Spawning Biology of Female Blue Crabs, *Callinectes sapidus*

by

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University Program in Ecology
Duke University

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University Program in Ecology in the Graduate School of Duke University

2009
ABSTRACT

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Abstract

This dissertation investigated spawning biology of female blue crabs, *Callinectes sapidus*. Females mate following the terminal molt and undertake a spawning migration seaward, producing multiple clutches of larvae. To examine lifetime reproductive potential of female crabs, individual crabs were confined in the field from terminal molt to death. Crabs produced up to 7 clutches over 1-2 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. Size at maturity was negatively correlated with water temperature on the day of the terminal molt. Most measurements of clutch quality and larval fitness were similar for all clutches. The percentage of embryos developing normally decreased 40% from clutch 1 to clutch 4 and clutch volume decreased 50% from clutch 1 to clutch 5. Thus, most of a crab’s reproductive output is from the first few clutches.

Using swimming and abdominal pumping assays, the roles of pheromones in larval release and migratory behavior were investigated. Following delivery of egg extract, bradykinin (a pheromone mimic), and trypsin (an enzyme that generates peptide pheromones), ovigerous crabs responded with increased abdominal pumping, indicating that peptide pheromones stimulate larval release in blue crabs. Ovigerous crabs responded with increased swimming following delivery of egg extract, but not
following delivery of a peptide pheromone mimic or an enzyme that produces peptide pheromones. These results suggest that some substance generated from the egg mass stimulates vertical swimming, but that peptides alone do not stimulate swimming. A blend of molecules, possibly including sugars, may be the cue that stimulates swimming behavior.

Endogenous rhythms in vertical swimming, a mechanism underlying migration in tidal estuaries, were examined in the laboratory under constant conditions in juvenile females, recently-molted females, and females with mature ovaries from Beaufort, NC. Rhythms were variable in each stage, though circatidal rhythms consistent with ebb tide transport were observed in juvenile females and recently-molted females. Crabs with mature ovaries typically swam around the time of high tide. Rhythms were also examined for ovigerous females collected from estuaries with three different tidal regimes: semi-diurnal, diurnal, and non-tidal. Crabs from the tidal estuaries had circatidal or circalunidian swimming rhythms with period lengths corresponding to the tidal period of their home estuary. Swimming occurred primarily on ebb tide. Crabs from the non-tidal estuary had a circadian rhythm of vertical swimming around the time of sunset. Such a rhythm has no obvious migratory significance and migration likely takes place though another mechanism.

Swimming behavior was also examined in the field in one non-tidal site and three tidal sites. Crabs were tethered in the field and swimming was monitored using
archival pressure tags. Crabs tethered in the non-tidal site did not swim, possibly due to
the lack of necessary environmental cues. Crabs at the tidal sites swam primarily on ebb
tides. Swimming was greatest at the deepest site, which also had the strongest currents.
This site is known to be a migratory area for spawning blue crabs. Decreased swimming
behavior was observed at the two shallower sites, including one site that is known to be
habitat for all stages of blue crabs. These results indicate that swimming behavior is
variable among different areas in a single estuary. In areas where swimming is reduced,
crabs may continue migrating seaward by walking or may spend additional time in that
area to forage. Within each site, peak swimming generally occurred during the time of
the most rapid decrease in water level, suggesting that hydrostatic pressure may serve
as a cue for swimming.

Mark-recapture studies were conducted in three rivers (North River, South
River, Adams Creek) in eastern North Carolina, and recently-molted female crabs were
tagged to ensure a relatively constant time since molting. Most crabs traveled relatively
short distances and were recaptured before producing a clutch of eggs. Individuals that
moved substantial distances typically moved down-estuary. The Adams Creek canal,
connecting Adams Creek with the Newport River estuary, functioned as a migratory
corridor, as crabs from both Adams Creek and South River migrated down the canal,
presumably using ebb tide transport. Many of the crabs that migrated down the canal
into the Newport River were recaptured while ovigerous. Results of this study support
the hypothesis that rapid long-distance migratory movements do not begin until production of the first clutch of eggs, though some down-estuary movement takes place by prior to production of the first clutch of eggs.

Female blue crabs mate following the terminal molt and begin moving seaward soon thereafter by walking and swimming. Once the appropriate salinity (> 22 ppt) is reached, the first clutch of eggs is produced and migration rate rapidly increases. Blue crab spawning biology should be similar throughout the range of the species. After taking latitudinal temperature variation and other local variables into account, results presented here should be applicable not only to blue crabs in North Carolina, but in other areas as well.
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1. Introduction

The blue crab, *Callinectes sapidus* Rathbun, is a commercially and ecologically important portunid crab native to western Atlantic estuaries and coastal waters. The natural range of *C. sapidus* extends from Nova Scotia to Argentina (Millikin & Williams 1984). Though native to the east coast of North and South America, the blue crab has been introduced in Europe, and a breeding population has been established in the Mediterranean and surrounding areas (Williams 1974). Blue crabs have a migratory life cycle, occupying a variety of habitats over the course of their life. Larval development occurs offshore and the life stages prior to mating are characterized by up-estuary migration and dispersal throughout estuaries. Most spawning female blue crabs migrate from low-salinity mating grounds to high-salinity spawning grounds (Van Engel 1958, Tankersley et al. 1998). Periodically during this migration, multiple clutches of larvae are released (Hines et al. 2003, Dickinson et al. 2006, Darnell et al. 2009). The purpose of this dissertation was to examine (1) the spawning biology of blue crabs, including reproductive timing and lifetime reproductive potential, and (2) the mechanisms underlying the blue crab spawning migration.

1.1 Life history of *C. sapidus*

seaward and develop offshore for 30-50 days (Epifanio et al. 1984, Millikin & Williams 1984). Following 7-8 larval stages (Costlow & Bookhout 1959), blue crab zoeae metamorphose into the postlarval or megalopal stage, which typically lasts from 6-11 days (Costlow et al. 1959, Costlow 1967). Megalopae are transported back into estuaries by surface currents and migrate to settlement sites using flood tide transport (FTT)(Epifanio & Garvine 2001). Once inside the estuaries, megalopae swim upward into the water column during nocturnal flood tides and remain on the bottom during ebb tides (Tankersley & Forward 1994, Forward et al. 1997, Welch et al. 1999). This results in a series of movements up-estuary. Blue crab megalopae settle and metamorphose into juvenile crabs (stage J1) in areas of submerged vegetation such as seagrass beds and salt marshes (Heck & Thoman 1984, Orth & Van Montfrans 1987, Etherington & Eggleston 2000). Juveniles remain in these areas until they begin to disperse throughout the estuary (Blackmon & Eggleston 2001, Forward et al. 2004, Reyns & Eggleston 2004, Forward et al. 2005b).

Blue crabs typically reach maturity 10-20 months after hatching, following 18-20 postlarval molts (Millikin & Williams 1984). Although males continue to molt several more times after reaching sexual maturity, females undergo a terminal pubertal molt. Mating is usually immediate, although incompletely mated females remain receptive for up to 10 days after the terminal molt (Rittschof et al., unpublished data).
Most sexually mature adult blue crabs mate in the low-salinity waters of upper estuaries, with peaks in mating occurring in the spring and fall in North Carolina (Hay 1905, Millkin & Williams 1984). Approximately 2 days before the female’s terminal molt, she pairs with a male who carries her in the precopulatory embrace, or cradle-carry position, which consists of the male carrying the female under his body with his first pair of walking legs. Once the female molts and mating occurs, the male continues to carry the female in the postcopulatory embrace for up to 2 d (Van Engel 1958, Jivoff 1997).

After completing the spawning migration to high salinity waters, female crabs do not return to the upper estuaries, but rather live out their lives in high salinity waters of the lower estuary and coastal ocean (Van Engel 1958, Forward et al. 2005a). The typical blue crab life span is estimated to be between 2 and 5 years (Van Engel 1958, Tagatz 1968), though this likely depends on latitudinal temperature variations.

### 1.2 Biological rhythms in *C. sapidus*

Blue crabs inhabit a variety of estuarine and offshore habitats during their life cycle. During many of these life stages, biological rhythms in general activity or specific behaviors (i.e. vertical swimming) contribute, along with various physical factors, to determining the distribution of that particular life stage. A number of biological rhythms have been identified in blue crabs, including rhythms in swimming speed, activity levels, vertical swimming, egg-maintenance behaviors, and larval release.
Zoeae exhibit a rhythmic cycle of swimming speeds under natural conditions (Sulkin et al. 1979). This rhythm was not present when zoeae were monitored under constant light, suggesting that the rhythmic behavior is exogenous in origin. Because an increase in activity generally results in an ascent in the water column, this rhythm in swimming speed should result in blue crab zoeae being distributed in the surface waters during the night and at depth during the day. This is a typical diel vertical migration pattern, seen in many species of zooplankton. Blue crab zoea, however, show no rhythm in vertical position in the water column under constant darkness (Forward et al., unpublished data). While several studies have demonstrated that blue crab zoeae are present in offshore surface waters (McConaugha 1988b, Epifanio et al. 1989), these studies have generally not looked at the temporal distribution of zoeae in the water column at a fine enough scale to detect such a vertical migration pattern in the field. Lochmann et al. (1995), who examined the vertical distributions of zoeae in a tidal inlet, found that zoeal abundance was similar during ebb and flood tides, and found no clear pattern in the vertical distribution of zoeae in relation to the diel cycle.

After metamorphosis in offshore waters, megalopae possess a circadian rhythm with peaks in swimming occurring during daylight hours (Tankersley & Forward 1994). This results in megalopae being distributed primarily near the surface during the day and lower in the water column at night when in offshore waters (McConaugha 1988b, Epifanio et al. 1989). Once exposed to estuarine water, chemical cues present in estuarine
water reverse the photobehavior of C. sapidus megalopae, causing them to be negatively-phototactic (Forward & Rittschof 1994). The circadian rhythm of diurnal swimming that occurs in offshore areas is thus suppressed in estuaries, and swimming in response to environmental cues (changes in salinity and turbulence) takes over as the major mechanism of transport (Forward et al. 1995, Tankersley et al. 1995, Welch et al. 1999).

Juvenile blue crabs undergo secondary dispersal throughout estuaries, using night-time FTT (Reyns & Eggleston 2004). Crabs in the first five juvenile stages (J1-J5) have a circadian rhythm in vertical swimming, with maximum swimming occurring during the night (Forward et al. 2004, 2005b, Forward et al. 2007). Because peak swimming under constant conditions was not related to tidal phase in juvenile crabs, an endogenous rhythm does not control the onset of vertical swimming during flood tide that is seen in FTT. Instead, it appears as though increased turbulence may be one of cues that initiates vertical swimming for FTT in blue crab juveniles (Blackmon & Eggleston 2001). The circadian rhythm in which peak swimming occurs at night limits FTT to night-time flood tides (Forward et al. 2004, Reyns & Eggleston 2004).

Ovigerous blue crabs possess a circatidal rhythm in vertical swimming that drives ebb tide transport during the spawning migration. Forward et al. (2003) examined this rhythm in vertical swimming and found that some crabs possessed a typical circatidal period around 12.4 h, whereas others possess a circalunidian rhythm with a period around 24.8 h. The circalunidian rhythm suggests that some crabs are
undergoing ETT during night-time ebb tides only. After releasing larvae, female crabs become arrhythmic, though the circatidal rhythm becomes re-entrained following exposure to the tidal cycle (Forward et al. 2005a). In addition to the rhythm in vertical swimming, circatidal rhythms in egg maintenance behavior and migratory restlessness (general locomotor activity) have been observed in spawning female blue crabs (Forward et al. 2003). Peak restlessness occurred during ebb tides, whereas egg-maintenance behaviors occurred during flood tide, suggesting that ovigerous females alternate between periods of vertical swimming and periods of egg-maintenance behavior.

Both a tidal and a diel rhythm in larval release have been identified in blue crabs, with release occurring on morning high tides, centered around sunrise (Ziegler 2002). The circatidal rhythm can be entrained by a step cycle in salinity, with release occurring approx. 2 h after the onset of high salinity. When subjected to a 12:12 light:dark cycle but isolated from tidal cues, a circadian rhythm in larval release is expressed, with release occurring around the onset of light. In contrast to the rhythm expressed by crabs from a semi-diurnal location, crabs from a diurnal location showed a peak in larval release centered around noon, and few release events in the early morning.

1.3 Spawning migration of C. sapidus

Some time after mating, female blue crabs undertake a spawning migration to high salinity water (Millikin & Williams 1984, Tankersley et al. 1998, Hench et al. 2004)
where they release multiple clutches of larvae (Hines et al. 2003, Dickinson et al. 2006). In the Chesapeake Bay, where reproductive activity occurs from June-September, females spend approximately 2 months near their molting/mating habitat and do not begin to migrate until the fall (Turner et al. 2003, Aguilar et al. 2005). Mature females do not appear in the lower bay until mid- to late October, suggesting that regardless of mating time, females are cued to migrate by some environmental signal such as temperature or photoperiod (Turner et al. 2003). Similarly, Medici et al., (2006) tagged a total of 2700 mature female crabs from late June-October in the Pamlico Sound, Albemarle Sound, and Neuse River, NC and found that many females do not start migrating until late September or October. It appeared as though females migrate somewhat synchronously, regardless of when they mate, and the authors hypothesized that the majority of crabs do not spawn until the following spring (Medici et al. 2006). Such a delay in migration following mating allows females to forage and allocate energy to muscle growth, ovarian development, and hepatopancreas reserves necessary for the migration and subsequent clutch production (Turner et al. 2003).

Ovigerous females (Tankersley et al. 1998, Forward et al. 2003) and females between clutches of eggs (Hench et al. 2004, Forward et al. 2005a) use ebb tide transport (ETT) to migrate. ETT is a form of selective tidal-stream transport consisting of periods of vertical swimming during ebb tide, which move the crabs seaward with the falling tide. ETT in spawning female crabs is driven by an endogenous circatidal rhythm in
vertical swimming, in which females swim upward into the water column during ebb tide and remain on the bottom during flood tide (Forward et al., 2003). This rhythm is present in ovigerous female crabs during all stages of embryo development, in many crabs between clutches of eggs, and continues as crabs cycle through successive clutches (Forward et al. 2003, Hench et al. 2004, Forward et al. 2005a). It is not currently known when female blue crabs first express this circatidal rhythm. Although crabs are typically observed swimming at the surface only at night (Tankersley et al. 1998), it is likely that many females also swim into the water column during daytime ebb tides, though possibly at greater depths as crabs in the laboratory swim during ebb tide regardless of the diel cycle or lighting conditions (Forward & Cohen 2004).

Carr et al. (2004) tracked migrating female blue crabs using ultrasonic telemetry, and found that crabs moved seaward during both ebb and flood tides. During ebb tides, the mean difference between crab movement vector and the current vector was $1^\circ \pm 3^\circ$, indicating that crabs were moving in the same direction as the tidal currents. During flood tides, the mean difference was $187^\circ \pm 3^\circ$, indicating that crabs are swimming or walking against the tidal currents during flood tides (Carr et al. 2004). Using oriented walking or swimming during flood tide and episodic vertical swimming during ebb tide, crabs are able to migrate $\sim 5.4$ km day$^{-1}$ in the strongly tidal region around Beaufort Inlet (Carr et al. 2004). At this speed, a migrating female could complete migration from low salinity areas out of Beaufort Inlet in less than three days.
To date there have been few studies conducted on migratory behavior of female blue crabs in non-tidal locations, where the lunar-tidal cycle is negligible. The mechanisms underlying the spawning migration in these areas, as well as differences in behavior and distribution between tidal and non-tidal estuaries remains unclear. A circatidal swimming rhythm would not be an effective transport mechanism in a non-tidal estuary. In tidal systems, it appears that changes in pressure may be the cue that synchronizes the circatidal rhythm and the tidal cycle. Peaks in ascents occur primarily when water level, and thus pressure, are falling (Hench et al. 2004). While a non-tidal estuary lacks a predictable cycle of pressure changes due to tides, decreasing water level could still function as a cue for an outgoing current and could cue a migrating crabs to swim vertically and take advantage of the seaward currents.

1.4 Organization and content of the dissertation

Each chapter of this dissertation is written as an independent manuscript. As a result, some introductory material is repeated. Chapter topics are as follows:

Chapter 2. Lifetime reproductive potential of female blue crabs, *Callinectes sapidus*, in North Carolina. This chapter has been published in Marine Ecology Progress Series (Darnell et al. 2009). With the exception of formatting changes, is included in the form it was accepted.

Chapter 3. Role of peptide pheromones in larval release and migratory behavior of blue crabs
Chapter 4. Endogenous swimming rhythms underlying ebb tide transport during the blue crab spawning migration

Chapter 5. Swimming behavior of ovigerous blue crabs in relation to local hydrologic variables

Chapter 6. Large-scale movements of female blue crabs in tidal and non-tidal estuaries

A Synthesis chapter appears at the end of the dissertation to integrate the results and conclusions of the preceding chapters.
2. Lifetime reproductive potential of female blue crabs, *Callinectes sapidus*, in North Carolina

2.1 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 (CBP 1997, Lipcius & Stockhausen 2002, Eggleston et al. 2004) and protection of the spawning stock is a common management strategy. To date, however, management decisions are 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 one to two days 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 days after the terminal molt (Rittschof et al., unpublished data).

Following mating, the male typically continues to carry the female in the postcopulatory embrace for 1-3 days (Van Engel 1958, Jivoff 1997). This post-copulatory embrace serves to reduce the risk of predation and sperm competition (Jivoff 1997). Thus, the duration
of the post-copulatory 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, 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 (Van Engel 1958, Hines et al. 2003). Wolcott et al. (2005) found that the number and viability of sperm transferred during mating is 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. 2005a) using both ebb tide transport and directed walking during flood tides (Forward et al. 2003, Carr et al. 2004). Spawning females produce multiple clutches of eggs 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. 2005a).

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, 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 per year from previously published studies. Number of broods per year ranged from 1-10, with an average of 3.1 broods per year. With the exception of the Majidae, which averaged 5.9 clutches per year, most species were estimated to produce <3 clutches per year (Hines 1982).

In addition to blue crabs, other species of Portunidae are also able to produce multiple clutches (Ingles & Braum 1989, Haddon & Wear 1993, 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 1-3 clutches of eggs (de Lestang et al. 2003), the New Zealand paddle crab *Ovalipes catharus* producing 1-5 clutches (Haddon & Wear 1993), and the swimming crab *Arenaeus cribrarius* producing up to six 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). Larger crab species, however, 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 six clutches of eggs 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. 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 seven clutches of eggs 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. Each study, however, monitored crabs that
were collected while already mature or ovigerous. Thus, it is possible that these crabs may have produced one or more clutches of eggs 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 crab, 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 two 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 ($T_{\text{min}}$), and are calculated by summing the differences between the daily temperature means and $T_{\text{min}}$ 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.

2.2 Methods

2.2.1 Collection of animals

In each of two years (2006-2007), female blue crabs were collected by hand at night during the spawning season (May-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. 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 three 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 1-2 days (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 three days prior to capture, 10 were somewhat more (~50%) calcified and assigned a mating date of five days prior to capture, and 5 were almost fully (~75%) calcified and were assigned a mating date of seven days 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.

### 2.2.2 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 cm × 23 cm) buried halfway into the sediment on their long axis in the immediate
subtidal at the Duke University Marine Lab, approximately 0.5 km from the collection site. 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 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 seven days (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.

2.2.3 Clutch quality analysis

Beginning in August 2007, clutch quality was assessed for each clutch produced, using four 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-posterior), width, and depth (dorsal-ventral) of the sponge. Egg diameter was measured for four samples, one 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 48-8 hours 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 four samples of 20 eggs from each clutch, at the same time as the egg diameter measurements. The percentage of embryos in each of the four samples developing normally were averaged to calculate a mean percentage developing normally for each clutch. Lipid content of early-stage eggs (50-100% yolk) was determined using the colorimetric sulphophosphovanillin method (Barnes & Blackstock 1973). Pre-weighed, lyophilized samples of eggs from each clutch were analyzed and percentage of lipids by weight was calculated.

Larval viability was assessed for each clutch using two 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 1-3 days after placement in the bucket. Water was changed daily for crabs held in the bucket for more than 24 hours. Within 6 hours 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 L:D cycle. Swimming ability was assessed approximately every 12 hours by capping and inverting the tubes, stimulating swimming behavior. Preliminary investigation indicated that death typically followed within 12 hours 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.

2.2.4 Data analysis

Water temperature data were extracted from the NOAA NOS station BFTN7 at the Duke University Marine Lab in Beaufort, NC (NOAA 2008). 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 $T_{\text{min}}$. This estimate is the mean of previously reported temperatures at which growth and/or feeding cease in *C. 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 clutch\(^{-1}\). 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.

### 2.3 Results

#### 2.3.1 Size at maturity

Female crabs used in this study ranged from 86-174 mm (mean = 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 (Figure 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, \( p = 0.181 \)).

![Graph showing relationship between carapace width and water temperature](image)

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

### 2.3.2 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 one 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 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 one month after the terminal molt.

2.3.3 Clutch production

Timing of clutch production varied depending on mating season. Crabs mating in the summer months (May-August) began spawning the same season, 23-82 d after mating (Figure 2a). One crab of four that mated in August and survived to spawn 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 Figure 2. Spawning during two 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-October) did not begin producing eggs until the following April or May (218-240 d after mating), and spawned only during a single season (Table 1).
Table 1: Predicted relationships between production of first clutch, years spawning, and year of death for different mating seasons.

