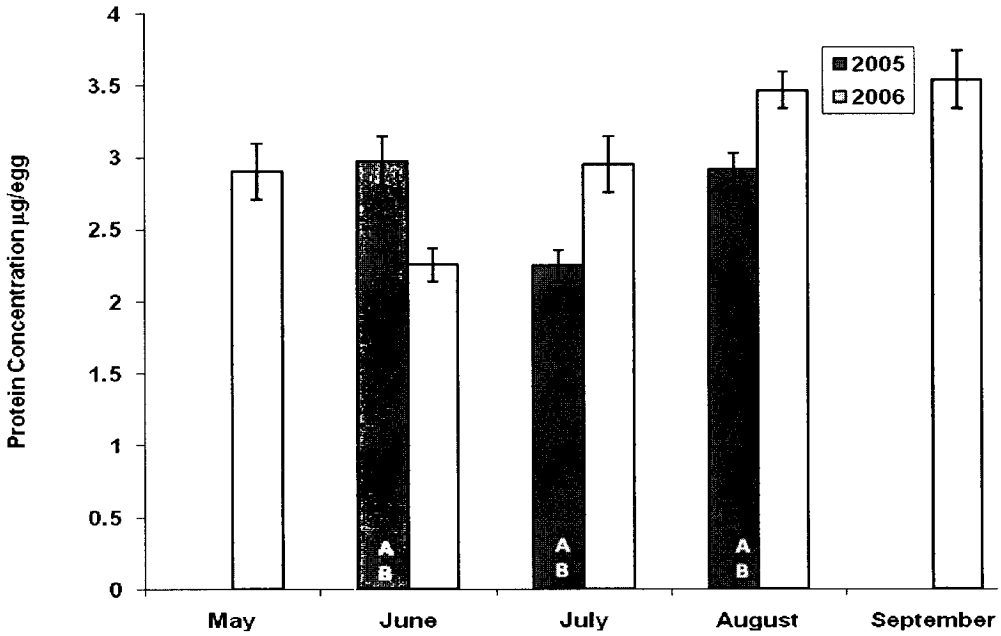


Table 2.1. Estimated egg energy per µg of lipid and protein, using conversions of 9.3 kcal/g for lipid, and 5.5 kcal/g for protein. (Vollenweider 2000).

Sampling Period	Mean Lipid Concentration (µg)	Lipid Energy (J) per egg	Mean Protein Conc. (µg)	Protein Energy (J) per egg	Total Energy (J)
June 2005	1.58	6.15×10^{-2}	2.97	6.83×10^{-2}	12.98×10^{-2}
July 2005	1.77	6.89×10^{-2}	2.25	5.18×10^{-2}	12.07×10^{-2}
August 2005	1.00	3.89×10^{-2}	2.91	6.70×10^{-2}	10.59×10^{-2}
May 2006	2.75	10.70×10^{-2}	2.90	6.67×10^{-2}	17.37×10^{-2}
June 2006	2.18	8.48×10^{-2}	2.26	5.20×10^{-2}	13.68×10^{-2}
July 2006	1.82	7.08×10^{-2}	2.95	6.79×10^{-2}	13.87×10^{-2}
August 2006	1.55	6.03×10^{-2}	3.46	7.96×10^{-2}	13.99×10^{-2}
September 2006	1.95	7.59×10^{-2}	3.54	8.15×10^{-2}	15.73×10^{-2}

The value of potential energy from lipids and protein within the eggs was calculated using values determined by Vollenweider (2000) for other crustaceans. Table 2.1 shows the estimated energy per egg. Lipids (9.3 kcal/g) contribute a greater amount of energy per μg than do proteins (5.5 kcal/g) (Vollenweider 2000). In times when lipid concentrations decreased protein concentrations increased in the eggs, which provided some compensation for energy lost due to low lipid. Eggs produced in 2006 had much greater energy available than the eggs produced in 2005. In August of 2005 the increase in protein was not great enough to counteract the reduction in lipid, and there was a decrease in the amount of energy available to the embryo from earlier in the season. May 2006 had high concentrations of lipids and proteins, which equated to the greatest amount of energy available in the eggs throughout the study. In 2006, reductions in lipid concentration were counteracted by increases in protein (Table 2.1).

Discussion

Observed changes in the fecundity and egg size of the Chesapeake Bay mature female blue crab indicate that the reproductive output of this population has decreased in recent years (see Chapter 2). In the past, females exhibited a strong allometric relationship between carapace width and fecundity with a high number of eggs produced per brood (Prager et al. 1990). At no time during our study did egg production reach the high numbers observed per brood in the late 1980's, and the lack of a significant relationship between female size and fecundity suggests that external as well as internal factors may be controlling reproduction in this population (Prager et al. 1990; see Chapter 2).

Females have the ability to allocate resources for a given brood differently by changing the number and size of eggs produced. This has been observed in the blue crab. Broods with a higher number of eggs had smaller eggs, and those with larger eggs had a lower total number of eggs in the brood (see Chapter 2). Resource allocation changes suggest that there is an external factor controlling the allocation. Recent changes in the availability of resources necessary for reproduction are a likely external factor. Lipid and protein concentrations in the hepatopancreas, ovaries, and eggs of mature females demonstrated further inter- and intra-annual variability indicative of changes in available resources for female reproduction, or a diversion of energy resources away from reproduction. Changing age at maturity may also play a role. Maturing earlier in the life cycle involves trade-offs and one of these may be a reduction in the time available for individual resource stockpiling (Stearns 1992; Shertzer and Ellner 2002). Female blue crabs are maturing at smaller average sizes than in the past, suggesting that the average age of maturity has also decreased (Stearns 1992; Lipcius and Stockhausen 2002; Shertzer and Ellner 2002).

Lipids and proteins are used throughout the crustacean body for growth, cell maintenance, and metabolic energy (Allen 1972; Conklin et al. 1983; Hasek and Felder 2006; Sanchez-Paz et al. 2006). This is especially true for developing embryos and the subsequent larvae. These resources must be apportioned from the mother (Spaargaren and Haefner 1994; Walker et al. 2003; Hasek and Felder 2006; Lee et al. 2006; Sanchez-Paz et al. 2006). Just prior to extrusion, oocytes go through vitellogenesis (Lee and Walker 1995). Vitellogenesis forms what will become the yolk in the extruded egg, and requires further lipid and protein resources to complete (Lee and Walker 1995). In this

study, prior to the onset of spawning, hepatopancreas and ovarian lipid and protein concentrations were high and significantly decreased with the extrusion of eggs. The decrease in protein and lipid concentration within the hepatopancreas indicates that stored resources are the primary energetic source for first brood production. This demonstrates that energy is mobilized from the hepatopancreas to the ovaries for the preparation of the first brood of the season. This is further supported by the data from the gonadal index, which showed an increase in ovarian weight prior to egg extrusion, and a precipitous drop in ovarian weight following extrusion.

The hepatopancreas and ovarian protein and lipid concentrations remained low in this study while brood production continued throughout the spawning season. The production of multiple broods of eggs within a spawning season requires that oocytes must also be developed for each brood. This requires sufficient energetic reserves for the embryos of each brood (Wolin et al. 1973; Sibert et al. 2004). The low concentrations of lipids and proteins detected in the hepatopancreas throughout the spawning season suggest that the necessary resources for oocyte and egg production beyond the first brood are not stored prior to gamete development. This is a common occurrence among crustaceans that brood multiple times within a reproductive season such as *Penaeus semisulcatus* (Ravid et al. 1999), *Uca longisignalis* (Hasek and Felder 2006), and *Carcinus maenas* (Heath and Barnes 1970). Lipids and proteins for later season broods are often derived directly from food ingested when stored resources are lacking (Teshima 1986 a, b; Ravid et al. 1999). Mature female food consumption increases as the spawning season progresses and later broods are produced, but lipid and protein

concentration do not, suggesting that the resources necessary for reproduction are quickly utilized (Teshima 1986 a, b; Ravid et al. 1999).

Concentrations of lipids in the hepatopancreas and ovaries increased following the conclusion of the spawning season for both years. Protein concentrations also increased at the same time in the ovaries, but not the hepatopancreas. Increases in concentrations following the spawning period may be attributed to resource stockpiling by existing females in the spawning stock. New females just entering the spawning stock could also increase the average lipid and protein concentrations as they would have unused stored resources (Churchill 1919; McConaugha 1995; Turner et al. 2003). Because most females do not survive to spawn a second year, most likely due to fishing pressure, it is more likely that the post-spawning increases can be attributed to newly matured females (Jones et al. 1990; Lipcius and Stockhausen 2002).

The discrepancy between lipid and protein accumulation in the hepatopancreas following spawning may indicate that equivalent lipids and proteins are not being ingested by the females. Females forage throughout the season as they make their trek to the spawning grounds (Turner et al. 2003). The density of the primary prey sources (i.e. *Macoma balthica, Mya arenaria*) dramatically decrease as the spawning season progresses, reaching the yearly low in July and August (Eggleston et al. 1992). There are fewer resources available to the mature females later in the season, which may force the crabs to forage for inferior prey items such as amphipods and isopods (Hines et al. 1990; Mansour and Lipcius 1991).

Proteins and lipids are necessary for tissue growth in embryos, as well as metabolic energy (Garcia-Guererro et al. 2003; Graeve and Wehrtmann 2003; Sibert et al.

