ESTUARINE INGRESS OF THE BLUE CRAB *CALLINECTES SAPIIDUS*

by

Matthew Bryan Ogburn

Nicholas School of the Environment and Earth Sciences
Duke University

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Approved:

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Richard B. Forward, Jr., Supervisor

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Daniel Rittschof

___________________________
Larry B. Crowder

___________________________
Richard T. Barber

___________________________
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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Environment in the Graduate School of Duke University

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ABSTRACT

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Abstract

This dissertation investigated ingress of postlarval blue crabs *Callinectes sapidus* to the Newport River estuary, North Carolina, USA. Data from *C. similis*, *Menippe mercenaria*, *Pachygrapsus transversus*, and *Arenaeus cribrarius* are included in some chapters for comparison. Changes in tolerance to low salinity were examined by: 1) exposing postlarvae (megalopae) collected in coastal and estuarine areas to a range of salinities and 2) determining the cue that stimulates acclimation of coastal megalopae to low salinities, the time to acclimation, and the decrease in salinity necessary for acclimation. Coastal megalopae were less tolerant to salinities of 5 and 10 than megalopae from the estuary. Coastal megalopae became acclimated to low salinities within 12 h when salinity was reduced from 35 to 31.

Spatial patterns in abundance during ingress were investigated simultaneously in coastal and estuarine areas. Coastal distributions were determined using nighttime surface plankton tows at slack water after ebb tide and slack water after flood tide on four nights; two each during spring and neap tides. Estuarine distributions were determined using nightly settlement on ‘hog’s hair’ collectors. *C. sapidus* megalopae were most abundant at the coast east of Beaufort Inlet, but settlement was restricted to western channels of the estuary. Species-specific patterns in abundance were
maintained during two spring/neap cycles, possibly due to interactions between larval behavior and physical forcing.

Biophysical mechanisms of estuarine ingress were investigated by comparing nightly abundance in coastal and estuarine areas with environmental variables. Comparisons were made using cross-correlation and cross-fourier analyses. High estuarine abundances were associated with wind-driven estuarine inflow and nighttime flood tides. The seasonal pattern of estuarine ingress was strongly associated with the seasonal pattern of alongshore wind stress, suggesting that inter-annual variations in atmospheric forcing may determine the yearly abundance of megalopae arriving in estuarine nursery habitats.

The effect of sampling interval on annual megalopal abundance estimates was determined using an 11-year dataset of nightly settlement. Variability in abundance estimates increased with increasing sampling interval. Switching from a one day to two day sampling interval resulted in a 20 % decrease in the likelihood of detecting a significant correlation between annual abundance and CPUE in the North Carolina blue crab pot fishery.
Acknowledgements

I am grateful to my advisor, Richard Forward, who shares my interest in studying the recruitment of blue crab postlarvae to estuarine nursery habitats. We each had received funding for similar projects, and our collaboration resulted from my need for a suitable advisor and his need for a student to take on the project. His support has been essential to the successful and timely completion of this research. I wish him the best of luck in his upcoming retirement, but I hope he never retires from science. I must also thank Humberto Diaz, my ‘technician’ and second advisor, for always challenging me to work hard, explore new possibilities, and enjoy life while doing it.

I would also like to thank the members of my committee for their contributions to my education. Dan Rittschof is a friend and collaborator with whom I have had countless discussions about blue crab biology and fisheries management, and who got me interested in blue crabs in the first place. Dick Barber was my first oceanography professor and has always challenged me to think broadly. Larry Crowder has inspired me to consider the effects of fishing not only on populations, but on entire ecosystems. Last, but not least, Rick Luettich has helped me understand how my critters interact with the physical environment they live in.

Each of my family members, friends, and fellow students have supported me in this work, which I will strive to thank them for in person. I am indebted to my lab
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My final thanks go to the fishermen and women who made Triple S and Sportsman’s fishing piers wonderful places to work. This dissertation would not have been possible if you hadn’t been interested in the project and taken time to make sure nothing happened to my collectors. I am indebted to you and share the loss you feel after both piers were dismantled to make room for a few more condominiums.
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1. Introduction

The blue crab *Callinectes sapidus* is an ecologically and economically important species that inhabits estuaries of the western Atlantic from Nova Scotia to Argentina (Williams 1984). One of many estuarine crustacean species that undergoes larval development in continental shelf waters, *C. sapidus* migrate back to estuaries as postlarvae prior to metamorphosis to the first juvenile stage. During this migration, the relatively weak-swimming postlarval stage must adapt to low salinities and take advantage of wind-driven and tidal currents to successfully recruit to estuarine nursery habitats. Understanding these processes will add insight into *C. sapidus* physiology, behavior, and population dynamics. Monitoring the number of postlarvae entering estuaries each year may yield data that help explain large variations in annual fishery landings. The purpose of this dissertation was to investigate: 1) the physiological changes that occur as *C. sapidus* postlarvae enter estuaries, 2) the biophysical mechanisms underlying postlarval supply to estuarine nursery habitats, and 3) the effect of sampling interval on annual estimates of post larval abundance. Data from *C. similis*, *Menippe mercenaria*, *Pachygrapsus transversus*, and *Areneaus cribrarius* are often included to provide additional insight into the physiology and behavior of *C. sapidus* postlarvae.
1.1 Life History

Adult *C. sapidus* live primarily in low salinity areas at the upper reaches of estuaries. After mating, ovigerous females migrate to high salinity water near the coast where they release several clutches of larvae (Turner et al. 2003, Carr et al. 2004, Dickinson et al. 2006). Larvae are released at the surface near the time of slack water after flood tide at sunrise and are transported seaward during ebb tide by remaining near the surface (Epifanio 1988, Ziegler 2002). They can be rapidly advected to the coastal ocean in estuarine plume water and mixed seaward during coastal upwelling (Natunewicz et al. 2001). Such transport of larvae from estuarine to oceanic habitats is known as the ‘export strategy’ (Strathmann 1982).

In North Carolina, spawning begins in mid-March, reaches a peak from June to August, and declines to low levels by October (Dudley and Judy 1971). Larval development lasts about 30-50 days and consists of seven zoeal stages and one postlarval (megalopal) stage (Costlow and Bookhout 1959). Zoeae develop near the surface in continental shelf waters, whereas megalopae migrate from the shelf back to estuaries where they settle in submerged aquatic vegetation or other structured habitats and metamorphose to the first juvenile stage (Johnson 1985, Orth and van Montfrans 1987, Mense and Wenner 1989, Epifanio et al. 2003). Juveniles move up-estuary toward adult habitat at approximately 25 mm carapace width and reach maturity after 10-20
months (Millikin and Williams 1984, Orth and van Montfrans 1987, Reyns and Eggleston 2004). Most adults live one additional year for an average life span of 2-3 years (Millikin and Williams 1984).

1.2 Osmoregulation

*Callinectes sapidus* experience relatively constant salinity during zoeal development in the coastal ocean, but megalopae, juveniles and adults encounter physiologically challenging conditions in estuarine habitats where salinity is low or variable. Ontogenetic changes in osmoregulation are one set of adaptations that allow euryhaline crabs such as *C. sapidus* to survive in estuaries. Zoeae are osmoconformers, exchanging ions by osmosis to match their environment, and are least tolerant of low salinities (Costlow and Bookhout 1959, Kalber 1970). Gills, the site of active ion exchange via ion pumps in brachyuran crabs, first appear in megalopae and facilitate moderate hyperosmotic regulation at low salinities (Cieluch et al. 2004). Juvenile and adult *C. sapidus* are capable of strong hypo- and hyperosmotic regulation (Guerin and Stickle 1997).

