Vol. 394: 153–163, 2009
doi: 10.3354/meps08295

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 18

Lifetime reproductive potential of female blue crabs *Callinectes sapidus* in North Carolina, USA

M. Zachary Darnell[1,*], Dan Rittschof[1], Kelly M. Darnell[1,2], Ruth E. McDowell[1,3]

[1]Duke University Marine Laboratory, Nicholas School of the Environment, 135 Duke Marine Lab Rd., Beaufort, North Carolina 28516, USA

[2]*Present address:* Marine Science Institute, The University of Texas at Austin, 750 Channel View Drive, Port Aransas, Texas 78373, USA

[3]*Present address:* Department of Biology, University of Alabama at Birmingham. 1530 3rd Ave. S., CH 103, Birmingham, Alabama 35294, USA

ABSTRACT: We examined lifetime clutch production and size at maturity for blue crabs *Callinectes sapidus* Rathbun in North Carolina, USA. Female crabs were collected at terminal molt and confined individually in the field for the duration of their lifetime. Crabs were monitored weekly for the presence of eggs. Clutch quality and larval viability were assessed for each clutch. Crabs produced up to 7 clutches over 1 to 2 spawning seasons and survived up to 394 d after the terminal molt. Time to first clutch and time between clutches were positively correlated with carapace width and best described by degree-days, physiological time calculated as a thermal integral. Size at maturity was negatively correlated with water temperature on the day of the terminal molt. Egg lipid content (mean = 79.2% of dry mass), egg diameter (mean = 267.5 µm), larval carapace width (mean = 278.4 µm), and larval survival time without food (mean = 3.4 d) were similar for all clutches. The percentage of embryos developing normally decreased 40% from Clutch 1 to 4, and clutch volume decreased 50% from Clutch 1 to 5. Thus, most of a crab's reproductive output is from the first few clutches. Realistic estimates of fecundity and reproductive potential are essential for accurate spawning stock assessment and population modeling.

KEY WORDS: Blue crab · *Callinectes sapidus* · Reproductive potential · Spawning biology · Fecundity · Multiple clutches · Degree-days

INTRODUCTION

The blue crab *Callinectes sapidus* Rathbun is a commercially and ecologically important brachyuran crab common along the western Atlantic coast from Cape Cod to northern Argentina (Williams 1974). Major commercial fisheries exist along the Atlantic and Gulf coasts of the United States (Millikin & Williams 1984). Positive spawning stock–recruitment relationships have been identified for blue crabs (Chesapeake Bay Program 1997, Lipcius & Stockhausen 2002, Eggleston et al. 2004), and protection of the spawning stock is a common management strategy. To date, however, management decisions have been based on incomplete understanding of blue crab spawning biology. If recent population declines (e.g. Lipcius & Stockhausen 2002) are to be mitigated and the fishery is to be effectively managed, accurate knowledge of blue crab spawning biology would be helpful.

The process of mating begins 1 to 2 d before the terminal, pubertal molt. A pre-molt female pairs with a male who carries her under his body with his first pair of walking legs in the pre-copulatory embrace until she molts. Mating usually occurs immediately after molting, although incompletely-mated females remain receptive for approximately 10 d after the terminal molt (D. Rittschof et al. unpubl. data). Following mating, the male typically continues to carry the female in the postcopulatory embrace for 1 to 3 d (Van Engel 1958, Jivoff 1997). This post-copulatory embrace

*Email: mzd2@duke.edu

serves to reduce the risk of predation and sperm competition (Jivoff 1997). Thus, the duration of the postcopulatory embrace depends on the presence of conspecific predators, sex ratio, and male size and density (Jivoff 1997). Approximately 12% of females mate with at least a second male (Jivoff 1997). Females mate only following the terminal molt (Van Engel 1958); thus, all clutches produced by a female must be fertilized by stored sperm. Sufficient sperm is stored to fertilize up to a dozen clutches of eggs (Hines et al. 2003). Wolcott et al. (2005) found that the number and viability of sperm transferred during mating are independent of male and female body size. Female crabs then forage, develop mature ovaries, extrude a first clutch, and undertake a seaward spawning migration (Van Engel 1958, Tankersley et al. 1998, Turner et al. 2003, Forward et al. 2005) using both ebb-tide transport and directed walking during flood tides (Forward et al. 2003, Carr et al. 2004). Spawning females produce multiple clutches, and migratory behavior continues between clutches, ensuring that spawning females are continually moving seaward throughout the spawning season (Hench et al. 2004, Forward et al. 2005).

Studies of crab and lobster reproductive potential have traditionally examined fecundity for only a single clutch (e.g. Pillay & Nair 1971, Hines 1982, Campbell & Robinson 1983, Jones & Simons 1983, Dugan et al. 1994, Medina Mantelatto & Fransozo 1997), presumably due to the difficulty of observing crabs for multiple clutches. While such studies provide valuable information on single-clutch fecundity and size–fecundity relationships, they do not allow assessment of lifetime reproductive potential for species that spawn multiple times. A number of crabs are known to be capable of producing multiple clutches from a single mating (e.g. Hines 1982, Morgan et al. 1983, Paul 1984, Haddon & Wear 1993, Pinheiro & Fransozo 1999, de Lestang et al. 2003, Hines et al. 2003). For these species, spawning patterns and fecundity must be assessed over multiple clutches in order to make accurate assessments of reproductive potential.

Hines (1982) analyzed reproductive output of 20 species of brachyuran crabs based on single-clutch fecundity measurements and estimates of average number of broods yr^{-1} from previously published studies. Number of broods yr^{-1} ranged from 1 to 10, with an average of 3.1 broods yr^{-1}. With the exception of the Majidae, which averaged 5.9 clutches yr^{-1}, most species were estimated to produce <3 clutches yr^{-1} (Hines 1982).

In addition to blue crabs, other species of Portunidae are also able to produce multiple clutches (Ingles & Braum 1989, Haddon & Wear 1993, Medina Mantelatto & Fransozo 1997, Pinheiro & Fransozo 1999, de Lestang et al. 2003). Clutch number varies by species, with the blue swimmer crab *Portunus pelagicus* producing an estimated 1 to 3 clutches (de Lestang et al. 2003), the New Zealand paddle crab *Ovalipes catharus* producing 1 to 5 clutches (Haddon & Wear 1993), and the swimming crab *Arenaeus cribrarius* producing up to 6 clutches (Pinheiro & Fransozo 1999). In general, single-clutch fecundity (clutch volume or number of eggs per clutch) is size-dependent within a species, with larger crabs producing larger clutches (Hines 1982, Ingles & Braum 1989, de Lestang et al. 2003). However, larger crab species are not necessarily more fecund than smaller crab species, due to differences in egg sizes among species (Hines 1982). Blue crabs produce a large number of very small eggs, resulting in the highest size-adjusted fecundity of the 20 species examined by Hines (1982).