<table>
<thead>
<tr>
<th>Mating season (year t)</th>
<th>Production of first clutch</th>
<th>Years spawning</th>
<th>Year of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>spring</td>
<td>summer, year t t</td>
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<tr>
<td>summer</td>
<td>fall, year t t +1</td>
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<tr>
<td>fall</td>
<td>spring, year t+1</td>
<td>t+1</td>
<td>t+1</td>
</tr>
</tbody>
</table>

Physiological time to first clutch averaged $747.1 \pm 34.3$ 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 (Figure 2b). The regression was not significantly improved by fitting separate lines to summer- and fall-mated crabs.
Figure 2: Time to first clutch in (a) days and (b) degree-days plotted against carapace width (mm).
Production of multiple clutches was common for crabs used in this study (Figure 3). 94.3% (33 of 35 crabs) of crabs produced at least two clutches of eggs 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 that survived longer produced more clutches. Crabs that spawned during two 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.

![Histogram of lifetime clutch production.](image)

**Figure 3: Histogram of lifetime clutch production.**

Clutch production interval was positively correlated with carapace width. The relationship was strongest for crabs spawning during a single season (linear regression, \( r^2 = 0.602, p < 0.001 \)), though the significant relationship persisted when crabs spawning during two seasons were included in the analysis (linear regression, \( r^2 = 0.124, p = \))
Clutch production interval averaged $262.6 \pm 9.1$ degree-days clutch$^{-1}$ (30.1 $\pm$ 2.9 d clutch$^{-1}$).

![Figure 4: Clutch production interval (CPI, degree-days clutch$^{-1}$) plotted against carapace width (mm) for crabs (a) crab spawning during one season and (b) crabs spawning during two seasons. Best-fit lines have been added.](image)

### 2.3.4 Mature lifespan

High mortality was seen during the first 30 days after mating. Mature lifespan ranged from 59 to 394 d after the terminal molt (Figure 5). Mean lifespan from terminal molt to death was $134.76 \pm 15.09$ d (1092.4 $\pm$ 101.64 degree-days). For crabs surviving to spawn, mean mature lifespan was $249.11 \pm 18.63$ d (1986.6 $\pm$ 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 two seasons were significantly larger than crabs that survived one season (t-test, $p = 0.038$)(Table 1).
2.3.5 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, mean ± SEM), larval carapace width (278.4 ± 4.5 μm, mean ± SEM), and larval survival time without food (3.4 ± 0.2 d, mean ± SEM) were similar for all clutches (GLMM, p > 0.05)(Figure 6) and were not significantly correlated with carapace width (GLMM, p > 0.05). The percentage of embryos developing normally (82.1 ± 4.1%, mean ± SEM) decreased with increasing clutch number, decreasing approximately 41% from clutch 1 to clutch 4 (Figure 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 cm³, mean ± SEM) increased with
increasing crab size and decreased with increasing clutch number (Figure 6a). 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 clutch 1 (Tukey HSD, \( p = 0.019 \)).
Figure 6: Measurements of clutch quality and larval fitness (mean ± SEM) for clutches 1-5: (a) clutch volume (cm³), (b) percentage lipids by weight of early-stage eggs, (c) percentage of embryos developing normally, (d) egg diameter (µm), (e) larval carapace width (µm).
2.4 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 1-2 seasons, and produced up to seven 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 in Dickinson et al. (2006). In the absence of fishery-dependent mortality, egg predation, parasitism, or predation, we predict that most female blue crabs in North Carolina produce between 3-7 clutches of eggs 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 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 (Figure 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 clutch 5 (Figure 6a). The first three clutches were similar in size and normal development, and decreases in these two measurements did not occur until clutch 4 (Figure 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 70-85% of a female crab’s reproductive output is from the first three clutches of eggs 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 over a year 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 weeks after mating, either through loss or degradation of dead sperm. None of the crabs used by Wolcott et al. (2005) spawned in this 12 week interval. Decreasing sperm viability with sperm age, generally evidenced by decreased fertilization potential or motility, has been demonstrated in numerous taxa including molluscs (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 and Courtney (1984) and Begon and 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. This condition is not met by blue
crabs, which 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 still 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 (Figure 5a), it appears that female blue crabs produce up to three 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 the same year. Those beginning spawning in the late summer frequently spawned during two years. Crabs mating in the fall (September to November) did not spawn until the following year and usually spawned in only a single year (Figure 2a, Table 1). The single regression line fit to the degree-day data (Figure 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 two 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 (Figure 5a). Expressing lifespan in degree-days resulted in a more unimodal lifespan distribution (Figure 5b), again suggesting that temperature is an important determinant of lifespan. Crabs that matured from July-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 survive two 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 North Carolina (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 lifespan range from 1-4 years (Chesapeake: Churchill 1919, Van Engel 1958, Florida: Tagatz 1968, North Carolina: Judy & Dudley 1970). In this 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 of 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 North Carolina have a similar lifespan to those confined in this study, surviving less than 2 years 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 a free-ranging crab's lifetime. 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), though 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 for many exploited species, including blue crabs, is lacking. 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.
3. Role of peptide pheromones in larval release and migratory behavior of blue crabs

3.1 Introduction

Larval release in many brachyuran crabs is precisely timed to correspond with tidal, diel, or lunar cycles (Saigusa & Hidaka 1978, Devries et al. 1983, Forward 1987, Morgan & Christy 1995, Tankersley et al. 1995, Saigusa & Kawagoye 1997). Fertilized eggs are attached to the pleopods, where they develop for days to months. Releasing larvae during favorable phases of these environmental cycles can decrease predation pressure on both larvae and females (DeCoursey 1981, Christy 1982, Morgan 1990, Morgan & Christy 1995, Hovel & Morgan 1997, Morgan & Christy 1997), maximize the chances of transport to appropriate nursery grounds (Saigusa & Hidaka 1978, Christy 1982, Morgan & Christy 1995), avoid larval stranding (Saigusa 1981, Christy 1986), and decrease physiological stress on the larvae (Saigusa 1981, Forward et al. 1982). Larval release is often highly synchronous within a given release event, lasting only a few minutes (e.g. Saigusa & Hidaka 1978, Forward et al. 1982, DeVries et al. 1991). At the time of larval release, the female elevates herself on her walking legs and vigorously flexes (pumps) her abdomen, releasing larvae relatively synchronously. Synchronous larval release can decrease the amount of time females are exposed to predators and can swamp potential larval predators (Morgan & Christy 1995)

Hatching synchrony in subtidal crabs is explained in a conceptual model, proposed by Forward and Lohmann (1983) for the estuarine crab Rhithropanopeus harrisii,
in which embryos control the timing of hatching and females synchronize hatching. At the time of hatching, a few eggs hatch, releasing pheromones that stimulate larval release behaviors (abdominal pumping) by the female. This abdominal pumping causes egg membranes to rupture, increasing the concentration of the pheromone, resulting in more abdominal pumping by the female. In this way, egg hatching is synchronized through a positive feedback loop.

Peptide pheromones released by hatching eggs are similar to peptide pheromones involved in other crustacean signaling systems, including hermit crab shell signaling and barnacle settlement (reviewed by Rittschof 1993, Rittschof & Cohen 2004). Larval release pheromones appear to be short peptides, <500 Daltons in size, with one or more neutral amino acids (Gly, Cys, Leu, etc.) preceding arginine or lysine at the carboxyl-terminus (Rittschof et al. 1985, Forward et al. 1987, Rittschof et al. 1989, Rittschof & Cohen 2004). Solutions of water in which eggs had hatched or water in which eggs had been homogenized induce larval release behaviors in a number of crabs (e.g. Forward & Lohmann 1983, DeVries et al. 1991, Tankersley et al. 2002). Peptide pheromone mimics with the correct carboxyl-terminus sequence have also been shown to be biologically active in larval release behaviors (reviewed by Rittschof & Cohen 2004). In all cases, however, it appears that there are other molecules, in addition to peptides, that are involved, as pure peptides typically induce much weaker responses than natural larval release pheromones (Rittschof et al. 1985, Rittschof & Cohen 2004).
Proteolytic enzymes also play a role in crustacean egg hatching. Proteolytic enzymes are released from the egg mass around the time of larval release of *Neopanope sayi* (DeVries & Forward 1991) and *Sesarma hematocheir* (Saigusa 1996, Saigusa & Iwasaki 1999, Gusev et al. 2004). Incubation of ovigerous females in exogenous trypsin stimulates abdominal pumping in the crab *R. harrisii* (Rittschof et al. 1990) and the spiny lobster *Panulirus argus* (Ziegler 2007). Rittschof et al. (1990) examined the responses of *R. harrisii* to exogenous trypsin and trypsin inhibitors. Increased abdominal pumping and premature loss of embryos and eggs were observed following treatment with trypsin. These results suggest abdominal pumping is stimulated by peptides generated by proteolytic cleavage of proteins in the egg membrane. Six trypsin inhibitors also stimulated increased abdominal pumping. The trypsin inhibitors tested were from three families of large protein trypsin inhibitors. The only common feature among these proteins was the ability to bind to the catalytic site of trypsin. Simulaneous treatment with trypsin and trypsin inhibitor did not stimulate abdominal pumping, indicating that the portion of the inhibitor molecules that stimulates abdominal pumping is the portion that binds with the trypsin catalytic site. Rittschof et al. (1990) hypothesized that the binding site of the pheromone receptor resembles the catalytic site of trypsin (Rittschof et al. 1990). Similar results were observed in the spiny lobster *P. argus* (Ziegler 2007).

To date, much of the work on brachyuran larval release pheromones has been focused on two Xanthid crabs (*R. harrisii*: Forward & Lohmann 1983, Rittschof et al.
1985, Forward et al. 1987, Rittschof et al. 1989, Rittschof et al. 1990, N. sayi: DeVries & Forward 1991, DeVries et al. 1991) and one Grapsid crab, *S. hematocheir* (Saigusa 1994, 1996, Saigusa & Iwasaki 1999, Gusev et al. 2004). Blue crabs, *Callinectes sapidus* Rathbun, present an interesting model system for the study of larval release pheromones. Blue crabs exhibit synchronous egg hatching, both across tidal cycles and within a given release event. Larvae are released around the times of morning high tides and the rhythm in larval release is endogenous in nature (Ziegler 2002). Within a larval release event, blue crabs synchronously release all larvae within a very short period of time (Tankersley et al. 2002, Ziegler 2002). Both water in which eggs have hatched and water in which eggs have been homogenized stimulate abdominal pumping in blue crabs (Tankersley et al. 2002), and blue crab egg hatching appears to follow the conceptual model of Forward and Lohmann (1983).

unpublished data), though the cues that initiate migratory behavior are unknown. Tankersley et al. (2002) speculated that a chemical cue, possibly the same pheromone(s) that stimulates abdominal pumping, may initiate the spawning migration.

The purpose of this study was to test the hypothesis that peptides, released from the egg mass, induce both larval release behavior and migratory behavior in ovigerous blue crabs. Abdominal pumping and swimming assays were conducted to test the responsiveness of ovigerous blue crabs to egg extract, a peptide pheromone mimic (bradykinin), and a serine protease (trypsin) which generates peptide pheromones within egg masses.

3.2 Methods

3.2.1 Definitions

The terminology of Giese and Pearse (1974) as used by DeVries and Forward (1991) and Forward and Lohmann (1983) will be used throughout. The term “embryo” refers to the developmental stages occurring within the egg. The term “egg” refers to the combination of egg membrane, developing embryo, and all other material enclosed within the egg membrane. The term “larvae” refers to the developmental stages that occur outside of the egg.

3.2.2 Collection and maintenance of crabs

Ovigerous blue crabs were captured at night around the time of low tide using dip nets in the Rachel Carson National Estuarine Research Reserve, Beaufort, NC.
(34°42.65’ N, 76°40.40’ W). Crabs were transported to the Duke University Marine Lab individually in buckets containing ~2.5 cm of estuarine seawater. Crabs were held in 250 gallon tanks with running, estuarine seawater and were fed seasonal fish (primarily pinfish, spot, and croaker) daily until use in assays. Each crab was used for either the swimming or pumping assay and was tested twice at each volume or concentration of one test solution, once during the time of flood tide the field and once during the time of ebb tide. Concentrations of each test solution were delivered in increasing order, beginning with the appropriate control. All crabs were used within 7 days of collections.

3.2.3 Preparation of test solutions

3.2.3.1 Egg extract

The first test solution was an extract of late-stage eggs homogenized in seawater (Forward & Lohmann 1983) and was used to determine if one or more substances generated by the eggs stimulate larval release and vertical swimming behaviors. Females possessing late-stage (black, stages 8-9 of Devries et al. 1983) eggs were collected as above and eggs were removed from the pleopods using forceps and a scalpel. Eggs were homogenized with a mortar and pestle in an equal volume of seawater filtered to remove particles > 5 μm. The homogenate was centrifuged at 15,000 rpm for 5 min and the supernatant was collected and frozen in 15 mL aliquots at -20°C until use. This procedure resulted in a concentration of ~19,000 eggs ml⁻¹. Filtered seawater was used as a control solution in the egg extract experiments.
3.2.3.2 Bradykinin

Bradykinin is a vertebrate peptide hormone with a neutral phenylalanine residue preceeding a basic arginine residue at the carboxyl terminus. Bradykinin is biologically active in a number of crustacean signaling systems, including barnacle settlement and crustacean larval-release behavior (reviewed by Rittschof & Cohen 2004). A stock solution of $10^{-3}$ M bradykinin (B3259, Sigma-Aldrich) in deionized water was prepared and frozen at -20°C in 1.5 mL aliquots until use. The stock solution was diluted to the test concentration ($10^{-8}$ to $10^{-6}$ M) in 5 μm-filtered seawater immediately before use. Filtered seawater was used as a control solution in bradykinin experiments.

3.2.3.3 Trypsin

Trypsin stimulates abdominal pumping in ovigerous mud crabs *Rhithropanopeus harrisi* (Rittschof et al. 1990) and ovigerous spiny lobsters *Panulirus argus* (Ziegler 2007). Test solutions were bovine trypsin (9820 BAEE units mg$^{-1}$, T8003, Sigma-Aldrich) in NaCl isotonic to seawater and 1 mM HCl. Two concentrations of trypsin were prepared: ~17,000 BAEE units mL$^{-1}$ and ~44,000 BAEE units mL$^{-1}$. These concentrations are similar to concentrations of trypsin found within the egg mass of crabs incubating late-stage embryos (Hinshaw et al. In Review). Trypsin solutions were stored in 15 ml aliquots at ~4°C until use. Control solutions used in trypsin experiments were filtered seawater and 0.1 mM HCl in an NaCl solution isotonic to seawater.
3.2.4 Behavioral assays

3.2.4.1 Abdominal pumping assay

The purpose of the abdominal pumping assay was to determine if the test solutions stimulate larval release behaviors (i.e. abdominal pumping, Forward & Lohmann 1983, Rittschof et al. 1985, Tankersley et al. 2002). Ovigerous crabs with eggs containing embryos in all stages of development were used in this assay. Each crab was held in a small plastic aquarium (30 cm × 18 cm) containing ~3 L of aerated, ambient estuarine water filtered to remove particles > 5 μm in diameter. To minimize potential responses of crabs to the experimenter, aquaria were illuminated continuously with dim red light, as blue crabs are insensitive to this wavelength based on their visual pigments (Cronin & Forward 1988).

Approximately 50 cm of 0.86 mm inner-diameter polyethylene tubing (PE/6 tubing, Scientific Commodities Inc., Lake Havasu City, AZ) was attached to the carapace using cyanoacrylate glue such that the one end of the tubing extended 2-3 mm into the egg mass and the other end extended outside of the aquarium. In this way, the test solutions could be delivered directly into the egg mass. Plastic-coated, 18-gauge copper wire was wrapped around the large lateral spines and used to provide support for the tubing and to hold the free tubing away from the crab, preventing the crab from pinching or becoming entangled in the tubing.

In the pumping assay, each crab was observed for two consecutive 3 min intervals. First, each crab was observed for 3 min and the number of abdominal pumps
recorded. A test solution was then delivered through the tubing into the egg mass over 5-10 s and pumping activity was quantified for up to 3 min more. A response was classified as positive if pumping activity was greater following delivery of the test solution than before delivery. Observation was ceased before the end of the 3 min time period if a positive response was observed. The before- and after- protocol was used to control for variations in baseline pumping activity with egg stage or time of day (Rittschof et al. 1989, Tankersley et al. 2002). Delivery volumes ranged from 10-500 μL for the homogenized eggwater solution. For the bradykinin and trypsin assays, delivery volume was held constant at 100 μL and different concentrations were tested.

3.2.4.2 Swimming assay

The purpose of the swimming assay was to determine if the test solutions stimulate vertical swimming behavior. Only crabs with stage 6-9 embryos (Devries et al. 1983) were used. Each crab was held in a large, transparent cylindrical tube (1.23 m tall × 44 cm diameter, Aquatic Eco-Systems model T8) containing aerated, ambient estuarine water filtered to remove particles > 5 μm in diameter and illuminated continuously with red light. Approximately 1.6 m of tubing was attached to the carapace as described for the abdominal pumping assay. Crabs were monitored using a video camera (Panasonic WV-BP330) and time-lapse recorder (Panasonic AG-RT850).

In the swimming assay, each crab was observed for 30 min. The number of ascents into the water column was recorded. An ascent was recorded when a crab swam
above the bottom 1/3 (~41 cm high) of the column. The test solution was then delivered through the tubing over 5 min, delivering 1/10 of the total delivery volume every 30 s. Ascents were then quantified for 30 min, including the 5 min of delivery time. A response was classified as positive if the number of ascents was greater following delivery of the test solution than before delivery. Delivery volumes ranged from 250-1500 μL for the homogenized eggwater solution. For the bradykinin and trypsin assays, 750 μL of $10^{-6}$ M bradkyin or 17,000 BAEE units mL$^{-1}$ trypsin was used.

### 3.2.5 Data analysis

For each test solution, 10-25 crabs were tested in each assay during the times of both ebb and flood tides. Crabs were held in constant conditions, however, and were not exposed to the tidal cycle. Thus, ebb tide and flood tide refers to the time of those tidal phases at the collection site. Proportion of crabs responding positively to each test solution was compared to the appropriate control using a $Z$ statistic for testing differences between two proportions (Walpole 1968). If egg extract assays indicated that there was no significant difference in the proportion responding positively between the times of ebb and flood tide, then either ebb or flood tide was randomly chosen for each crab and those responses were used in analyses.
3.3 Results

3.3.1 Pumping assay

3.3.1.1 Egg extract

The percentage of crabs responding positively with increased pumping to the seawater control solution was 40% during the time of ebb tide in the field and 26% during the time of flood tide in the field. Percentages responding positively to the egg extract solution varied from 57-75% during the time of ebb tide and 41-72% during the time of flood tide (Figure 7). Delivery of egg extract significantly increased abdominal pumping activity during the times of both ebb and flood tides. During the time of ebb tide, a significant increase in the proportion responding positively above the seawater control was observed at delivery volumes above 250 μL (n = 16, Z = 2.1, p < 0.05). During the time of flood tide, a significant increase was observed at delivery volumes above 100 μL (n=15, Z = 1.86, p < 0.05). Although the threshold volumes differed between ebb and flood tides, the proportions of crabs responding positively did not differ significantly between tidal phases (n = 15, Z = 0.36, p > 0.05). Thus, while each crab was tested in the bradykinin and trypsin assays on both tidal phases, only one response for each concentration from each crab, chosen at random, was used for analyses. Egg extract stimulated increased abdominal pumping in ovigerous crabs and responses did not differ between the times of flood and ebb tides.
Figure 7: Percentage of crabs increasing pumping following delivery of egg extract. * indicates proportions significantly different from seawater controls at $p < 0.05$.

3.3.1.2 Bradykinin

The percentage of crabs responding positively to the seawater control solution was 33.3%. The percentage responding positively to the bradykinin test solutions ranged from 44.4-89.4 (Figure 8). Delivery of $10^{-7}$ M bradykinin significantly increased abdominal pumping activity ($n = 17$, $Z = 2.96$, $p < 0.01$). The proportion responding positively to the highest concentration tested ($10^{-6}$ M) was not significantly different from the seawater control ($n = 17$, $Z = 1.26$, $p > 0.05$). Bradykinin stimulated increased abdominal pumping by ovigerous blue crabs when delivered at a concentration of $10^{-7}$ M.
Figure 8: Percentage of crabs increasing pumping following delivery of bradykinin. Numbers indicate sample sizes. * indicates proportions significantly different from seawater control at \( p < 0.05 \).