2004; Calado et al. 2005). Protein concentrations were high in eggs in the first month of spawning, significantly decreased in the second month, and then significantly increased as the season progressed. Lipid concentrations demonstrated a slightly different pattern with higher concentrations at the beginning of the spawning season similar to the proteins, but decreasing to the lowest concentrations near the end of the spawning season. The lipid concentration of the eggs decreased significantly late in the spawning season of each year, at the time when protein concentrations reached their peak. More energy can be obtained per mg of lipid than from protein in crustaceans, and almost double the amount of protein is needed to equal the metabolic energy input from lipids in crustaceans (Pandian and Schumann 1967; Vollenweider 2000). The concentrations measured in this study indicate that protein increased when lipid decreased, but did not increase enough in 2005 to counteract the energy lost from the decline in lipids. This demonstrates late season smaller eggs, with significantly lower lipid concentrations (i.e. August 2005, Table 2.1), may have significantly less energy available for embryos than early season eggs.

Eggs of marine invertebrates generally are higher in protein concentration than other components as proteins are necessary for cellular structures, amino acids, enzymes, and can be used as an energetic source (Holland et al. 1987; Graeve and Wehrtmann 2003). In this study the protein concentrations were slightly higher than the lipids in all months of 2005. The first two months of spawning in 2006 had equivalent lipid and protein concentrations, but protein concentrations were higher in all other months. The embryos from eggs produced in the first month of spawning had far greater energy available to them than eggs produced later in 2006. Eggs produced that are significantly

greater in energetic quality allow the embryo and subsequent larvae to grow larger in a shorter amount of time, or allow the larvae to survive longer without food (Pernet et al. 2003; Lee et al. 2006). Egg production in 2006 was of a greater energetic quality than the previous year. Lower quality eggs between spawning seasons may have grave implications for larval survival, but at this time, egg hatching and larval survivability data are lacking.

Egg production in 2006 was also greater than 2005 in both the average number of eggs produced per brood and the average egg diameter (see Chapter 2). 2006 had a longer reproductive season than 2005 by starting a month earlier (May) and ending a month later (September). The changes detected in egg lipid and protein concentrations between 2005 and 2006 demonstrated that there was considerable variation in the amount of reproductive effort expended by mature females based on the amount of lipids and proteins apportioned to each egg. Overall, 2006 egg lipid and protein concentrations were significantly higher than in 2005. This is opposite from what was seen for the hepatopancreas and ovaries that had higher concentrations of both in 2005. This suggests that a greater amount of resources were allocated in 2006 than in 2005. It also suggests that although greater stored resources were available in 2005, they were not all allocated to reproduction. Protein concentration in the eggs exhibited the same trend in the first three months of spawning for both years, but the higher lipid concentrations throughout 2006 demonstrated a greater maternal investment per egg that season. An earlier start to the reproductive season may increase an individual's reproductive output for the year by increasing the number of broods produced (Stearns 1992; Hines et al. 2003; Dickinson et al. 2006). Increased reproductive effort in 2006 in conjunction with an elongated

reproductive season suggests that the reproductive output for this year was considerably higher than 2005. This could lead to a subsequent increase in recruitment back to the Chesapeake Bay population, and a potential increase in the population size.

Each egg must be apportioned with sufficient lipids and proteins for the embryo to form all of the tissues and cellular structures needed to reach the feeding stage (Mourente and Rodriguez 1997; Garcia-Guererro et al. 2003; Pernet et al. 2003). In this study, in the latter part of each year, eggs had lower lipid concentrations, but higher protein concentrations. It is likely that the embryos from these eggs would have to rely more heavily on proteins for metabolic energy (Rosa et al. 2003). At times when the lipid and protein concentrations were higher, such as the beginning of 2006, excess energy could be utilized for growth (Brooks et al. 1997; George 1999). In other crustacean species (*Chasmagnathus granulata, Penaeus vannamei*, and others) reduced necessary concentrations of lipids and proteins reduced hatchability and survivability of larvae (Palacios et al. 1999; Giminez and Anger 2001). This study showed that larger eggs had higher amounts of lipids and proteins. Larger eggs also suggest growth rates of those larvae will be greater which may reduce predation compared with their smaller counterparts (George 1990, 1999; Brooks et al. 1997). The larger eggs produced in 2006, with higher lipid and protein concentrations, were of a better quality than those produced in 2005, suggesting that the larvae hatched from the eggs produced in 2005 may be less viable.

The changing concentrations of lipids and proteins throughout each spawning season demonstrate that broods are variable. The amount of lipids and proteins in individual broods are adjusted, both inter- and intra-annually. This may be dependent on

the food supply and the energy demand from other metabolic systems or changing environmental conditions. The preferred prey species of the blue crab have experienced reductions in population sizes that have led to changes in the feeding strategies and efficiencies of the crab (Clark et al. 1999). Patches of prey such as clams and oysters have not only become smaller, but are farther apart and require more energy to get to (Clark et al. 1999). In addition to the increased time and energy it takes to obtain the food, the reduction in prey patches also leads to greater interaction with other blue crabs. These interactions are often aggressive and require the expenditure of energy that could otherwise be devoted to reproduction (Goss-Custard 1977; Goss-Custard et al. 1984; Clark et al. 1999). Blue crabs have demonstrated that they will change their feeding strategies in the presence of competitors. Crabs often shorten the time spent on the feeding patch and make repeated trips to the patch in short bursts to minimize the interaction with other crabs (Clark et al. 1999). This greatly reduces the feeding efficiency of the crab and increases the amount of energy that must be expended to forage. Changes in the metabolic rate of mature females could cause a significant diversion of energetic resources away from reproduction (Stearns 1992).

Fluctuations in temperature, salinity and oxygen in the water column could result in dramatic changes in metabolism that would affect the concentrations of lipids and proteins allocated to reproduction (Leffler 1972; Guerin and Stickle 1992; Taylor and Eggleston 2000). Changes in lipid and protein concentrations within and between seasons seen in this study suggest that there were significant changes in energy utilization and/or food supply experienced. These external factors are most likely contributing to the reproductive output in this population.

The goal of most blue crab fishery management models is to protect the relationship between the spawning stock and recruitment to the population (Lipcius and Stockhausen 2002). A depression in the spawning stock will ultimately decrease larval recruitment, and a significant decrease in the spawning stock has been observed in recent years (Lipcius and Stockhausen 2002). Conserving egg production among the remaining spawning stock will allow the maximum number of larvae to recruit into the population. This study demonstrates that the biochemical composition of the eggs needs to be accounted for when modeling larval recruitment. Since I do not know the minimum concentrations of lipids and proteins necessary for larval hatchability and survival, I also do not know if any of the concentrations measured in this study were below the minimums. There is considerable variation in the number of eggs produced per brood, the size of the eggs, and the concentrations of necessary lipids and proteins. It seems likely that eggs produced early in the spawning season are higher in quality than later season eggs. This will lead to an increased rate of larval survival earlier in the season. This is not accounted for in any fisheries models to our knowledge, and may lead to an overestimate of recruitment for this exploited population.

CHAPTER IV

INTER- AND INTRA-ANNUAL CHANGES IN BLUE CRAB, *CALLINECTES SAPIDUS*, REPRODUCTIVE OUTPUT BASED UPON LIPID FRACTION CONCENTRATIONS

Introduction

Negative changes in blue crab reproductive strategies, as well as reproductive allocation, have been recently observed (see Chapter 2). A previously determined relationship between mature female size and fecundity is no longer significant for this population (see Chapter 2). The number of eggs produced per brood has fluctuated, but in all years studied (2002-2006) was significantly less than reported 25 years ago (Prager et al. 1990; see Chapter 2). Females are maturing at smaller sizes, producing fewer eggs per brood, and seasonal differences in total lipid and protein concentrations within those eggs have been observed (see Chapter 3). Changes in lipid and protein concentrations within eggs produced later in the spawning season suggest that those eggs are of a lower quality than earlier season eggs (see Chapter 3). Changes in egg quality may have an adverse affect on hatchability resulting in a decrease in larval survival (Pletl 1992), and ultimately a reduction in recruitment back to the population.

Observed changes in reproductive output and energy allocation to eggs in this population suggested a need for further study of the quality of the eggs produced. The main goal of this study was to determine if the previously observed variation in total lipid concentration within and between seasons resulted in differences in concentrations of the key lipid fractions. It was hypothesized that triglyceride, phospholipid, and cholesterol

concentrations measured in the hepatopancreas, ovaries, and eggs of mature female blue crabs from the Chesapeake Bay, would exhibit both inter- and intra-annual variation. We compared these changes in the specific lipid fractions with the patterns observed for the total lipid concentrations in the same organs. Concentrations of total lipid and lipid fractions within the eggs of this population address questions of changing egg quality.

Materials and Methods

Mature female blue crabs were collected from Graham & Rollins, Inc., a crab processing house in Hampton, VA, which routinely harvests crabs from pots and dredging throughout the lower Chesapeake Bay. This allowed us to sample the spawning population in the southern portion of the Bay, without biasing the results by concentrating samples from any one area. Crabs were collected monthly throughout the year as available, and biweekly throughout the spawning season. The spawning season was defined as the time when extruded eggs were present. Biweekly sampling began when eggs were first observed and continued until sponges were no longer seen. Fifteen crabs were collected at each sampling date, which provided statistical significance, as determined by power analysis and preliminary data. The hepatopancreas and ovaries were dissected and a subsample of each was taken for lipid fraction analysis. A subsample of eggs from the sponge of each female was also taken for fraction analysis. These samples were stored under nitrogen at -80^{o} C until analyzed to prevent oxidation of tissues.

Concentrations of the lipid fractions, triglyceride, phospholipids, and cholesterol, within the hepatopancreas, ovaries, and eggs were measured. Each of these analyses utilized three 1 mg replicates of tissue from the ovary and hepatopancreas of individual

crabs. For egg measurements, three replicates of 100 eggs were used for each of the tests so that results could be reported on a per egg basis. All tissues were sonicated for a total of 10 minutes in Milli-pore water, and subsamples of the sonicated tissue were used for each fractionation test.