Blue crab spawning biology and clutch production have been observed for captive crabs during a single spawning season. In Florida, Hines et al. (2003) observed up to 6 clutches produced by captive females. The authors estimated a maximum lifetime clutch production of 18 clutches for Florida crabs, 7 clutches for crabs from the lower Chesapeake Bay, and 6 clutches for crabs from the upper Chesapeake Bay, USA. These differences in estimates of total clutches produced reflect differences in the length of the spawning season, which decreases with increasing latitude, differences in the timing and rate of clutch production at different water temperatures, and differences in estimates of total lifespan. Dickinson et al. (2006) examined blue crab fecundity in one spawning season in North Carolina (NC). Crabs produced up to 7 clutches in a single season. Clutch size was related to body size, and generally decreased with increasing clutch number. Because there is an inverse relationship between body size and clutch production interval, reproductive potential was similar for most size classes of crabs.

The studies of Dickinson et al. (2006) and Hines et al. (2003) provided valuable information on blue crab spawning biology. However, each study monitored crabs that were collected while already mature or ovigerous. Thus, it is possible that these crabs may have produced 1 or more clutches prior to collection. In order to make accurate estimates of lifetime clutch production and total fecundity, it is necessary to observe clutch production for individual crabs from maturity to death including multiple spawning seasons. Water temperature and body size must also be taken into account. For ectotherms such as blue crabs, the rates of most physiological processes, including growth, development, and presumably egg production, vary with temperature. Thus, calendar time (i.e. days) is not physiologically appropriate for assessing clutch timing because physiological processes slow at low temperatures. Temporal patterns of clutch production in one location may not be valid in another location along the species' range due to

latitudinal differences in temperature. Physiological time, expressed in degree-days, is a relevant metric as it accounts for the temperature dependence of the processes. The concept of degree-days, also referred to as growing degree-days or heat units, has been used routinely in agriculture and phenology for over 2 centuries to predict flowering, fruiting, or harvesting dates (Wang 1960) and has more recently been applied to study fish (Neuheimer & Taggart 2007) and blue crab (Brylawski & Miller 2006) growth. Degree-days are the integral of daily temperatures above a minimum temperature threshold (T_{min}), and are calculated by summing the differences between the daily temperature means and T_{min} over a time period of interest. When the average temperature is less than the minimum threshold, no degree-days are accumulated.

The purpose of this study was to examine the spawning biology of female blue crabs in the vicinity of Beaufort Inlet, NC. Lifetime clutch production was determined for newly mature female blue crabs that were confined in the field for the duration of their lifetimes. Clutch quality and larval viability were assessed for each clutch to determine if clutch quality decreased with successive clutches.

MATERIALS AND METHODS

Collection of animals. In each of 2 yr (2006 and 2007), female blue crabs were collected by hand at night during the spawning season (May to November) from the Rachel Carson National Estuarine Research Reserve (34° 42.83′ N, 76° 40.52′ W), Beaufort, NC. The collection site is approximately 2 km from Beaufort Inlet. When possible, crabs were captured in the pre- or post-copulatory embrace or while mating, and both the male and female were returned to the Duke University Marine Lab, approximately 0.5 km from the collection site. Crabs collected in the pre-copulatory embrace were held as a pair until the female molted and mating was complete. Crabs collected while mating usually ceased mating upon capture, but resumed mating once placed in a tank together. Mating date was noted. After the pair separated, carapace width was measured as the distance between the tips of the large lateral spines for the male, female, and the shed female exoskeleton. In 3 cases, female crabs were placed with a second male immediately after mating. Crabs collected in the post-copulatory embrace were immediately separated and measured as above. Mating was assumed to have taken place within the previous 1 to 2 d (Van Engel 1958, Jivoff 1997). Males were released after being measured.

Additional females (24 of 107 total) were collected as recently molted, unpaired female crabs. Recently molted females were identified by incomplete calcification of the cuticle such that manual depression of the carapace below the large lateral spines was possible. An approximate mating date was determined for each of these crabs based on the degree of calcification of the cuticle. Of these 24 crabs, 9 were still soft (~25% calcified) and assigned a mating date of 3 d prior to capture, 10 were somewhat more (~50%) calcified and assigned a mating date of 5 d prior to capture, and 5 were almost fully (~75%) calcified and were assigned a mating date of 7 d prior to capture. These crabs were measured as above. Each female crab was marked with an individually numbered plastic poker chip or printed tag secured with 18-gauge coated copper wire wrapped around the large lateral spines.

Field confinement. The field confinement study ran from June 2006 through August 2008. Crabs were held and monitored using a slight modification of the procedures used by Dickinson et al. (2006). Females were confined individually in plastic minnow traps (42 × 23 cm) buried halfway into the sediment on their long axis in the immediate subtidal at the Duke University Marine Lab. The collection site and the confinement site are typical of high-salinity spawning habitat, with salinities remaining relatively constant at 35 (Ramach et al. 2009). When water temperatures were above 14.5°C, crabs were fed daily with seasonal fish (typically pinfish, spot, and croaker) and shrimp through the end of the trap that protruded into the water column. Small fish that swam into the traps and bivalves such as oysters and scallops that settled in the traps supplemented daily rations. Crabs confined in this way show high survival rates and do not mutilate their sponges, a common response of ovigerous blue crabs to stress (Dickinson et al. 2006).

In 2006, each crab was checked weekly for the presence of a sponge, and if present, the developmental state of eggs was noted. This sampling interval was chosen to ensure that all clutches were observed, as egg development takes at least 7 d (Hines et al. 2003). In 2007 and 2008, each crab was checked twice weekly. Regular monitoring ceased from mid-November through early March, during which time the crabs ceased clutch production and remained buried in the sediment. Monitoring continued until death.

Clutch quality analysis. Beginning in August 2007, clutch quality was assessed for each clutch produced, using 4 measurements: clutch volume, egg diameter, percentage of embryos developing normally, and lipid content of the eggs. Clutch volume was calculated as the product of the length (anterior to posterior), width, and depth (dorsal to ventral) of the sponge. Egg diameter was measured for 4 samples, 1 from each quadrant of the sponge, of 20 eggs from each clutch using an optical microscope fitted with an ocular micrometer.

All egg diameter measurements were made between 48 and 8 h before larval release, and only fertilized, normally developing eggs were measured. Egg diameters were averaged for all 80 measured eggs to calculate a mean egg diameter for each clutch. The percentage of embryos developing normally was visually assessed for 4 samples of 20 eggs from each clutch, at the same time as the egg diameter measurements. The percentages of embryos in each of the 4 samples developing normally were averaged to calculate a mean percentage developing normally for each clutch. Lipid content of early-stage eggs (50 to 100% yolk) was determined using the colorimetric sulfophosphovanillin method (Barnes & Blackstock 1973). Preweighed, lyophilized samples of eggs from each clutch were analyzed, and the percentage of lipids by weight was calculated.