3.3.1.2 Trypsin

The percentage of crabs responding positively by pumping to the seawater control solution was 12.5% (Figure 9). The percentage of crabs responding positively to 0.1 mM HCl in an NaCl solution isotonic to seawater was 39.1%, significantly higher than the response to the seawater control (\( n = 23, Z = 1.82, p < 0.05 \)). The lower trypsin concentration (~17,000 BAEE units mL\(^{-1}\)) resulted in a 75.0% positive response, significantly higher than both the seawater (\( n = 16, Z = 3.56, p < 0.001 \)) and the HCl controls (\( n = 16, Z = 2.21, p < 0.05 \)). The higher trypsin concentration (~44,000 BAEE units mL\(^{-1}\)) resulted in 50.0% responding positively, significantly higher than the seawater control.
control (n = 16, Z = 2.28, p < 0.05), but statistically similar to the HCl control (n = 16, Z = 0.67, p > 0.05). Trypsin increased abdominal pumping by ovigerous blue crabs when delivered at concentrations above ~17,000 BAEE units mL\(^{-1}\).

![Graph showing the percentage increasing pumping of crabs following delivery of trypsin](image)

**Figure 9**: Percentage of crabs increasing pumping following delivery of trypsin. Numbers indicate sample sizes. * indicates proportions significantly different from seawater control and † indicates proportions differently from HCl control at p < 0.05.

### 3.3.2 Swimming assay

#### 3.3.2.1 Egg extract

Delivery of egg extract stimulated vertical swimming (Figure 10). Following delivery of filtered seawater, 25% and 24% of females responded positively during the times of ebb and flood tide, respectively. Following delivery of egg extract, the
percentages of positive responses ranged from 50-67% during the time of ebb tide and 46-66% during the time of flood tide. All volumes of egg extract tested (250 μL-1500 μL) stimulated swimming during both tidal phases, as the proportions of crabs responding positively were significantly (n = 11-24, Z > 1.72, p < 0.05) greater for all egg extract treatments than for the seawater controls. The proportion responding positively at the threshold volume (250 μL) was statistically similar between ebb and flood tides (n = 14, Z = 0.76, p > 0.05). Egg extract stimulated increased swimming in ovigerous crabs and responses did not differ between the times of flood and ebb tides. Thus, for bradykinin and trypsin assays, only one response for each concentration from each crab, chosen at random, was used for analyses.

Figure 10: Percentage of crabs increasing swimming following delivery of egg extract. * indicates proportions significantly different from seawater control at p < 0.05.
3.3.2.2 Bradykinin

Delivery of the filtered seawater control resulted in 18.18% responding. Delivery of $10^{-6}$ M bradykinin resulted in 35.3 responding positively (Figure 11). Though delivery of bradykinin resulted in increased swimming behavior, this increase was not statistically significant ($n = 17$, $Z = 1.21$, $p > 0.05$). Bradykinin did not stimulate increased vertical swimming behavior at the concentration tested.

![Figure 11: Percentage of crabs increasing swimming following delivery of bradykinin. Numbers indicate sample sizes.](image)

3.3.2.3 Trypsin

The percentage of crabs responding positively by swimming in response to the seawater control solution was 21.4%, while the percentage of crabs responding
positively to the HCl control solution was 16.7% (Figure 12). Delivery of 17,000 BAEE units mL\(^{-1}\) trypsin resulted in 30.0% responding positively. The proportions of crabs responding positively by swimming to the three solutions tested (filtered seawater control, HCl control, and trypsin) were all statistically similar (n = 14-20, Z < 1.33, \(p > 0.05\)). Trypsin did not stimulate increased vertical swimming behavior at the concentration tested.

![Graph showing percentage of crabs increasing swimming](image)

**Figure 12:** Percentage of crabs increasing swimming following delivery of trypsin. Numbers indicate sample sizes.

### 3.4 Discussion

The purpose of this study was to test the hypothesis that peptides, released from the egg mass, induce larval release behavior and swimming behavior in ovigerous blue
crabs. Egg extract, bradykinin, and trypsin were delivered into the egg mass of ovigerous females to assess the effects of these solutions on abdominal pumping and vertical swimming.

A conceptual model of egg hatching was proposed for *Rhithropanopeus harissii* by Forward and Lohmann (1983) and has since been supported by research on other subtidal crabs including *Neopanope sayi* (DeVries & Forward 1991, DeVries et al. 1991) and *C. sapidus* (Tankersley et al. 2002). In this model, the embryos determine the time of hatching while the female synchronizes hatching through a positive-feedback loop. Upon completion of development, at a time related to the diel and tidal cycles, a few eggs hatch, liberating larval release pheromones. These pheromones stimulate vigorous abdominal pumping by the female and this pumping ruptures more egg membranes, liberating more pheromones, which in turn stimulate more abdominal pumping. The process results in highly synchronous larval release in many crustaceans (Forward & Lohmann 1983, Forward et al. 1987, DeVries & Forward 1991, DeVries et al. 1991, Ziegler & Forward 2007).

Delivery of egg extract resulted in increased abdominal pumping (Figure 7), indicating that larval release pheromones can be generated from *C. sapidus* eggs. This result confirms the findings of Tankersley et al. (2002), indicating that delivery of the test solutions directly into the egg mass is a viable delivery method for the assays. The freeze-thaw cycle and subsequent homogenization liberates enzymes including serine
proteases (Rittschof 1980). After removal of large solids through centrifugation, these enzymes and their respective substrates remain in the extract, generating a variety of substances including larval release peptides.

The proportions of crabs responding with increased pumping following delivery of egg extract were similar during both tidal phases for all volumes tested. Threshold volumes differed during the two tidal phases, with a lower threshold volume during the time of flood tide. This suggests that female sensitivity to larval release pheromones may be greatest during flood tide. At higher delivery volumes, however, this difference in sensitivity was overcome (Figure 7). Forward et al. (2003) identified a circatidal rhythm in abdominal pumping, with maximum pumping occurring during the time of flood tide. This pumping rhythm may result from increased sensitivity to larval release pheromones during the time flood tide. Concentrations of larval release pheromones generated from the egg mass are unknown, and may be high enough to overcome the tidal variation in sensitivity. If that is the case, some other mechanism must generate the abdominal pumping rhythm. It is possible that the female circatidal activity rhythm, in which females are more active during ebb tides (Forward et al. 2003, Forward et al. 2005a) contributes to the abdominal pumping rhythm. Swimming into the water column, as well as walking along the bottom, would increase flow around the egg mass, possibly diluting the chemical cues to levels below that necessary to induce abdominal
pumping. During flood tides, when movement is decreased, flow around the egg mass would be decreased and higher concentrations of the pheromone would be present.

Bradykin (Figure 8) and trypsin (Figure 9) stimulated abdominal pumping compared to controls and the highest response to each was similar to the highest response to egg extract. This suggests that blue crab larval release pheromones includes one or more peptide generated from the eggs, with one or more neutral amino acids preceeding arginine or lysine at the carboxyl terminus, similar to the larval release pheromone in *Rhithropanopeus harissii* (Rittschof et al. 1989) and *Panulirus argus* (Ziegler 2007). Bradykinin has the correct amino acid sequence at the carboxyl terminus, with phenylalanine preceeding arginine, and is larval release pheromone mimic.

Trypsin is a serine protease that cleaves peptide bonds following a positively-charge amino acid (arginine or lysine), producing peptides with the correct carboxyl terminus sequence to stimulate larval release behaviors. Forward et al. (1987) assessed abdominal pumping responses of *R. harissii* to pure peptides, and found that all neutral-basic dipeptides and tripeptides induced a pumping response, at threshold concentrations of $1.5 \times 10^{-10}$ M (L-Leucyl-L-arginine) to $1.8 \times 10^{-6}$ M (Glycyl-L-lysine). In contrast, dipeptides with other combinations of neutral, acidic, and basic amino acids did not induce pumping responses (Forward et al. 1987).

Proteolytic enzymes released around the time of hatching have been identified in several crab species. DeVries and Forward (1991) demonstrated that proteolytic
enzymes are released from the eggs of *N. sayi* near the time of larval release. These enzymes, along with osmotic swelling of the eggs, cause the egg membranes to rupture. Proteolytic enzymes are also released from the eggs of *Sesarma haematocheir* around the time of hatching (Saigusa 1996), though the authors concluded that these enzymes do not dissolve the main components of the egg capsule. While these enzymes have not yet been identified, it is likely that digestion of the egg membrane by one or more of these enzymes would produce larval release pheromones, including peptides with the correct neutral-basic amino acid sequence. Saigusa (1994, 1996) also identified a second substance present in *S. haematocheir* hatch water that strips the egg attachment structures (investment coat, funiculus) from the ovigerous hairs following larval release. This substance, termed ovigerous hair-stripping substance (Saigusa 1996) has since been identified as a trypsin-like serine protease (Saigusa & Iwasaki 1999, Gusev et al. 2004) and its action would thus produce peptides with the correct amino acid sequence to stimulate abdominal pumping.

In addition to the three test solutions, delivery of 1 mM HCl resulted in increased abdominal pumping compared to filtered seawater, though the response was significantly lower than the response to trypsin (Figure 9). Abdominal pumping serves multiple purposes for ovigerous blue crabs. During larval release, abdominal pumping serves to synchronize egg hatching by mechanically rupturing the egg membranes (Davis 1968, 1981, Forward & Lohmann 1983, DeVries & Forward 1991). Abdominal
pumping occurs throughout embryo development (Tankersley et al. 2002) and serves to ventilate the egg mass, ensuring that embryos near the center of the egg mass receive adequate oxygen (Fernandez et al. 2002, Fernandez et al. 2006) and carrying away waste products. Tankersley et al. (2002) found that abdominal pumping increased as the embryos developed. This trend has also been observed for Neopanope sayi (DeVries et al. 1991) and is likely the result of increased respiration of more developed embryos. Increased respiration would result in decreased oxygen concentrations in the egg mass, which stimulates abdominal pumping in crabs (Fernandez et al. 2002, Fernandez et al. 2006). Increased respiration would also result in increased CO₂ production, which would decrease the pH of the water in and around the egg mass. I hypothesize that a pH decrease of the water in and around the egg mass may serve as a cue for abdominal pumping. Delivery of the HCl solution would similarly decrease the pH, simulating increased embryonic respiration and stimulating abdominal pumping. Thus, while delivery of HCl did stimulate abdominal pumping, this response was likely based on the egg-maintenance function of the behavior rather than the larval release function.

Delivery of egg extract also stimulated vertical swimming (Figure 10), indicating that some substance present in or derived from the eggs stimulates migratory behavior. As was the case with the abdominal pumping assays, female sensitivity to egg extract did not vary with tidal phase (Figure 10). Delivery of bradykinin (Figure 11) and trypsin
(Figure 12) both resulted in slight increases in vertical swimming, though these
difference were not significant.

Although it is clear that substances present in or derived from from the egg mass
stimulate swimming behavior, the identity of these substances is unknown. Neutral-
basic peptides alone did not stimulate increased vertical swimming as bradykinin and
trypsin failed to induce a significant response. I hypothesize that other compounds, in
addition to neutral-basic peptides, are necessary to induce vertical swimming. In
addition to peptide receptors, crustaceans possess receptors for detecting other
hydrolysis products including sugars (Forward & Rittschof 1999, Rittschof & Cohen
2004). In addition to trypsin-like serine proteases, amylase and lysozyme are also
produced and released by ovigerous females (Hinshaw et al. In Review). Amylase and
lysozyme act on glycosidic bonds, cleaving carbohydrates and producing sugars. These
sugar molecules may play an important role in stimulating swimming behavior.

Ovigerous blue crabs possess a circatidal rhythm in vertical swimming, with
peak swimming occurring during ebb tides (Forward et al. 2003, Forward et al. 2005a).
This behavior is the basis for ebb tide transport and the seaward spawning migration.
These results indicate that some substance present in or derived from the eggs is
responsible for stimulating this swimming behavior. Responses did not, however, differ
between the times of ebb and flood tides (Figure 10), indicating that female sensitivity to
these compounds is constant throughout the tidal cycle. Thus, the swimming rhythm
may arise from rhythmic release of enzymes from the female or, less likely, from rhythmic release of peptides or enzymes from the eggs.

I hypothesize that a blend of molecules produced through enzymatic digestion of the egg membranes and the glue that attaches the eggs to the pleopods stimulate vertical swimming in ovigerous *C. sapidus*. Upon production of the first clutch of eggs, enzymatic digestion of the egg membranes and glue begins to produce the these compounds and stimulate vertical swimming behavior. The seaward spawning migration thus begins. Following release of a clutch of eggs, some of the glue that had been used to attach the eggs as well as remnants of the egg membranes remains on the pleopods for several days to several week. Continued hydrolysis of the residual residual glue and egg remnants continues to release the compounds, though signal production decreases over time. Thus, the rhythm persists for many crabs between clutches of eggs, though not as strongly as during clutches.
4. Endogenous swimming rhythms underlying ebb tide transport during the blue crab spawning migration

4.1 Introduction

Marine organisms often use tidal currents to enhance migratory ability or decrease the energetic cost of migrating. Selective tidal stream transport is a common mechanism used by many marine organisms for horizontal transport (reviewed by Forward & Tankersley 2001). Selective tidal-stream transport (STST) occurs when an organism ascends into the water column during one phase of the tide and is transported by tidal currents. During the opposite tidal phase, the organism remains on or near the bottom (Forward & Tankersley 2001). Using STST, small organisms such as larvae are able to use tidal currents for long-distance migrations, while larger organisms such as crabs and fish save substantial amounts of energy compared to active migration against tidal currents (Metcalfe et al. 1990).

Selective tidal-stream transport can be described as either ebb tide transport (ETT) or flood tide transport (FTT), depending on the timing of vertical migration into the water column. The behavior underlying STST is a tidal vertical migration into the water column during one phase of the tide. While this behavior can be in response to exogenous environmental cues (e.g. Welch et al. 1999), it is often driven by an endogenous circatidal rhythm (Cronin & Forward 1979, Zeng & Naylor 1996, Forward & Tankersley 2001, Lopez-Duarte & Tankersley 2007). Species inhabiting different habitats at different life history stages may undergo ontogenic changes in the direction or
mechanism of STST. Because STST is driven by endogenous rhythms in vertical swimming or vertical migration in many species, studies of ontogenic changes in these rhythms can provide valuable information on the movements of these species.

Blue crabs have a complex, migratory life history. Different life stages inhabit a variety of estuarine and offshore habitats and migration between these habitats takes place using a number of mechanisms, including FTT and ebb tide transport (ETT). Larvae are released in the lower estuaries and coastal ocean (Millikin & Williams 1984). After hatching, zoeae are transported offshore where they develop throughout the zoeal stages. Zoeae are transported passively by currents, with movement offshore determined primarily by the time of their hatching and release.

Following development through the zoeal stages, larvae metamorphose into megalopae, which migrate back into the estuaries where they settle into nursery habitats and metamorphose into juveniles (Heck & Thoman 1984, Orth & Van Montfrans 1987). The megalopal migration onshore takes place using two mechanisms. While offshore, the megalopae exhibit a circadian rhythm in vertical swimming in which they remain near the surface during the day and near the bottom at night (McConaugha 1988a, Forward et al. 1997). This rhythmic behavior serves to enhance shoreward transport by wind-driven surface currents. Once inside the estuary and exposed to estuarine chemical cues, the circadian swimming rhythm is suppressed and transport takes place via FTT driven by environmental cues (increases in salinity, turbulence)(Forward & Rittschof

Adults mate in the low-salinity waters of upper estuaries (Millikin & Williams 1984). Some time after mating, females undertake a spawning migration to high-salinity waters of the inlets and coastal ocean, during which multiple clutches of larvae are released (Tankersley et al. 1998, Forward et al. 2003, Dickinson et al. 2006, Darnell et al. 2009). Ovigerous females (Tankersley et al. 1998) and females between clutches of eggs (Hench et al. 2004, Forward et al. 2005a) use ETT to migrate. ETT consists of periods of vertical swimming during ebb tide, which move the crabs seaward with the falling tide.

While FTT in blue crab megalopae is driven by behavioral responses to environmental cues, ETT in spawning female crabs is driven by an endogenous rhythm in which females swim upward into the water column during ebb tide and remain on the bottom during flood tide (Forward et al. 2003). This rhythm is present in ovigerous female crabs during all stages of egg development, in many crabs between clutches of eggs, and continues as crabs cycle through successive clutches (Hench et al. 2004, Forward et al. 2005a). The rhythm takes one of two forms: a circatidal rhythm with a period of ~12.4 h or a circalunidian rhythm with a period of ~24.8 h (Forward et al. 2003,
Forward et al. 2005a). Crabs with a circatidal rhythm have one peak in swimming each tidal cycle, during ebb tide. Crabs with a circalunidian rhythm have one peak in swimming every lunar day, corresponding to every other ebb tide. While the movements and behaviors of ovigerous crabs have been examined extensively (Forward et al. 2003, Carr et al. 2004, Hench et al. 2004, Forward et al. 2005a), little is known about the movements and behaviors of adult crabs prior to oviposition. Information is lacking on the ontogeny of the circatidal rhythm in vertical swimming that drives the blue crab spawning migration and at what stage the circatidal rhythm is expressed and ETT begins.

Because blue crabs use STST for the spawning migration, vertical swimming behavior should be synchronized to the tidal cycle in the home estuary. The tidal regime, however, is vastly different over much of the blue crab’s range, with semi-diurnal tides along the majority of the east coast of the United States and South America, diurnal tides in the Gulf of Mexico, mixed tides in the Caribbean (Garrison 2005), and some estuaries which are essentially non-tidal (Roelofs & Bumpus 1953, Luettich et al. 2002). To date, all studies of the circatidal swimming rhythm in ovigerous blue crabs have been conducted in an estuary with semi-diurnal tides (Forward et al. 2003, Forward & Cohen 2004, Forward et al. 2005a), and the behavior of ovigerous crabs from other tidal regimes, including estuaries with negligible tidal cycles, remains unknown.
The purpose of this study was to examine endogenous rhythms in vertical swimming of female blue crabs that underlie ebb tide transport. Specific objectives were as follows:

1. Assess the ontogeny of the circatidal swimming rhythm by examining vertical swimming behavior of multiple adult life-history stages.

2. Analyze endogenous swimming rhythms in ovigerous blue crabs from three different tidal regimes: semi-diurnal, diurnal, and non-tidal.

### 4.2 Methods

To examine the ontogeny of the circatidal rhythm in vertical swimming that underlies ebb tide transport during the spawning migration, vertical swimming behavior was monitored under constant conditions for: (1) immature females > 50 mm carapace width prior to initiation of physical changes related to the terminal molt, (2) females after the terminal molt but not yet showing visible mature ovaries, (3) females showing mature ovaries but not yet ovigerous, and (4) ovigerous females. Ovigerous females were tested both with- and without food to determine if food availability altered the circatidal swimming rhythm. Recently-molted crabs were identified by incomplete calcification of the cuticle such that manual depression of the carapace below the large lateral spines was possible. Most of these crabs were within 14 d after the terminal molt. Crabs showing mature ovaries were identified by the orange
crescent visible at the base of the lateral spines. Ovigerous crabs were identified by the large, external egg mass.

Crabs were collected from the Rachel Carson National Estuarine Research Reserve, Beaufort, NC (34°42.65′ N, 76°40.40′ W) which has semi-diurnal tides. Crabs were transported individually to the Duke University Marine Laboratory in buckets containing 5-10 cm of ambient estuarine water. Crabs that had not produced a clutch of eggs were obtained by collecting mating pairs from the same location. After mating was complete, females were confined individually in minnow traps buried half way into the sediment on their long axis in the high subtidal using established procedures (Dickinson et al. 2006, Darnell et al. 2009) until mature ovaries were visible. Ovigerous crabs were collected by hand in the Reserve.

To analyze endogenous rhythms in spawning female crabs from different tidal regimes, vertical swimming behavior was observed for ovigerous females collected from: (1) St. Andrews Bay, FL (30° 8.95′ N, 85°42.79′W), and (2) South River, NC (34° 57.37′N, 76° 34.48′W). St. Andrews Bay is located near Panama City, FL and has a diurnal tidal cycle with a tidal range of approximately 0.5 m. South River is is a primarily wind-driven system with a very weak lunar tidal cycle (Roelofs & Bumpus 1953).

Crabs with early-stage eggs from St. Andrews Bay, FL were collected by hand with dip nets during nocturnal low tides. Individual crabs were confined in plastic
minnow traps near the collection site until transport. Crabs were transported individually to the Duke University Marine Laboratory by truck in perforated, plastic containers within large coolers of aerated seawater from the collection site. Transport time was 15-18 h, and water in the coolers was changed approximately half way into the trip. Water temperature in the coolers was maintained near the ambient temperature at the collection site using sealed containers of ice as necessary.

Females with mid- to late-stage eggs from South River were collected in crab pots with the assistance of local commercial crabbers. Crabs were transported individually to the Duke University Marine Laboratory in perforated, plastic containers within a large cooler of aerated seawater from the collection site. Transport time was ~0.75 h and water was not changed during transport.

Each set of crabs was collected in multiple batches, as the number of columns in which crabs that could be monitored simultaneously were limited. Collection trips occurred throughout the summer months, and when possible, were timed such that the phasing between the tidal cycle and the diel cycle varied. This procedure ensures that a circadian and circatidal rhythm can be differentiated.