Triglycerides were analyzed using a triglyceride quantification kit, with a lipid calibrator used for standards (Wako Chemicals USA, Inc.; Ohkubo and Matsubara 2002). The procedure provided with the kit was modified for microplate analysis so that smaller amounts of tissue and eggs could be utilized. Each mg of tissue previously weighed was analyzed in triplicate to determine an average concentration. After sonication, 10 μL of sample was pipetted onto a 96-well microplate, and 80 μL of color reagent A was added to each well. The plate was incubated at 37° C for five minutes and then read on a Bio-Tek EL-800 spectrophotometric microplate reader at 600 nm. 40 μL of color reagent B was then added to each well, incubated, and read again at 600 nm. The concentration was determined from the standard curve generated by the program KC Junior that operates the microplate reader.

Phospholipids were determined using the phospholipids C quantification kit with choline chloride provided with the kit for standards (Wako Chemicals USA, Inc.; Ohkubo and Matsubara 2002). This procedure was also modified for microplate analysis, and each mg of tissue weighed was analyzed in triplicate. After sonication, 10 μL of sample was pipetted onto a 96-well microplate, and 300 μL of phospholipids buffer was added to each well. The plate was incubated at 37° C for ten minutes, and then read on a

spectrophotometric microplate reader at 600 nm. The concentration for each sample was determined from the standard curve generated with each run.

Cholesterols were determined with the cholesterol E quantification kit with cholesterol standards provided (Wako Chemicals USA, Inc.; Ohkubo and Matsubara 2002). The basic procedure was modified for use with the microplate reader, and each mg of tissue was analyzed in triplicate. After sonication, 10 μL of sample was pipetted onto a 96-well microplate. 300 μL of color reagent was added to each well. The plate was incubated at 37° C for five minutes, and immediately read at 600 nm on a spectrophotometric microplate reader. These concentrations were also determined from the standard curve generated on each plate.

The average concentrations of the three subsamples analyzed for each test were used for statistical analysis. To examine all of the concentrations for significant differences within and among the reproductive seasons a one-way ANOVA with a GT2 post hoc test was used. Correlations were also run to determine if there were relationships between the lipid fractions.

Results

During 2005 and 2006 the three lipid fractions, cholesterol, phospholipids and triglyceride, were measured in the ovaries and hepatopancreas prior to spawning, throughout the spawning period, and at the completion of spawning. Spawning for this study was defined as the presence of extruded eggs in the population. Prespawning is defined as the time, during spring, prior to the extrusion of eggs. Postspawning is defined as the time directly following the conclusion of spawning. All three lipid fractions were

measured in the eggs during each month of the spawning period. The 2005 spawning season covered a three-month period from June-August. Spawning for 2006 began in early May and lasted three weeks into September.

Fig 3.1, a-c. Triglyceride, phospholipids and cholesterol concentrations in the hepatopancreas of mature female blue crabs during 2005 and 2006. N = 10 for each sampling period per year. Triglyceride (a) concentrations were lowest during spawning for both years, and phospholipids (b) concentrations were highest at the same time. Cholesterol (c) demonstrated differing trends for each year with the highest concentration observed in 2005 prior to spawning, and the highest concentration in 2006 was after spawning was complete. Different letters within the bars of each figure indicate significant differences. Bars that have the same letter(s) are not significantly different. Absence of letters within a figure indicates that none of the bars are significantly different.

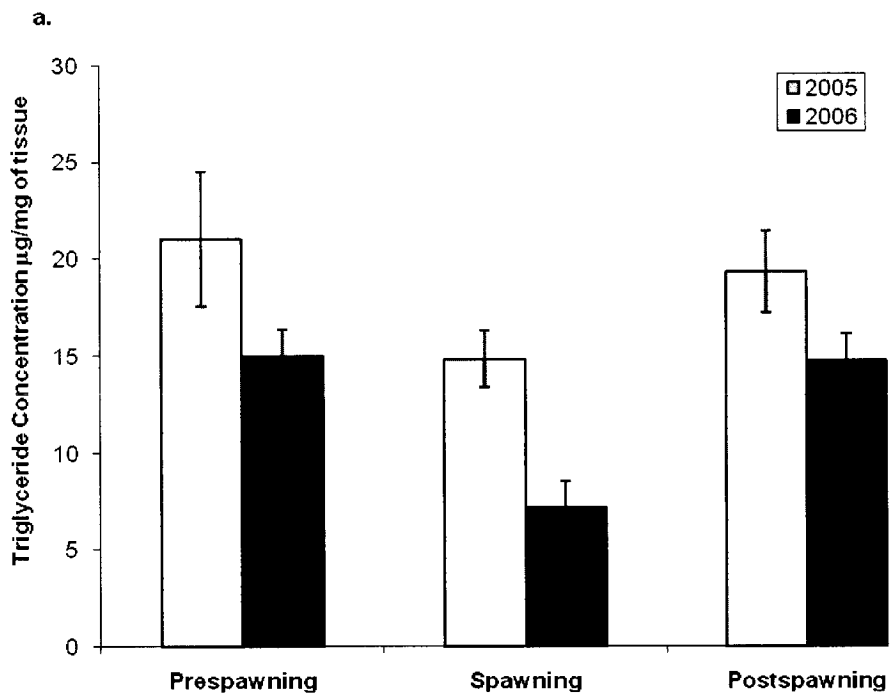
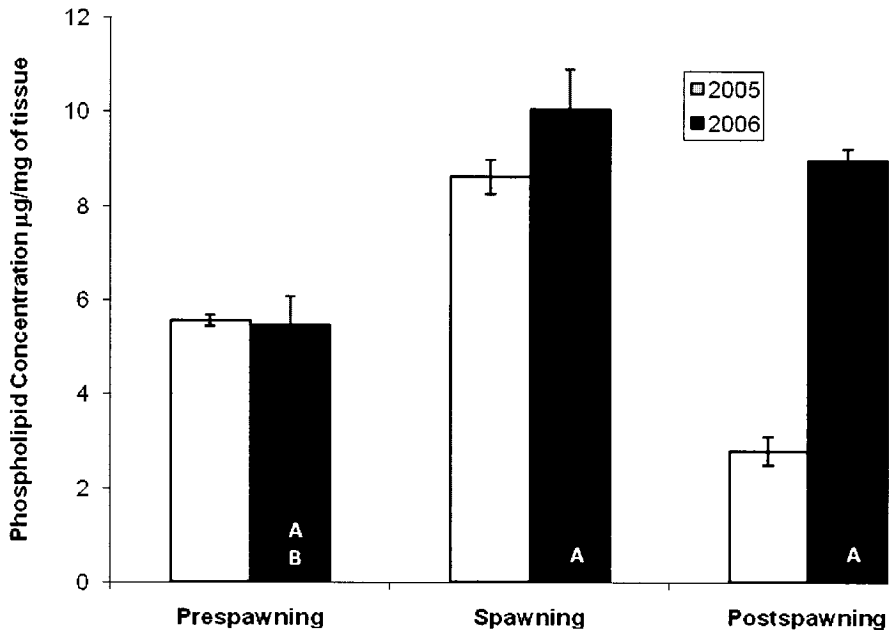


Fig 3.1 cont.

b.



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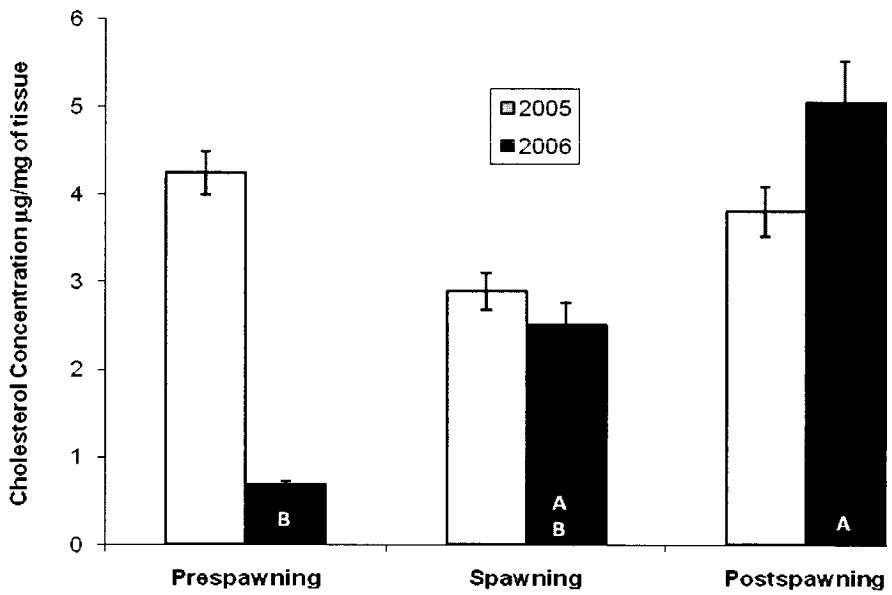


The triglyceride concentrations in the hepatopancreas exhibited the same trends in both years, but overall the 2005 concentrations were significantly higher than in 2006 (ANOVA, n = 60, F = 4.361, P = 0.044) (Fig 3.1a, Table 3.1). No significant differences for triglyceride concentration between the prespawning, spawning, and postspawning periods of 2005 (ANOVA, n = 30, F = 0.643, P = 0.539), or 2006 (ANOVA, n = 30, F = 3.665, P = 0.051)

Phospholipid concentrations within the hepatopancreas were significantly different between 2005 and 2006 (ANOVA, n = 60, F = 4.996, P = 0.032), and the trends were slightly different for each year (Fig 3.1b, Table 3.1). Both years had the highest phospholipids concentration in the middle of the spawning period. The lowest concentration for 2005 was after the completion of spawning, but the lowest concentration in 2006 was prior to the onset of spawning. The spawning period was significantly higher than post-spawning (GT2 multiple pairwise comparison, n = 30, F = 13.833, $P \leq 0.001$) in 2005. In 2006 there were no significant differences between the spawning periods of that year (GT2 multiple pairwise comparison, n = 30, F = 1.621, P = 0.230).