Megalopae are capable of migrating from high salinity water in the coastal ocean to low salinity areas of estuaries within a few days. During this transition, ontogenetic changes in osmoregulation may be sufficient for survival. Alternatively, megalopae may become more tolerant to low salinity by changing their physiology in response to
environmental cues. For example, Charmantier et al. (2002) observed increased survival of late stage zoeae and megalopae of *Chasmagnathus granulata*, another estuarine crab that uses the export strategy, when previous stages were exposed to reduced salinity. Chapter 2 investigates salinity tolerance of *C. sapidus* megalopae after acclimation to reduced salinity and estuarine chemical cues during the transition from coastal to estuarine areas.

**1.3 Coastal and Estuarine Distributions**

Successful migration of megalopae from nearshore coastal areas to estuarine nursery habitats, known as estuarine ingress, is a critical step in the life history of *C. sapidus*. Hare et al. (2005) proposed a two step model for estuarine ingress in which larvae first are transported from coastal areas to the estuarine tidal prism and subsequently are transported into the estuary. Hydrodynamic models suggest that each channel of an estuary is connected to a specific source region of the tidal prism (Churchill et al. 1999, Brown et al. 2004). If megalopae acted as passive particles during ingress, their success in reaching estuarine areas with suitable nursery habitat would depend on the location of their arrival in the estuarine tidal prism.

Horizontal gradients in larval density have been observed across estuarine inlets during ingress (e.g., Forward et al. 1999). Such gradients in larval density may result from arrival in the inlet from a particular coastal source area to one side of the inlet.
(Forward et al. 1999). This link between nearshore coastal distribution and relative abundance across an estuary is supported by particle tracking models (Luettich et al. 1999, Brown et al. 2004), but has not been explicitly investigated in the field. In Chapter 3, I compare the distribution of *C. sapidus* megalopae in nearshore coastal areas with their relative abundance across an estuary and make inferences based on existing hydrodynamic models and larval behavior to explain the distributions observed.

### 1.4 Mechanisms of Estuarine Ingress

The return migration of *C. sapidus* from the continental shelf to estuaries must be achieved by transport in currents because megalopae are not capable of swimming across large horizontal distances. On the continental shelf, megalopae are collected primarily in the neuston (Johnson 1985), suggesting that most transport occurs in surface currents. Shoreward transport of megalopae in the South Atlantic Bight (SAB) probably occurs primarily during southwestward directed winds which drive coastal downwelling and onshore flow near the surface (Blanton et al. 1995). Megalopae can also be transported in frontal zones that develop during coastal upwelling (caused by northeastward winds in the SAB) and move shoreward following relaxation of upwelling favorable winds (Shanks et al. 2000). Shanks (1983, 1988) proposed an additional mechanism of transport in shoreward moving surface convergence zones over tidally generated internal waves.
Within estuaries, *C. sapidus* megalopae move upstream against net downstream flow using nocturnal Flood Tide Transport (FTT). Megalopae swim up into the water column at night in response to increasing salinity during flood tide (Tankersley et al. 1995). They continue swimming in response to turbulent flood tide currents and actively select benthic habitats when turbulence declines at slack water after flood (Welch et al. 1999, Welch and Forward 2001, van Montfrans et al. 2003). Megalopae remain on the bottom during the day due to negative phototaxis and during ebb tide (DeVries et al. 1994, Forward and Rittschof 1994). The result of nocturnal FTT is saltatory movement up an estuary using tidal currents.

The abundance of *C. sapidus* megalopae in an estuary is indicative of shoreward transport and estuarine ingress because megalopae undergo metamorphosis to the first juvenile stage within 2-5 days of ingress (Forward et al. 1994). During the recruitment season, the estuarine abundance of *C. sapidus* megalopae has typically been estimated using settlement on artificial ‘hog’s hair’ collectors. Throughout this dissertation, settlement is defined as settlement on a substrate from the water column, but does not indicate that megalopae undergo metamorphosis. Settlement is highly episodic, can vary by an order of magnitude from one day to the next, and has often been associated with potential wind driven and tidal transport mechanisms (Rabalais et al. 1995, van Montfrans et al. 1995, Forward et al. 2004). Because these studies and others did not always test explicit mechanistic hypotheses based on larval behavior and often
employed different analysis techniques, there remains little consensus in the literature on the relative importance of various transport mechanisms. Interactions between mechanisms of shoreward transport and estuarine ingress may also obscure the interpretation of estuarine settlement data. One way to account for the latter problem may be to compare settlement data collected simultaneously at coastal and estuarine sites. In Chapter 4, I investigate mechanisms of estuarine ingress inferred from a three year record of daily settlement collected at nearby coastal and estuarine sites.

1.5 Effect of Sampling Interval on Annual Postlarval Abundance Estimates

Monitoring the annual estuarine abundance of *C. sapidus* megalopae during the recruitment season may provide an estimate of year class strength within the population. Collected over many years, these data can be compared with fishery landings or fishery independent surveys to determine the extent to which variability in the annual abundance of megalopae predicts future population size. Forward et al. (2004) observed a positive relationship between annual megalopal settlement in the Newport River estuary, North Carolina and catch per unit effort in the North Carolina crab pot fishery. Continued monitoring of annual megalopal settlement will be necessary to investigate this relationship further, but such data are costly and time-consuming to collect.
Reducing the frequency of sampling may be necessary to limit costs or effort but can lower the accuracy of annual abundance estimates. Understanding the potential variability in estimates at different sampling intervals is critical to generating useful estimates of annual abundance. Hettler et al. (1997) determined that annual estimates of larval Atlantic menhaden abundance were acceptable when the sampling interval was seven days or less. Because their analysis was derived from a single year of daily abundance data, it did not account for inter-annual variations in the pattern of daily abundance. In Chapter 5, I assess the effect of inter-annual variation in the pattern of abundance on estimates of annual *C. sapidus* megalopal abundance.

### 1.6 Organization and Content of the Dissertation

The chapters of the dissertation are written as independent manuscripts. As a result, some introductory material is repeated. Chapter topics include:

- **Chapter 2.** Comparison of low salinity tolerance in *Callinectes sapidus* Rathbun and *Callinectes similis* Williams postlarvae upon entry into an estuary.

- **Chapter 3.** Ingress of brachyuran crab postlarvae to the Newport River estuary, North Carolina.

- **Chapter 4.** Shoreward transport and estuarine ingress of brachyuran crab postlarvae inferred from daily coastal and estuarine settlement in North Carolina, USA.

- **Chapter 5.** Effect of sampling interval on estimates of annual blue crab postlarval abundance.
A summary and conclusions section appears at the end to integrate the ideas presented in the preceding chapters.
2. Comparison of Low Salinity Tolerance in *Callinectes sapidus* Rathbun and *Callinectes similis* Williams Postlarvae upon Entry into an Estuary

2.1 Introduction

Planktonic larvae of crustaceans that inhabit rivers and estuaries as adults often are exported to the continental shelf for development and subsequently return to estuaries as postlarvae or juveniles. This ‘export strategy’ reduces the exposure of some developmental stages to low or variable salinity conditions (Strathmann 1982). The returning stage is not afforded this protection but must survive during the transition from oceanic to estuarine conditions. Adaptations to changing physiological conditions are critical for estuarine ingress but have received little attention in the literature.