Endogenous rhythms in vertical swimming were analyzed for at least 10 females from each life history stage and collection site using the methods described by Forward et al. (2003). Crabs were placed individually into transparent vertical columns (1.22 m x 30.5 cm diameter, Aquatic Eco-Systems, Inc. model T4), filled with estuarine water
diluted to the salinity of the collection site. The water was filtered to remove particles > 5 μm and aerated for the duration of the experiment. Columns were continuously illuminated with dim (20-30 lux) red light. Blue crabs are insensitive to this wavelength, so red light approximates constant darkness (Cronin & Forward 1988). Vertical swimming behavior was monitored for 5-6 d and recorded with a video camera (Panasonic WV-BP330) and time-lapse video recorder (Panasonic AG-RT850). With the exception of six ovigerous crabs from the Beaufort Inlet drainage, crabs were not fed for the duration of the experiment. The six ovigerous females tested with food were fed 10 fiddler crabs *Uca pugilator* at the initiation of monitoring and 6 *U. pugilator* daily thereafter, at haphazard times. Vertical swimming behavior was quantified by counting the number of ascents per 30 minute interval. A swimming bout was considered an ascent if the crab ascended 40 cm above the bottom of the column.

Swimming behavior was analyzed for periodicity using autocorrelation and maximum entropy spectral analysis (MESA). If larval release occurred before the end of the monitoring period, monitoring was ceased, as Forward et al. (2003, 2005a) found that female blue crabs become arrhythmic following larval release unless re-entrained to the tidal cycle. Rhythmicity was assessed using autocorrelation, which plots the autocorrelation coefficients as a function of lag at 0.5 hour intervals. Peaks with autocorrelation coefficients exceeding ±2/√N, where N is number of 0.5 hour intervals, indicate statistical significance at p < 0.05 (Dowse & Ringo 1989). Period estimates were
obtained using MESA, which fits an autoregressive model to the data and uses Fourier analysis to construct a power spectrum, from which period estimates can be obtained (Levine et al. 2002). Peaks in the MESA spectrum were validated by comparison with peaks in the correlogram.

Rhythmic activity was compared to the expected tidal cycle at the collection site using cross-correlation, by plotting cross-correlation as a function of lag at 0.5 hour intervals. Peaks exceeding $\pm 2/\sqrt{N}$ are statistically significant ($p < 0.05$) and indicate the relationship between maximum activity and the time of high tide. Predicted tidal data were obtained from Tides & Currents Pro Version 3.3 (Nobeltech Corp., Portland, OR). Rhythmic activity was compared to the diel cycle using standard circular statistical techniques to determine mean times of activity for each crab.

Because the ranges of free-running period lengths for circalunidian (~24.8 h) and circadian (~24 h) rhythms often overlap, circular statistics were used to differentiate between the two types of rhythms when MESA produced a period estimate near 24 h. To test for a circadian rhythm, the mean time of activity for each crab was converted to an angle, with $0^\circ$ representing 00:00 (midnight) and $180^\circ$ representing 12:00 (noon). Rao’s spacing test was used to test the uniformity of the distribution of mean times of activity for each group of crabs (Batschelet 1981). A significant deviation from a uniform distribution suggests a circadian activity rhythm.
To test for a circatidal rhythm, the mean cross-correlation lag for each crab was converted to an angle with 0° representing high tide and 180° representing low tide. Rao’s spacing test was again used to test the uniformity of the distribution of mean crosscorrelation lags for each group of crabs. In this case, a significant deviation from a uniform distribution suggests a circalunidian activity rhythm.

Mean swimming frequency for each crab was determined by dividing the total number of ascents by the length of the observation period. Data were log-transformed to meet assumptions of normality and homoscedasticity. Swimming frequency was compared among life history stages, and among collection sites for ovigerous crabs, using analyses of variance (ANOVA), followed by Holm-Sidak multiple comparison tests when ANOVA indicated a significant difference at $p < 0.05$. A t-test was used to compare swimming frequency of ovigerous crabs with and without food.

4.3 Results

4.3.1 Juveniles

Eleven intermolt juvenile females from Beaufort, NC were tested for rhythmicity. Swimming frequency averaged $5.59 \pm 1.36$ ascents h$^{-1}$. Seven crabs displayed significant rhythmicity (Table 2). The remaining 4 crabs were arrhythmic. Distribution of period estimates was bimodal. Two crabs had period estimates in the circatidal range (~12.4 h). Period estimates for these crabs were 12.4 h and 13.0 h. Cross-correlation lags indicated
that peak activity took place 0 and 5.5 h after high tide, respectively. Mean time of peak activity was 00:00 and 14:41, respectively.

Figure 13: Actogram for representative juvenile female with circalunidian rhythm of swimming during the time of ebb tide. Top right panel is autocorrelation output, bottom right panel is MESA output.

Five crabs had period estimates in the circadian/circalunidian range (~24-24.8 h, Figure 13). Period estimates for these crabs averaged 24.44 ± 0.39 h. Cross-correlation analysis indicated that peak swimming occurred 1.24 ± 0.90 h after high tide. Times of peak swimming averaged 20:39 ± 1:40. Both cross-correlation lags and times of peak activity were significantly different from a uniform circular distribution (Rao’s spacing test, $p < 0.05$).

4.3.2 Recently-molted

Fourteen recently-molted and mated female crabs from Beaufort, NC were tested for rhythmicity. Swimming frequency averaged 1.61 ± 0.43 ascents h$^{-1}$. Thirteen
displayed significant rhythmicity and one was arrhythmic (Table 2). Distribution of period estimates was bimodal. Period estimates for 4 of the crabs were in the circatidal range and averaged 12.00 ± 0.48 h. Three of these crabs had cross-correlation lags of 0.5-1.5 h. The fourth crab did not swim during the first two days. Thus, a cross-correlation lag could not be calculated. Times of peak activity ranged from 10:23-23:21.

Period estimates for the remaining 9 crabs were in the circalunidian or circadian range and averaged 24.11 ± 1.06 h (Figure 14). Two of these did not swim during the first 2 d in the columns and were excluded from cross-correlation and circular statistical analyses. The distribution of peak swimming times, determined by cross-correlation analysis, was uniform with respect to the tidal cycle (Rao’s spacing test, $p > 0.05$). Times of peak swimming averaged 20:00 ± 1:25, and the distribution of times of peak swimming was significantly different from uniform (Rao’s spacing test, $p < 0.05$).
Figure 14: Actogram for representative recently-molted female with a circadian rhythm of swimming around the time of sunset. Top right panel is autocorrelation output, bottom right panel is MESA output.
Table 2: Summary of swimming rhythms observed. \( n \) is the number of crabs tested. Numbers in each rhythm column indicate the number of crabs with that type of rhythm, followed by the time of peak swimming.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Collection site</th>
<th>Tidal regime</th>
<th>( n )</th>
<th>Circatidal ((~12.4\ h))</th>
<th>Circlunadian ((~24.8\ h))</th>
<th>Circadian ((~24\ h))</th>
<th>Arrhythmic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile</td>
<td>Beaufort, NC</td>
<td>semi-diurnal</td>
<td>11</td>
<td>2, ebb tide</td>
<td>5*, ebb tide</td>
<td>5*, sunset</td>
<td>4</td>
</tr>
<tr>
<td>Recently-molted</td>
<td>Beaufort, NC</td>
<td>semi-diurnal</td>
<td>14</td>
<td>4, ebb tide</td>
<td>0</td>
<td>9, sunset</td>
<td>1</td>
</tr>
<tr>
<td>Mature ovaries</td>
<td>Beaufort, NC</td>
<td>semi-diurnal</td>
<td>12</td>
<td>5, high tide</td>
<td>2, high tide</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Ovigerous</td>
<td>Beaufort, NC</td>
<td>semi-diurnal</td>
<td>12</td>
<td>4, ebb tide</td>
<td>6, ebb tide</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Ovigerous, fed</td>
<td>Beaufort, NC</td>
<td>semi-diurnal</td>
<td>6</td>
<td>4, ebb tide</td>
<td>2, ebb tide</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovigerous</td>
<td>St. Andrews Bay, FL</td>
<td>diurnal</td>
<td>16</td>
<td>1, high tide</td>
<td>12, ebb tide</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ovigerous</td>
<td>South River, NC</td>
<td>non-tidal</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>9, sunset</td>
<td>2</td>
</tr>
</tbody>
</table>

*Ambiguous results. Rhythms in these 5 crabs may be circlunidian with swimming during the time of ebb tide or circadian with swimming around the time of sunset.
4.3.3 Mature ovaries

Twelve females with mature ovaries from Beaufort, NC were tested for rhythmicity. Swimming frequency averaged $1.67 \pm 0.37$ ascents h$^{-1}$. Seven exhibited significant rhythmicity, 3 were arrhythmic, and 2 did not swim during the observation period (Table 2). Distribution of period estimates was bimodal. Period estimates for 5 of the crabs were in the circatidal range and averaged $13.72 \pm 0.74$ h (Figure 15). Cross-correlation analysis indicated that peak swimming occurred $0.18 \pm 0.68$ h after high tide, and the distribution of cross-correlation lags was significantly different from uniform (Rao’s spacing test, $p < 0.05$). Times of peak swimming averaged $20:47 \pm 1:39$, though the distribution of times of peak swimming was not significantly different from uniform (Rao’s spacing test, $p > 0.05$).

Figure 15: Actogram for representative female with mature ovaries with a circatidal swimming rhythm of swimming around the time of high tide. Top right panel is autocorrelation output, bottom right panel is MESA output.
Period estimates for the remaining 2 crabs were in the circalunidian or circadian range and averaged $26.30 \pm 1.30$ h. Cross-correlation analysis indicated that peak swimming for both crabs occurred at the time of high tide (lag = 0 h). Times of peak swimming were 17:03 and 20:00.

### 4.3.4 Ovigerous from Beaufort, NC

Twelve ovigerous females collected from Beaufort, NC were tested for rhythmicity. This estuary experiences semi-diurnal tides. Swimming frequency averaged $2.84 \pm 1.21$ ascents h$^{-1}$. Ten exhibited significant rhythmicity while 2 were arrhythmic (Table 2). Distribution of period estimates was bimodal. Period estimates for 4 of the crabs were in the circatidal range and averaged $12.40 \pm 0.24$ h. Cross-correlation analysis indicated that peak swimming occurred $0.75 \pm 0.20$ h after high tide, and the distribution of cross-correlation lags was significantly different from uniform (Rao’s spacing test, $p < 0.05$). Times of peak swimming averaged $20:17 \pm 1:48$, though the distribution of times of peak swimming was not significantly different from uniform (Rao’s spacing test, $p > 0.05$).

Period estimates for the remaining 6 rhythmic crabs were in the circalunidian or circadian range and averaged $23.58 \pm 0.60$ h (Figure 16). Two of these crabs appeared to switch from a circatidal rhythm to a circalunidian/circadian rhythm. Cross-correlation analysis indicated that peak swimming occurred $1.48 \pm 0.77$ h after high tide, and the distribution of cross-correlation lags was significantly different from uniform (Rao’s spacing test, $p < 0.05$).
spacing test, $p < 0.05$). Times of peak swimming averaged 20:26 ± 1:25, and the
distribution of times of peak swimming was significantly different from uniform (Rao’s
spacing test, $p < 0.05$).

**Figure 16:** Actogram for representative ovigerous female from Beaufort, NC. This crab
switched from a circatidal rhythm to a circalunidian rhythm, with peak swimming
during the time of ebb tide. Top right panel is autocorrelation output, bottom right pane

### 4.3.5 Ovigerous from Beaufort, NC with food

A further six ovigerous crabs collected from the Rachel Carson National
Estuarine Research Reserve were tested for rhythmicity with food present. Swimming
frequency averaged 3.16 ± 0.70 ascents h⁻¹. All 6 displayed significant rhythmicity (Table
2). Distribution of period estimates was bimodal. Period estimates for 4 of the crabs were
in the circatidal range and averaged 12.63 ± 0.06 h (Figure 17). One of these crabs
appeared to switch from a circatidal rhythm to a circalunidian/circadian rhythm. Cross-
correlation analysis indicated that peak swimming occurred 0.49 ± 0.03 h after high tide, and the distribution of cross-correlation lags was significantly different from uniform (Rao’s spacing test, $p < 0.05$). Times of peak swimming averaged 18:40 ± 2:41, though the distribution of times of peak swimming was not significantly different from uniform (Rao’s spacing test, $p > 0.05$).

Figure 17: Actogram for representative ovigerous female from Beaufort, NC tested with food. This crab had a circatidal rhythm with peak swimming during the time of ebb tide. Top right panel is autocorrelation output, bottom right pane

Period estimates for the remaining 2 crabs were in the circalunidian or circadian range and averaged 25.30 ± 0.40 h. Cross-correlation analysis indicated that peak swimming for both crabs occurred 0.5 h after high tide. Times of peak swimming were 15:52 and 22:00.
4.3.6 Ovigerous from St. Andrews Bay, FL

Sixteen ovigerous females from St. Andrews Bay, FL were tested for rhythmicity. This estuary experiences diurnal tides. Swimming frequency averaged $3.44 \pm 0.86$ ascents h$^{-1}$. Thirteen crabs exhibited significant rhythmicity, while 3 were arrhythmic (Table 2). One of the rhythmic crabs had a period of 11.9 h, though this crab ceased swimming after 2 d. Thus, the accuracy of the period estimate for this crab is low, as accuracy increases with increasing time series length. Period estimates for the remaining 12 crabs were in the circalunidian or circadian range and averaged $25.82 \pm 0.54$ h (Figure 18). One of these did not swim during the first 2 d in the columns and was excluded from cross-correlation and circular statistical analyses. Cross-correlation analysis indicated that peak swimming occurred $9.94 \pm 1.01$ h after high tide, and the distribution of cross-correlation lags was significantly different from uniform (Rao’s spacing test, $p < 0.05$). Times of peak swimming averaged 18:23 ± 00:56, and the distribution of times of peak swimming was significantly different from uniform (Rao’s spacing test, $p < 0.05$).
Figure 18: Actogram for representative ovigerous female from St. Andrews Bay, FL. This crab had a circalunidian rhythm with peak swimming during the time of ebb tide. Top right panel is autocorrelation output, bottom right pane

4.3.7 Ovigerous from South River, NC

Eleven ovigerous crabs from South River, NC were tested for rhythmicity. This estuary does not experience lunar tides. Swimming frequency averaged 2.52 ± 0.66 ascents h⁻¹. Nine crabs exhibited significant rhythmicity, while 2 were arrhythmic (Table 2). Period estimates were in the circadian range and averaged 25.06 ± 0.28 h (Figure 19). Times of peak swimming averaged 19:59 ± 00:47 and the distribution of times of peak swimming was significantly different from uniform (Rao’s spacing test, \( p < 0.05 \)).
Figure 19: Actogram for representative ovigerous female from South River, NC. This crab had a circadian rhythm with peak swimming around the time of sunset. Top right panel is autocorrelation output, bottom right pane.

4.3.8 Comparisons of swimming frequency

Swimming frequency (ascents h⁻¹) varied significantly among life history stages (ANOVA, p = 0.021)(Figure 20). Swimming frequency was significantly higher for juvenile females than for recently-molted females or females with mature ovaries (Holm-Sidak, p < 0.05). Ovigerous females’ swimming frequency was intermediate, statistically similar to the 3 other stages (Holm-Sidak, p > 0.05). Swimming frequency did not vary significantly among collection locations for ovigerous females (ANOVA, p = 0.683)(Figure 21) and was similar between ovigerous females from Beaufort, NC both with and without food (t-test, p = 0.322).
Figure 20: Swimming frequency of female crabs at different life history stages from Beaufort, NC. Different letters indicate statistically significant differences at $p < 0.05$. 
4.4 Discussion

Endogenous rhythms in vertical swimming were present in all life history stages of crabs tested, as well as in crabs from each of the three collection sites. Female blue crabs from an area having semi-diurnal tides experience ontogenic changes in vertical swimming rhythms over the range of life-history stages tested. There was also variations in rhythm type and period within stages. These ontogenic changes in endogenous swimming rhythms have consequences for the distribution and movements of each life history stage.

Intermolt juvenile female crabs typically exhibited a circatidal or circalunidian rhythm with peak swimming occurring during the time of ebb tide, though 36% of crabs
tested were arrhythmic. Swimming frequency was the highest of any stage. For crabs with a circalunidian (~24.8 h) rhythm, there was also evidence of a circadian rhythm, as the times of peak swimming were not uniform relative to the diel cycle. Average time of peak swimming (20:39) was around the time of sunset (~20:30) at the collection site. Further study is necessary to determine if this rhythm is actually circadian or circalunidian. A circatidal circalunidian rhythm with swimming on ebb tides suggests that these crabs may be undergoing ebb tide transport and moving seaward. A circadian rhythm of swimming around the time of sunset would not result in net movement with the tides, but would allow movement within foraging areas and would reduce the risk of predation by diurnal visual predators.

Recently-molted females, tested within ~14 d of the terminal molt, displayed one of two rhythms. The majority (9 of 13 rhythmic crabs) exhibited a circadian rhythm with peak swimming occurring at ~20:00, very close to the time of sunset. The remaining 4 crabs exhibited a circatidal rhythm with peak swimming occurring during ebb tide. Thus, similar to juvenile females, recently-molted females have rhythms that are suggestive of either non-migratory movements within foraging areas or, to a lesser extent, ebb tide transport. Swimming frequencies were relatively low compared to juvenile females and ovigerous females. Mark-recapture studies of recently-molted female blue crabs indicate that following mating, females typically forage for a period of
weeks to months before beginning the seaward spawning migration (Turner et al. 2003, Aguilar et al. 2005).

Female crabs with mature ovaries typically displayed a circatidal or circalunidian swimming rhythm with peak swimming occurring around the time of high tide. There was no evidence of a circadian rhythm. Swimming frequencies remained low during this stage. Swimming centered around the time of high tide would result in no net movement with the tides, but could be used for movements during foraging.

Ovigerous female crabs from an estuary with semi-diurnal tides exhibited a very clear circatidal or circalunidian rhythms in vertical swimming, with peak swimming occurring during the time of ebb tide, and this rhythm was not altered in the presence of food. This rhythm of swimming on ebb tides is the basis for the spawning migration (Forward et al. 2003, Forward et al. 2005a), in which ovigerous female blue crabs migrate seaward from generally low-salinity mating areas to high-salinity areas where larvae are release. Qualitatively, the circatidal/circalunidian rhythm of ovigerous crabs was much more clearly defined than any circatidal or circalunidian rhythms observed in earlier life-history stages. Mean swimming frequency of ovigerous crabs was 70% higher than females with mature ovaries and 76% higher than recently molted females. The lack of a statistical difference in swimming frequency between ovigerous crabs and these two prior stages may be due to a relatively low sample size (11-14 for each stage). These
results suggest that the seaward spawning migration begins in earnest once a female becomes ovigerous.

There was some evidence of a circadian rhythm in ovigerous crabs, tested without food, with ~24 h periods, though this may be an artifact of the collection dates. These crabs were collected on June 6 and June 18, 2008. High tide on the collection dates occurred at 23:15 and 20:47, respectively. Thus, the relationship between the tidal and diel cycles were similar for both sets of ovigerous crabs tested. Previous studies of ovigerous blue crab swimming rhythm found no evidence of a circadian rhythm (Forward et al. 2003, Forward et al. 2005a).

Ovigerous blue crabs possess an endogenous rhythm in vertical swimming, though the type of rhythm present, and the period length of the rhythm, depend on the tidal regime at the collection site. Ovigerous female crabs collected from Beaufort, NC, where a semi-diurnal tidal cycle is present, exhibited circatidal rhythms in vertical swimming, with peak swimming occurring during ebb tides. Periods were variable, with crabs swimming either every ebb tide (circatidal rhythm) or every other ebb tide (circalunidian rhythm). Either swimming rhythm would move crabs seaward. Swimming on every ebb tide would result in a faster rate of movement, though crabs would be in the water column during daytime ebb tides as well as nighttime ebb tides, subjecting them to predation risk from diurnal visual predators. Swimming only on nocturnal ebb tides would reduce the predation risk by diurnal visual predators, but
would result in slower seaward movement. Circalunidian rhythms, i.e. those with periods of ~24.8 h, would also be more easily synchronized to the tidal cycle, as deviations from the single-cycle 12.4 h period are much greater than deviations from the dual-cycle 24.8 h period in nature (Palmer 1995).

Ovigerous females were tested both with and without food to determine if food availability affects swimming behavior. Ovigerous blue crabs tethered in the field in high-salinity areas display variable amounts of vertical swimming, depending on the tethering site. I hypothesize that in high-salinity (≥22 ppt, Rittschof et al., In Review), some areas may serve as foraging habitat while others may serve as migratory corridors, and that food availability may be one cue that contributes to determine swimming frequency. No obvious differences were apparent in the form, period, or amplitude of the circatidal rhythm between ovigerous crabs tested with food and those tested without food. Additionally, swimming frequencies were similar, indicating that food availability does not affect the circatidal swimming rhythm. Further tests of more females, and perhaps a greater amount of food, are necessary to confirm this observation, as sample size was low (n = 6).