The annual means for the cholesterol concentrations in the hepatopancreas were not significantly different (ANOVA, n = 60, F = 0.015, P = 0.905) (Table 3.1), but the trends seen each year were very different (Fig 3.1c). In 2005 there were no significant differences among spawning periods (GT2 multiple pairwise comparison, n = 30, F = 2.054, P = 0.163). The cholesterol concentration in the 2006 pre-spawning period was very low, increased with the onset of spawning to the highest concentration detected for the year after spawning. Among the spawning periods of 2006, pre-spawning

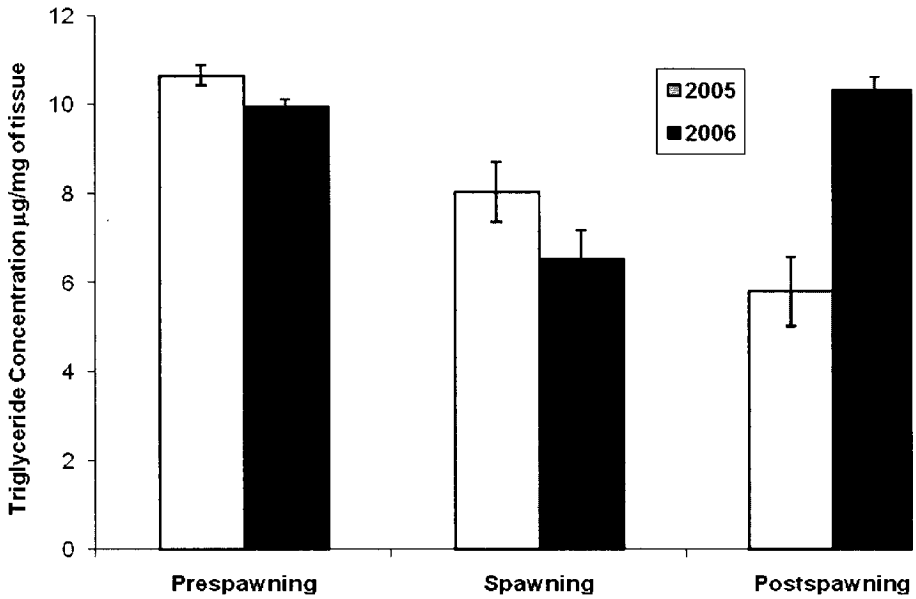
hepatopancreas cholesterol was significantly lower than what was measured for post-spawning (GT2 multiple pairwise comparison, n = 30, F = 6.076, P = 0.029).

Table 3.1. Hepatopancreas mean lipid fraction concentrations for 2005 and 2006.

Lipid Fraction/ Spawning Period	2005 Concentration (μg/mg of tissue)	Standard Error (SE)	2006 Concentration (μg/mg of tissue)	SE
Triglyceride				
Prespawning	21.013	6.905	14.975	5.845
Spawning	14.827	2.886	7.179	1.520
Postspawning	19.294	4.254	14.716	2.748
Phospholipids				
Prespawning	5.566	0.255	5.472	1.222
Spawning	8.624	0.748	10.060	1.720
Postspawning	2.800	0.621	8.977	0.475
Cholesterol				
Prespawning	4.243	0.496	0.689	0.071
Spawning	2.895	0.413	2.517	0.502
Postspawning	3.809	0.567	5.051	0.929

Fig 3.2, a-c. Triglycerides, phospholipids, and cholesterol concentrations within the ovaries of mature female blue crabs during 2005 and 2006. N = 10 per sampling period per year. Triglyceride (a) concentrations had differing trends between 2005 and 2006 with high concentrations in 2005 before spawning and the low for the year was after spawning. 2006 triglycerides were lowest during spawning. Phospholipids (b) concentrations in the ovaries showed similar trends as the triglycerides between years. The trend in cholesterol (c) concentrations was very similar to what was observed in the hepatopancreas for each year. Different letters within the bars of each figure indicate significant differences. Bars that have the same letter(s) are not significantly different. Absence of letters within a figure indicates that none of the bars are significantly different.

a.



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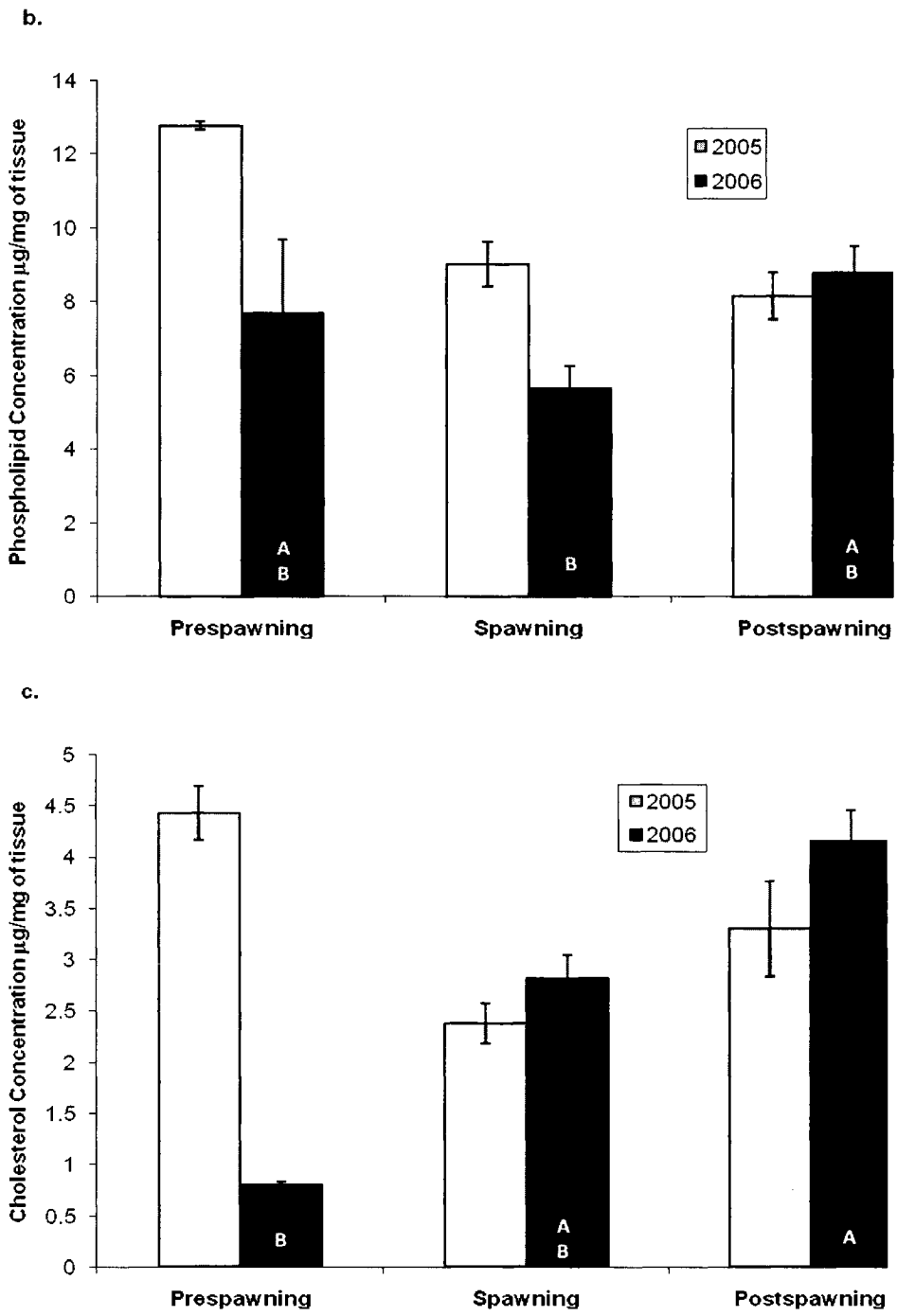


The ovarian triglyceride concentrations showed a slightly different trend in 2005 than in 2006, but the mean concentrations for each year were not significantly different

(ANOVA, n = 60, F = 0.447, P = 0.508) (Fig 3.2a, Table 3.2). The 2005 triglyceride concentrations were not significantly differences between spawning periods (ANOVA with GT2 post hoc, n = 30, F = 1.824, P = 0.195). The 2006 triglyceride concentrations were also not significantly different between periods (ANOVA with GT2 post hoc, n = 30, F = 2.803, P = 0.092). Correlation analysis demonstrated no significant correlation between hepatopancreas and ovarian triglyceride concentration for either year sampled (Correlation, n = 120, r = 0.055, P = 0.830).

The phospholipid concentrations in the ovaries followed a pattern similar to the triglycerides in both years (Fig 3.2b, Table 3.2). There was an overall significant difference in concentration between the two years (ANOVA, n = 60, F = 4.189, P = 0.048). The highest phospholipids concentration was prior to spawning in 2005. There was a slight decrease once spawning began, and another smaller decrease following spawning. There were no significant differences detected in phospholipid concentrations in 2006 (GT2 multiple pairwise comparison, n = 30, F = 1.678, P = 0.220). There was also no significant correlation detected for phospholipids concentration between the hepatopancreas and the ovaries for 2005 or 2006 (Correlation, n = 120, r = 0.139, P = 0.582).