The ontogeny of osmoregulation has been described for a variety of estuarine crustaceans that export their larvae to the ocean. Salinity tolerance generally increases during development for the lobster *Homarus americanus* (Charmantier et al. 1988), the shrimp *Penaeus japonicus* (Charmantier et al. 1988), the freshwater prawn *Macrobrachium petersi* (Read 1984) and a variety of crabs including *Chasmagnathus granulata* (Charmantier et al. 2002), *Sesarma curacaense* (Anger and Charmantier 2000), *Carcinus maenas* (Cieluch et al. 2004) and *Callinectes sapidus* (Costlow and Bookhout 1959, Kalber 1970). In euryhaline crabs, a switch from osmoconforming to hyperosmotic regulation at low salinities typically occurs at the transition to the postlarva (megalopa) stage.
concurrent with the development of gills and activation of the ion transport pumps located therein (Cieluch et al. 2004), suggesting that megalopae should be capable of surviving in low salinity water. Stenohaline crabs are unable to acclimate to low salinities at any point in their life cycle (Henry et al. 2000), indicating that acclimation to low salinity is an evolved characteristic of euryhaline species.

Prior salinity exposure can lead to differences in salinity tolerance and osmoregulatory capacity within a developmental stage. In *C. granulata*, an estuarine crab with larvae that can remain in brackish water for a day or more before leaving the estuary, acclimation of embryos to salinities of 15 and 20 increased the time to 50% mortality of 1st zoea at a salinity of 5 over individuals raised at a salinity of 32 (Charmantier et al. 2002). This adaptation allows them to survive until they exit the estuary. Zoea II-IV and the megalopa exhibited increased osmoregulatory capacity when the previous stage was acclimated to moderate salinities over individuals maintained in seawater at a salinity of 35 (Charmantier et al. 2002), although adaptability to reduced salinities decreased in these later larval stages (Gimenez and Anger 2003). Gimenez and Anger (2003) suggested that since *C. granulata* megalopae are strong osmoregulators, they should be resistant to reduced salinities, but instead the authors observed low survival in megalopae that had remained in water with a salinity of 15 throughout larval development.
Juvenile and adult crabs generally have a higher osmoregulatory capacity and show a greater capacity to acclimate to low salinities (Cervino et al. 1996, Guerin and Stickle 1997, Henry 2005, Piller et al. 1995). Estuarine crabs at various life history stages are capable of acclimatizing to a range of salinities according to the environment in which each stage is found (Gimenez and Anger 2003). Thus, the recruitment stage that returns to an estuary should be capable of undergoing physiological changes that increase salinity tolerance upon reaching brackish water.

The purpose of this study was to determine whether the recruitment stage of an estuarine crab that exports its larvae to the continental shelf for development possesses adaptations, independent of larval stage, that allow it to survive when it returns to an estuary in contrast to a similar species that recruits to the coast but generally does not enter estuaries. The blue crab *C. sapidus* is found primarily in estuaries of the western Atlantic as an adult but develops through about seven zoeal stages in shelf waters before returning to estuaries as a megalopa (Costlow and Bookhout 1959, Johnson 1985, Nichols and Keney 1963). *C. similis* is a closely related species that shares much of the same range but is typically found as an adult in coastal areas or in the lower, relatively high salinity portions of estuaries (Hsueh et al. 1993, Williams 1984). *C. similis* also develops through about seven zoeal stages in shelf waters (Bookhout and Costlow 1977) and megalopae of both species can be found along the North Carolina (USA) coast in high abundances (Chapter 4). I hypothesized that megalopae of *C. sapidus* should be
better adapted for survival in brackish water upon entry into an estuary than megalopae of *C. similis*.

Cues encountered at the mouth of an estuary that might trigger an increase in low salinity tolerance could be reduced salinity or a chemical component of estuarine water. Salinity is the logical cue for changes in osmoregulatory capacity, especially since *C. sapidus* megalopae are highly sensitive to changing salinity (Forward 1989). *C. sapidus* megalopae also exhibit a variety of responses to chemical cues (humic acids, seagrass odors, conspecific odors, etc.) including reduced time to metamorphosis (e.g., Forward et al. 1996), changes in swimming orientation in flow (Forward and Rittschof 1994), settlement site selection (van Montfrans et al. 2003) and changes in endogenous rhythms at the time of metamorphosis (Forward et al. 2007).

The first objective of this study was to determine whether *C. sapidus* megalopae collected within an estuary were more tolerant to low salinities than megalopae collected outside the estuary along the coast. The second objective was to characterize changes in salinity tolerance by determining the cue (salinity vs. estuarine chemical cues) that induces changes in salinity tolerance, the time required for changes to occur and the salinity at which changes were induced. The third objective was to compare the salinity tolerance of *C. similis* megalopae to the values determined for *C. sapidus*. *C. sapidus* megalopae collected in an estuary were found to have an increased tolerance to low salinity that was induced within 12 hrs by a moderate reduction in salinity. *C.
similis megalopae were initially less tolerant of and were unable to acclimate to low salinity water.

2.2 Materials and Methods

During September and October 2005, C. sapidus megalopae were collected at a coastal and an estuarine location near Beaufort Inlet, NC. ‘Coastal megalopae’ were collected using passive ‘hog’s hair’ larval samplers at Triple S fishing pier on Atlantic Beach, NC. This pier was located about 3 km west of the entrance (Beaufort Inlet) to the Newport River estuary. ‘Hog’s hair’ collectors were suspended at mid-depth (about 1 m depth at low tide) for 24 h and retrieved in the morning. Megalopae were removed by shaking the collectors in a bucket of seawater from the collection site. Salinity varied from 33–34 at the time of collection. ‘Estuarine megalopae’ were collected about 2 km inside the Newport River estuary at the Duke University Marine Laboratory at night during flood tide using plankton nets (505 µm mesh size). Salinity at the time of collection varied from 27 to 32 with a 1–7 difference between coastal and estuarine sites on a given night. After collection, C. sapidus megalopae were identified and sorted using a dissecting microscope (Costlow and Bookhout 1959). Megalopae were maintained unfed in large fingerbowls containing water from the collection sites at room temperature (23 °C) prior to experiments.
During Fall 2006, coastal *C. sapidus* and *C. similis* megalopae were collected at a coastal site at Sportsman’s fishing pier located approximately 1 km to the west of Triple S fishing pier on Atlantic Beach, N.C. This sampling site was chosen because Triple S fishing pier was dismantled during spring 2006. Because lower numbers of megalopae were caught on ‘hog’s hair’ collectors at this site, megalopae were collected at night using a light trap and transported to the Duke University Marine Laboratory in water from the collection site. Megalopae were identified and sorted using a dissecting microscope (Costlow and Bookhout 1959, Bookhout and Costlow 1977) and kept unfed in large fingerbowls of offshore water with a salinity of 35 prior to experiments. Offshore water was collected about 25 km seaward of Beaufort Inlet. This collection site is beyond the estuarine plume and in an area where estuarine chemical cues are not present (Forward et al. 2003). Water was stored in acid washed glass and polycarbonate carboys.

2.2.1 Salinity Tolerance of Animals Collected in the Field

The first set of experiments in 2005 investigated the survival of coastal and estuarine collected *C. sapidus* megalopae exposed to a range of salinities. To approximate the exposure a megalopa might experience during the course of a tidal cycle, we chose an assay that consisted of a 6 h exposure to salinity treatments followed by a return to the respective collection site water for 24 h after which mortality was
determined. Treatment water with salinities of 5, 10, 15, 20, and 30 was made by mixing offshore seawater (salinity of 35) and nano-pure deionized water. Megalopae were tested at 23 °C in groups of 10 in fingerbowls containing 500 mL of treatment or collection site water. During all experiments, megalopae were exposed to an ambient light:dark cycle in the laboratory. Due to a limited number of megalopae in the field after hurricane Ophelia passed in mid-September, 2005, we focused on treatment salinities of 5 and 10 for which the greatest mortality differences were observed. Because data were not normally distributed and sample sizes were small (n = 10), a Mann-Whitney Rank Sum Test was used to determine differences (α = 0.05) among the number of coastal and estuarine megalopae surviving after exposure to salinities of 5 and 10.