Ovigerous females from St. Andrews Bay, FL displayed a circalunidian rhythm, with peak swimming occurring during ebb tides. Tides in St. Andrews Bay are diurnal, with a single ebb tide and single flood tide per lunar day. Thus, a circalunidian rhythm corresponds to one peak in swimming each tidal cycle, during ebb tide. Periods for all
but one of the rhythmic crabs averaged 25.8 h, indicating that the period of the circatidal rhythm approximates that of the tidal cycle in St. Andrews Bay. There was some evidence of a circadian rhythm in ovigerous crabs from St. Andrews Bay. Time of peak activity averaged 18:23 and the distribution of times of peak swimming was significantly different from a uniform distribution. I hypothesize that this is not due to a circadian swimming rhythm, but is instead an artifact of the relationship between the tidal and diel cycle at the collection site. Crabs from St. Andrews Bay were collected on June 13, June 25, and July 25, 2007. On those dates, the phasing between the tidal and diel cycles were similar, as high tide occurred at 08:26, 06:49, and 06:45, respectively. It is also possible, though unlikely, that these crabs possess a circadian rhythm in swimming that is used for seaward movement. During the months of June and July, 2007, the times of high tide in St. Andrews Bay ranged from 04:44 to 14:39 and averaged 09:39 ± 00:21. High tide generally occurs in the morning. A circadian rhythm of peak swimming in the late afternoon would, in general result in seaward movement, as that time of day generally corresponds to ebb tide.

Ovigerous females from the non-tidal South River exhibited circadian rhythms in vertical swimming with peak swimming occurring around the time of sunset. The time of peak swimming occurred at 19:59. The time of sunset during this component of the study was ~18:00. While the period of the circadian rhythm seen in South River crabs is similar to the period of the circalunidian rhythm seen in crabs from Florida, peak
swimming for South River crabs consistently occurred around sunset rather than during a specific tidal phase. Any seaward movement resulting from such a circadian rhythm would be due only to wind-driven currents and residual flow seaward at the surface of the estuary (Forward & Tankersley 2001). Thus, I hypothesize that seaward migration in non-tidal estuaries takes place using some method other than STST.

Differences in endogenous rhythms in crabs from different tidal regimes could result from either genetic differences between the areas, or more likely, from phenotypic plasticity in the rhythm. Genetic differences among different tidal regimes is unlikely, as blue crabs exhibit extremely high levels of genetic diversity and lack spatial population structure (McMillen-Jackson & Bert 2004). It is more likely that the clock mechanism in blue crabs is phenotypically plastic, such that it can be entrained to either semi-diurnal or diurnal tidal cycles, optimizing migratory behavior in each type of estuary. Phenotypic plasticity in rhythm form has been demonstrated in fiddler crab hatching (Weaver & Salmon 2002, Christopher et al. 2008) and activity (Barnwell 1968) rhythms. Translocation experiments between the two tidal regimes would provide further clarity on this hypothesis. I also hypothesize that the circadian rhythm seen in crabs from South River may be based on the same clock mechanism as the circatidal and circalunidian rhythms in crabs from tidal areas. Due to the lack of a tidal cycle, the rhythm is not entrained to tides, and the diel light:dark cycle takes over as the primary entrainment cue. While no direct evidence of single-clock control of both types of rhythms is
available for blue crabs, circumstantial evidence from a variety of other marine
organisms supports the single-clock hypothesis (Palmer 1990, 1995)

Although female blue crabs at each life history stage and from each collection site
had endogenous circadian or circatidal rhythms in vertical swimming in the laboratory,
further study is necessary to test these observations in the field. It is possible that what
manifests as a vertical swimming rhythm in the columns is actually a general locomotor
rhythm. In the columns used for this study, walking space is severely limited, so an
activity rhythm may manifest as a swimming rhythm. If that is the case, such a rhythm
would not necessarily result in horizontal transport. The presence of a vertical
swimming rhythm in ovigerous blue crabs has been confirmed by field observations of
tethered crabs (Hench et al. 2004) as well as observations of crabs swimming at the
surface during ebb tides (Tankersley et al. 1998, Hench et al. 2004). Observations of
female behavior in the field at the life history stages preceeding oviposition is necessary
to determine if the swimming rhythms of other stages persist outside of laboratory
conditions.
5. Swimming behavior of ovigerous blue crabs in relation to local hydrologic variables

5.1 Introduction

Female blue crabs, *Callinectes sapidus* Rathbun, undertake a spawning migration to high salinity water (Van Engel 1958, Tankersley et al. 1998) where they release multiple clutches of larvae (Hines et al. 2003, Dickinson et al. 2006, Darnell et al. 2009). Larvae are transported passively by currents, with movement offshore determined primarily by the time and location of larval release. The female spawning migration moves females into the lower estuaries and coastal ocean where salinity is suitable for larval survival and the chances of larvae being transported offshore are high.

In tidal estuaries, ovigerous females (Tankersley et al. 1998, Forward et al. 2003, Forward et al. 2005a) and females between clutches of eggs (Forward et al. 2005a) use ebb tide transport (ETT) to migrate. ETT is a form of selective tidal-stream transport consisting of episodic vertical swimming during ebb tide and remaining on the bottom during flood tide. This results in stepwise movements seaward on each falling tide. This pattern of vertical swimming during ebb tide continues when crabs are monitored under constant laboratory conditions, in the absence of any tidal or cues, indicating that ETT in spawning female crabs is driven by an endogenous circatidal rhythm (Forward et al. 2003). This rhythm is present in ovigerous female crabs during all stages of embryo development (Forward et al. 2003, Forward et al. 2005a) and in many crabs between clutches of eggs (Forward et al. 2005a), ensuring that spawning females continue to
migrate seaward throughout the spawning season. By August, in small strongly tidally driven systems such as the Beaufort Inlet Drainage, North Carolina (Luettich et al. 1999), most spawning females have moved into the ocean and produce clutches of eggs until late October (Dickinson et al. 2006, Darnell et al. 2009).

In addition to ETT, female crabs also move seaward during flood tides by walking at speeds of up to 0.25 m s$^{-1}$, with a mean difference between crab movement vector and flood current vector of 187 ± 3º, indicating that the walking is directed and not random movement (Carr et al. 2004). Using both oriented walking during flood tide and the episodic vertical swimming during ebb tide, crabs are able to migrate 5.4 km day$^{-1}$ on average, in the strongly tidal region around Beaufort Inlet (Carr et al. 2004). At this speed, a migrating female could complete migration out of Beaufort Inlet in less than three days.

Hench et al. (2004) tethered ovigerous crabs individually in a flat, sandy area of Bogue Sound, NC. The tethering site was approximately 2.2 m deep (Hench et al. 2004) and was near Beaufort Inlet, NC, in an estuary with strong semi-diurnal tides. Pressure loggers, attached to the carapace and sampling at a frequency of 0.091 Hz, were used to monitor vertical swimming behavior. These data showed a tidal rhythm in vertical swimming. Peak vertical swimming generally corresponded to the the time of the most rapid decrease in water level. Crabs migrated on both night and day ebb tides and migratory behavior continued after larval release (Hench et al. 2004). In addition to the
endogenous circatidal swimming rhythm identified by Forward et al. (2003, 2005), results of Hench et al. (2004) indicate that decreasing hydrostatic pressure may be a cue for vertical swimming.

In the field (Carr et al. 2004, Hench et al. 2004) and in constant laboratory conditions (Forward et al. 2003, Forward et al. 2005a), spawning female migratory behavior is characterized by a series of brief swimming bouts, lasting seconds to minutes, interspersed with periods of time spent on the bottom. Hench et al. (2004) hypothesized that, if decreasing hydrostatic pressure functions as a cue for vertical swimming, this time spent on the bottom may be necessary for the crab to sense hydrostatic pressure changes. If the appropriate change in hydrostatic pressure was experienced, the crab would ascend for another swimming bout. This hypothesis does not, however, account for the high swimming frequency of crabs held under constant conditions, which experience no changes in hydrostatic pressure. Hench et al. (2004) further hypothesized that migrating crabs may use decreasing hydrostatic pressure as a cue to time swimming precisely, and that the circatidal swimming rhythm may be essential for maintaining ETT in the presence of ambiguous external cues.

Migrating female crabs experience a variety of environmental conditions as they move seaward, including varying salinities, depths, bottom types, and tidal conditions. Because vertical swimming during ebb tide results from both an endogenous rhythm and exogenous environmental cues, swimming behavior should vary among different
habitats as the magnitudes and patterns of these environmental cues vary. Although much of the work on migratory behavior to date has focused on an estuary with strong semi-diurnal tides (Forward et al. 2003, Hench et al. 2004, Forward et al. 2005a), crabs in some areas begin their spawning migration in areas that do not experience a lunar tidal cycle. Although female blue crabs in non-tidal estuaries make the same seaward migration as crabs in tidal estuaries, little is known about the behavioral basis for this migration.

The purpose of this study was to examine vertical swimming behavior of ovigerous blue crabs in the field in four locations that represent different habitats encountered by blue crabs. A combined bio-physical study was conducted, consisting of simultaneous measurements of both physical parameters of the water column and vertical swimming behavior of individual crabs in the water column using custom-built archival pressure tags.

5.2 Methods

5.2.1 Study sites

Vertical swimming behavior of ovigerous blue crabs was monitored in the field at three sites within the strongly-tidal Beaufort Inlet drainage and one site in the non-tidal Albemarle-Pamlico Estuarine System (Figure 22): (1) a high-salinity embayment within the Rachel Carson National Estuarine Research Reserve (34°42.711’N, 76°40.390’W), (2) south of Kirby-Smith Island (34°42.957’N, 76°40.494’W), (3) east of the
Pivers Island Bridge (34°43.216’N, 76° 40.326’W), and (4) West Bay (34°58.233’N, 76°19.723’W). The Rachel Carson site is a high-salinity embayment that is habitat for blue crabs in all adult life history stages including mating and spawning females (Ramach et al. 2009). Water depth at the embayment tethering site averaged ~0.8 m (Table 3). Kirby-Smith Island is a small, primarily intertidal sand island located between Radio Island and Pivers Island. It is not consistently frequented by juvenile or adult life stages. The tethering site was ~20 m south of island at low tide and water depth averaged ~1 m (Table 3). The Pivers Island Bridge tethering site was located ~100 m east of the bridge. This channel is a migratory corridor for spawning female crabs, as ovigerous females are often seen swimming at the surface during nocturnal ebb tides (Tankersley et al. 1998). Water depth at the bridge site averaged ~4.3 m. The West Bay tethering site was ~150 m east of Green Point. Water depth at the West Bay site averaged ~ 1.8 m (Table 3).

**Table 3: Physical parameters of the four tethering sites**

<table>
<thead>
<tr>
<th>Tidal regime</th>
<th>Embayment</th>
<th>Kirby-Smith Island</th>
<th>Pivers Island bridge</th>
<th>West Bay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tidal regime</td>
<td>semi-diurnal</td>
<td>semi-diurnal</td>
<td>semi-diurnal</td>
<td>non-tidal</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>0.8</td>
<td>1</td>
<td>4.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>35-36</td>
<td>35-36</td>
<td>36-37</td>
<td>21.8-24</td>
</tr>
<tr>
<td>Max ebb V (m s⁻¹)</td>
<td>0.15</td>
<td>0.04</td>
<td>0.21</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean ebb V (m s⁻¹)</td>
<td>0.09</td>
<td>-0.09</td>
<td>0.15</td>
<td>n/a</td>
</tr>
<tr>
<td>Max pos. dS/dt (ppt s⁻¹)</td>
<td>2.35 × 10⁻⁴</td>
<td>9.64 × 10⁻⁵</td>
<td>5.98 × 10⁻⁵</td>
<td>1.48 × 10⁻⁴</td>
</tr>
<tr>
<td>Mean ebb dS/dt (ppt s⁻¹)</td>
<td>1.79 × 10⁻⁵</td>
<td>-4.76 × 10⁻⁶</td>
<td>-6.05 × 10⁻⁶</td>
<td>n/a</td>
</tr>
<tr>
<td>Max neg. dη/dt (dbar s⁻¹)</td>
<td>-6.80 × 10⁻⁵</td>
<td>-1.10 × 10⁻⁴</td>
<td>-5.11 × 10⁻⁵</td>
<td>-1.2 × 10⁻⁵</td>
</tr>
<tr>
<td>Mean ebb dη/dt (dbar s⁻¹)</td>
<td>4.21 × 10⁻⁵</td>
<td>5.05 × 10⁻⁵</td>
<td>3.54 × 10⁻⁵</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Figure 22: Locations of tethering sites: (a) overview, (b) Beaufort Inlet drainage, (c) West Bay.
5.2.2 Archival pressure tags

Internally-recording pressure tags were custom built for this study (Figure 23, Figure 24). Tags were based around a PIC16LF88 microcontroller (Microchip Technology, Inc., Chandler, AZ) and SM5420 pressure transducer (Silicon Microstructures, Inc., Milpitas, CA)(Table 4). Data were recorded to a 1 MB internal, flash memory chip (M45PE80, Numonyx B.V, Rolle, Switzerland) and power was provided by a 120 mAh lithium battery (CR2032, Sanyo North America Corp., San Diego, CA). Tags were programmed to record pressure every 6 s (0.17 Hz). This logging interval was chosen because it would ensure that nearly all ascents into the water column would be sampled at least once (Hench et al. 2004). The internal memory of the tags was sufficient for up to 34 days of deployment. Internal circuitry was encased in low-density potting epoxy (RBC-3600, RBC Industries, Inc., Warwick, RI). Prior to initial deployment, the tags were placed in a lyophylizer with the pressure transducer port submerged in silicone oil (200 Fluid, Dow Corning Corp., Midland, MI). A near-vacuum (~60 mTorr) was created to remove air from the port, and pressure was slowly increased, filling the port with silicone oil to isolate the electronics from the seawater. Communication with a laptop computer was possible through an RS232 serial connection, which was sealed with melted vaseline prior to each deployment. External tag dimensions measured 51 mm × 30.5 mm × 10 mm (Figure 24).
Figure 23: Schematic for archival pressure tags.
<table>
<thead>
<tr>
<th>Qty</th>
<th>Ref Des</th>
<th>Description</th>
<th>Package</th>
<th>Mounting</th>
<th>Part #</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>C1, C2</td>
<td>33 pF capacitor</td>
<td>C1206</td>
<td>SMT</td>
<td>C1206C330J5GACTU</td>
<td>KEMET Corporation</td>
</tr>
<tr>
<td>4</td>
<td>C3 – C7</td>
<td>0.1 uF capacitor</td>
<td>C0805</td>
<td>SMT</td>
<td>C0805C104J5RACTU</td>
<td>Kemet</td>
</tr>
<tr>
<td>1</td>
<td>IC1</td>
<td>AD627 instrumentation amplifier</td>
<td>SO-08</td>
<td>SMT</td>
<td>AD627ARZ-R7CT-ND</td>
<td>Analog Devices, Inc.</td>
</tr>
<tr>
<td>1</td>
<td>IC2</td>
<td>M45PE80 1 Mbit flash memory</td>
<td>SO-08W</td>
<td>SMT</td>
<td>M45PE80-VMW6G</td>
<td>Numonyx, Inc.</td>
</tr>
<tr>
<td>1</td>
<td>IC3</td>
<td>MAX4501 analog switch</td>
<td>SOT23-5</td>
<td>SMT</td>
<td>MAX4501EUK+</td>
<td>Maxim Integrated Products</td>
</tr>
<tr>
<td>1</td>
<td>IC4</td>
<td>PIC16LF88</td>
<td>SSOP-20</td>
<td>SMT</td>
<td>PIC16LF88-1/SS-ND</td>
<td>Microchip Technology, Inc.</td>
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<td>1</td>
<td>J1</td>
<td>JST 7-pin connector</td>
<td>n/a</td>
<td>SMT</td>
<td>SM07B-SRSS-TB</td>
<td>JST Sales America, Inc.</td>
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<td>1</td>
<td>PSENS1</td>
<td>SM5420 pressure sensor</td>
<td>SO-08</td>
<td>SMT</td>
<td>SM5420</td>
<td>Silicon Microsystems</td>
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<tr>
<td>1</td>
<td>Q1</td>
<td>P-MOSFET</td>
<td>SOT23</td>
<td>SMT</td>
<td>TP0610K-T1-E3</td>
<td>Vishay/Siliconix</td>
</tr>
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<td>1</td>
<td>R10</td>
<td>2 kOhm resistor</td>
<td>R0805</td>
<td>SMT</td>
<td>ERJ-6ENF2001V</td>
<td>Panasonic - ECG</td>
</tr>
<tr>
<td>3</td>
<td>R2, R3, R4</td>
<td>10 kOhm resistor</td>
<td>R0805</td>
<td>SMT</td>
<td>RR1220P-103-D</td>
<td>Sasumo Co. Ltd.</td>
</tr>
<tr>
<td>2</td>
<td>R5, R6</td>
<td>1 MOhm resistor</td>
<td>R0805</td>
<td>SMT</td>
<td>ERJ-6ENF1004V</td>
<td>Panasonic - ECG</td>
</tr>
<tr>
<td>1</td>
<td>R7</td>
<td>3 mm trimpot, 1kOhm</td>
<td>3 mm trimpot</td>
<td>SMT</td>
<td>TC73X-1-102E</td>
<td>Bourns Inc.</td>
</tr>
<tr>
<td>1</td>
<td>R9</td>
<td>2 MOhm resistor</td>
<td>R0805</td>
<td>SMT</td>
<td>ERJ-6GEYJ205V</td>
<td>Panasonic - ECG</td>
</tr>
<tr>
<td>1</td>
<td>R1</td>
<td>510 Ohm resistor</td>
<td>R0805</td>
<td>SMT</td>
<td>ERJ-6ENF510W</td>
<td>Panasonic - ECG</td>
</tr>
<tr>
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<td>R8</td>
<td>3 mm trimpot, 500 Ohm</td>
<td>3 mm trimpot</td>
<td>SMT</td>
<td>TL73X-501ECT-ND</td>
<td>Bourns Inc.</td>
</tr>
<tr>
<td>1</td>
<td>U$1</td>
<td>Reed switch, NO</td>
<td>thru-hole</td>
<td></td>
<td>HE503-ND</td>
<td>Hamlin Electronics</td>
</tr>
<tr>
<td>1</td>
<td>XT1</td>
<td>32.768 kHz crystal</td>
<td>thru-hole</td>
<td></td>
<td>AB38T-32.768kHz</td>
<td>Abracon Corp.</td>
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<tr>
<td>1</td>
<td>B1</td>
<td>120 mAh battery</td>
<td>thru-hole</td>
<td></td>
<td>CR2032</td>
<td>Sanyo NA Corp.</td>
</tr>
</tbody>
</table>
Figure 24: Archival pressure tags: (a) top view, (b) bottom view, (c) ovigerous blue crab with archival pressure tag attached. In panel b, tp indicates the pressure transducer port and sp indicates the serial port connection.

5.2.3 Laboratory testing of pressure tags

To ensure that the pressure tags were functional, and to estimate swimming frequency of ovigerous females held in constant laboratory conditions, three ovigerous female crabs, collected from the Rachel Carson embayment, were fitted with pressure tags and placed individually into transparent vertical columns (1.22 m x 30.5 cm diameter, Aquatic Eco-Systems, Inc. model T4), filled with ambient estuarine water. The water was filtered to remove particles > 5 μm and aerated for the duration of the experiment. Columns were continuously illuminated with dim (20-30 lux) red light. Blue crabs are insensitive to this wavelength, so red light approximates constant darkness (Cronin & Forward 1988). Swimming frequency was monitored for ~ 2 d (Table 5), at
which time the crabs were removed from the columns and data were downloaded from the tags.

**5.2.4 Monitoring swimming behavior in the field**

Ovigerous blue crabs were collected from areas near the tethering site, either by hand on nocturnal low tides or with the assistance of local commercial crabbers. Within 72 h of collection, each crab was fitted with a pressure tag and tethered in the field. Tags were attached to the carapace with 18 gauge plastic-coated copper wire wrapped around the large lateral spines (Figure 24) using a modification of the procedure used by Hench et al. (2004). A loop in the wire allowed attachment of the tether. Tethers were constructed of ~3 m lengths of 0.81 mm diameter nylon-coated braided stainless steel leader material (60 lb test Surflon, American Fishing Wire, Coatesville, PA) with stainless steel snap swivels at each end to allow attachment. Tethers were anchored to the sediment in one of two ways. In the shallower sites (Rachel Carson Embayment and Kirby-Smith Island), tethers were anchored to 0.5 m PVC stakes that were driven into the sediment such that only ~3 cm protruded above the surface. In the deeper sites (West Bay and Pivers Island Bridge), tethers were anchored to 3 lb lead weights. A 10 m nylon groundline connected each of these weights to cinder blocks, from which a second nylon line ran to a buoy on the surface.

Data were downloaded from the tags to a laptop computer every 1-3 d. During data downloads, each crab was held in a bucket containing 2.5-5 cm of ambient estuarine
water for 5-10 minutes. After the data were downloaded, the serial port was re-sealed, tags were re-attached, and crabs were returned to the water. Crabs were monitored for 1-10 d (Table 5).