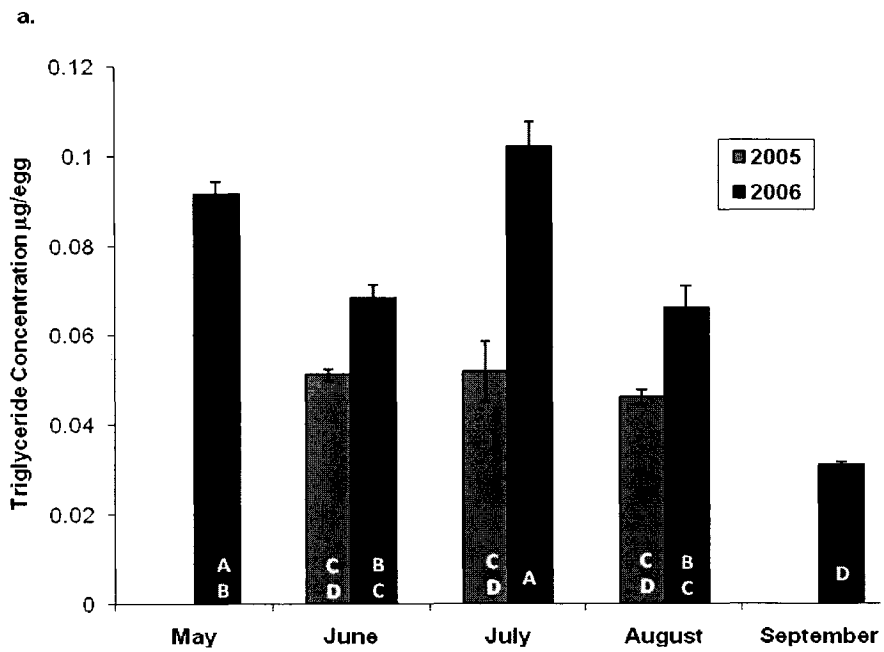
Cholesterol annual mean concentrations in the ovaries were not significantly different between 2005 and 2006 (ANOVA, n = 60, F = 0.003, P = 0.956) (Table 3.2). As with the hepatopancreas cholesterol concentrations, the trends in cholesterol concentration were quite different between years. The trends seen in the ovary were very similar to that seen in the hepatopancreas for each year (Fig 3.2c). The cholesterol concentrations in 2005 were highest before spawning. 2006 was very different with the

lowest concentration of the year occurring when the concentration was highest in 2005 (pre-spawning). The cholesterol concentration increased in 2006 during spawning, and post-spawning. The post-spawning period concentration was significantly higher than the pre-spawning concentration (GT2 multiple pairwise comparison, $n = 30$, $F = 4.975$, $P = 0.032$). Unlike the other two lipid fractions that were measured in this study, there was a highly significant correlation for cholesterol concentration between the hepatopancreas and the ovary for 2005 (Correlation, $n = 60$, $r = 0.594$, $P = 0.009$) and 2006 (Correlation, $n = 60$, $r = 0.865$, $P \leq 0.001$).

Table 3.2. Ovarian mean lipid fraction concentrations for 2005 and 2006.

Lipid Fraction/ Spawning Period	2005 Concentration (μg/mg of tissue)	Standard Error (SE)	2006 Concentration (μg/mg of tissue)	SE
Triglyceride				
Prespawning	10.646	0.454	9.944	0.333
Spawning	8.039	1.342	6.520	1.305
Postspawning	5.805	1.550	10.335	0.580
Phospholipids				
Prespawning	12.769	0.244	7.682	3.991
Spawning	9.018	1.218	5.650	1.240
Postspawning	8.158	1.246	8.788	1.454
Cholesterol				
Prespawning	4.427	0.522	0.801	0.063
Spawning	2.381	0.397	2.813	0.467
Postspawning	3.306	0.929	4.157	0.603

Fig 3.3, a-c. Triglyceride, phospholipids, and cholesterol concentrations in the eggs for each month of spawning in 2005 and 2006. N = 8 per month per year. The 2006 spawning season began a month earlier, and lasted a month longer than the 2005 season. Triglyceride (a) concentrations were relatively constant in 2005, but were significantly lower in June, August, and September of the 2006 season. Phospholipids (b) concentrations were highest in June of 2005 and significantly decreased in July, but the concentrations exhibited similar trends as the triglycerides in 2006. Cholesterol (c) concentrations varied throughout 2005 with the peak concentration in July. The 2006 cholesterol concentrations were relatively constant throughout the year with the exception of a low concentration detected in June. Different letters within the bars of each figure indicate significant differences. Bars that have the same letter(s) are not significantly different.



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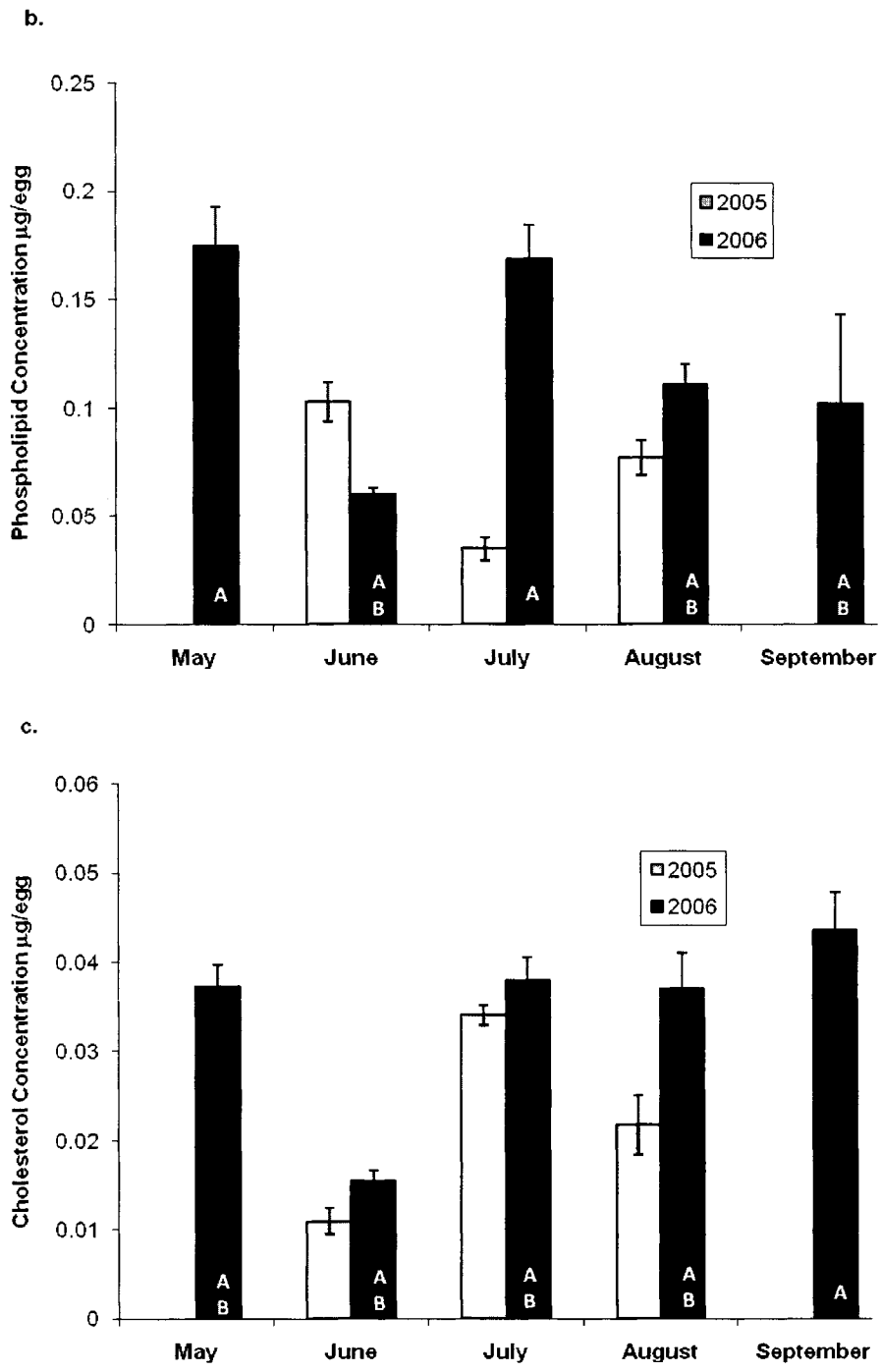


The triglyceride concentrations in eggs were overall significantly higher in 2006 than in 2005 (ANOVA, n = 64, F = 16.855, $P \leq 0.001$) (Fig 3.3a, Table 3.3). There were