During 2006, C. similis megalopae collected at the coastal pier were exposed to salinity treatments of 5, 10, 15, 23 and 35. A salinity treatment of 23 was included instead of 20 because data from acclimation salinity experiments were used (see below). The experimental procedures were the same as described for C. sapidus above. Insufficient numbers were collected for statistical analyses (n = 3 groups of 10 per treatment) but data are included for comparison. Very few C. similis were collected inside the estuary and were not included in this study.
2.2.2 Acclimation Cue

The next series of experiments tested whether the cue for increased salinity tolerance of *C. sapidus* was low salinity or an estuarine chemical cue. Coastal *C. sapidus* megalopae collected at Triple S pier were exposed in groups of 10 to three treatments: offshore water (salinity 35), offshore water plus deionized water (salinity 23) and estuarine water from the Newport River estuary (salinity 23). Megalopae were transferred from collection site water into bowls containing 500 mL of treatment water for a 24 h acclimation period. Changes in low salinity tolerance were tested by subsequently transferring megalopae to bowls having diluted offshore water at a salinity of 5 for 6 h. Megalopae were then transferred back into collection site water for 24 h after which mortality was determined. Ten groups (100 individuals) were exposed to each treatment. A Kruskal-Wallis One-way Analysis of Variance on Ranks was used to determine differences in the number of surviving megalopae between treatments ($\alpha = 0.05$). Post hoc pair-wise multiple comparisons were conducted using Dunn’s Method to determine differences among groups.

2.2.3 Acclimation Time

To determine the time required for low salinity acclimation, coastal *C. sapidus* megalopae were exposed in groups of 10 (10 groups per treatment) to dilute offshore water with a salinity of 23 for acclimation treatments of 0 h (control), 12 h and 24 h.
Following acclimation, megalopae were transferred into dilute offshore water having a salinity of 5 for 6 h and were subsequently transferred back to offshore water having a salinity of 35 for 24 h. The number surviving in each group was counted. Data were analyzed using the methods described above.

2.2.4 Acclimation Salinity

The salinity reduction necessary for inducing the tolerance to low salinity was determined for coastal *C. sapidus* megalopae. Megalopae collected coastally at Sportsman’s fishing pier were exposed in groups of 10 (n = 10 per treatment) to acclimation water made from offshore water diluted with nano-pure deionized water with salinities of 35, 31, 27 and 23 for 12 h. Following acclimation, megalopae were returned to offshore water having a salinity of 35 for 12 h to allow internal ion concentrations to equilibrate among treatments prior to exposure to low salinities and to control for the magnitude of salinity change. Megalopae were transferred to water at a salinity of 5 for 6 h and then returned to offshore water (salinity 35) for 24 h. The number of megalopae surviving in each group was recorded. Data were analyzed using the methods described above.

*C. similis* megalopae were included in the acclimation experiment as a comparison to low salinity acclimation ability in *C. sapidus*. Megalopae were collected in the same samples as for *C. sapidus* acclimation experiments and exposed to an identical
experimental regimen. Due to limited numbers of individuals, only the 23 salinity acclimation treatment (n = 7 groups of 10) was used because acclimation was most likely to occur at this salinity based on results for \( C. sapidus \). Differences in survival among \( C. sapidus \) and \( C. similis \) megalopae were determined using a Mann-Whitney Rank Sum test.

### 2.3 Results

#### 2.3.1 Salinity Tolerance of Animals Collected in the Field

Coastal \( C. sapidus \) megalopae collected at Triple S fishing pier exhibited lower survival when exposed to low salinity water as compared to megalopae collected in the estuary. Survival was significantly lower (\( p < 0.001 \)) for coastal megalopae (5 %) versus estuarine megalopae (46 %) exposed to a salinity of 5 and to a salinity of 10 (66 % vs. 91 %; \( p < 0.001 \)) (Fig. 1). Survival was 98 % or greater for both groups of megalopae at salinities of 15, 20 and 30. The number of surviving megalopae from the estuarine site was more variable than from the coastal site when exposed to a salinity of 5. This difference may be due to the greater degree of salinity variability of the water from which megalopae were collected in the estuary. Alternatively, molt stage differences in megalopae collected on different nights within the estuary could have resulted in increased variability.
Figure 1: The mean percent (+1 SE) of offshore *C. sapidus* (black bars), estuarine *C. sapidus* (striped bars) and offshore *C. similis* (white bars) megalopae surviving a 6 h exposure to various salinities followed by a 24 h return to collection site water. Asterisks denote significant differences (p < 0.001) between offshore and estuarine *C. sapidus* at a given salinity. Too few *C. similis* were collected to make statistical comparisons.

*C. similis* megalopae were less tolerant of low salinities than *C. sapidus* megalopae. No *C. similis* megalopae survived after a 6 h exposure to water with a salinity of 5 whereas 13% survived at a salinity of 10 and 23% survived at a salinity of 15 (Fig. 1). Survival was 100% at salinities of 23 and above. The number of animals collected was insufficient for statistical comparison.
2.3.2 Acclimation Cue

Survival of *C. sapidus* megalopae in low salinity water increased after acclimation in water of a moderate salinity. Coastal megalopae exposed to water with a salinity of 5% after a 24 h acclimation in estuarine water or diluted offshore seawater with salinities of 23% exhibited significantly higher survival (94% and 94% vs. 21%; *H* = 20.918, d.f. = 2, *p* < 0.001) than those that did not experience an acclimation period (Fig. 2). There was no difference in survival among groups exposed to estuarine water and low salinity offshore water, which indicates the acclimation cue was low salinity.

![Figure 2: The mean percent of offshore *C. sapidus* megalopae (+1 SE) surviving after 24 h acclimation to offshore water (salinity 35), estuarine water (salinity 23), or offshore plus deionized water (salinity 23), a 6 h exposure to water at a salinity of 5 and returned to 35 salinity water for 24 h. Letters denote statistically different groups identified using post-hoc tests.](image-url)
2.3.3 Acclimation Time

The number of coastal *C. sapidus* megalopae surviving after exposure to low salinity water increased with acclimation time in moderate salinity water. With no previous exposure to moderate salinity water, 21% survived a 6 h exposure to water with a salinity of 5 (Fig. 3). Coastal megalopae acclimated in diluted seawater with a salinity of 23 were significantly more likely to survive \( (H = 22.151, \text{ d.f.} = 2, p < 0.001) \) after acclimation times of 12 h \((83 \pm 3\%)\) and 24 h \((94 \pm 3\%)\). There was no significant difference in survival after acclimation times of 12 h and 24 h. Thus, the minimum

Figure 3: The mean percent survival (+1 SE) of offshore *C. sapidus* megalopae after acclimation for 0, 12 and 24 h to diluted offshore water (salinity 23) before exposure to water at a salinity of 5 for 6 h and returned to water at a salinity of 35 for 24 h. Letters denote statistically different groups identified using post-hoc tests.
amount of time needed for *C. sapidus* megalopae to acclimate to low salinities was less than 12 h.