**Table 5: Dates of monitoring at each site.** INST indicates the dates instruments were deployed at the site.

<table>
<thead>
<tr>
<th>Crab</th>
<th>Site</th>
<th>Start date</th>
<th>End date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Laboratory columns</td>
<td>7/3/2009</td>
<td>7/5/2009</td>
</tr>
<tr>
<td>2</td>
<td>Laboratory columns</td>
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<td>7/10/2009</td>
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5.2.5 Physical measurements of the water column

Currents at each tethering site were measured using a bottom mounted 2MHz Aquadopp current profiler (NortekUSA, Inc., Annapolis, MD). The profiler sampled velocity along 3 axes (East, North, Up) every 5 min., averaging for 60 seconds. Vertical bin size was 20 cm (Embayment, Kirby-Smith Island, West Bay) or 40 cm (Pivers Island Bridge). Velocity data were rotated into along- and across- channel components for analyses. Salinity at the tethering sites were monitored using a YSI 6600 V2-4 multiparameter sonde (YSI, Inc. Yellow Springs, OH). The sonde sampled salinity ~10 cm above the bottom simultaneous with current measurements by the profiler. Due to the predictable tidal currents in the Beaufort Inlet drainage, instruments were deployed for 27-72 h at each of the tethering sites. The APES (West Bay) tethering site, however, is not subjected to a predictable tidal regime. Thus, the profiler and sonde were deployed for the duration of the tethering study at that site. Instrument deployments were not done concurrently at the four sites, but were conducted over a period of ~ 5 weeks (Table 5).

5.2.6 Data analysis

Pressure tag data were converted to depth using known tidal heights for the tethering locations. A crab was counted as swimming if it ascended ≥ 30 cm above the sediment. This depth was chosen to minimize false ascents due to variations in local bathymetry and noise in the data. Swimming frequency was quantified as the number of
6 s logging intervals spent swimming, summed into 30 min bins. For crabs from the tidal sites, each bin was then classified based on time relative to high tide.

Profiles of along-channel current velocity, averaged into hourly bins as a function of time after high tide, indicated that there was little variation in along-channel current velocity relative to depth above the bottom (Figure 25, Figure 26). Thus, the along-channel velocity bin centered 0.5 m above the bottom was used for all analyses. Flow, pressure, and salinity data were averaged every 30 min and, for the three tidal sites, each bin was then classified based on its time relative to the tidal cycle. Data from the non-tidal West Bay site could not be binned based on the tidal cycle, so are presented as a time series of 30 min bins from the start to the end of the experiment.

Changes in hydrostatic pressure, along-channel current velocity, and salinity were examined as possible cues for vertical swimming behavior. Pressure tendency (d\eta/dt, dbar s\(^{-1}\)) is the rate of change of hydrostatic pressure and is negative for decreasing water level and positive for increasing water level. Velocity tendency (dV/dt, m s\(^{-2}\)) is the rate of change in along-channel current velocity and is positive when velocity is increasing and negative when velocity is decreasing. Salinity tendency (dS/dt, ppt s\(^{-1}\)) is the rate of change of salinity and is positive when salinity is increasing and negative when salinity is decreasing.
Figure 25: Mean along-channel velocity profiles, relative to the time of high tide, for (a) the Rachel Carson embayment, (b) Kirby-Smith Island, and (c) Pivers Island bridge. Positive current velocities indicate ebb flow. Vertical lines indicate 0 m s\(^{-1}\) velocity for each hour of the tidal cycle. Dashed line indicates water depth.
Figure 26: Mean along-channel velocity profiles for West Bay. Each row represents 24 h and the two panels represent different deployments. Negative current velocities indicate ebb flow. Vertical lines indicate 0 m s⁻¹ velocity for each hour of the day. Dashed line indicates water depth. Gray bars indicate hours of darkness.
Average time spent swimming was regressed against along-channel current velocity ($V$), salinity ($S$), and velocity, pressure, and salinity tendencies. Swimming frequency was compared between day and night using a paired t-test. Swimming frequency was compared between ebb and flood tide for each site using a Mann-Whitney Rank Sum test. Ebb tide was defined as time period during which water level was decreasing. For the tidal sites, this corresponded to the 6 h following high tide. Flood tide was defined as the period during which water level was increasing, corresponding to 6.5-12.5 h after high tide. Swimming frequency was compared among sites for both flood and ebb tides using analysis of variance (ANOVA) followed by Dunn’s multiple comparison test where appropriate.

Swimming frequency for each crab was binned into 24 h bins, based on the number of days before or after larval release, and normalized by calculating the percentage of total swimming occurring on each day. Percentages of total swimming on each day relative to larval release were averaged for all crabs from each tethering site and plotted to examine trends in swimming frequency over time.

**5.3 Results**

**5.3.1 Laboratory columns**

Three crabs were tagged with archival pressure tags and monitored in columns under constant laboratory conditions for 2 d. Swimming frequency did not differ between day and night (paired t-test, $p = 0.789$). Crabs in the columns swam primarily
during ebb tides (Figure 27). On average, crabs in the columns swam 4.28 ± 0.67 intervals per 30 min during ebb tide and 0.60 ± 0.20 intervals per 30 min during flood tide. Crabs swam significantly more during ebb tide than flood tide (Mann-Whitney Rank Sum Test, \( p < 0.001 \)). Peak swimming occurred ~1.5-3 h after high tide. Swimming frequency did not differ between day and night (paired t-test, \( p = 0.789 \)).

5.3.2 Rachel Carson embayment

In the Rachel Carson embayment, 4 crabs were tethered, each for 3-10 d. Swimming frequency did not differ between day and night (paired t-test, \( p = 0.419 \)). Vertical swimming occurred primarily during ebb tides, though occasional ascents occurred on flood tides (Figure 27, Figure 28). On average, crabs in the embayment swam 0.10 ± 0.03 intervals per 30 min during ebb tide and 0.01 ± 0.01 intervals per 30 min during flood tide. Crabs swam significantly more during ebb tide than flood tide (Mann-Whitney Rank Sum Test, \( p = 0.003 \))(Table 6). Peak swimming occurred ~4.5-5.5 h after high tide. Average along-channel current velocities ranged from 0.15 ± 0.01 m s\(^{-1}\) ebb, approximately 4.5 h after high tide, to 0.21 ± 0.03 m s\(^{-1}\) flood, approximately 10.5 h after high tide (Figure 28).

Swimming frequency was negatively correlated with pressure tendency (linear regression, \( p = 0.002 \)), but was not significantly correlated with along-channel current velocity (linear regression, \( p = 0.369 \)) or velocity tendency (linear regression, \( p = 0.899 \))(Table 6). Pressure tendency in the embayment ranged from \(-6.80 \times 10^{-5}\) to \(6.81 \times 10^{-5}\).
Salinity was highest ~5-6 h after high tide, though the change in salinity was relatively small and salinity remained between 35-36 ppt. Time spent swimming was positively correlated with salinity (linear regression, \( p = 0.029 \)) and salinity tendency at the tethering site (linear regression, \( p = 0.013 \)). Salinity tendency in the embayment ranged from \(-8.98 \times 10^{-3}\) to \(2.35 \times 10^{-4}\) ppt s\(^{-1}\).

### Table 6: Results of statistical tests for correlations between swimming frequency and physical variables at the three site. NS indicates a non-significant result.

<table>
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5.3.3 Kirby-Smith Island

At Kirby-Smith Island, 5 crabs were tethered, each for 2-9 d. Swimming frequency did not differ between day and night (paired t-test, $p = 0.148$) (Table 6). Vertical swimming behavior occurred mainly during ebb tides (Figure 25), though 1 crab began swimming during flood tide after larval release. Additionally, one crab that was tethered for 2 d swam only once, during flood tide. On average, crabs at Kirby-Smith Island swam $0.07 \pm 0.02$ intervals per 30 min during ebb tide and $0.03 \pm 0.01$ intervals per 30 min during flood tide (Figure 25, Figure 26). There was no significant difference in time spent swimming between ebb and flood tide (Mann-Whitney Rank Sum Test, $p = 0.225$) (Table 6). The difference remains non-significant if the crab that switched to swimming on flood tides is excluded from the analysis. Peak swimming occurred ~2-3 h after high tide. Average along-channel current velocities ranged from $0.04 \pm 0.01$ m s$^{-1}$ ebb, approximately 1 h after high tide, to $0.37 \pm 0.01$ m s$^{-1}$ flood, approximately 8.5 h after high tide (Figure 28).
Figure 27: Mean swimming frequency, relative to the time of high tide, of crabs (a) monitored in columns under constant laboratory conditions, (b) tethered in the Rachel Carson embayment, (c) tethered at Kirby-Smith Island, and (d) tethered at the Pivers Island bridge. Error bars indicate ±1 SEM.
Swimming frequency was negatively correlated with the pressure tendency (linear regression, $p = 0.02$), but was not significantly correlated with along-channel current velocity (linear regression, $p = 0.856$) or velocity tendency (linear regression, $p = 0.255$) (Table 6). Pressure tendency at Kirby-Smith Island ranged from $-1.10 \times 10^{-4}$ to $7.63 \times 10^{-5}$ dbar s$^{-1}$. Salinity was highest ~9-10 h after high tide, though the change in salinity was relatively small and salinity remained between 35-36 ppt. Time spent swimming was not significantly correlated with either salinity (linear regression, $p = 0.868$) or salinity tendency at the tethering site (linear regression, $p = 0.196$). Salinity tendency at Kirby-Smith Island ranged from $-3.83 \times 10^{-5}$ to $9.64 \times 10^{-5}$ ppt s$^{-1}$.

5.3.4 Pivers Island bridge

Six crabs were tethered near Pivers Island Bridge, each for 2-8 d. Swimming frequency did not differ between day and night (paired t-test, $p > 0.05$) (Table 6). Vertical swimming occurred primarily during ebb tides, though occasional ascents occurred on flood tides. On average, crabs tethered near the bridge swam $0.87 \pm 0.14$ intervals per 30 min during ebb tide and $0.07 \pm 0.02$ intervals per 30 min during flood tide (Figure 25, Figure 26). Crabs swam significantly more during ebb tide than flood tide (Mann-Whitney Rank Sum Test, $p < 0.001$) (Table 6). Peak swimming occurred ~1.5 h after high tide and remained high until ~4.5 h after high tide (Figure 25). Average along-channel current velocities ranged from $0.21 \pm 0.02$ m s$^{-1}$ ebb, approximately 3.5 h after high tide, to $0.33 \pm 0.03$ m s$^{-1}$ flood, approximately 10 h after high tide (Figure 26).
Figure 28: Mean swimming frequency, current velocity, and pressure at (a) Rachel Carson embayment, (b) Kirby-Smith Island, and (c) Pivers Island bridge, relative to the time of high tide. Filled circles represent along-channel current velocity, and positive velocity indicates ebb flow. Dotted horizontal line indicates 0 m s\(^{-1}\) velocity. Open triangles represent pressure at the tethering site. Error bars indicate ±1 SEM.
Swimming frequency was negatively correlated with the pressure tendency (linear regression, \( p < 0.001 \)), but was not significantly correlated with along-channel current velocity (linear regression, \( p = 0.118 \)) or velocity tendency (linear regression, \( p = 0.330 \))(Table 6). Pressure tendency at the Pivers Island Bridge ranged from \(-5.11 \times 10^{-5}\) to \(8.01 \times 10^{-5}\) dbar s\(^{-1}\). Salinity was highest ~9 h after high tide, though the change in salinity was relatively small and salinity remained between 36-37 ppt. Time spent swimming was not significantly correlated with either salinity (linear regression, \( p = 0.232 \)) or salinity tendency at the tethering site (linear regression, \( p = 0.159 \)). Salinity tendency at the bridge ranged from \(-2.62 \times 10^{-5}\) to \(5.98 \times 10^{-5}\) ppt s\(^{-1}\).

**5.3.5 West Bay**

Eight ovigerous crabs were tethered in West Bay. Four of these crabs were each tethered for 2 d while the remaining 4 were tethered for 1 d. All crabs remained on the bottom for the duration of the tethering period and did not swim. Along-channel current velocities 0.5 m above the sediment ranged from 0.11 m s\(^{-1}\) ebb to 0.11 m s\(^{-1}\) flood. Current velocities 1.5 m above the bottom, within 0.5 m of the surface, ranged from 0.23 m s\(^{-1}\) ebb to 0.24 m s\(^{-1}\) flood. Pressure tendency in West Bay ranged from \(-1.2 \times 10^{-5}\) to \(1.31 \times 10^{-5}\) dbar s\(^{-1}\). Salinity in West Bay ranged from 21.8-24.0 ppt. Salinity tendency in West Bay ranged from \(-1.02 \times 10^{-4}\) to \(1.48 \times 10^{-4}\) ppt s\(^{-1}\).
5.3.6 Inter-site comparisons

Crabs collected from the tidal Beaufort Inlet drainage swam primarily on ebb tides, both in the columns and tethered in the field in the three tidal tethering sites, though swimming frequency varied by site (Figure 29). Crabs collected from and tethered in the non-tidal West Bay site did not swim and were thus excluded from inter-site analyses of swimming frequency. Crabs monitored in the columns under laboratory conditions swam significantly more during ebb tides than crabs tethered at any of the field sites (Kruskal-Wallis ANOVA, $p < 0.001$; Dunn’s multiple comparison test, $p < 0.05$). Crabs tethered near the Pivers Island Bridge swam significantly more during ebb tides than crabs tethered in the Rachel Carson embayment or at Kirby-Smith Island (Dunn’s multiple comparison test, $p < 0.05$)(Figure 6). Vertical swimming during ebb tides was statistically similar between the embayment and Kirby-Smith Island (Dunn’s multiple comparison test, $p > 0.05$) During flood tides, crabs monitored in the columns swam significantly more than crabs tethered in the embayment (Kruskal-Wallis ANOVA, $p < 0.001$; Dunn’s multiple comparison test, $p < 0.05$). There was no difference in swimming behavior during flood tides among the tidal tethering sites (Dunn’s multiple comparison test, $p > 0.05$)(Figure 29).

Mean along-channel current velocity during ebb tide varied among the tidal tethering sites (Kruskal-Wallis ANOVA, $p < 0.001$). Mean ebb velocity at Pivers Island bridge ($0.15 \pm 0.01 \text{ m s}^{-1}$) was statistically similar to mean ebb velocity in the Rachel
Carson embayment (0.10 ± 0.01 m s\(^{-1}\)). Mean ebb velocity at both Pivers Island bridge and the embayment were significantly higher than at Kirby-Smith Island (-0.10 ± 0.03 m s\(^{-1}\))(Tukey test, \(p < 0.05\)). Mean ebb tide pressure tendency was statistically similar between sites (Kruskal-Wallis ANOVA, \(p = 0.100\)).

**Figure 29:** Mean swimming frequency for crabs tethered at the three tidal sites and monitored in the columns under constant laboratory conditions. Error bars indicate ±1 SEM. Different letters indicate significant differences at \(p < 0.05\).

Crabs were monitored from 8 d prior to larval release to 7 d after larval release.

Data were variable, but swimming frequency generally increased in the days preceeding larval release (Figure 30). Swimming decreased slightly following larval release.

Swimming was observed as early as 7 d prior to larval release.
Figure 30: Daily mean swimming frequency relative to the day of larval release for all crabs combined. Error bars indicate ±1 SEM and numbers above error bars indicate the number of crabs tethered.

5.4 Discussion

Ovigerous female blue crabs were tethered in four locations to monitor swimming behavior in the field. Crabs in the three tidal tethering sites swam primarily on ebb tides and swimming frequency generally increased as embryonic development progressed. Crabs swam during both the day and night. Swimming frequency varied greatly by site. Crabs tethered near the Pivers Island bridge, in an area that is known to serve as a migratory corridor for spawning blue crabs (Tankersley et al. 1998), swam ~10 times as much during ebb tide than crabs tethered in the Rachel Carson embayment or
near Kirby-Smith Island. The embayment is known to be habitat for blue crabs at all life history stages, including juveniles of both sexes, males, and mating and spawning females (Ramach et al. 2009). Kirby-Smith Island is of similar depth and bottom type as the embayment, though relatively few crabs are observed in the area (Rittschof, pers. comm.). Swimming behavior during flood tides was similar among the three sites and was ~10-35% of ebb tide swimming. In the non-tidal West Bay tethering site, eight crabs were tethered, none of which swam during the study period.

When held in columns in constant laboratory conditions, ovigerous blue crabs from both the Beaufort Inlet drainage and the Albemarle-Pamlico Estuarine System, display endogenous rhythms in swimming. Crabs from the Beaufort Inlet drainage have a circatidal rhythm, with peak swimming occurring during ebb tide (Forward et al. 2003, Forward et al. 2005a) and this rhythm persists between clutches (Forward et al. 2005a). Ovigerous crabs from the Albemarle-Pamlico Estuarine system have a circadian rhythm in swimming, with peak swimming occurring around the time of sunset (see Chapter 3). The difference in swimming behavior among sites is not explained by differences in swimming rhythms, as all crabs from the tidal sites were collected from the same location, the Rachel Carson Embayment, and the crabs tethered in West Bay were collected from the same area as crabs previously used in rhythms experiments. Thus, I hypothesize that these differences in swimming frequency among sites is due to exogenous environmental cues as opposed to endogenous rhythmicity.
At each of the three tidal sites, swimming frequency was negatively correlated with the pressure tendency ($d\eta/dt$, Table 6). Crabs swam more when water level was decreasing at a high rate, which corresponds to ebb tide. This suggests that decreasing hydrostatic pressure may be an important cue for female blue crab swimming behavior. Regardless of a crab’s location within an estuary, decreasing hydrostatic pressure corresponds to ebb tide, and swimming into the water column during periods of decreasing hydrostatic pressure would result in seaward movement. Although swimming frequency was negatively correlated with $d\eta/dt$ within each site, there is no trend in swimming frequency and maximum or mean ebb tide $d\eta/dt$ among all the sites. Kirby-Smith Island had the greatest ebb tide $d\eta/dt$ ($-1.1 \times 10^{-4}$ dbar s$^{-1}$) and the lowest ebb tide swimming frequency (0.07 ± 0.02 intervals per 30 min) while Pivers Island bridge had the second-lowest ebb tide $d\eta/dt$ ($-5.1 \times 10^{-5}$ dbar s$^{-1}$) and the greatest ebb tide swimming frequency (0.87 ± 0.14 intervals per 30 min). West Bay had the lowest ebb tide $d\eta/dt$ ($-1.23 \times 10^{-5}$ dbar s$^{-1}$) and crabs did not swim at all. Mean ebb tide pressure tendencies were statistically similar among the three tidal tethering sites. Thus, while decreasing hydrostatic pressure may serve as a cue for swimming, it does not explain differences in swimming frequency between the sites.

Swimming frequency of crabs in the Rachel Carson embayment was negatively correlated with both salinity and salinity tendency ($dS/dt$). Peak swimming occurred when salinity was increasing rapidly. This relationship was not seen for crabs tethered at
the Pivers Island Bridge or Kirby-Smith Island. Animals undergoing ETT would generally not be expected to swim during times of salinity increase. For most estuarine locations, salinity increases correspond to flood tides. In the embayment, however, salinity increased towards the end of ebb tide, and peaked 5 h after high tide. The embayment is a shallow, enclosed body of water. Evaporation could result in a salinity increase, especially during the summer months in the shallower areas. This high-salinity water would then be carried out of the embayment, past the tethering location, with the falling tide. Because this pattern of salinity variation would only be seen in certain locations, it is not a likely cue for swimming, and the observed relationship may simply be an artifact of the co-variation between salinity and other variables such as pressure. Additionally, the maximum salinity tendency observed in the embayment (2.35 × 10^{-4} ppt s^{-1}), while higher than at the other three sites (Kirby-Smith Island: 9.64 × 10^{-5} ppt, Pivers Island bridge: 5.98 × 10^{-5} ppt s^{-1}, West Bay: 1.25 × 10^{-4} ppt s^{-1}) is less than half of the threshold detectable salinity tendency determined for blue crab megalopae (was 5.53× 10^{-4} ppt s^{-1}) calculated by Tankersley et al. (1995). These results indicate that, while the pressure tendency and possibly salinity can serve as cues for swimming and explain the timing of swimming within a site, these two factors do not explain variation in swimming behavior between sites.

Ebb current velocity was not a significant predictor of swimming frequency at any of the four sites (Table 6). There was a trend, however, among the three tidal sites of...
increasing swimming frequency with increasing maximum and mean ebb current velocity. Ebb tide swimming frequency was greatest at the Pivers Island bridge, which was subjected to stronger ebb currents (max: 0.21 m s\(^{-1}\), mean: 0.15 ± 0.01 m s\(^{-1}\)) than either the Rachel Carson embayment (max: 0.15 m s\(^{-1}\), mean: 0.10 ± 0.01 m s\(^{-1}\)) or Kirby-Smith Island (max: 0.04 m s\(^{-1}\), mean: -0.10 ± 0.03 m s\(^{-1}\)), though the difference in mean ebb velocity between Pivers Island bridge and the embayment was not statistically significant. In West Bay, where crabs did not swim at all, peak ebb currents were 0.05 m s\(^{-1}\). I hypothesize that ebb current velocity, or some other factor such as turbulence that varies with current velocity, is used by spawning crabs as a cue for swimming, and that swimming will be greatest in areas with high ebb current velocities. Hench et al. (2004) tethered females in a mid-sound channel that possessed similar flow conditions to those found near the Pivers Island Bridge, and found that crabs frequently ascended into the water column on ebb tides.

Based on the results of these experiment, I hypothesize that some areas may serve as migratory corridors for spawning blue crabs, while others serve as foraging habitat. When in high salinity (>22 ppt; Rittschof et al., In Review) areas, vertical swimming behavior may decrease or even cease in foraging areas. These foraging areas are likely defined by a combination of cues, including current velocity, food availability, and presence or absence of predators. The spawning migration serves to move females to high-salinity water so that zoeae can be transported offshore for development.
Decreased swimming in shallow, low-energy foraging habitat would allow the crabs to replenish energy stores and reduce predation risk by large fish predators that may be present in other areas. Because the crabs are already in high-salinity water, larval success would not likely be affected, as the salinity is appropriate for larval development and larvae would be rapidly transported offshore on ebb tides following larval release.