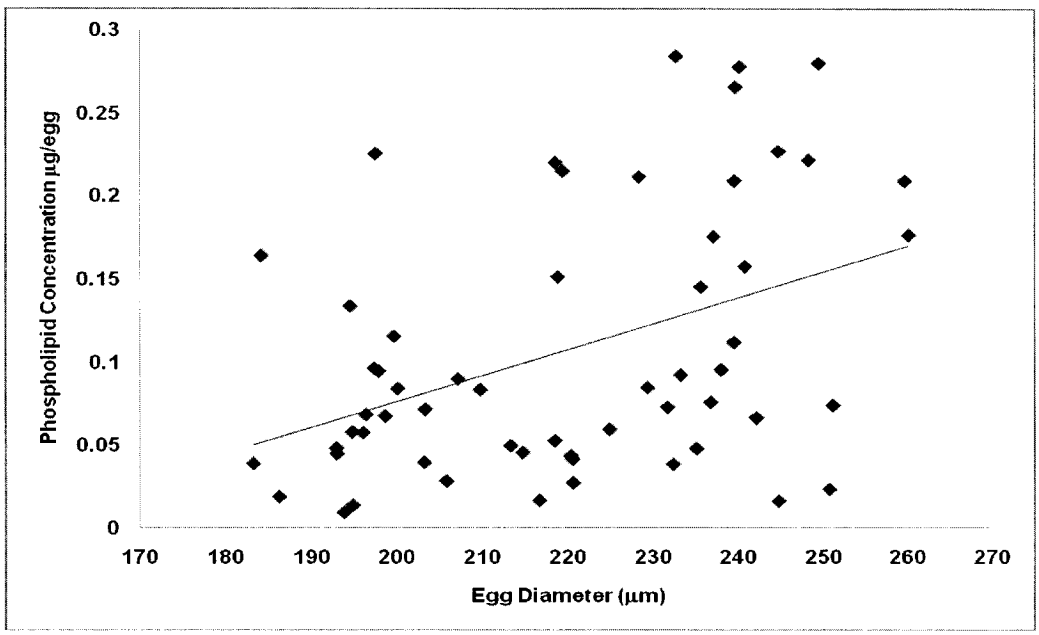
very small changes in concentration throughout the 2005 spawning season, indicating that eggs throughout the season were apportioned triglycerides similarly. The elongated 2006 spawning season was very different with peak triglyceride concentrations in May and July. The lowest concentration in eggs was detected in September. May was significantly higher in triglyceride concentration than September (GT2 multiple pairwise comparison, $n = 40$, $F = 7.524$, $P = 0.002$). July was also significantly higher than June (GT2 multiple pairwise comparison, $n = 40$, $F = 7.524$, $P = 0.040$), August (GT2 multiple pairwise comparison, $n = 40$, $F = 7.524$, $P = 0.019$), and September (GT2 multiple pairwise comparison, $n = 40$, $F = 7.524$, $P \leq 0.001$).

Egg phospholipids concentrations were also significantly different overall between 2005 and 2006 (ANOVA, $n = 64$, $F = 5.626$, $P = 0.021$). 2005 and 2006 showed a different trend in phospholipids concentration compared with triglyceride concentration (Fig 3.3b, Table 3.3). 2005 phospholipid concentrations were not significantly different between months (GT2 multiple pairwise comparison, $n = 24$, $F = 3.371$, $P = 0.056$). There were also no significant differences detected between the months of 2006 (GT2 multiple pairwise comparison, $n = 40$, $F = 2.807$, $P = 0.069$).

Like the other lipid fractions, the cholesterol concentration in the eggs was significantly different between 2005 and 2006 (ANOVA, $n = 64$, $F = 7.143$, $P = 0.010$) (Fig 3.3c, Table 3.3). In 2005 June was significantly lower in cholesterol concentration than July (GT2 multiple pairwise comparison, $n = 24$, $F = 4.094$, $P = 0.030$), but no other significant differences were detected between the months. Cholesterol concentrations showed very little variation for most of the 2006 spawning season, but there was a significant decrease in June followed by a rebound for the rest of the season. June was

significantly lower than all months in the season (GT2 multiple pairwise comparison, n = 40, F = 2.753, P = 0.046).

Fig 3.4. The relationship between phospholipid concentration and egg diameter in eggs from 2005 and 2006. Phospholipid concentration was significantly correlated with egg diameter, with larger eggs having more phospholipid than smaller eggs.



Egg diameter and egg number per brood had previously been determined for these samples (see Chapter 2). Phospholipid concentration of the eggs was significantly correlated with the average egg diameter (Correlation, n = 64, r = 0.416, P = 0.001) (Fig 3.4), but triglyceride (Correlation, n = 64, r = 0.005, P = 0.485) and cholesterol (Correlation, n = 64, r = 0.118, P = 0.188) were not. When examining the mean number of eggs produced per brood, there was a significant correlation between the cholesterol concentration per egg (Correlation, n = 64, r= 0.383, P = 0.002). Triglyceride (Correlation, n = 64, r = 0.098, P = 0.233) and phospholipid (Correlation, n = 64, r =

0.151, P = 0.131) concentrations in eggs were not significantly correlated with egg number.

Table 3.3. Egg mean lipid fraction concentrations throughout the 2005 and 2006 spawning seasons. "---" indicates that there was no eggs available for those months.

Lipid Fraction/ Spawning Period	2005 Concentration (µg/egg)	Standard Error (SE)	2006 Concentration (µg/egg)	SE
Triglyceride				
May	---	---	9.163×10^{-2}	5.607×10^{-3}
June	5.117×10^{-2}	2.576×10^{-3}	6.833×10^{-2}	5.872×10^{-3}
July	5.194×10^{-2}	1.316×10^{-2}	1.022×10^{-1}	1.074×10^{-2}
August	4.617×10^{-2}	3.231×10^{-3}	6.663×10^{-2}	9.541×10^{-3}
September	---	---	3.097×10^{-2}	1.187×10^{-3}
Phospholipids				
May	---	---	1.747×10^{-1}	3.600×10^{-2}
June	1.027×10^{-1}	1.838×10^{-2}	5.999×10^{-2}	6.074×10^{-3}
July	3.489×10^{-2}	1.083×10^{-2}	1.684×10^{-1}	3.207×10^{-2}
August	7.698×10^{-2}	1.610×10^{-2}	1.106×10^{-1}	1.915×10^{-2}
September	---	---	1.017×10^{-1}	8.220×10^{-2}
Cholesterol				
May	---	---	3.718×10^{-2}	4.979×10^{-3}
June	1.094×10^{-2}	2.902×10^{-3}	1.540×10^{-2}	2.425×10^{-3}
July	3.402×10^{-2}	2.241×10^{-3}	3.793×10^{-2}	5.206×10^{-3}
August	2.171×10^{-2}	6.746×10^{-3}	3.690×10^{-2}	8.152×10^{-3}
September	---	---	4.346×10^{-2}	8.485×10^{-3}

Discussion

Previous results from studying reproduction in this population have shown that the blue crab has experienced changes in fecundity and reproductive output (see Chapter 2 and 3). Fecundity has decreased in recent years, and a previously determined allometric relationship between size and fecundity is no longer valid (Prager et al. 1990;

see Chapter 2). The lack of a size dependent relationship has strongly suggested that there is another external or internal factor controlling reproduction in the blue crab. Variations in the amount of resources allocated to each egg have been detected, leading to tradeoffs between egg number and size (see Chapter 2). Inter-and intra-annual variations detected for total lipid and protein concentrations in the hepatopancreas, ovaries, and eggs, indicate that changes in reproductive output may be attributed to variability in food supply or energetic requirements (see Chapter 3).

Lipids and other components are mobilized from the hepatopancreas to the ovaries in mature blue crab females to be used in oocytes (Spaargaren and Haefner 1994; Walker et al. 2003; Hasek and Felder 2006; Lee at al. 2006; Sanchez-Paz et al. 2006). The process of vitellogenesis results in the synthesis of vitellogenin, which will ultimately become the yolk of each egg (Lee and Walker 1995). Vitellogenin is produced in the ovaries and requires further resources for production. This includes more lipids, which are again primarily mobilized from the hepatopancreas. The lipids can be broken down into types, or fractions, that play important roles in egg production and subsequent larval survival (Lee and Puppione 1988; Ravid et al. 1999; Graeve and Wehrtmann 2003; Sibert et al. 2004). These fractions (triglyceride, phospholipid, and cholesterol) must be allocated in sufficient quantities to allow larvae to reach the feeding stage (Mourente and Rodriguez 1997; Garcia-Guererro et al. 2003; Pernet et al. 2003).

Mobilization was observed in the early concentrations of triglyceride that significantly decreased in the hepatopancreas with the onset of spawning. This precipitous decrease suggests that the majority of triglyceride in the hepatopancreas had been allocated for reproduction. The patterns of triglyceride concentration that were

observed in the hepatopancreas were similar to the patterns detected in the ovaries. This indicates that triglyceride was moved to the ovary from the hepatopancreas in direct proportions for reproduction. The slight recovery in hepatopancreas triglyceride concentrations following the spawning period indicates that there was a reaccumulation of resources in females that had spawned at least one time. Another stronger possibility is that newly matured females were entering the spawning stock. This is more likely because few females are able to survive to spawn a second year, most likely due to intense fishing pressure (Jones et al. 1990). Also the ovarian triglyceride concentrations in 2005 did not rebound as they did in the hepatopancreas following spawning, suggesting that resources are not moved to the ovaries until closer to the commencement of spawning. There was an increase in the ovarian triglyceride concentration in the postspawning period of 2006, which supports the idea that there are new females entering the spawning stock late in the season with greater stored resources than those that have already spawned.

The hepatopancreas had the highest phospholipid concentrations during spawning, when concentrations were the lowest in the ovaries presumably due to egg extrusion. Although ovarian phospholipid was lowest during spawning the concentrations were higher in the ovaries than the hepatopancreas, which suggests that the phospholipids were derived from another source besides the hepatopancreas. The most likely source would be directly from ingested food that passed immediately through the hepatopancreas and into the ovaries (Teshima 1986 a, b; Ravid et al. 1999). The increase in triglyceride concentration following spawning was not observed for phospholipid in either year of this study. Also, the quantity of phospholipid that was

mobilized to the ovaries was less than the amount of triglyceride. This is to be expected as many crustaceans contain significantly higher concentrations of triglyceride as compared to phospholipid (Mourente and Rodriguez 1997; Morais et al. 2002; Sibert et al. 2004).

The concentrations of cholesterol in the hepatopancreas and ovaries showed similar trends within each year of this study, but the trends were very different between the two years sampled. 2005 concentrations in both organs were highest during prespawning, decreased with the onset of spawning, and had a slight recovery after spawning. The lowest 2006 concentrations were detected prior to spawning, and cholesterol concentrations increased to the year's high after spawning was complete. The mean concentrations for the hepatopancreas were not significantly different from the ovarian concentrations at any time during this study. All of these factors were markedly different from what was observed for the other lipid fractions in these two organs, which is most likely attributable to changes in ingested food. Cholesterol cannot be synthesized by crustaceans, and must be ingested (Ravid et al. 1999; Sanchez-Paz et al. 2006). Cholesterol must have been consumed in higher quantities in 2005, and in the postspawning period of 2006. Higher quantities of cholesterol could originate from prey items that contained greater amounts, such as oysters, or from a greater amount of prey available to the mature females (Wen et al. 2002).