### 2.3.4 Acclimation Salinity

Survival of coastal *C. sapidus* megalopae in low salinity water increased as acclimation salinity decreased. There were significant differences in survival among acclimation salinity treatments after a 6 h exposure to dilute offshore water with a salinity of 5 ($H = 41.196$, d.f. = 4, $p < 0.001$). Survival was only 5% after an acclimation salinity of 35 but increased to 86% after an acclimation salinity of 23 (Fig. 4). Post-hoc analysis suggested that treatments were divided into three groups with acclimation

![Bar graph](image)

**Figure 4:** The mean percent survival (+1 SE) of offshore *C. sapidus* (black bars) and *C. similis* (white bar) megalopae after 12 h acclimation to treatment salinities, exposure to water at a salinity of 5 for 6 h and returned to water at a salinity of 35 for 24 h. Letters at the top denote statistically different groups identified using post-hoc tests.
salinities of 23 and 27 forming one group, 27 and 31 forming a second group and 35 making up the final group. All megalopae were returned to water having a salinity of 35 for 12 h prior to immersion in water having a salinity of 5, suggesting that the observed differences were due to the degree of acclimation among treatments and not due to the magnitude of salinity reduction from acclimation treatment salinities to test water at a salinity of 5.

*C. similis* megalopae collected coastally were unable to acclimate to low salinity water. Survival of *C. similis* megalopae was 3 %, which was significantly lower than *C. sapidus* megalopae exposed to the same treatment salinity of 23 (p < 0.001). This survival level was indistinguishable from coastal *C. sapidus* megalopae that were never exposed to reduced salinity water prior to immersion in test water at a salinity of 5 (Fig. 4).

**2.4 Discussion**

Megalopae of the blue crab *C. sapidus* collected inside the Newport River estuary, NC, were more tolerant to low salinities than those collected outside the estuary along the coast. For species that export planktonic larvae to the continental shelf during postembryonic development, arrival at an estuary is a critical life stage at which a physiological transformation must occur when individuals transition from relatively constant high salinities to brackish water with fluctuating salinities. After a relatively short period of time inside the estuary (the estuarine collection site could be reached
within one tidal excursion from the estuary mouth), *C. sapidus* megalopae survived short-term exposure to salinities of 5 and 10 that caused mortality in individuals collected just outside the estuary. Increased salinity tolerance was achieved in the field despite relatively small differences in salinity (1-7) between the coastal and estuarine collection sites. During the transition from the coastal ocean to estuaries, a component of estuarine water induces increased low salinity tolerance in *C. sapidus* megalopae and equips them physiologically to invade a variety of brackish settlement habitats.

*Callinectes sapidus* megalopae collected along the coast exhibited increased low salinity tolerance after exposure to dilute offshore water. Chemical cues associated with estuarine water were not necessary to induce the changes we observed in field-caught animals, as there was no difference in survival at low salinities after acclimation to dilute offshore and estuarine water of the same salinity. Our results are consistent with those of Torres et al. (2006) for *Armases roberti*, a freshwater crab from the Caribbean region with megalopae that may return to the adult freshwater habitat up to 10 km from the sea. Megalopae of *A. roberti* reared at a salinity of 25 were unable to survive when placed directly into salinities of 3 or less, but successful development to metamorphosis was achieved at salinities as low as 0.17 after a gradual (over several days) reduction in salinity (Torres et al. 2006).

Survival of *C. sapidus* megalopae in low salinity water increased four-fold after a 12 h acclimation to a reduced salinity. Acclimation times shorter than 12 h resulted in
smaller increases in survival rate (M. Ogburn, unpublished data). Such short acclimation times are consistent with our field observations that low salinity tolerance increases within the first few km of the Newport River estuary. Megalopae undergo flood tide transport (FTT) for movement into and up an estuary. During FTT they ascend into the water column during flood tide and are transported with a parcel of water. At the end of flood tide, they settle out of the water column and remain on or near the bottom during ebb tide (Forward et al. 2003). Since this behavioral sequence exposes them to a range of salinities over a tidal cycle, they would quickly acclimatize and adjust their osmoregulatory capabilities before reaching low salinity water.

The acclimation times of megalopae are consistent with those for acclimation of adult *C. sapidus* to low salinities. Hyperosmotic ionic regulation at reduced salinities in crustaceans occurs by active ion transport in posterior gills and is regulated by proteins whose expression is mediated by salinity (Henry 2001, Henry and Cameron 1982, Neufeld et al. 1980, Towle et al. 1976). Carbonic anhydrase (CA) induction, a critical component of physiological adaptation to low salinity in crustaceans (Henry 2001) is under regulatory control by the eyestalk (Henry 2006) and occurs in the posterior gills of *C. sapidus* after a minimum acclimation time of 12 h (Henry 2005). Henry (2005) speculates that this timing is related to gene activation and CA mRNA production. That we observed a marked increase in low salinity tolerance after a 12 h acclimation to
reduced salinities suggests that megalopae may be subject to similar physiological constraints as adults during low salinity acclimation.

A salinity decrease of 4 was sufficient to induce changes in the low salinity tolerance of *C. sapidus* megalopae. This result corresponds well to field observations of increased salinity tolerance at the estuarine collection site with salinities between 27 and 32 versus offshore animals collected at salinities of 33 to 34. Sensitivity to slight decreases in salinity is important, as megalopae entering an estuary will at first encounter only small reductions in salinity. Henry (2005) observed increases in CA production at a salinity of 27, the salinity at which *C. sapidus* adults shift from osmoconforming to osmoregulating. If CA induction only occurs at or below a salinity of 27 (Henry, 2005), increased survival after acclimation to a salinity of 31 may be due to cell volume regulation or other physiological changes. Our data do not allow us to distinguish among these hypotheses.

The low salinity tolerance of *C. similis* megalopae collected coastally was much less than that of *C. sapidus* megalopae from the same collection site. This difference is not surprising considering that juvenile and adult *C. similis* have a lower osmoregulatory capability in brackish water than *C. sapidus* (Engel 1977, Guerin and Stickle 1997, Piller et al. 1995). Acclimation of *C. similis* to reduced salinity also did not increase survival upon exposure to low salinity water. One explanation for this observation could be that reduced salinities do not induce an increased tolerance to low
salinity in *C. similis*. An alternative explanation could be that *C. similis* megalopae are simply unable to survive in such low salinity water. We find the second explanation to be more likely given that adult *C. similis* do exhibit measurable physiological changes during acclimation to reduced salinities (Engel 1977, Guerin and Stickle 1997, Piller et al. 1995). If this is the case, *C. similis* should exhibit some acclimation at salinities of 10 - 15 in which a few megalopae survived during our salinity tolerance experiment (Fig. 1).

The transition from the continental shelf to estuaries is a key stage during the development of many estuarine crustacean species, representing the time when individuals must shift from being osmoconformers to osmoregulators. *C. sapidus* megalopae are well-equipped for this transition, exhibiting an increased salinity tolerance shortly after entering an estuary. The increase in salinity tolerance was induced by experimental reductions in salinity of 4 and occurred within the time of one tidal cycle (12.4 h). *C. similis* megalopae were less tolerant of low salinities than *C. sapidus* and were unable to survive exposure to very low salinities even after acclimation to reduced salinity. Thus, *C. sapidus* megalopae are better adapted to make use of estuarine settlement habitats and likely represent a more advanced stage in the evolutionary process of estuarine invasion.
3. Ingress of Brachyuran Crab Postlarvae to the Newport River Estuary

3.1 Introduction

Many estuarine organisms have planktonic larvae that are exported to the continental shelf during development. Potential benefits of this ontogenetic migration include pursuit of optimal physical conditions, reduced predation, increased dispersal and increased prey availability, but export necessitates a return migration of weak or non-swimming offspring. The proportion of individuals successfully returning from oceanic to estuarine nursery habitats can significantly impact population growth rate (Quinlan and Crowder 1999). Thus, connectivity between coastal and estuarine areas may affect the recruitment of larvae to the juvenile cohort. Understanding transport pathways between coastal and estuarine areas may also provide insight into the relative importance of spatially segregated estuarine nursery habitats or help prioritize sites to monitor for invasive species.