Larval viability was assessed for each clutch using 2 measurements: larval size and duration of larval survival without food. As each crab approached the time of larval release, it was removed from the minnow trap and placed in a bucket containing aerated ambient seawater. Crabs were held in these conditions until larval release, typically 1 to 3 d after placement in the bucket. Water was changed daily for crabs held in the bucket for more than 24 h. Within 6 h after larval release, Stage I zoeae were collected. Carapace width, measured between the tips of the lateral spines, was measured on 20 Stage I zoeae from each clutch using a light microscope fitted with an ocular micrometer. Larval carapace widths were averaged for each sample of 20 zoeae to calculate a mean larval carapace width for each clutch. To determine the duration of larval survival without food, 10 zoeae from each clutch were placed into individual glass test tubes containing 20 ml of aged seawater filtered to remove particles >1 µm. Tubes were stored in racks, loosely covered with plastic wrap, and held at 25°C on a 12:12 h light:dark cycle. Swimming ability was assessed approximately every 12 h by capping and inverting the tubes, stimulating swimming behavior. Preliminary investigation indicated that death typically followed within 12 h of loss of swimming ability for zoeae held in this manner. Larval survival times were averaged for each group of 10 larvae to calculate a mean survival time for each clutch.

Data analysis. Water temperature data were extracted from the National Oceanic and Atmospheric Administration National Ocean Service (NOAA NOS) station BFTN7 at the Duke University Marine Lab in Beaufort, NC (www.ndbc.noaa.gov). This station is located approximately 0.7 km from the collection site and approximately 10 m from the field confinement site. Daily mean water temperatures were calculated for the entire study period. For degree-day calculations, a value of 12.2°C was used for T_{min}. This estimate is the mean of previously reported temperatures at which growth and/or feeding cease in *Callinectes sapidus* (Churchill 1919, Van Engel 1958, Leffler 1972, Brylawski & Miller 2006).

Timing of clutch production in relation to body size was assessed using linear regression. Clutch production interval (CPI) was calculated for each crab by dividing the physiological time interval over which clutches were produced by the number of clutches produced in that interval, producing a measurement of degree-days per clutch. CPI and physiological time to first clutch were individually regressed against carapace width. Linear regression techniques were also used to assess the relationship between size at maturity and water temperature.

Estimates of clutch quality and larval viability were modeled in relation to carapace width and clutch number with generalized linear mixed models (GLMM; Green 1987, Breslow & Clayton 1993). Clutch number and carapace width were fixed effects, crab number was the random effect, and the benchmark measurement was the response variable. When GLMM analysis indicated that a clutch quality assessment varied significantly with clutch number, analysis of variance (ANOVA) followed by a Tukey HSD test was used to compare means for each clutch.

RESULTS

Size at maturity. Female crabs used in this study ranged from 86 to 174 mm (mean ± SEM = 133.3 ± 1.7 mm) carapace width at maturity. Mean water temperature on the day of the terminal molt was a significant predictor of size at maturity (linear regression, $p < 0.001$), and the relationship appears to be linear over the range of temperatures during the study period (Fig. 1). Average size increase at the terminal molt for crabs mated in captivity was 37.5 ± 1.1% (mean ± SEM). There was no significant relationship between size increase at the terminal molt and water temperature on the day of the molt (linear regression, $p = 0.181$).

Fate of confined crabs. Of the 107 females collected for this study, 51 (47.7%) survived to spawn at least once. Of these, 35 died seemingly natural deaths while in the minnow traps. These 35 crabs were used for analyses of mature lifespan and total clutch production. An additional 2 crabs died due to handling outside the minnow traps after producing at least 1 clutch, 11 escaped, and 3 died during winters. These 16 crabs were included in analyses of time to first clutch and clutch production interval but were excluded from analyses of mature lifespan and total clutch production, unless otherwise noted. Crabs that did not spawn

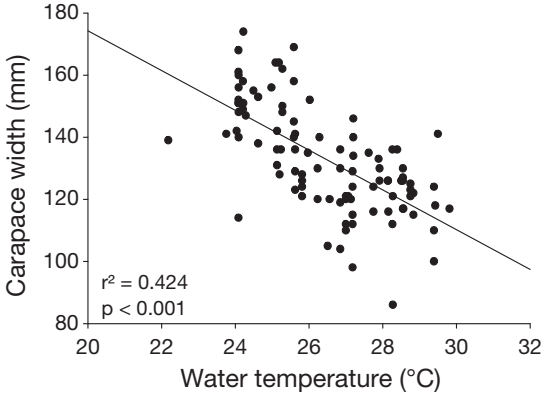

Fig. 1. *Callinectes sapidus*. Carapace width at maturity (mm) plotted against mean water temperature on the day of the terminal molt. A best-fit line has been added

were excluded from all analyses, including 5 that escaped and 51 that died before production of their first clutch. Of the crabs that died before production of the first clutch, 68.6% (35 of 51) survived less than 1 mo after the terminal molt.

Clutch production. Timing of clutch production varied depending on mating season. Crabs mating in the summer months (May to August) began spawning during the same season, 23 to 82 d after mating (Fig. 2a). Of the 4 crabs that mated in August and survived to spawn one did not spawn until the following May, 291 d after mating. Because a carapace width measurement was never obtained for that crab, it is not included in Fig. 2. Spawning during 2 spawning seasons was common for crabs maturing in June, July, and August, as 3 of 10 crabs mating in June, 9 of 21 crabs mating in July, and 2 of 4 crabs mating in August produced clutches during a second season. Clutches produced in the second spawning season for these crabs were fertilized and developed normally. Crabs mating in the fall (September to October) did not begin producing eggs until the following April or May (218 to 240 d after mating), and spawned only during a single season (Table 1).

Physiological time to first clutch averaged 747.1 ± 34.3 degree-days (mean ± SEM) after mating and was significantly correlated with carapace width (linear regression, $p < 0.001$). This relationship is linear over the range of carapace widths used for this study (Fig. 2b). The regression was not significantly improved by fitting separate lines to summer- and fall-mated crabs.

Production of multiple clutches was common for crabs used in this study (Fig. 3); 94.3% (33 of 35 crabs) of crabs produced at least 2 clutches, and 48.6% (17 of 35) produced at least 5 clutches in their lifetime, for an average of 4.14 ± 0.26 clutches (mean ± SEM). Total number of clutches produced was significantly correlated with lifespan (linear regression, $p < 0.001$). Crabs

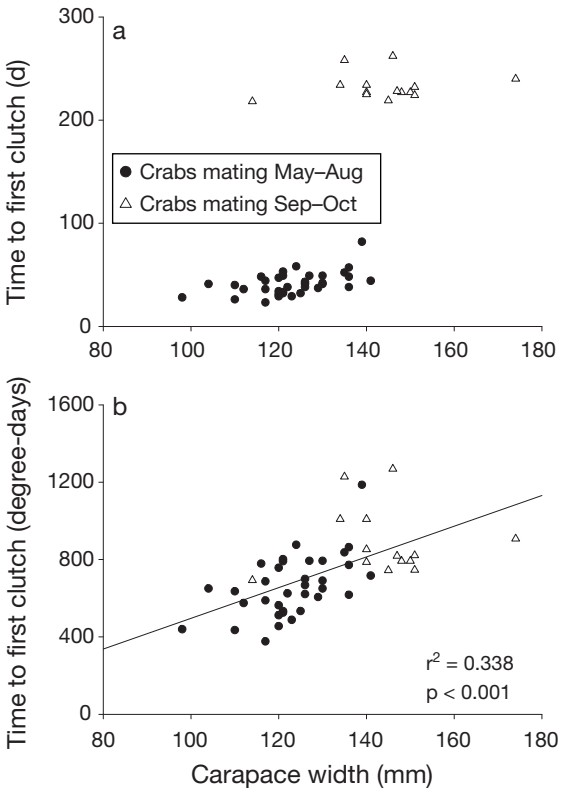

Fig. 2. *Callinectes sapidus*. Time to first clutch in (a) days after mating and (b) degree-days after mating plotted against carapace width (mm)

that survived longer produced more clutches. Crabs that spawned during 2 seasons were significantly smaller (*t*-test, $p = 0.049$) and produced significantly more clutches (*t*-test, $p = 0.003$) than crabs spawning in a single season.