All three of the tidal tethering sites were located in a high-salinity (~35 ppt) area within 3 km of Beaufort Inlet. Based on the results of this study, as well as previous observations of blue crab habitat use in the area (Ramach et al. 2009), I hypothesize that the Rachel Carson embayment and the shallow areas surrounding Kirby-Smith Island serve as foraging areas for ovigerous blue crabs during their seaward spawning migration and that migrating female crabs decrease or cease vertical swimming while in these areas so that energy stores can be replenished to produce future clutches of eggs. The channel around Pivers Island bridge, however, clearly serves as a migratory corridor for ovigerous blue crabs, indicated by high levels of vertical swimming as well as previous observations of ovigerous blue crabs migrating on the surface near the bridge on ebb tides (Tankersley et al. 1998).

Crabs tethered in the non-tidal West Bay remained on the bottom for the duration of the study (1-2 d per crab). I propose two hypotheses to explain the lack of swimming exhibited by crabs tethered in West Bay. First, it is possible that West Bay serves as a high-salinity foraging habitat, similar to the Rachel Carson embayment or the
area surrounding Kirby-Smith Island. Salinity in West Bay ranged from 21.8-24.0 ppt. While substantially lower than the salinity at the three tidal sites, this salinity is suitable for larval development (Costlow & Bookhout 1959) and is in the range of suitable salinities for ovigerous blue crabs as determined by Rittschof et al. (In Review). West Bay, however, is > 30 km from the nearest inlet (Ocracoke Inlet), so is unlikely that larvae hatching in West Bay would be transported into the coastal ocean. Another possible explanation is that, in non-tidal estuaries, ovigerous blue crabs rely on environmental cues such as salinity changes or flow conditions to stimulate vertical swimming, rather than on an endogenous circatidal rhythm. If suitable environmental cues were not encountered during the tethering period, vertical swimming would not occur.

Though crabs tethered in the field swam most in areas with high ebb current velocities, crabs held in constant still-water laboratory conditions, frequently swam to the surface in the absence of any flow cues (Figure 27). Swimming frequency during ebb tides was significantly greater in the columns than at any of the field tethering sites. Such swimming behavior under laboratory conditions may be a manifestation of a general activity (locomotion, not necessarily vertical swimming) rhythm in addition to a swimming rhythm. In the field, the activity rhythm would result in walking movements along the bottom, and would not be detected by pressure-sensitive tags. In laboratory conditions, in 30.5 cm diameter columns, where walking is severely limited,
this activity may manifest as vertical swimming, contributing to the high swimming frequency under laboratory conditions. Crabs monitored in the columns were not attached to a tether, while crabs monitored in the field were tethered to the bottom. Thus, some tethering effect may have resulted in decreased swimming frequencies in the field. Crabs monitored in the columns also swam more during flood tides than did crabs tethered in the field, though the difference was only significant between crabs in the columns and crabs tethered in the embayment. Much of the swimming behavior in the columns during flood tide was in the 1-2 h before high tide, and may be a result of a free-running rhythm. Crabs in the columns were not exposed to any tidal cues and thus their swimming rhythm would not be entrained to the tidal cycle and would free-run. If the period length was slightly less than 12.4 h, some swimming would occur before high tide, and would occur earlier each day the crab was held in constant conditions.

Spawning blue crabs swim into the water column during ebb tide, using ETT to migrate seaward. Swimming frequency varies substantially among sites, suggesting that some areas serve as migratory corridors while others serve as foraging habitats. Ebb current velocities may explain some of these differences, but other cues also likely play a role in determining swimming frequency. Food availability, presence of predators, bottom type, and salinity may also be important and should be further investigated.
6. Large-scale movements of female blue crabs in tidal and non-tidal estuaries

6.1 Introduction

Most sexually mature adult blue crabs, *Callinectes sapidus* Rathbun, mate in the low-salinity waters of upper estuaries. Although mating is continuous throughout the active season, there are peaks in mating in the spring and fall in North Carolina (Hay 1905, Millikin & Williams 1984). Some time after mating, females migrate seaward to high salinity water (Millikin & Williams 1984, Tankersley et al. 1998, Hench et al. 2004) where they release multiple clutches of larvae (Hines et al. 2003, Dickinson et al. 2006, Darnell et al. 2009). In tidal estuaries, many ovigerous crabs (Tankersley et al. 1998, Forward et al. 2003) and females between clutches of eggs (Hench et al. 2004, Forward et al. 2005a) use ebb tide transport (ETT) to migrate, swimming into the water column during ebb tide and remaining on the bottom during flood tide. This results in step-wise movement seaward with each clutch of eggs. Females also move seaward during flood tides by walking down-estuary (Carr et al. 2004). Using walking during flood tide and walking and episodic vertical swimming during ebb tide, crabs are able to migrate ~ 5 km day\(^{-1}\) in the strongly tidal region around Beaufort Inlet, NC (Carr et al. 2004).

The majority of research on adult blue crab migratory behavior has been conducted in tidal systems, such as the Beaufort Inlet drainage and Chesapeake Bay. Tidal regimes, however, are vary over the blue crab’s range. Some estuaries are non-tidal (Roelofs & Bumpus 1953, Luettich et al. 2002).
System, the second largest estuary in the USA and the major blue crab fishing grounds in North Carolina, is a wind-driven system with a weak to nonexistent lunar tidal cycle (Roelofs & Bumpus 1953, Luettich et al. 2002). With respect to tidal systems, there are semi-diurnal tides along the majority of the east coast of the United States and South America, diurnal tides in the Gulf of Mexico, and mixed tides in the Caribbean (Garrison 2005). In non-tidal systems, local wind patterns are the primary driver of water circulation and may excite seiches and complex flow patterns, including opposing surface and bottom currents (Luettich et al. 2002). Since spawning females in tidal systems use directed walking and rely on currents for ebb tide transport, this raises questions about mechanisms of migratory behavior in non-tidal systems. In tidal systems, ETT is driven by a circatidal rhythm in vertical swimming (Forward et al. 2003, Forward et al. 2005a). In an estuary lacking predictable tidal currents, a tidal swimming rhythm is less effective than in an estuary with strong lunar tides. Swimming has the potential to result in down-estuary movement, as net flow at the surface of estuaries is generally seaward. Thus, in such an estuary with negligible lunar tides, vertical swimming results in movement seaward, regardless of the timing of the ascents. The rate of movement seaward, however, would be substantially slower than the rate of movement in a tidal estuary. Walking down-estuary is still an effective mechanism in non-tidal systems, assuming similar directional cues are present.
The timing of initiation of the spawning migration by individuals is not thoroughly understood. Turner et al. (2003) found that in the Chesapeake Bay, where the peak in reproductive activity is from June-September, females spend approximately 2 months near their molting/mating habitat and do not begin to migrate until the fall. Mature females do not appear in large numbers in the lower Bay until mid- to late October, suggesting that regardless of mating time, females are cued to migrate by some environmental signal such as temperature or photoperiod (Turner et al. 2003). Similarly, Medici et al., (2006) tagged a total of 2700 mature female crabs from late June-October in the Pamlico Sound, Albemarle Sound, and Neuse River, and found that many females do not start migrating until late September or October. It appeared as though females migrate somewhat synchronously, regardless of when they mate, and the authors hypothesized that the majority of crabs do not spawn until the following spring (Medici et al. 2006). Recent research into the circatidal swimming rhythm of female blue crabs indicates that some crabs possess a circatidal rhythm of ebb tide swimming in the reproductive stages prior to production of the first clutch, suggesting that some seaward movement may occur in these stages (see Chapter 4).

Previous studies aimed at determining when mated females begin migrating have relied on tagging mature females, with no reliable way of determining when these females mated. In addition, previous studies did not begin tagging females until June or later (Turner et al. 2003, Aguilar et al. 2005, Medici et al. 2006). In North Carolina there is
a large peak in mating in the spring (Millikin & Williams 1984). For crabs mating in the spring or summer, production of the first clutch of eggs generally occurs in the same year, as early as 3 weeks after mating (Darnell et al. 2009, see Chapter 2). I hypothesize that these crabs mating early in the year begin migrating soon thereafter rather than waiting until the fall to migrate down-estuary.

The purpose of this study was to assess large-scale movements of mature female blue crabs in tidal and non-tidal estuaries. Mark-recapture studies were conducted in three rivers in eastern North Carolina, and recently-molted and mated female crabs were tagged to ensure a relatively constant time since molting.

6.2 Methods

6.2.1 Study areas

Mark-recapture studies were conducted in three rivers in eastern North Carolina: North River, South River, and Adams Creek (Figure 31). North River is a component of the strongly-tidal Beaufort Inlet Drainage, which also consists of the Newport River estuary, Bogue Sound, and Back Sound, and is connected to Onslow Bay and the Atlantic Ocean by the 1 km wide Beaufort Inlet. North River experiences semi-diurnal tides with a ~0.5-1 m tidal range. Salinity in North River generally ranges from ~20-30 ppt.
Figure 31: Tagging locations in eastern North Carolina

South River is a tributary of the Neuse River, a sub-estuary of the Albemarle Pamlico Estuarine System. The Albemarle Pamlico Estuarine System has an area of approximately 6600 km² and is the second largest estuary in the United States. Exchange between Albemarle Pamlico Estuarine System and the Atlantic Ocean takes place through three ~1 km wide inlets: Oregon, Hatteras, and Ocracoke. Due to the large size of the system and the small size and number of inlets, the Albemarle Pamlico Estuarine System is primarily a non-tidal system, with negligible astronomical tides beyond a few kilometers from the inlets. The long axis of the system is aligned with the prevailing
winds and wind stress is the primary forcing mechanism on water level (Roelofs & Bumpus 1953, Luettich et al. 2002). Salinity in South River generally ranges from 10-20 ppt.

Adams Creek, a tributary of the Neuse River, has been modified by dredging into part of the Intracoastal Waterway (ICW) system. The ~12 km Adams Creek Canal serves to connect Adams Creek and the Neuse River to the Newport River and is strongly tidal. Tidal range in the canal is ~0.5 m. This tidal cycle extends into Adams Creek and the predominant currents in Adams Creek are tidal currents.

### 6.2.2 Tagging

Recently molted and mated female blue crabs were tagged in each of the three areas over two years (2007-2008). In 2007, tagging took place from June-October. Tagging in 2008 began in April and continued through August. Recently molted female blue crabs were identified by their clean carapace, lack of visible mature ovaries, and by the ability to manually depress the carapace at the base of the lateral spines. Crabs were captured in crab pots and fitted with 2.54 cm × 5.08 cm individually-numbered plastic across-the-back tags (Floy Tag, Inc. Seattle, WA), attached with 18 gauge plastic-coated copper wire looped around the large lateral spines (Figure 32). Each tag was numbered and printed with a telephone number, the text “student project”, and a request for capture date, location, and egg mass color (if present).
Crabs were tagged and released immediately upon capture, typically within 1 km of the capture location. Location (latitude and longitude) was recorded for each release. All tagging was done with the assistance of local commercial crabbers, and the number of crabs tagged in each location in any given time period depended on the number of crabs caught as well as fishing effort (Figure 33). A total of 3000 recently-molted female crabs were tagged over the course of the study.
6.2.3 Recaptures

Recapture data were obtained from commercial and recreational crabbers and fishers. A $5 reward was paid for each recapture reported. Many of the crabbers reporting recaptures did not have GPS units on their boats and were only able to report general locations based on water bodies and landmarks. For these recaptures, latitude and longitude were approximated. In cases where only the name of a body of water was given (typically small rivers or creeks), the center of that body of water was assigned as the recapture location. Though most crabs were harvested after capture, some crabbers released recaptured crabs alive so that multiple recaptures were possible.
6.2.4 **Analysis**

For each recapture, time at liberty, movement direction, and distance traveled were calculated. Time at liberty was calculated as the time interval (in days) between tagging and recapture. Movement direction and distance traveled were calculated using ArcView GIS 3.2 (Environmental Science Research Institute, Redlands, CA) and the Distances and Bearings between Matched Features extension (Jenness 2007). Mean distances and directions traveled for each of the tagging areas were calculated using circular statistics (Batschelet 1981). Time at liberty, movement direction, and distance traveled were used as response variables and analyses of variance (ANOVA) were used to compare each response variable among seasons (spring: April-May, summer: June-August, fall: September-October) and tagging areas (North River, South River, Adams Creek). When significant differences were detected by ANOVA at \( p < 0.05 \), a Holm-Sidak test was used for all pairwise comparisons (Zar 1999). For each river, the distribution movement directions was tested for uniformity using Rao’s Spacing Test (Batschelet 1981).

6.3 **Results**

6.3.1 **North River**

During 2007 and 2008, recently molted female crabs were tagged in the tidal North River which drains primarily through Beaufort Inlet. Recaptures of crabs tagged in North River occurred in North River \( (n = 308) \), Core Sound \( (n = 14) \), Newport River \( (n \)
Adams Creek Canal (n = 4), Bogue Inlet (n = 2), South River (n = 1), Back Sound (n = 1), Stump Sound (n = 1), and the Intracoastal Waterway near Wilmington, NC (n = 1) (Figure 34). The crab recaptured near Wilmington, NC was recaptured 141 km from the tagging location after being at liberty for 271 d. Of the 1322 crabs tagged, 290 (21.9%) were recaptured at least once and 33 (2.4%) were recaptured at least twice. Crabs tagged in North River were recaptured up to 4 times. Three crabs were recaptured while ovigerous. These recaptures occurred in the Newport River, Back Sound, and Adams Creek canal (Figure 35).

Time at liberty between tagging and final recapture averaged 21.1 ± 2.0 d. Time at liberty varied with season in which crabs were tagged (Figure 36) (ANOVA, p < 0.001). Crabs tagged in the fall were at liberty significantly longer than crabs tagged during the spring or summer (Holm-Sidak, p < 0.05) (Figure 36). Straight-line distances between tagging locations and recapture locations ranged from 0.1-140.8 km. Average straight-line distance traveled was 3.3 ± 1.2 km, and varied with tagging season (ANOVA, p = 0.029). Crabs tagged in the fall traveled farther than crabs tagged in the summer (Holm-Sidak, p < 0.05). Distances traveled were similar between spring and summer and spring and fall (Holm-Sidak, p > 0.05). Distance traveled also varied significantly with time at liberty (ANOVA, p < 0.001). Crabs at liberty for > 60 days traveled significantly farther than crabs at liberty for <30 or 30-60 d (Holm-Sidak, p < 0.05). Movement rate, determined based on straight-line distance traveled and time at liberty, averaged 0.3 ±
0.1 km d\(^{-1}\). Movement rate varied among tagging seasons (ANOVA, \(p = 0.004\))(Figure 36). Movement rate for crabs tagged in the spring was significantly higher than for crabs tagged in the summer (Holm-Sidak, \(p < 0.05\)). Movement rate was similar between summer and fall and spring and fall (Holm-Sidak, \(p > 0.05\)). Movement rate did not vary significantly with time at liberty (ANOVA, \(p = 0.06\)). The distribution of directions traveled by crabs tagged in North River was not uniform but was significantly oriented (Rao’s Spacing Test, \(p < 0.01\)). Mean direction traveled was approximately SSE (175.1 ± 6.2°).

### 6.3.2 South River

During 2007 and 2008, recently molted female crabs were tagged in the non-tidal South River, which drains into the Neuse River. Recaptures of crabs tagged in South River occurred in South River (\(n = 252\)), Neuse River and its tributaries (\(n = 21\)), Adam’s Creek (\(n = 9\)), Newport River (\(n = 8\)), Pamlico Sound (\(n = 7\)), Bogue Sound (\(n = 2\)), near Hatteras Inlet (\(n = 2\)), Core Sound (\(n = 1\)), Adams Creek canal (\(n = 1\)), and near Ocracoke Inlet (\(n = 1\))(Figure 34). Of the 1194 crabs tagged in South River, 214 (17.9%) were recaptured at least once and 57 (4.8%) were recaptured at least twice. Crabs tagged in South River were recaptured up to 8 times. Of the 214 crabs recaptured, 6 were recaptured while ovigerous. These ovigerous crabs were recaptured in the Newport River, Adams Creek canal, and Neuse River (Figure 35).
Figure 34: Recapture locations for crabs tagged in (a) North River, (b) South River, and (c) Adams Creek, grouped by general area. The size of each circle indicates the number of crabs.
Time at liberty between tagging and final recapture averaged 23.0 ± 2.8 d. Time at liberty varied with season in which crabs were tagged (ANOVA, \( p < 0.001 \)) (Figure 36). Crabs tagged in the fall were at liberty significantly longer than crabs tagged during the spring or summer (Holm-Sidak, \( p < 0.05 \)). Straight-line distances between tagging locations and recapture locations ranged from 0-78.8 km. Average straight-line distance traveled was 5.8 ± 0.9 km, and varied with tagging season (ANOVA, \( p = 0.029 \)) (Figure 36). Crabs tagged in the spring traveled significantly farther than crabs tagged in the summer or fall (Holm-Sidak, \( p < 0.05 \)). Distance traveled also varied significantly with time at liberty (ANOVA, \( p < 0.001 \)) as longer times at liberty resulted in increasing
distances traveled (Holm-Sidak, $p < 0.05$). Movement rate, determined based on straight-line distance traveled and time at liberty, averaged $0.3 \pm 0.04$ km d$^{-1}$. Movement rate varied among tagging seasons (ANOVA, $p < 0.001$)(Figure 36). Movement rates for crabs tagged in the spring and summer were similar (Holm-Sidak, $p > 0.05$), and significantly higher than for crabs tagged in the fall (Holm-Sidak, $p < 0.05$). Movement rate did not vary significantly with time at liberty (ANOVA, $p = 0.568$). The distribution of directions traveled by crabs tagged in South River was uniform (Rao’s Spacing Test, $p > 0.05$).

6.3.3 Adams Creek

During 2007 and 2008, recently molted female crabs were tagged in Adams Creek, which connects the tidal Newport River to the non-tidal Neuse River. Recaptures of crabs tagged in Adams Creek occurred in Adams Creek (n = 219), Newport River (n = 35), Adams Creek canal (n = 10), Neuse River and its tributaries (n = 6), South River (n = 3), Bogue Sound (n = 1), and near the Emerald Isle beach (Figure 34). Of the 484 crabs tagged in Adams Creek, 190 (39.3%) were recaptured at least once and 52 (10.7%) were recaptured at least twice. Crabs tagged in Adams Creek were recaptured up to 5 times. Of the 190 recaptured crabs, 16 were recaptured while ovigerous, in Adams Creek canal (n = 3) and Newport River (n = 13)(Figure 35).
Figure 36: Time at liberty (a, d), distance traveled (b, e), and movement rate (c, f) for crabs tagged during the spring, summer, and fall. Data are grouped by tagging area in panels a-c. Panels d-f represent all crabs, regardless of tagging area.
Time at liberty between tagging and final recapture averaged 30.5 ± 3.8 d. Time at liberty varied with season in which crabs were tagged (ANOVA, \( p < 0.001 \))(Figure 36). Crabs tagged in the fall were at liberty significantly longer than crabs tagged during the spring or summer (Holm-Sidak, \( p < 0.05 \)). Straight-line distances between tagging locations and recapture locations ranged from 0-36.8 km. Average straight-line distance traveled was 6.1 ± 0.6 km, and varied with tagging season (ANOVA, \( p < 0.001 \))(Figure 36). Crabs tagged in the spring traveled significantly farther than crabs tagged in the summer (Holm-Sidak, \( p < 0.05 \)). Distance traveled was similar between spring and fall and between summer and fall (Holm-Sidak, \( p > 0.05 \)). Distance traveled also varied significantly with time at liberty (ANOVA, \( p < 0.001 \)). Crabs at liberty 30-60 or >60 d traveled similar distances (Holm-Sidak, \( p > 0.05 \)), both significantly greater than crabs at liberty for <30 d (Holm-Sidak, \( p < 0.05 \)). Movement rate, determined based on straight-line distance traveled and time at liberty, averaged 0.5 ± 0.1 km d\(^{-1}\). Movement rate was statistically similar among tagging seasons (ANOVA, \( p = 0.204 \))(Figure 36). Movement rate did not vary significantly with time at liberty (ANOVA, \( p = 0.377 \)). The distribution of directions traveled by crabs tagged in Adams Creek was not uniform but was significantly oriented (Rao’s Spacing Test, \( p < 0.01 \)). Mean direction traveled was approximately SSW (188.6 ± 10.1°).
6.3.4 Inter-site comparisons

Straight-line distances traveled by crabs tagged in this study ranged from 0-140.8 km (mean = 4.9 ± 0.4 km), with the vast majority (85.9%) of crabs recaptured within 10 km of the tagging location. Time at liberty ranged from 0-271 d (mean = 24.3 ± 1.6 d). Most crabs (64.1%) were recaptured within 14 d of tagging. Movement rate ranged from 0-18.7 km d⁻¹ (mean = 0.4 ± 0.04 km d⁻¹), though 99.7% of crabs moved < 4.5 km d⁻¹ and 91.1% of crabs moved < 1 km d⁻¹.