Hare et al. (2005) propose a 2-step process for estuarine ingress. Larvae must first be transported from oceanic development habitats to the tidal prism of an estuary. Once within the tidal prism, inlet forcing mechanisms result in actual ingress into the estuary. During each step of ingress, larval behavior interacts with physical forcing mechanisms to produce temporal and spatial patterns of abundance. The degree to
which larvae alter estuarine ingress over underlying physical processes is species-specific (Hare et al. 2005) and is probably related to orientation and swimming abilities.

Few studies have examined the spatial patterns of estuarine ingress or attempted to link ingress with the abundance of larvae along the coast. Gradients in the density of ingressing larvae across estuarine inlets have been observed for a variety of species including fish (Forward et al. 1999, Brown et al. 2004), shrimp (Wenner et al. 1998) and crabs (Eggleston and Armstrong 1995), but were not compared to coastal distributions. In model simulations, the path of particles approaching an inlet often determines the subsequent estuarine distribution because water entering each channel of the estuary typically comes from a specific source region of the tidal prism (Luettich et al. 1999, Brown et al. 2004). Verifying this connection between source region and estuarine distribution will require field studies that simultaneously sample both coastal and estuarine distributions of larvae.

In this study, I investigated estuarine ingress of brachyuran crab postlarvae (megalopae) to the Newport River estuary, North Carolina, USA. The blue crab *Callinectes sapidus* Rathbun, the fiddler crabs *Uca* species (*U. minax*, *U. pugilator*, and *U. pugnax*) each occur in the study area and are morphologically indistinguishable), and the mottled shore crab *Pachygrapsus transversus* each undergo larval development in coastal areas and enter estuaries as megalopae (Johnson 1985, Epifanio et al. 1988, Cuesta and Schubart 1998). *C. sapidus* was chosen as the focal species of the study because of its
ecological and commercial importance. Megalopae of *C. sapidus* preferentially settle in submerged aquatic vegetation, but also settle in marshes, mud flats and other estuarine habitats (e.g., Orth and van Montfrans 1987, Mense and Wenner 1989). *Uca* megalopae settle in estuarine marshes and sand flats (O’Connor 1993), whereas *P. transversus* megalopae may preferentially settle on coastal rocky shores (Flores et al. 1998) even though settlers are collected in the study estuary.

Field and laboratory studies suggest that behaviors associated with up-estuary transport of megalopae may be different for each species. *C. sapidus* and *Uca* move up-estuary via nocturnal Flood Tide Transport (FTT). For *C. sapidus*, megalopae swim up into the water column at night in response to increasing salinity (Tankersley et al. 1995) and remain there until water turbulence declines at the end of flood tide (Welch et al. 1999, Welch and Forward 2001). They settle out of the water column prior to ebb tide, and remain on or near the bottom during ebb tide and during the day (DeVries et al. 1994, Tankersley et al. 2002). Nocturnal FTT of *Uca* megalopae differs because it is based on an endogenous circatidal swimming rhythm (Tankersley and Forward 1994). *P. transversus* megalopae may undergo reverse diel vertical migration (Moreira et al. 2007), although behaviors have not been investigated in the laboratory.

Ingress of crab megalopae to estuaries is often measured as the daily number of megalopae collected on passive ‘hog’s hair’ settlement substrates. This method has been used in the past for inter-regional comparisons (Rabalais et al. 1995, van Montfrans et
Table 1: Schedule of sampling efforts conducted to investigate study objectives.

<table>
<thead>
<tr>
<th>Coastal and Estuarine Distributions</th>
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<tbody>
<tr>
<td>August 13-14, 2007</td>
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<tr>
<td>August 23-24, 2007</td>
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<tr>
<td>August 28-29, 2007</td>
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<tr>
<td>September 4-5, 2007</td>
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<tr>
<th>Settlement during the spring/neap tidal cycle</th>
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<tr>
<td>August 14-24, 2006</td>
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<td>September 20 to October 3, 2006</td>
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<th>Diel sampling at the coast</th>
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<tr>
<td>July 21-22, 2005</td>
</tr>
<tr>
<td>August 28-29, 2005</td>
</tr>
<tr>
<td>October 4-5, 2006</td>
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</tbody>
</table>

al. 1995) and is reasonable for comparisons across a single estuary. In tidal flow, settlement of *C. sapidus* occurs only during slack water (Tankersley et al. 2002), presumably from larvae available near the settlement location. In this study, ‘hog’s hair’ collectors were used both inside a tidal estuary and along the coast, where tidal currents are much weaker. To compare settlement at the two sites, it was necessary to determine the duration of settlement at the coastal site relative to the estuary.

The purpose of this study was to determine the coastal and estuarine distributions of brachyuran crabs during estuarine ingress. The primary objectives
were: 1) to compare the distribution of postlarvae in nearshore coastal areas to the
distribution of estuarine settlers, 2) to determine whether spatial patterns in estuarine
settlement are maintained throughout the spring/neap tidal cycle, and 3) to determine
the diel timing of coastal settlement over the 24 h day for comparison with estuarine
settlement. The data are presented in the order of the objectives listed above, not in
chronological order, to maintain a logical progression from coastal to estuarine areas
that is consistent with estuarine ingress (Table 1).

3.2 Materials and Methods

3.2.1 Coastal and Estuarine Distributions

To relate the distribution of megalopae in nearshore coastal areas to the
distribution of estuarine settlers, thirteen coastal sampling locations were established at
1 km intervals across the inlet to the Newport River estuary, North Carolina, USA
(Beaufort Inlet) at a distance of 0.5 km offshore (Fig. 5). Beaufort Inlet was the center of
the 12 km transect. Sampling began west of the inlet and ended east of the inlet. Each
site was sampled twice each night at the times of slack water after flood tide and slack
water after ebb tide with each sampling lasting about 3 h (1.5 h before to 1.5 h after slack
water). Sampling occurred at times in the lunar month corresponding to spring and
neap tides. Spring tide sampling occurred on the nights of August 13-14, 2007 and
Figure 5: Map of the Newport River estuary with letters corresponding to settlement collector locations in Morehead Channel (M), Newport River Channel (NR), Radio Island Channel (RI), Shackleford Channel (S), Triple S fishing pier (TS), and Sportsman’s fishing pier (SP). Plankton sampling stations are numbered according to distance (in km) away from the center of the Beaufort Inlet (Station 0).

August 28-29, 2007, with slack water after flood sampling beginning just after dusk followed by slack water after ebb sampling in the early morning. Neap tide sampling occurred on the nights of August 23-24, 2007 and September 4-5, 2007, with slack water after ebb sampling beginning just after dusk followed by slack water after flood sampling in the early morning.

At each location, surface plankton tows were conducted from the R/V Susan Hudson by towing paired 0.67 m diameter plankton nets due south (in an offshore
direction) for 5 min at 2 kts. Each net was fitted with a General Oceanics flowmeter (model 2030R) for calculation of the volume of water sampled. Salinity was measured at each station using a refractometer. Large organisms (primarily fish and ctenophores) and debris were removed using a hand net (8 mm diameter mesh) and samples were preserved in 70% ethanol. Because this study was originally designed to focus exclusively on blue crabs, *C. sapidus* megalopae only were identified under a dissecting microscope and enumerated (Costlow and Bookhout 1959). Samples with large numbers of portunid megalopae were sub-sampled prior to identification by splitting 1-3 times using a Folsom plankton splitter.