Clutch production interval was positively correlated with carapace width. The relationship was strongest for crabs spawning during a single season (linear regression, $r^2 = 0.602$, $p < 0.001$), although the significant relationship persisted when crabs spawning during 2 seasons were included in the analysis (linear regression, $r^2 = 0.124$, $p = 0.024$; Fig. 4). Clutch production interval averaged 262.6 ± 9.1 degree-days per clutch (30.1 ± 2.9 d per clutch).

Table 1. *Callinectes sapidus*. Predicted relationships between production of first clutch, years spawning, and year of death for different mating seasons

Mating season (year *t*)	Production of first clutch	Years spawning	Year of death
Spring	Summer, year *t*	*t*	*t*
Summer	Fall, year *t*	*t* and *t* + 1	*t* or *t* + 1
Fall	Spring, year *t* + 1	*t* + 1	*t* + 1

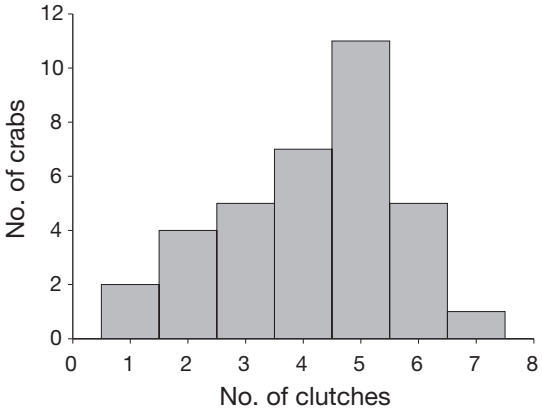

Fig. 3. *Callinectes sapidus*. Lifetime clutch production

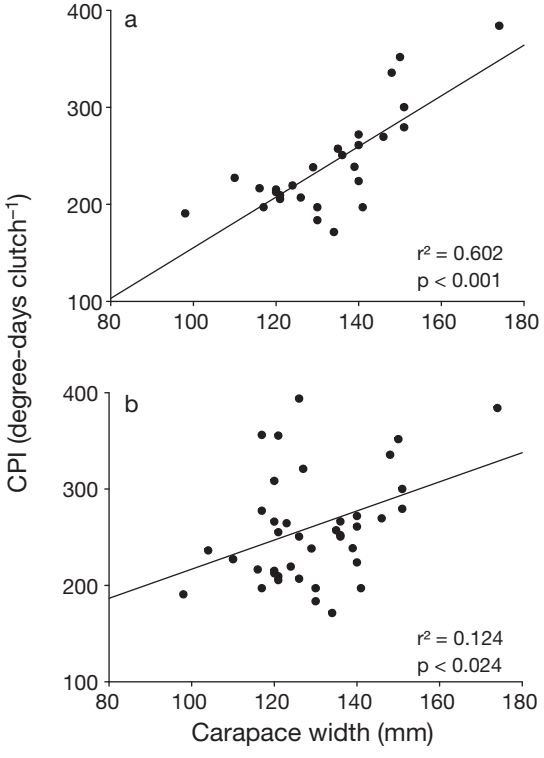

Fig. 4. *Callinectes sapidus*. Clutch production interval (CPI, degree-days per clutch) plotted against carapace width (mm) for crabs (a) spawning during 1 season and (b) spawning during 2 seasons. Best-fit lines have been added

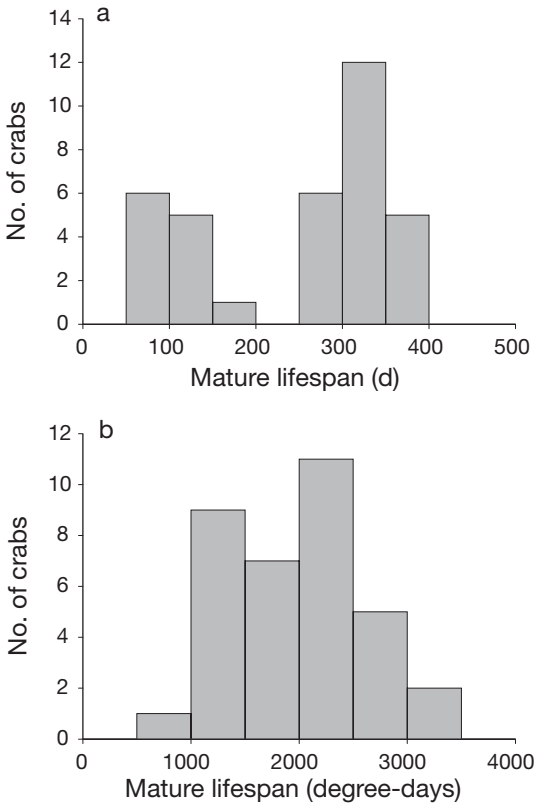

Fig. 5. *Callinectes sapidus*. Mature lifespan in (a) days after terminal molt and (b) degree-days after terminal molt

Mature lifespan. High mortality was seen during the first 30 d after mating. Mature lifespan of females surviving to produce at least 1 clutch ranged from 59 to 394 d after the terminal molt (Fig. 5). Mean lifespan from terminal molt to death was 134.76 ± 15.09 d (1092.4 ± 101.64 degree-days). For crabs surviving to spawn, mean mature lifespan was 249.11 ± 18.63 d (1986.6 ± 569.88 degree-days). While there was no linear relationship between lifespan as days or degree-days and carapace width (linear regression, $p = 0.110$), crabs that survived 2 seasons were significantly larger than crabs that survived 1 season (*t*-test, $p = 0.038$; Table 1).

Clutch quality and larval fitness. Early-stage egg lipid content (79.2 ± 2.1% of dry egg mass, mean ± SEM), egg diameter (267.5 ± 1.9 µm), larval carapace width (278.4 ± 4.5 µm), and larval survival time without food (3.4 ± 0.2 d) were similar for all clutches (GLMM, $p > 0.05$; Fig. 6) and were not significantly correlated with carapace width (GLMM, $p > 0.05$). The percentage of embryos developing normally (82.1 ± 4.1%) decreased with increasing clutch number, decreasing approximately 41% from Clutch 1 to Clutch 4 (Fig. 6c). Clutch number was a significant (GLMM, $p < 0.001$) predictor of the percentage of embryos developing normally. ANOVA indicated significant differences among clutches (ANOVA, $p = 0.009$), with Clutch 4 being significantly lower than Clutch 1 (Tukey HSD, $p = 0.005$) and Clutch 2 (Tukey HSD, $p = 0.05$). The percentage developing normally did not vary with carapace width (GLMM, $p > 0.05$). Clutch volume (19.5 ± 1.2 cm^3) increased with increasing crab size and decreased with increasing clutch number (Fig. 6a).