Distance traveled, time at liberty, and movement rate all varied significantly among seasons (ANOVA, p < 0.01)(Figure 36). Crabs tagged in the fall were at liberty significantly longer than crabs tagged in either the spring or summer (Holm-Sidak, p < 0.05)(Figure 5b). Crabs tagged in the spring and fall traveled similar distances, significantly higher than crabs tagged in the summer (Holm-Sidak, p < 0.05)(Figure 6b). Movement rate was highest for crabs tagged in the spring, significantly lower for crabs tagged in the fall (Holm-Sidak, p < 0.05), and intermediate for crabs tagged in the summer (Holm-Sidak, p > 0.05).

Distance traveled, time at liberty, and movement rate also varied among tagging areas (ANOVA, p < 0.05)(Figure 37). Crabs tagged in Adams Creek and South River traveled similar distances (Holm-Sidak, p > 0.05), both greater than crabs tagged in North River (Holm-Sidak, p < 0.05). Time at liberty was significantly greater for crabs from Adams Creek than for crabs from North River (Holm-Sidak, p < 0.05). Time at
liberty was similar between crabs from Adams Creek and South River and between crabs from South River and North River (Holm-Sidak, $p < 0.05$). Movement rate followed the same pattern, with Adams Creek being significantly higher than North River (Holm-Sidak, $p < 0.05$), but statistically similar to South River (Holm-Sidak, $p > 0.05$).

Figure 37: Distance traveled (a), time at liberty (b), and movement rate (c) for crabs tagged in each of the 3 areas. Different letters indicate statistically significant differences at $p < 0.05$. 

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6.4 Discussion

To gain insight into movements of female blue crabs in different tidal regimes, 3000 recently-molted and mated female blue crabs were tagged in three NC rivers. Results suggest that significant seaward migration does not take place until several weeks after mating, presumably upon production of a clutch of eggs. Straight-line distances traveled ranged from 0-140.8 km. Overall mean straight-line distance traveled (4.9 ± 0.4 km) was nearly an order of magnitude less than observed by Aguilar et al. (2005) for mature female crabs tagged in the Chesapeake Bay (35.7 ± 4.06 km). While time at liberty was slightly lower for crabs tagged here (24.3 ± 1.6 d v. 32.1 ± 4.59 d), this would not explain the large difference in distance traveled unless movement rate increased substantially 3-4 weeks after mating. Because only recently-molted females were tagged in this study, mating can be assumed to have taken place ≤ 14 d before crabs were tagged. This is in contrast to the mark-recapture study conducted by Aguilar et al. (2005), who tagged mature females but were unable to determine when these females had mated. Recapture rates (17.9-39.3%) were relatively high compared to previous blue crab tagging efforts (5-21%: Turner et al. 2003, 11.6%: Aguilar et al. 2005, 0.3-20%: Medici et al. 2006). While greater fishing pressure would result in higher recapture rates, our own qualitative observations indicate that fishing pressure was likely less during the study period than during previous years in the same areas, as many crabbers were shifting to other fisheries because of low blue crab catches. I
hypothesize that the high recapture rates are due to the limited movement of crabs soon after their molt to maturity. Many crabs remained in areas where they were tagged until recaptured.

Though most crabs traveled relatively short distances, the direction of movement was significantly oriented for crabs from North River and Adams Creek. Crabs tagged in North River traveled at a heading of ~ 175°. This is within 1-2° of the orientation of the long axis of North River, in a down-estuary direction. Crabs tagged in Adams Creek traveled at a heading of ~189°. Flow in Adams Creek is dominated by the lunar tidal cycle due to its connection with the Adams Creek Canal. This heading is in the direction of ebb tide flow in Adams Creek. These results suggest that crabs in these two areas are moving down-estuary, albeit at much slower rates than ovigerous females. This hypothesis is supported by observations on endogenous swimming rhythms of recently-molted female blue crabs (see Chapter 4), as many recently-molted females display a circatidal swimming rhythm with peak swimming occurring during ebb tides. This would serve to move crabs down-estuary on falling tides. The distribution of directions traveled by crabs tagged in South River was not significantly different from uniform. The crabs from South River that traveled the farthest, however, generally traveled in a seaward direction, either into Pamlico Sound or down the Adams Creek canal into Newport River (Figure 34). Thus, crabs tagged in South River also generally moved seaward. Observations on swimming rhythms of recently-molted females from South
River suggest that a circadian rhythm, rather than a circatidal rhythm, may be present, and that peak swimming occurs around the time of sunset (Darnell, unpublished data). A similar rhythm is seen in ovigerous crabs collected from South River (see Chapter 4). Such a rhythm would not result in rapid seaward movement. Thus, seaward movement of crabs in non-tidal areas likely results from walking or swimming in response to environmental cues that indicate seaward flow.

Several crabs were recaptured while ovigerous. Three crabs tagged in North River were ovigerous when recaptured in the Newport River (n = 2), and on the south side of Harkers Island in Back Sound (n = 1). Of 16 crabs tagged in Adams Creek and recaptured while ovigerous, all were in the Newport River. The 6 crabs tagged in South River and recaptured while ovigerous were recaptured in the Newport River (n = 5) and the Neuse River, just east of the mouth of South River (n = 1). These results clearly indicate that the Adams Creek Canal serves as a migratory corridor for crabs from Adams Creek, South River, and potentially other tributaries of the Neuse River. Crabs maturing in Adams Creek likely moved down the canal using ebb tide transport driven by a circatidal swimming rhythm. Crabs tagged in South River may have moved into Adams Creek, and upon exposure to a tidal cycle, their rhythm may have become entrained to the tidal cycle. Thus, crabs maturing in tributaries of the Neuse River that move into Adams Creek and encounter the tidal cycle would become entrained to this cycle and move down-estuary into the Newport River using ebb tide transport.
In addition to the crabs that were recaptured while ovigerous, many crabs were likely between clutches of eggs when recaptured. The crab that traveled the greatest distance, 141 km, was tagged in September, 2007 and recaptured in late June, 2008, 271 d after being tagged. Thus, it had likely produced at least one clutch of eggs (Darnell et al. 2009, see Chapter 2). Other crabs that were at liberty for substantial lengths of time had also likely spawned at least once prior to being recaptured, as time from mating to production of the first clutch can be as low as 21 d, though this interval is dependent on crab size and the time of year that the crab mated (Darnell et al. 2009, see Chapter 2). While it may be possible to determine whether or not a female has spawned previously based on pleopod condition (Rittschof, unpublished data), the crabbers providing recapture data in this study were neither trained nor instructed to make this assessment. Thus, no data are available on the spawning history of crabs between tagging and recapture.

Distance traveled, time at liberty, and movement rate all varied among tagging areas (Figure 37). On average, crabs tagged in South River and Adams Creek traveled farther than crabs in North River. Time at liberty was highest for crabs tagged in Adams Creek and lowest for crabs tagged in North River, while crabs tagged in South River were at liberty for an intermediate length of time. Movement rate followed the same pattern. I hypothesize that this difference in movement rate between Adams Creek and North River, both tidal systems, may be due to the salinities of the two areas during the
study period. The two years of this study, 2007 and 2008, were both drought years. Salinity in North River ranged from 20-30 ppt. The ultimate destination of the blue crab spawning migration is high-salinity water, with salinities above ~ 22 ppt (Rittschof et al., In Review). Thus, crabs tagged in North River were already in or near high-salinity water suitable for spawning. Adams Creek, however, is much lower in salinity (10-20 ppt). Thus, crabs tagged in Adams Creek had to move to higher salinity water prior to spawning.

Distance traveled, time at liberty, and movement rate also varied with season (Figure 36). Crabs tagged in the spring and fall moved the greatest distances. The large distances moved by crabs tagged in the fall is likely due to the long time at liberty for these crabs. Fishing pressure decreases substantially in the fall and winter, resulting in fewer recaptures and longer time periods when crabs are at liberty. Crabs tagged in the spring, however, were recaptured relatively quickly, as evidenced by low times at liberty. Thus, longer times at liberty do not explain the larger distances traveled by crabs tagged in the spring. Crabs mating in the spring rapidly produce the first clutch of eggs the same year, due to warmer temperatures during the summer (Darnell et al. 2009, see Chapter 2). Warmer temperatures may allow crabs tagged in the spring to more rapidly accumulate resources and prepare for migration, thus resulting in higher movement rates. Similar patterns were seen in each of the three tagging areas, with the exception of movement rate in the strongly tidal North River, which was similar in spring and fall.
Results of this study support the hypothesis that, for most crabs, long-distance migratory movements do not begin until near the time of production of the first clutch of eggs. I hypothesize that down-estuary movement occurs before production of the first clutch, though at a much slower rate than while ovigerous. In tidal systems, this movement appears to be accomplished using directed walking and ebb tide transport driven by a circatidal rhythm in vertical swimming, similar to the spawning migration of ovigerous females. In non-tidal estuaries, however, seaward movement may depend on down-estuary walking and swimming in response to environmental cues.

Crabs tagged in this study moved slowly, but once the seaward migration began in earnest, crabs rapidly moved seaward. Although most crabs were recaptured before moving out of the area in which they were tagged, several crabs moved great distances, up to 144 km from the tagging site. Results from previous tagging studies have suggested that migration does not begin until the fall (e.g. Aguilar et al. 2005). Here, movement rate was greatest in the spring and lowest for crabs tagged in the fall, and distance traveled was similar between spring and fall. Although crabs in the Chesapeake Bay may not appear in the lower Bay until October, it is likely that they are spawning prior to that point and are actually between clutches or on their second or later clutch by the time they reach the mouth of the Bay.
7. Synthesis

This dissertation investigated the spawning biology of female blue crabs *Callinectes sapidus*. Reproductive timing and lifetime reproductive potential were examined to provide a timeline for female blue crabs maturing at different times of the year. Migratory behavior was investigated in the laboratory and in the field, in a variety of habitats and tidal regimes, to provide insight into the mechanisms underlying the spawning migration.

Female blue crabs molt to maturity and mate immediately thereafter. Size at maturity is influenced by water temperature, with cooler temperatures resulting in larger crabs. This results in a U-shaped pattern of female size throughout the molting season, with larger crabs in the spring and fall and smaller crabs in the summer. After mating, female blue crabs undertake a spawning migration seaward. Results from this dissertation indicate that, depending on the season, some seaward movement takes place soon after mating, prior to oviposition of the first clutch (Chapters 4, 6). In tidal estuaries, this seaward movement occurs by ebb tide transport, driven by an endogenous swimming rhythm of swimming during ebb tides, and down-estuary walking. Endogenous swimming rhythms were present in all pre-ovigerous stages examined, though the type of rhythm and timing of activity was variable. Recently-molted crabs had either a circadian rhythm of swimming around the time sunset or a circatidal rhythm of swimming on ebb tides. Females with mature ovaries typically had
circatidal or circalunidian rhythms of swimming around the time of high tide. This rhythm was similar for crabs tested with food and crabs tested without food. These results suggest that some seaward movement, driven by ebb tide transport, occurs prior to initial oviposition. Swimming frequencies were generally lower for recently-molted crabs and crabs with mature ovaries than for ovigerous crabs, indicating that seaward movement is not as rapid in the stages prior to oviposition. These results were supported by results of the mark-recapture study (Chapter 6). Recently-molted female crabs were tagged and released, and recapture data were obtained from local crabbers. Movements of crabs in all three areas (North River, Adams Creek) were generally seaward, though movement rates were much lower than those observed by previous mark-recapture studies of mature female movements (e.g. Aguilar et al. 2005).

For crabs molting to maturity and mating in low-salinity areas, seaward movement prior to oviposition is necessary to ensure that oviposition occurs at salinities suitable for embryonic and larval development. Rittschof et al. (In Review) examined the distribution of ovigerous females in the Albemarle-Pamlico Estuarine System and found that ovigerous crabs were primarily found in areas with salinity > 22 ppt, suggesting that crabs are moving to higher salinities prior to oviposition. This salinity is very close to the minimum salinity in which successful larval development occurs (20.1 ppt, Costlow & Bookhout 1959).
Once in high-salinity areas, female blue crabs produce multiple clutches of eggs over the course of their lifetime. The timing of these clutches is dependent on mating season and crab size (Dickinson et al. 2006, Chapter 2). Crabs mating in the spring begin spawning in the summer and often complete spawning in the fall. Crabs mating the summer begin spawning in the same year, and often overwinter before resuming spawning in the spring. Crabs mating in the fall delay spawning until late spring and complete spawning by the following fall. This seasonal variation is due primarily due to temperature variation. Cooler temperatures slow egg production.

Size at maturity also plays a role in determining the timing of clutch production. Time to first clutch and time between clutches were positively correlated with carapace width. Smaller crabs more rapidly produce their first clutch and all subsequent clutches. Larger crabs, however, produce larger clutches of eggs (Hines 1982, Dickinson et al. 2006), so reproductive potential is similar for most sizes of blue crabs.

Time to first clutch, clutch production interval, and mature lifespan are best described by degree-days, physiological time calculated as a thermal integral. Using degree-days, the effects of seasonal temperature variations can be reduced, allowing assessment of the effects of other variables such as body size. Degree-days may be a useful metric for future studies of blue crab reproductive patterns and migratory movements.
Crabs confined in the field produced up to 7 clutches of eggs in their lifetime. Egg lipid content, egg diameter, larval carapace width, and larval survival time without food were similar for all clutches. The percentage of embryos developing normally decreased 40% from clutch 1 to clutch 4, likely due to decreasing egg and sperm viability over time. Clutch volume decreased 50% from clutch 1 to clutch 5. 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 reproductive strategy maximizes potential reproductive output. These results indicate that most of a crab’s reproductive output is from the first few clutches. Thus, it would seem advantageous for a female blue crab to be as far seaward as possible before production of the first clutch of eggs, to ensure that the first clutch is successfully transported offshore where predation is reduced (Morgan 1990, Hovel & Morgan 1997, Morgan & Christy 1997) and conditions are appropriate for larval development (Saigusa 1981, Forward et al. 1982).

Upon production of the first clutch of eggs, tidal swimming rhythm become more apparent, and swimming activity increases (Chapter 3). Migration rate increases, from ~0.4 km d\(^{-1}\) for recently-molted females (Chapter 6) to 3-6 km d\(^{-1}\) for ovigerous females and females between clutches (Carr et al. 2004, Aguilar et al. 2005). This increase in swimming behavior appears to stimulated by a blend of molecules generate from the egg mass (Chapter 4). Upon production of the first clutch, enzymatic digestion of the egg membranes and the glue that attaches the eggs to the pleopods generates various
molecules, including peptides and sugars. This blend of molecules stimulates increased swimming behavior by the female.

Following embryonic development, egg hatching follows the conceptual model for subtidal crabs developed by Forward and Lohmann (1983) for *Rhithropanopeus harrisi*, in which the embryos control the time of hatching, while the female controls hatching synchrony. Hatching is synchronized through a positive feedback loop. Following hatching of a few eggs, peptide pheromones are generated from the egg mass, which stimulate abdominal pumping, thus rupturing more eggs and releasing more peptides (Forward & Lohmann 1983). These pheromones have been studied in detail in *R. harrisi*, and consist of short peptides with a neutral amino acid preceding arginine or lysine at the carboxyl-terminus (Rittschof et al. 1989, Rittschof & Cohen 2004). Similar peptides generated from the egg mass stimulate larval release behavior in blue crabs, as delivery of egg extract, bradykinin (a pheromone mimic), or trypsin (a pheromone-generating enzyme) stimulated increased abdominal pumping. Female sensitivity to these pheromones is slightly greater during the time of flood tide, though the difference in sensitivity is overcome at higher concentrations.

Following release of a clutch of eggs, some of the glue that had been used to attach the eggs as well as remnants of the egg membranes remains on the pleopods for several days to several weeks. Continued enzymatic digestion of these remnants continues to release the compounds, though production decreases over time as the
pleopods becomes clean. Thus, the swimming rhythm persists for many crabs between clutches of eggs, though not as strongly as in females incubating a clutch.

Throughout the blue crab reproductive period, female crabs are continually moving seaward using ebb tide transport driven by a circatidal or circalunidian rhythm in vertical swimming (Forward et al. 2003, Forward et al. 2005a) and seaward walking (Carr et al. 2004). Swimming behavior varies based on the tidal cycle present in the estuary (Chapter 3). The blue crab range includes estuaries with semi-diurnal tides, diurnal tides, and some estuaries with no appreciable lunar tidal cycle. Endogenous vertical swimming rhythms, monitored under constant conditions in the laboratory, reflect the tidal cycle in the crab’s home estuary. Crabs in estuaries with semi-diurnal tides such as those along the east coast of the US have one of two rhythms: (1) a circatidal swimming rhythm of swimming on every ebb tide, or (2) a circalunidian swimming rhythm of swimming on every other ebb tide. Both rhythms would successfully move crabs seaward, with possible trade-offs between migration rate and predation risk. In estuaries with diurnal tidal cycles, such as those in the northern Gulf of Mexico, ovigerous crabs have a circulunidian rhythm, corresponding to the diurnal tidal in the home estuary. Endogenous swimming rhythms were also examined for crabs from South River, a non-tidal estuary. Ovigerous crabs from South River, NC exhibited a circadian rhythm with peak swimming occurring around the time of sunset. Any seaward movement resulting from such a rhythm would be due only to wind-driven
currents and residual flow seaward at the surface of the estuary. Thus, crabs migrating in non-tidal estuaries may migrate seaward by swimming in response to environmental cues that would indicate seaward flow or by walking in a seaward direction. In the tidal Beaufort Inlet drainage, peak swimming occurred during the times of maximum decreasing water level (Chapter 5), suggesting that decreasing hydrostatic pressure may serve as the entrainment cue for synchronizing the circatidal swimming rhythm with the actual tidal cycle. In non-tidal estuaries, decreasing hydrostatic pressure may serve as a cue for vertical swimming. Crabs tethered in non-tidal West Bay did not swim during the duration of the study period, preventing assessment of possible cues for swimming. Decreasing pressure tendency was lower in West Bay than at the three tidal sites near Beaufort Inlet. It is possible that crabs tethered in West Bay did not experience the appropriate conditions necessary to stimulate vertical swimming. Future studies of blue crab swimming behavior in non-tidal estuaries may clarify the mechanisms underlying migration in these areas.

Vertical swimming behavior also varies within estuaries, based on a crab’s position within the estuary and the environmental conditions experienced (Chapter 5). When tethered in the field under natural conditions, not all crabs swim. The decision to swim may be based on energetics. In certain areas with low ebb current velocities, it may not be energetically favorable to swim, and walking down-estuary may be energetically favorable. For crabs tethered near Beaufort Inlet, NC, swimming frequency was greatest
in the ~4.5 m deep channel near Pivers Island bridge, and was significantly lower in the shallow (~0.8 m deep) Rachel Carson embayment and the ~1 m deep sand flat near Kirby-Smith Island. Among the sites, swimming followed a trend of increasing swimming frequency with increasing ebb tide current velocity. Additionally, in some areas it may be advantageous to remain in the area foraging rather than continuing seaward migration. I hypothesize that, once in high-salinity areas of the lower estuary, some areas may serve as migratory corridors for spawning blue crabs, while others serve as foraging habitat. Decreased swimming in shallow, low-energy foraging habitat would allow the crabs to replenish energy stores and reduce predation risk by large fish predators that may be present in other areas. It is likely a combination of factors, including current velocity (or some other variable such as turbulence that varies with velocity), and possibly others such as food availability, presence of predators, and water depth, that contribute to determining swimming frequency of crabs at a given site, and define areas as foraging or migratory areas.

In the three tidal tethering sites examined, swimming frequency was significantly lower than swimming frequency of crabs monitored under constant laboratory conditions in plexiglass columns. This difference may be due in part to a tether effect, which may reduce the time spent swimming by tethered crabs. Additionally, the high frequency of swimming observed in the columns may be an escape response, as crabs are limited in their walking area. It is possible that a
swimming rhythm observed in the laboratory may be expressed in the field as a locomotor rhythm in which activity levels, but not necessarily vertical swimming frequency, varies based on the tidal or diel cycle. Thus, observations of vertical swimming behavior in constant laboratory conditions should be verified in the field.

Tankersley et al. (1998) developed a conceptual model for the blue crab spawning migration, in which females re-enter estuaries using flood tide transport following larval release. The results of this dissertation do not support post-release flood tide transport. Although one crab tethered at Kirby-Smith Island began swimming during flood tide after larval release, most crabs continued to swim during ebb tides following larval release (Chapter 5). Hench et al. (2004) found similar swimming patterns, with ebb tide swimming continuing after larval release. Although endogenous vertical swimming rhythms in post-release crabs were not examined here, Forward et al. (2005) determined that females between clutches of eggs become arrhythmic following larval release in constant conditions, but again display a circatidal rhythm of ebb tide swimming following exposure to a tidal cycle. Female crabs releasing larvae in the estuary would be expected to continue migrating seaward rather than returning to the estuary, though swimming behavior would decrease or cease if suitable high-salinity foraging habitat was encountered. Thus, some females likely complete the spawning migration within estuaries, while others migrate out the inlets into the coastal ocean.
Further research is necessary to examine behavior and movements of spawning females that reach the coastal ocean.
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