To determine if there were differences in megalopal density across Beaufort Inlet, a Mann-Whitney Rank Sum test was performed after combining all data from samples to the west (sites -6 to -1) and east (sites 1 to 6) of the inlet (Zar 1999). Non-parametric methods were used because the data were not normally distributed nor did transformations result in a normal distribution.

On the nights of plankton sampling, settlement of *C. sapidus* megalopae was monitored simultaneously at four sites located in each major channel of the Newport River estuary. The four estuarine sites were, from west to east, Morehead channel (34°43.15’N, 76°42.31’W), Newport River channel (34°43.24N, 76°41.31’W), Radio Island channel (34°43.01’N, 76°40.23’W), and Shackleford channel (34°41.22’N, 76°38.62’W). In the morning prior to plankton sampling, ‘hog’s hair’ settlement collectors were
deployed within the estuary. Three replicate cylindrical ‘hog’s hair’ collectors were deployed at each site. Collectors were suspended approximately 1 m below the surface at low tide except for the Morehead channel site where collectors were deployed from a floating dock and remained at 1 m depth throughout the tidal cycle. This difference is not likely to affect settlement because previous studies indicated that *C. sapidus* megalopae were distributed throughout the water column in the Newport River estuary (De Vries et al. 1994). Each of the collector sites was chosen to be within one tidal excursion of the inlet (Leuttich et al. 1999), such that the megalopae collected each morning likely entered the estuary on the night before. Collectors were retrieved in the morning and washed according to Metcalf et al. (1995) to remove megalopae. *C. sapidus* were identified under a dissecting scope and counted (Costlow and Bookhout 1959). The relationship between planktonic abundance and estuarine settlement was investigated using regression analysis after averaging all plankton and collector data for each night. Local wind data were obtained for Buoy 41035 from NOAA’s National Data Buoy Center (approximately 65 km west of Beaufort inlet) because data were not available for the nearby Cape Lookout station during the sampling period. Westward directed wind stress was calculated following Large and Pond (1981) using daily averaged wind speed and direction for the 24 h period from 07:00 the morning of collector deployment to 06:00 the morning of retrieval. At the Radio Island channel site,
settlement was also monitored daily from August 9 to September 13, 2007 to determine the trends in settlement over the period during which plankton samples were collected.

**3.2.2 Settlement during the Spring/Neap Tidal Cycle**

To determine whether the pattern of estuarine settlement was maintained throughout the spring/neap tidal cycle, crab megalopae were collected at five sites near Beaufort inlet during two spring/neap tidal cycles. The four estuarine sites described above were sampled again, and a fifth site was added on the coast as an estimate of the coastal abundance of megalopae available for ingress (Fig. 5). The coastal site was located about 3 km west of Beaufort inlet at Triple S fishing pier (34°41.60′N, 76°42.67′W) in 2004 and 2005, but was moved about 1.5 km west to Sportsman’s fishing pier (34°41.68′N, 76°43.81′W) in 2006 after Triple S pier was demolished. Collectors were deployed from August 14-24, 2006 and September 20 to October 3, 2006 and retrieved each morning. Samples were processed as described above. Megalopae of *C. sapidus*, *Uca* species (*U. minax*, *U. pugilator* or *U. pugnax*) and *P. transversus* were identified under a dissecting scope and counted (Hyman 1920, Costlow and Bookhout 1959, Flores et al. 1998). Local wind data were obtained for Cape Lookout, NC (Station CLKN7) from NOAA’s National Data Buoy Center (approximately 15 km southeast of Beaufort inlet), and westward directed daily wind stress was calculated as described above.
Differences in median settlement among channels inside Beaufort inlet were determined for each species using Kruskal-Wallis One Way Analysis of Variance (ANOVA) on Ranks (Zar 1999). Non-parametric methods were used because the data were not normally distributed nor was a normal distribution achieved by data transformations. For *C. sapidus* and *Uca*, data from both sampling periods were pooled to increase sample size. For *P. transversus*, only data from the September sampling period were used due to very low abundance during the August sampling period. A Tukey Test was used to perform pairwise comparisons when significant differences were identified by the ANOVA (Zar 1999). Differences in settlement among sites for each species and among species at each site were investigated with the Spearman Rank Order Correlation using pooled data from the two sampling periods. The relationship between coastal and estuarine sites each night was investigated using regression analysis after calculating the average nightly settlement across all estuarine collector sites.

### 3.2.3 Diel Sampling at the Coast

To determine the diel timing of *C. sapidus* settlement over the 24 h day at a coastal site, three replicate ‘hog’s hair’ collectors were sampled throughout the day and night on three occasions. Collectors were retrieved every 2 h and alternated with a second set. Megalopae were removed and identified using the techniques described
above for the settlement survey. In 2005, sampling was conducted at Triple S fishing pier during both spring tides (07:25 July 21 – 07:25 July 22; time of high tide = 20:55) and neap tides (09:30 August 28 – 07:30 August 29; time of high tide = 04:41) (Fig. 5). Tide times were obtained from tide prediction software (Nobeltec). It was unclear from the first two diel collections whether the time of settlement at night was related to tidal height. Additional samples were collected in 2006 to further examine this possibility, but sampling was only conducted at night to reduce sampling effort after the first two studies indicated settlement primarily occurred at night. Sampling was conducted at Sportsman’s fishing pier between spring and neap tides from 19:30 October 4 – 07:30 October 5 (time of high tide = 23:38) (Fig. 5).

For the two sampling periods in 2005, data were grouped as day (n = 15) and night (n = 8), with night samples being those collected entirely during hours of darkness. Day-time and night-time settlement were compared using a Student’s t-test (Zar 1999). A square-root transformation was performed to meet assumptions of normality and equal variance.

### 3.3 Results

#### 3.3.1 Coastal and Estuarine Distributions

The distribution of *C. sapidus* megalopae in nearshore coastal areas differed from the distribution of estuarine settlers. Megalopae occurred throughout the coastal transect,
with increasing abundance at later dates. There were no significant differences in the
density of megalopae collected on the east vs. the west side of Beaufort inlet. Density in
paired samples ranged from 0 to 720 megalopae 100 m$^{-3}$ during the four sampling dates
(Fig. 6). Average density ranged from 8 ± 4 megalopae 100 m$^{-3}$ (mean ± 1 SD) during the
August 13-14, 2007 sampling to 252 ± 226 megalopae 100 m$^{-3}$ during the September 4-5,
2007 sampling. The lowest densities were consistently recorded within 1 km of the
center of the inlet during slack water after ebb, except for the August 23-24, 2007
sampling period (Fig. 6b) when even lower densities were recorded there during slack
after flood. During the September 4-5 sampling (Fig. 6d), megalopal density was
significantly greater at low tide (372 ± 252) than high tide (132 ± 109; T = 129, p = 0.018),
but no differences associated with tides were observed on the other sampling dates.
Due to extreme drought across the southeast US, the salinity was 35 at all sampling
stations including the center of the inlet during slack after ebb.