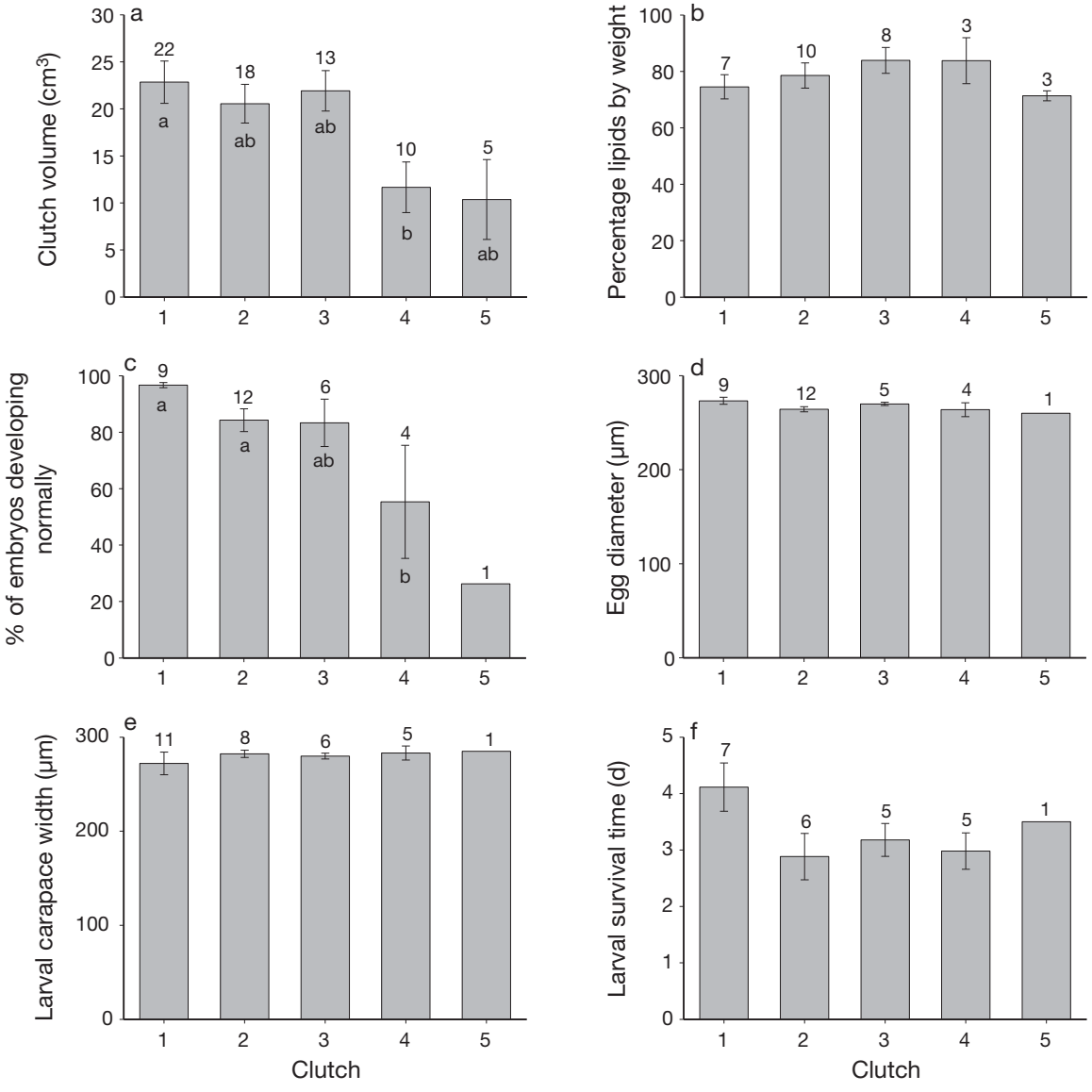

Fig. 6. *Callinectes sapidus*. Benchmarks of clutch quality and larval fitness (mean ± SEM) for Clutches 1 to 5: (a) clutch volume (cm^3), (b) percentage lipids by weight of early-stage eggs, (c) percentage of embryos developing normally, (d) egg diameter (µm), (e) larval carapace width (µm), and (f) larval survival time (days). Numbers above bars indicate sample size. Different letters within a panel indicate significant differences between values ($p < 0.05$)

Both carapace width (GLMM, $p = 0.002$) and clutch number (GLMM, $p < 0.001$, ANOVA, $p = 0.006$) were significant predictors of clutch volume. Volume of Clutch 4 was significantly lower than that of Clutch 1 (Tukey HSD, $p = 0.019$).

DISCUSSION

The primary objective of this study was to examine lifetime clutch production for mature female blue crabs. Crabs were collected around the time of mating, ensuring that no previous clutches had been produced. Crabs used in this study survived up to 394 d after the terminal molt, spawned during 1 to 2 seasons, and produced up to 7 clutches of eggs. Total clutch production was strongly correlated with lifespan, but clutch size and the timing of clutch production varied with water temperature as reported by Dickinson et al. (2006). In the absence of fishery-dependent mortality, egg predation, parasitism, or predation, we predict that most female blue crabs in NC produce between 3 and 7 clutches in their lifetime, depending on body size. This level of fecundity is relevant to estimates of spawning stock biomass and is consistent with prior estimates for blue crabs (Hines et al. 2003, Dickinson et al. 2006).

Despite relatively similar body sizes, blue crab total fecundity is likely higher than that of other portunids (Haddon & Wear 1993, de Lestang et al. 2003), based on higher single-clutch fecundity (Hines 1982) and greater number of clutches produced. Blue crabs are able to produce more eggs per clutch due to the small size of the eggs (267.5 ± 1.9 µm diameter, mean ± SEM) compared to other crabs (Hines 1982, Ingles & Braum 1989, Haddon & Wear 1993).

Many estimates of clutch viability and larval fitness (egg lipid content, egg size, larval size, and duration of larval survival without food) remained constant over all clutches, but the percentage of embryos developing normally and clutch volume decreased with successive clutches. The percentage of embryos developing normally decreased from 96.7 ± 0.9% for Clutch 1 to 55 ± 20.1% for Clutch 4 (Fig. 6c). While some of the abnormal eggs appeared to be unfertilized, others were fertilized but had ceased development. Clutch volume decreased by about 50% on average from Clutch 1 to 5 (Fig. 6a). The first 3 clutches were similar in size and normal development, and decreases in these 2 measurements did not occur until Clutch 4 (Fig. 6a,c). These findings indicate that earlier clutches are larger and contain a higher percentage of viable eggs, so the majority of a female crab's reproductive output comes from the first few clutches produced. Based on these results, we estimate that 70 to 85% of a female crab's reproductive output is from the first 3 clutches produced.

The decrease in the percentage of embryos developing normally may be due to factors relating to female age or to the number, age, or viability of stored sperm. Egg viability decreasing with female age is a generalized phenomenon across the animal kingdom, as older females often produce less viable eggs. This phenomenon, or some variation on the general trend, has been observed in a number of organisms including *Drosophila melanogaster* (Kern et al. 2001), chickens (Fasenko et al. 1992), and humans (Schwartz & Mayaux 1982).