The differences detected in lipid fractions in the hepatopancreas and ovaries demonstrate that there were considerable changes in energy consumption and storage. This was also seen in total lipid and protein concentration in the same females (see Chapter 3). Different prey items have varying amounts of energy available, and the

availability of prey to this population changes throughout a normal spawning season (Eggleston et al. 1992, see Chapter 3). As females migrate from the upper portion of the Chesapeake Bay to their spawning grounds in the lower Bay, they feed as they go (Turner et al. 2003). Nutrients are accumulated until spawning begins, and then must be ingested every time that a brood is produced (Wolin et al. 1973; Sibert et al. 2004). Blue crab preferred prey species often become depleted by July and August, which would require females to rely on subpar prey that provide varying concentrations of lipids (Eggleston et al. 1992). This would change the amount of triglyceride, phospholipid, and cholesterol available for mature females to allocate to reproduction, which will effect the amounts available for eggs.

Each oocyte developing within the ovaries must be apportioned with sufficient triglyceride, phospholipid and cholesterol for embryo and larval development (Mourente and Rodriguez 1997; Garcia-Guerrero et al. 2003; Pernet et al. 2003). Phospholipids are necessary for the development of membranes within the embryo (Graeve and Wehrtmann 2003), and are the precursor for lipovitellin in crustacean oocytes (Kerr 1969; Lee and Puppione 1988; Walker et al. 2006). Triglyceride is primarily used for metabolic energy, and is stored in large quantities (Coutteau et al. 1997; Garcia-Guerrero et al. 2003). Triglyceride can also be readily converted into phospholipid within the embryo when necessary poly-unsaturated fatty acids are supplied (Harrison 1990; Gonzalez-Felix et al. 2002). Cholesterol is necessary for membrane structure within the developing embryonic and larval cells, but it also serves another integral function in larvae (Kanazawa and Teshima 1971; Rosa et al. 2003). Hormones, including those necessary for molting and therefore growth, are derived from cholesterol (Kanazawa and Teshima 1971; Rosa et al.

2003). Each lipid fraction serves a distinct and imperative role within an egg. Without sufficient concentrations of each of these lipid fractions apportioned to each egg the viability of the embryos could decrease, and larval survival would decline (Graeve and Wehrtmann 2003).

Triglyceride concentrations in the eggs were not significantly different for any month of spawning in 2005, but there was significant variation in egg triglyceride concentration throughout 2006. The two peaks in concentration were observed in May and July of 2006, with significantly lower concentrations in all other months. This demonstrates that for 2006 there was considerable variation in egg quality based on triglyceride concentration. Triglyceride concentrations have been identified as the main source of energy for embryos and larvae in multiple crustacean species (Fraser 1989; Lavens and Sorgeloos 1991; Palacios et al. 1999). Significantly lower concentrations indicate that there was less energy available to those embryos and the subsequent larvae (Mourente and Rodriguez 1997; Palacios et al. 1999). While we do not know the threshold levels of triglyceride necessary for survival in the blue crab, reductions in available energy may be so low that larvae are unable to reach the feeding stage, and therefore will not survive (Mourente and Rodriguez 1997; Palacios et al. 1999). This would increase larval mortality and reduce the number available for recruitment.

Because we do not know the concentration of triglyceride that is necessary for larval survival, we must entertain other alternatives to the variations in concentration. Those eggs with significantly higher concentrations of triglyceride and therefore greater amounts of energy available may produce larvae that can grow larger in a shorter period of time (Brooks et al. 1997; George 1999; Pernet et al. 2003). Increased energy, beyond

the threshold levels needed, can also be utilized for survival during times of starvation (Fraser 1989; Mourente and Rodriguez 1997). This could lead to increased larval survival for the broods during the times of peak concentration.

Phospholipid egg concentrations exhibited the same trends as triglyceride in 2006, and the mean phospholipid concentrations were higher than the mean triglyceride concentrations. This is evidence that those eggs with higher triglyceride and phospholipid concentrations were of a greater quality than the other eggs with lower concentrations throughout the season. Greater amounts of phospholipid in conjunction with high triglyceride concentrations, suggests that less of the triglyceride would be needed to convert into phospholipid and could be devoted to energy. Other authors have found that phospholipid in crustacean eggs can be used as an energy source when the concentration exceeds that needed for cellular structures (Fraser et al. 1985; Wouters et al. 2001; Sibert et al. 2004). Excess energy could be used for growth, which could allow larvae to become too large to be ingested by some prey, or it could be used for nutrition in times of low larval food availability (Fraser 1989; Brooks et al. 1997; Mourente and Rodriguez 1997; George 1999; Pernet et al. 2003). On the other hand, 2005 had significant decreases in phospholipid concentrations that were not observed in the triglycerides. If these concentrations were below the threshold level of phospholipid that was necessary for the formation of membranes triglyceride would have to be converted into phospholipid and less energy would have been available to the embryos and larvae (Fraser 1989; Lavens and Sorgeloos 1991; Palacios et al. 1999).

The mean concentrations of cholesterol measured throughout the 2005 and 2006 spawning seasons were also variable within the eggs. The 2005 concentrations were

highest in July, which was when phospholipid was the lowest. 2006 concentrations were not significantly different throughout the spawning season, with the exception of a reduction in June. This indicates that the amount of cholesterol that was ingested in June was also significantly lower, or that ingested cholesterol was necessary for other processes within mature females. Because cholesterol is necessary for cellular processes and structures within blue crab embryos and larvae, as well as being the precursor for necessary hormones, reductions in cholesterol concentrations could have a significant impact on larval growth (Ravid et al. 1999; Graeve and Wehrtmann 2003; Rosa et al. 2003; Sibert et al. 2004). Larvae must molt in order to grow, and cholesterol is necessary for the production of the molting hormone that is a cue for each molt to begin (Kanazawa and Teshima 1971; Rosa et al. 2003). If the concentrations of cholesterol provided through maternal effort are not sufficient then the embryo will be unable to obtain cholesterol on its own and will not survive (Kanazawa et al. 1988; Wen et al. 2002).

The concentrations of the three lipid fractions measured in the eggs were higher in 2006 than in 2005, with the exception of the June 2005 phospholipid concentration. This was different than the hepatopancreas and ovarian lipid fractions. The amount of maternal effort put into each egg in 2006 was significantly greater than 2005. A similar trend was found in total lipid and protein concentrations in eggs from the same broods (see Chapter 3). As a whole, the reproductive effort was far greater in 2006 as evidenced by the extended spawning season, increased egg number and diameter, as well as increased protein, lipid, and lipid fraction concentrations (see Chapter 2 and 3). Investigations of other crustacean species have demonstrated that increased maternal effort and investment in reproduction results in significantly greater reproductive success

(Harrison 1990; Palacios et al. 1999; Lardies et al. 2004; Ouellet and Plante 2004; Sibert et al. 2004). Decreased effort in 2005, as compared to 2006, suggests that reproductive success was diminished. The substantial increase in all facets of maternal effort that were observed for 2006 demonstrates that reproductive success greatly exceeded 2005.

The significant relationship determined between egg diameter and phospholipid concentration for both years showed that larger eggs contain more phospholipid. This was not true for the other two lipid fractions, indicating that the lipid fractions are not mobilized proportionally, which is normally what is seen in crustacean oocytes and eggs (Clark 1977; Clark et al. 1990; Graeve and Wehrtmann 2003; Sibert et al. 2004). Generally, it has been found in multiple crustacean species that eggs that are larger contain greater quantities of triglyceride, and smaller eggs have more phospholipid than triglyceride (Herring 1974; Clarke 1977; Kattner et al. 1994; Graeve and Wehrtmann 2003). While there was no significant relationship found between triglyceride concentration and egg size, there was a greater amount of triglyceride in the larger eggs of 2006. Quantity and quality of food may play a more important role in how triglycerides and cholesterols are apportioned. Increased availability of food for the mature females would allow them to create larger eggs with greater energy (triglycerides), that would allow the larvae to be larger when hatching (Brooks et al. 1997; George 1999; Pernet et al. 2003).

Cholesterol was the only lipid fraction that was significantly correlated with the mean number of eggs produced per brood. Larger brood sizes had a greater cholesterol concentration and vice versa. This suggests that there is a set amount of cholesterol available to each brood, and that it is apportioned similarly between eggs within the

brood. The threshold level of cholesterol necessary for embryos and larvae is likely a significant limiting factor for the number of eggs produced per brood. At times when more cholesterol is available, a greater number of eggs can be produced. If cholesterol concentrations are low, then the number of eggs in the brood decreases. This has been shown in other species fed diets supplemented with cholesterol (Wen et al. 2002; Hassett 2004; Crockett and Hassett 2005). Increased cholesterol concentration in each instance resulted in increased egg production (Wen et al. 2002; Hassett 2004; Crockett and Hassett 2005). Egg production based upon available cholesterol concentration would reduce the amount of wasted reproductive effort expended by the female, as eggs containing less than the threshold limit of cholesterol would not be created.