The observed decline in normal development with successive clutches might also be due to sperm limitation. Unfertilized eggs were seen in some of the egg samples used for embryo viability. Sperm limitation in the form of unfertilized clutches has been reported in the Chesapeake Bay and Florida (Hines et al. 2003). No evidence of sperm limitation has been reported for blue crabs from NC (Wolcott et al. 2005). Female blue crabs store sperm for fertilization of all clutches from the single mating window after the terminal molt (Hines et al. 2003, Wolcott et al. 2005). Thus, sperm may be stored for months to >1 yr after mating. During this time, sperm cells are accumulating damage due to oxidative, osmotic, and temperature stress and ATP depletion, which may reduce their viability (e.g. Siva-Jothy 2000, Reinhardt 2007). Wolcott et al. (2005) found that in NC blue crabs, sperm number decreased by approximately 50% during the 12 wk after mating, either through loss or degradation of dead sperm. None of the crabs used by Wolcott et al. (2005) spawned in this 12 wk interval. Decreasing sperm viability with sperm age, generally evidenced by decreased fertilization potential or motility, has been demonstrated in numerous taxa including mollusks (e.g. Babcock & Keesing 1999), echinoderms (Levitan et al. 1991), crustaceans (Paul 1984), fish (e.g. Dreanno et al. 1999), and birds (e.g. Lodge et al. 1971, White et al. 2008).

The observed decrease in clutch size with age is consistent with models of clutch size in insects. Parker & Courtney (1984) and Begon & Parker (1986) modeled optimal clutch size as a function of age for insect species in which all resources needed for egg production are accumulated during the pre-reproductive phase, thus fixing reproductive potential before the start of reproduction. Although female blue crabs continually forage and mature their ovaries during the reproductive phase, we hypothesize that a female blue crab's lifetime complement of eggs are all present at the time of the terminal molt. Thus, female reproductive potential is fixed well before the start of spawning. Due to the fixed risk of death between each clutch, the probability of surviving to produce another clutch declines with clutch number. The optimal spawning strategy is thus to produce the maximum number of eggs as soon as possible. In the case of blue crabs, body cavity volume limits clutch size (Hines 1982), so larger females produce a larger first clutch of eggs. Based on data presented here (Fig. 5a), it appears that female blue crabs produce up to 3 full-sized clutches before clutch size decreases significantly. Given the observed decline in embryo viability with crab age, probable declines in sperm and egg viability over time, and the risk of death between clutches, this strategy maximizes potential reproductive output.

Crabs mating in the summer months generally began spawning in the same year. Those beginning spawning in the late summer frequently spawned during 2 yr. Crabs mating in the fall (September to November) did not spawn until the following year and usually spawned in only a single year (Fig. 2a, Table 1). The single regression line fit to the degree-day data (Fig. 2b) suggests that these seasonal patterns are due to temperature. Cooler water temperatures in the fall and winter likely slowed metabolism and egg production until the following spring. Because crabs maturing in the warmer summer months were generally smaller than crabs maturing in the fall months, crabs that produced clutches during 2 spawning seasons (crabs maturing and mating in the summer) were significantly smaller than crabs producing clutches

during a single spawning season (crabs maturing and mating in the spring and fall). Differences in the timing of maturation and mating likely led to the observed bimodal distribution of lifespans, with one mode at 105 d and another mode at 324 d (Fig. 5a). Expressing lifespan in degree-days resulted in a more unimodal lifespan distribution (Fig. 5b), again suggesting that temperature is an important determinant of lifespan. Crabs that matured from July to November were much more likely to survive a second season than were crabs that matured earlier in the year (Table 1), since they experience fewer days of warm temperatures between the terminal molt and the onset of winter. Due to seasonal (temperature-dependent) effects on carapace width, crabs that survived 2 seasons were significantly larger than those surviving a single season.

Size at maturity was strongly ($r^2 = 0.424$, $p < 0.001$) correlated with water temperature on the day of molting, with warmer temperatures producing smaller crabs. Based on seasonal trends in water temperature, the largest crabs molted to maturity in the spring and fall, while the smallest crabs molted to maturity during the warmer summer months. Because the relative increase in size at the molt was not related to temperature, the process is likely related to some other aspect of the physiology of the crabs. This could be switching of pathways from growth to sexual maturity or from growth to activity, or temperature-related changes in diet. It could also be a cumulative effect of higher temperatures resulting in smaller molt increments over many molts. Our results indicate that environmental factors play a major role in determining crab size. Similar negative relationships between temperature and size at maturity have been seen for blue crabs in the Gulf of Mexico (Fisher 1999) and other decapods including *Emerita analoga* (Dugan et al. 1994), *Helice crassa* (Jones & Simons 1983), *Homarus americanus* (Campbell & Robinson 1983), *Panulirus cygnus* (Melville-Smith & de Lestang 2006), and *Procambarus clarkii* (Alcorlo et al. 2008). The observed negative relationship between size at maturity and water temperature is the opposite of that seen for snow crabs and some other cold-water crabs, which are generally larger at higher temperatures (Somerton 1981, Ernst et al. 2005, Orensanz et al. 2007, Sainte-Marie et al. 2008).

Recent decreases in size at maturity, as seen in the Chesapeake Bay (Lipcius & Stockhausen 2002) and NC (Eggleston et al. 2004) may be due to a combination of genetic and environmental factors. Blue crabs exhibit extremely high levels of genetic diversity and lack spatial population structure (McMillen-Jackson & Bert 2004). Thus, if recent decreases in size are due to genetic factors, they must be induced by size-selective fishing pressure along the entire range of the blue crab. Due to the mixing occurring during the larval stages, localized selective pressures should have little effect on size. Future work using population genetics approaches and determining if there is population structure in large and small crabs will shed light on the relative importance of genetic and environmental components of size determination in blue crabs.

Previous estimates of blue crab mature lifespans range from 1 to 4 yr (Chesapeake: Churchill 1919, Van Engel 1958; Florida: Tagatz 1968; NC: Judy & Dudley 1970). In our study, crabs were given ample food and were housed in a predator-free environment. High mortality was seen in the first month after the terminal molt, likely due to handling stress, as the crabs were handled extensively in a short time period after molting. Survival was high following this vulnerable period, and no crabs were observed to mutilate their sponges, a behavior often observed in ovigerous females under stress, such as during pot capture. We observed that many crabs became incapacitated by shell fouling and were infected with gill parasites (gooseneck barnacle *Octolasmus muelleri*) or colonized by nemertean egg predators *Carcinonemertes carcinophila* (Dickinson et al. 2006). Lifespan in the wild may be greater if such a high degree of parasitism is a phenomenon related to the confinement site. Our qualitative observations do not suggest this, however, as we see many crabs that are completely fouled, parasitized, and colonized by *C. carcinophila* in the wild. Thus, we believe that crabs in the wild in NC have a similar lifespan to those confined in this study, surviving less than 2 yr after reaching maturity.