The changes observed in lipid fraction concentrations are further evidence that broods are highly variable in this population. Based upon the variation detected in triglyceride, phospholipid and cholesterol concentrations, this can primarily be attributed to the diet of the mature female. Negative changes in diet have been documented to have adverse effect on reproduction in crustaceans (Primavera et al. 1979; Kleppel and Burkhart 1995; Wen et al. 2002; Lardies et al. 2004). The results are often decreased egg number and size, a reduction in the number of broods produced in a season, and increased reproductive exhaustion of the female (Palacios et al. 1999; Pernet et al. 2003; Calado et al. 2005; Lee at al. 2006). In this study, 2005 was a year of reduced reproductive output and effort. We observed a shortened reproductive season, smaller and fewer eggs, as well as reduced quantities of total lipids, proteins and lipid fractions apportioned to the eggs. This strongly suggests that there was a dietary influence on that reproductive season. On the other hand, when quality prey items are plentiful, the increased food can

enhance the reproductive effort and the quality of the offspring (Kleppel and Burkhart 1995; Wen et al. 2002; Lardies et al. 2004). Other studies have shown that when females are exposed to increased food availability egg production is greater, and there is a direct correlation with the survivability of the larvae (Kleppel and Burkhart 1995; Wen et al. 2002; Lardies et al. 2004). The data from this study for 2006 shows that there was an increase in all of these reproductive parameters, likely as a result of increased food availability.

In recent years, the organisms found in the Chesapeake Bay have been subjected to multiple forms of stress which has lead to reduced populations of many species (Clark et al. 1999; Lipcius and Stockhausen 2002). Included in the reductions are blue crabs and their preferred prey species (Clark et al. 1999; Lipcius and Stockhausen 2002; Chesapeake Bay Commission Bi-State Blue Crab Technical Advisory Committee 2006). Variability in the amount of food available has lead to staggering changes in reproductive output of the blue crab in this study. Mature females must make trade-offs between increased egg numbers with the minimum biochemical contents or decreased numbers per brood with excess concentrations of the necessary components (Hartnoll 2006; Lee et al. 2006). This study has shown that there was more stored energy available in 2005, but less investment was put into reproduction than in 2006, strongly suggesting an internal trade-off. In times of energetic stress, the trade-off may be more critical and may lead to reduced numbers of eggs per brood with only the bare minimum of resources apportioned to the egg (Pernet et al. 2003; Calado et al. 2005; Hartnoll 2006; Lee et al. 2006). This in turn could cause reduced hatchability, reduced larval survival, and reduced recruitment back to the population. Obvious variation in reproductive effort was observed in this

study, leading to the conclusion that there was a significantly greater reproductive effort in the spawning population in 2006.

The Chesapeake Bay blue crab population and fishery has increasingly become the focus of management in recent years as the population has obviously declined. Much of that focus has been on mature females and egg production. Previously unknown variation in the biochemical content of eggs can have far reaching impacts on the population that include larval survivability and recruitment. Until the threshold levels of triglyceride, phospholipid, and cholesterol are determined we cannot know the full impact of the variations detected. We can conclude that during periods in the reproductive seasons, eggs are being produced with significantly greater energy than at other times. This also translates to significantly greater maternal effort, and increased reproductive success. On the other hand, there are also times when energy is very low in the eggs, indicating reductions in maternal effort primarily due to environmental stressors such as food. These changes in reproductive effort and output must be factored into decision making that will allow for effective population management.

CHAPTER V

CONCLUSIONS

The results of this study demonstrated that the Chesapeake Bay blue crab experiences significant variation in fecundity and reproductive output. Fecundity has dramatically and significantly declined in the past two decades. The shift in size frequencies and the increase in absolute number of smaller mature females support the hypothesis that females are maturing at smaller sizes. A decline in fecundity due to declining carapace width would be expected in this population based on the previously determined relationship between size and fecundity (Prager et al. 1990). The observed decline in fecundity per brood and the lack of a strong size-fecundity relationship suggests that population fecundity has declined further than expected.

Changes in size at maturity might suggest a diversion of resources from growth into other systems, or that maturity is occurring earlier in the life cycle (Stearns 1992; Shertzer and Ellner 2002). Selective pressure on the population can cause a phenotypically plastic reaction driving females toward early maturation and earlier reproduction. The most likely driving forces for this are increased mortality and decreased density within the population (Stearns 1992), both of which have been experienced by the Chesapeake Bay blue crab population. Other heavily exploited populations have shifted the size and age at maturity toward smaller, younger individuals. These individuals then contribute to the population reproductive effort earlier but with reduced individual fecundity per brood (Gadgill and Bossert 1970; Ermande, Dieckman and Heino 2004). Assuming they survive long enough under natural conditions, larger

individuals may divert more resources into growth and would have more internal space for oocyte storage, but the reproductive period would be shorter than an individual maturing earlier. For the blue crab, some of the reduced fecundity may be compensated for by producing more broods within her lifetime (Dickinson et al. 2006). In the Chesapeake Bay fishery, females can be harvested once they reach maturity, regardless of size, making it less likely that a female will survive to produce multiple broods. Harvesting females before they can produce multiple broods eliminates the potential to counteract lowered brood fecundity. This trend toward early maturation at reduced size substantially decreases population fecundity and the number of larvae available for recruitment.

Observations in this study indicated inter- and intra-annual differences in egg number and diameter suggesting tradeoffs between the two, and differences in the allocation of available reproductive energy. At times when eggs were larger, but there were fewer of them per brood, more of the available energy was apportioned to individual eggs, which can increase larval survival. Producing a greater number of eggs with fewer resources would increase the number of larvae that hatch, but survivability of the larvae may be reduced.

2006 was a reproductive season with high egg numbers but the eggs were also larger than in the previous two years. This suggests that 2006 was a season with few limitations on the availability of energy used for reproduction. The data from the other four years suggest that there were limitations on egg production other than volume resulting in a tradeoff between egg size and number. These tradeoffs, in conjunction with smaller mature females, and the lack of a strong size-fecundity relationship, strongly

suggests that allometric regulation is no longer the primary factor determining egg production and reproductive variability in this population. The more likely controlling factor is the availability of energy for reproduction.

The changing concentrations of lipids, proteins, and lipid fractions throughout each spawning season also demonstrated that the amount of each in individual broods are adjusted, both inter- and intra-annually. This may be dependent on the food supply, the energy demand from other metabolic systems of the female, or changing environmental conditions. Negative changes in diet have been documented to have adverse effect on reproduction in crustaceans (Primavera et al. 1979; Kleppel and Burkhart 1995; Wen et al. 2002; Lardies et al. 2004). The results are often decreased egg number and size, a reduction in the number of broods produced in a season, and increased reproductive exhaustion of the female (Palacios et al. 1999; Pernet et al. 2003; Calado et al. 2005; Lee at al. 2006). The availability of prey to this population changes throughout a normal spawning season (Eggleston et al. 1992). Females feed as they migrate from the upper portion of the Chesapeake Bay to their spawning grounds in the lower Bay (Turner et al. 2003). The density of the primary prey sources (i.e. *Macoma balthica, Mya arenaria*) dramatically decrease as the spawning season progresses, reaching the yearly low in July and August (Eggleston et al. 1992). There are fewer resources available to the mature females later in the season, which may force the crabs to forage for inferior prey items such as amphipods and isopods (Hines et al. 1990; Mansour and Lipcius 1991). This could reduce the amount of lipids, proteins, and lipid fractions available for reproduction, which can reduce how much is apportioned to an egg or reduce the number of eggs that can be produced at one time.

Changes in the metabolic rate of mature females could also cause a significant diversion of energetic resources away from reproduction. Fluctuations in temperature, salinity and oxygen in the water column could result in dramatic changes in metabolism that would affect the concentrations of lipids and proteins allocated to reproduction (Leffler 1972; Guerin and Stickle 1992; Taylor and Eggleston 2000). Changes in lipid, protein, and lipid fraction concentrations of the eggs within and between seasons seen in this study suggest that there were significant changes in energy utilization and/or food supply experienced that affected the amount of each available for reproduction.

As a whole, the reproductive effort was far greater in 2006 as evidenced by the extended spawning season, increased egg number and diameter, as well as increased protein, lipid, and lipid fraction concentrations. This data also suggests that 2006 was a season with fewer limitations in regards to energy for mature females, which allowed for greater energy to be devoted to reproduction. Eggs produced in 2006 were provided with greater energy than at any time in 2005, likely resulting in increased larval survival in 2006. Assuming that the larger eggs of 2002 and 2003 also had increased biochemical concentrations similar to that observed in 2006, the eggs produced in those two years would also result in larvae with greater survivability but reduced numbers because significantly fewer eggs were produced. Investigations of other crustacean species have demonstrated that increased maternal effort and investment in reproduction results in significantly greater reproductive success (Harrison 1990; Palacios et al. 1999; Lardies et al. 2004; Ouellet and Plante 2004; Sibert et al. 2004). Decreased effort in 2005, as compared to 2006, suggests that reproductive success was diminished that year. The substantial increase in all facets of maternal effort in 2006 suggests that reproductive

success greatly exceeded that of 2005. Assuming normal recruitment for 2006 larvae, there should have been a large increase in recruits and a subsequent increase in population size.

The Chesapeake Bay blue crab population and fishery has increasingly become the focus of management in recent years as the population has obviously declined. Much of that focus has been on mature females and egg production. Decreases in population fecundity in conjunction with variations in the biochemical content of eggs can have far reaching impacts on the population including larval survivability and recruitment. We can conclude that during some periods in the reproductive seasons, eggs are being produced with significantly greater energy than at other times. This also translates to significantly greater maternal effort and increased reproductive success with greater numbers of larvae hatching, surviving and recruiting back to the population. On the other hand, there are also times when energy is very low in the eggs, indicating reductions in maternal effort likely due to changes in the availability of resources for reproduction and environmental stressors. Mature females are reducing the number of eggs produced as compared to previously reported estimates of fecundity (Prager et al. 1990). Fluctuations in energy availability in an embryo, as well as the production of fewer embryos have significantly lowered the reproductive output of this population. These changes in reproductive effort and output must be factored into decision making that will allow for effective population management.

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