Crabs monitored in this study were confined in plastic minnow traps, rather than free-ranging in the wild. While monitoring free-ranging crabs would theoretically provide superior data, it is not logistically feasible to observe each clutch produced during the lifetime of a free-ranging crab. The confinement method used here was chosen because it allows the crabs to be held in the field and exposed to the natural tidal and diel cycles. While confined in the minnow traps, crabs were unable to migrate as they would in the wild (Forward et al. 2003, Carr et al. 2004, Hench et al. 2004), although we feel that this would have little, if any, effect on clutch production. Crabs were collected from and confined in high-salinity (~35) areas that are both mating habitat and spawning habitat for blue crabs (Ramach et al. 2009). Any environmental changes experienced by crabs migrating seaward from the sites would be minimal.

To successfully assess or manage the spawning stock of any species by present theory, an accurate knowledge of the life history and spawning biology of that species is necessary. Sufficient knowledge is lacking for many exploited species, including blue crabs. Blue

crab population models (e.g. Miller 2001, Bunnell & Miller 2005) have traditionally assumed that blue crabs produce a single clutch of eggs in their lifetime. In these models, fecundity estimates have a large effect on estimates of the intrinsic rate of population increase. It is now clear that female blue crabs produce multiple clutches of eggs, over multiple spawning seasons. Due to size-specific differences in timing and size of clutches, the relationship between total fecundity and crab size is likely not as simple as the linear relationship presented by Prager et al. (1990) and subsequently incorporated into population models (e.g. Miller 2001, Bunnell & Miller 2005) and stock assessments (Eggleston et al. 2004). Blue crab population models and management plans should be reworked to reflect multiple clutch production and total fecundity.

Acknowledgements. We thank J. Leonard for advice on lipid quantification protocols, J. Priddy for assistance with temperature data analysis, and J. E. Moore for providing valuable statistical advice. M. B. Ogburn provided insightful discussion about clutch quality analyses and valuable comments on this manuscript. Additionally, many DUML undergraduate students assisted with collecting and feeding the crabs used in this study. Three anonymous reviewers provided valuable comments on an early version of the manuscript. Funding for this study was provided by the North Carolina Sea Grant (Blue Crab Research Program grants 06-Biol-04 and 07-Biol-06).

LITERATURE CITED

Alcorlo P, Geiger W, Otero M (2008) Reproductive biology and life cycle of the invasive crayfish *Procambarus clarkii* (Crustacea: Decapoda) in diverse aquatic habitats of south-western Spain: implications for population control. Fundam Appl Limnol 173:197–212

Babcock R, Keesing J (1999) Fertilization biology of the abalone *Haliotis laevigata*: laboratory and field studies. Can J Fish Aquat Sci 56:1668–1678

Barnes H, Blackstock J (1973) Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanillin method for 'total' lipids. J Exp Mar Biol Ecol 12:103–118

Begon M, Parker GA (1986) Should egg size and clutch size decrease with age? Oikos 47:293–302

Breslow NE, Clayton DG (1993) Approximate inference in generalized linear mixed models. J Am Stat Assoc 88:9–25

Brylawski BJ, Miller TJ (2006) Temperature-dependent growth of the blue crab (*Callinectes sapidus*): a molt process approach. Can J Fish Aquat Sci 63:1298–1308

Bunnell DB, Miller TJ (2005) An individual-based modeling approach to spawning-potential per-recruit models: an application to blue crab (*Callinectes sapidus*) in Chesapeake Bay. Can J Fish Aquat Sci 62:2560–2572

Campbell A, Robinson DG (1983) Reproductive potential of three American lobster (*Homarus americanus*) stocks in the Canadian Maritimes. Can J Fish Aquat Sci 40: 1958–1967

Carr SD, Tankersley RA, Hench JL, Forward RB, Luettich RA (2004) Movement patterns and trajectories of ovigerous blue crabs *Callinectes sapidus* during the spawning migration. Estuar Coast Shelf Sci 60:567–579

Chesapeake Bay Program (1997) 1997 Chesapeake Bay blue crab fishery management plan. Chesapeake Bay Program Office, US Environmental Protection Agency, Annapolis, MD

Churchill EP (1919) Life history of the blue crab. Bull US Bur Fish 36:95–128

de Lestang S, Hall NG, Potter IC (2003) Reproductive biology of the blue swimmer crab (*Portunus pelagicus*, Decapoda: Portunidae) in five bodies of water on the west coast of Australia. Fish Bull (Wash DC) 101:745–757

Dickinson GH, Rittschof D, Latanich C (2006) Spawning biology of the blue crab, *Callinectes sapidus*, in North Carolina. Bull Mar Sci 79:273–285

Dreanno C, Suquet M, Fauvel C, Le Coz JR, Dorange G, Quemener L, Billard R (1999) Effect of the aging process on the quality of sea bass (*Dicentrarchus labrax*) semen. J Appl Ichthyol 15:176–180

Dugan JE, Hubbard DM, Wenner AM (1994) Geographic variation in life history of the sand crab, *Emerita analoga* (Stimpson) on the California coast: relationships to environmental variables. J Exp Mar Biol Ecol 181:255–278

Eggleston DB, Johnson EG, Hightower JE (2004) Population dynamics and stock assessment of the blue crab in North Carolina. Final Report for contracts 99-FEG-10 and 00-FEG-11 to the North Carolina Fisheries Resource Program, North Carolina Sea Grant, Raleigh, NC

Ernst B, Orensanz JM, Armstrong DA (2005) Spatial dynamics of female snow crab (*Chionoecetes opilio*) in the eastern Bering Sea. Can J Fish Aquat Sci 62:250–268

Fasenko GM, Hardin RT, Robinson FE, Wilson JL (1992) Relationship of hen age and egg sequence position with fertility, hatchability, viability, and preincubation embryonic development in broiler breeders. Poult Sci 71:1374–1383

Fisher MR (1999) Effect of temperature and salinity on size at maturity of female blue crabs. Trans Am Fish Soc 128: 499–506

Forward RB, Tankersley RA, Pochelon PN (2003) Circatidal activity rhythms in ovigerous blue crabs, *Callinectes sapidus*: implications for ebb-tide transport during the spawning migration. Mar Biol 142:67–76

Forward RB, Cohen JH, Darnell MZ, Saal A (2005) The circatidal rhythm in vertical swimming of female blue crabs, *Callinectes sapidus*, during their spawning migration: a reconsideration. J Shellfish Res 24:587–590

Green PJ (1987) Penalized likelihood for general semiparametric regression models. Int Stat Rev 55:245–259

Haddon M, Wear RG (1993) Seasonal incidence of egg-bearing in the New Zealand paddle crab *Ovalipes catharus* (Crustacea: Brachyura), and its production of multiple egg batches. NZ J Mar Freshw Res 27:287–293

Hench JL, Forward RB, Carr SD, Rittschof D, Luettich RA (2004) Testing a selective tidal-stream transport model: observations of female blue crab (*Callinectes sapidus*) vertical migration during the spawning season. Limnol Oceanogr 49:1857–1870

Hines AH (1982) Allometric constraints and variables of reproductive effort in brachyuran crabs. Mar Biol 69: 309–320

Hines AH, Jivoff PR, Bushmann PJ, van Montfrans J, Reed SA, Wolcott DL, Wolcott TG (2003) Evidence for sperm limitation in the blue crab, *Callinectes sapidus.* Bull Mar Sci 72:287–310

Ingles JA, Braum E (1989) Reproduction and larval ecology of the blue swimming crab *Portunus pelagicus* in Ragay Gulf, Philippines. Int Rev Gesamten Hydrobiol 74:471–490

Jivoff P (1997) The relative roles of predation and sperm competition on the duration of the post-copulatory association

between the sexes in the blue crab, *Callinectes sapidus.* Behav Ecol Sociobiol 40:175–185

Jones MB, Simons MJ (1983) Latitudinal variation in reproductive characteristics of a mud crab, *Helice crassa* (Grapsidae). Bull Mar Sci 33:656–670

Judy MH, Dudley DL (1970) Movements of tagged blue crabs in North Carolina waters. Commer Fish Rev 32:29–35

Kern S, Ackermann M, Stearns SC, Kawecki TJ (2001) Decline in offspring viability as a manifestation of aging in *Drosophila melanogaster.* Evolution 55:1822–1831

Leffler CW (1972) Some effects of temperature on growth and metabolic rate of juvenile blue crabs, *Callinectes sapidus*, in the laboratory. Mar Biol 14:104–110

Levitan DR, Sewell MA, Chia FS (1991) Kinetics of fertilization in the sea urchin *Strongylocentrotus franciscanus*: interaction of gamete dilution, age, and contact time. Biol Bull (Woods Hole) 181:371–378

Lipcius RN, Stockhausen WT (2002) Concurrent decline of the spawning stock, recruitment, larval abundance, and size of the blue crab *Callinectes sapidus* in Chesapeake Bay. Mar Ecol Prog Ser 226:45–61

Lodge JR, Fechheimer NS, Jaap RG (1971) Relationship of in-vivo sperm storage interval to fertility and embryonic survival in chicken. Biol Reprod 5:252–257

McMillen-Jackson AL, Bert TM (2004) Mitochondrial DNA variation and population genetic structure of the blue crab *Callinectes sapidus* in the eastern United States. Mar Biol 145:769–777

Medina Mantelatto FL, Fransozo A (1997) Fecundity of the crab *Callinectes ornatus* Ordway, 1863 (Decapoda, Brachyura, Portunidae) from the Ubatuba region, São Paulo, Brazil. Crustaceana 70:214–226

Melville-Smith R, de Lestang S (2006) Spatial and temporal variation in the size at maturity of the western rock lobster *Panulirus cygnus* George. Mar Biol 150:183–195

Miller TJ (2001) Matrix-based modeling of blue crab population dynamics with applications to the Chesapeake Bay. Estuaries 24:535–544

Millikin MR, Williams AB (1984) Synopsis of biological data on the blue crab *Callinectes sapidus* Rathbun. FAO Fish Tech Pap 138

Morgan SG, Goy JW, Costlow JD (1983) Multiple ovipositions from single matings in the mud crab *Rhithropanopeus harrisii*. J Crustac Biol 3:542–547

Neuheimer AB, Taggart CT (2007) The growing degree-day and fish size-at-age: the overlooked metric. Can J Fish Aquat Sci 64:375–385

Orensanz JM, Ernst B, Armstrong DA (2007) Variation of female size and stage at maturity in snow crab (*Chionoecetes opilio*) (Brachyura: Majidae) from the eastern Bering sea. J Crustac Biol 27:576–591

Parker GA, Courtney SP (1984) Models of clutch size in insect oviposition. Theor Popul Biol 26:27–48

Paul AJ (1984) Mating frequency and viability of stored sperm in the tanner crab *Chionocetes bairdi* (Decapoda, Majidae). J Crustac Biol 4:375–381

Pillay KK, Nair NB (1971) The annual reproductive cycles of *Uca annulipes, Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) from the South-west coast of India. Mar Biol 11:152–166

Pinheiro MAA, Fransozo A (1999) Reproductive behavior of the swimming crab *Arenaeus cribrarius* (Lamarck, 1818) (Crustacea, Brachyura, Portunidae) in captivity. Bull Mar Sci 64:243–253

Prager MH, McConaugha JR, Jones CM, Geer PJ (1990) Fecundity of blue crab, *Callinectes sapidus*, in Chesapeake Bay: biological, statistical and management considerations. Bull Mar Sci 46:170–179

Ramach SM, Darnell MZ, Avissar NG, Rittschof D (2009) Habitat use and population dynamics of blue crabs, *Callinectes sapidus*, in a high-salinity embayment. J Shellfish Res 28:635–640

Reinhardt K (2007) Evolutionary consequences of sperm cell aging. Q Rev Biol 82:375–393

Sainte-Marie B, Gosselin T, Sevigny JM, Urbani N (2008) The snow crab mating system: opportunity for natural and unnatural selection in a changing environment. Bull Mar Sci 83:131–161

Schwartz D, Mayaux MJ (1982) Female fecundity as a function of age: results of artificial insemination in 2193 nulliparous women with azoospermic husbands. N Engl J Med 306:404–406

Siva-Jothy MT (2000) The young sperm gambit. Ecol Lett 3: 172–174

Somerton DA (1981) Regional variation in the size of maturity of two species of tanner crab (*Chionoecetes bairdi* and *C. opilio*) in the Eastern Bering Sea, and its use in defining management subareas. Can J Fish Aquat Sci 38:163–174

Tagatz ME (1968) Biology of blue crab, *Callinectes sapidus* Rathbun, in St. Johns River, Florida. US Fish Wildl Serv Fish Bull 67:17–33

Tankersley RA, Wieber MG, Sigala MA, Kachurak KA (1998) Migratory behavior of ovigerous blue crabs *Callinectes sapidus*: evidence for selective tidal-stream transport. Biol Bull (Woods Hole) 195:168–173

Turner HV, Wolcott DL, Wolcott TG, Hines AH (2003) Post-mating behavior, intramolt growth, and onset of migration to Chesapeake Bay spawning grounds by adult female blue crabs, *Callinectes sapidus* Rathbun. J Exp Mar Biol Ecol 295:107–130

Van Engel WA (1958) The blue crab and its fishery in Chesapeake Bay. Part 1: Reproduction, early development, growth, and migration. Commer Fish Rev 20:6–16

Wang JY (1960) A critique of the heat unit approach to plant response studies. Ecology 41:785–790

White J, Wagner RH, Helfenstein F, Hatch SA, Mulard H, Naves LC, Danchin E (2008) Multiple deleterious effects of experimentally aged sperm in a monogamous bird. Proc Natl Acad Sci USA 105:13947–13952

Williams AB (1974) Swimming crabs of the genus *Callinectes* (Decapoda: Portunidae). Fish Bull (Wash DC) 72:685–798

Wolcott DL, Hopkins CWB, Wolcott TG (2005) Early events in seminal fluid and sperm storage in the female blue crab *Callinectes sapidus* Rathbun: effects of male mating history, male size, and season. J Exp Mar Biol Ecol 319: 43–55

Editorial responsibility: James McClintock, Birmingham, Alabama, USA

Submitted: May 19, 2008; Accepted: August 31, 2009
Proofs received from author(s): November 9, 2009