Settlement of *C. sapidus* megalopae during the nights when plankton sampling
took place was higher in western channels (Morehead, Newport and Radio Island) of the
Newport River estuary as opposed to the easternmost channel (Shackleford). The
highest settlement was observed in Morehead channel on two nights and in Radio
Island channel on one night, whereas few megalopae settled in Newport River or
Shackleford channels (Table 2). Settlement followed a pattern of increasing abundance
Figure 6: Density of *Callinectes sapidus* megalopae in nighttime surface plankton tows along the coast near Beaufort Inlet on the nights of a) August 13, b) August 23, c) August 28, and d) September 4, 2007. Solid lines denote samples collected around slack water after flood and dashed lines denote samples collected around slack water after ebb. Sampling was conducted during spring tides when the time of slack water after flood occurred in the early evening (a and c) and during neap tides when the time of slack water after flood occurred in the early morning (b and d).
Table 2: Settlement of *Callinectes sapidus* megalopae (mean number of megalopae per collector \[n = 3\]) on nights when plankton samples were collected. Dates correspond to the morning collectors were retrieved from the field. Decimals are used when megalopae were collected but rounding would result in a value of zero. The direction the wind was from (°) and its velocity (m/s) are reported for Cape Lookout, NC for the 24 h period from 7 am prior to sampling to 6 am the day of collection.

<table>
<thead>
<tr>
<th>Date</th>
<th>Morehead channel</th>
<th>Newport River channel</th>
<th>Radio Island channel</th>
<th>Shackleford channel</th>
<th>Wind (°, m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 13-14</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>242, 2.9</td>
</tr>
<tr>
<td>Aug. 23-24</td>
<td>6</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>61, 4.8</td>
</tr>
<tr>
<td>Aug. 28-29</td>
<td>5</td>
<td>0.3</td>
<td>2</td>
<td>1</td>
<td>60, 6.9</td>
</tr>
<tr>
<td>Sept. 4-5</td>
<td>28</td>
<td>6</td>
<td>180</td>
<td>2</td>
<td>53, 4.9</td>
</tr>
</tbody>
</table>

Figure 7: Time series of *Callinectes sapidus* megalopal settlement (mean number of megalopae per collector \[n = 3\]) during plankton sampling at the Radio Island channel site. Vertical dashed lines indicate the dates of plankton sampling.
with date similar to that observed in plankton samples, with the highest settlement (180 megalopae collector⁻¹) occurring during the September 4-5, 2007 sampling period (Fig. 7). There was a significant positive linear relationship between planktonic abundance at the coast and the abundance of estuarine settlers (Fig. 8a; p < 0.001, r² = 0.99).

### 3.3.2 Settlement during the Spring/Neap Tidal Cycle

Megalopae of *C. sapidus* consistently settled in higher abundance at the coastal site and in the three western channels of the Newport River estuary (Morehead, Newport River, and Radio Island) during two spring/neap tidal cycles (Table 3). *C. sapidus* were significantly more abundant at the coast and in Morehead and Radio Island
Table 3: Settlement of *Callinectes sapidus*, *Uca*, and *Pachygrapsus transversus* megalopae along the coast and at four sites within the Newport River estuary, NC during 2006. Data presented are minimum, mean (in parentheses) and maximum number of megalopae per collector (n = 3) for each sample period.

<table>
<thead>
<tr>
<th></th>
<th>Morehead</th>
<th>Newport River</th>
<th>Radio Island</th>
<th>Shackleford</th>
<th>Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. sapidus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 14–24</td>
<td>3 (19)</td>
<td>0 (1) 3</td>
<td>2 (11) 45</td>
<td>0 (0) 2</td>
<td>1 (7) 36</td>
</tr>
<tr>
<td>Sept. 20 – Oct. 3</td>
<td>1 (8) 41</td>
<td>0 (1) 3</td>
<td>3 (16) 30</td>
<td>0 (1) 2</td>
<td>3 (34) 159</td>
</tr>
<tr>
<td><em>Uca</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 14–24</td>
<td>0 (35)</td>
<td>0 (6) 15</td>
<td>6 (39) 85</td>
<td>0 (25) 90</td>
<td>0 (2) 7</td>
</tr>
<tr>
<td>Sept. 20 – Oct. 3</td>
<td>0 (34) 190</td>
<td>0 (2) 9</td>
<td>0 (12) 41</td>
<td>1 (13) 63</td>
<td>0 (4) 14</td>
</tr>
<tr>
<td><em>P. transversus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 14–24</td>
<td>0 (0) 0</td>
<td>0 (0) 0</td>
<td>0 (0) 0</td>
<td>0 (0) 10</td>
<td>0 (1) 2</td>
</tr>
<tr>
<td>Sept. 20 – Oct. 3</td>
<td>0 (0) 0</td>
<td>0 (0) 1</td>
<td>0 (0) 1</td>
<td>(2) 7</td>
<td>0 (4) 12</td>
</tr>
</tbody>
</table>

channels versus Newport River and Shackleford channels (Fig. 9; H = 78.767, d.f. = 4, p < 0.001). This settlement pattern was maintained during both westward and eastward directed winds (Fig. 9). Settlement was not correlated among coastal and estuarine sites, but was significantly correlated when Radio Island channel was compared to the adjacent Newport River and Shackleford channels (Table 4).

Settlement patterns of *Uca* and *P. transversus* megalopae differed from that of *C. sapidus*. *Uca* megalopae were relatively rare at the coastal site, but settled in abundance at all estuarine sites except Newport River channel (Fig. 9; Table 3). Megalopae were
Table 4: Correlation of all possible combinations of daily settlement among sampling sites for *Callinectes sapidus*, *Uca* and *Pachygrapsus transversus*. Data presented are correlation coefficient (r) and p-value (in parentheses) for significant correlations.

<table>
<thead>
<tr>
<th>T1</th>
<th>Newport River</th>
<th>Radio Island</th>
<th>Shackleford</th>
<th>Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. sapidus</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Uca</td>
<td>0.438 (0.029)</td>
<td>--</td>
<td>0.540 (0.006)</td>
<td>--</td>
</tr>
<tr>
<td>P. transversus</td>
<td>--</td>
<td>0.675 (&lt; 0.001)</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**Newport River**

| C. sapidus | 0.510 (0.010) | --          | --          | --    |
| Uca        | 0.508 (0.010) | 0.582 (0.002) | --          | --    |
| P. transversus | --           | --          | --          | --    |

**Radio Island**

| C. sapidus | 0.472 (0.017) | --          | --          | --    |
| Uca        | --            | --          | --          | --    |
| P. transversus | --           | --          | --          | --    |

**Shackleford**

| C. sapidus | --          | --          | 0.501 (0.011) |
| Uca        | --          | --          | --           |
| P. transversus | --          | --          | --           |
Figure 9: Nightly settlement of *Callinectes sapidus*, *Uca* (left axis) and *Pachygrapsus transversus* (right axis) during the August 14 – 24 and September 20 – October 3, 2006 sampling periods at a) Morehead channel, b) Newport River channel, c) Radio Island channel, d) Shackleford channel, and e) the coastal site. Data are mean number of megalopae per collector (n = 3) for *C. sapidus* (solid lines), *Uca* (dotted lines), and *P. transversus* (dashed lines). Wind stress (N m$^{-2}$) is depicted in top panels with positive values representing westward directed stress and negative values representing eastward directed stress.
significantly more abundant in Morehead, Radio Island and Shackleford channels than at the coast and in Newport River channel ($H = 31.046$, d.f. = 4, $p < 0.001$). Settlement was not correlated among coastal and estuarine sites, but was significantly correlated among four of six pairs of estuarine sites (Table 4).

Settlement of *P. transversus* was restricted to the coastal site and Shackleford channel (Fig. 9; Table 3). Megalopae were significantly more abundant at the coast and in Shackleford channel ($H = 50.945$, d.f. = 4, $p < 0.001$) and were rare at the other estuarine sites. Unlike *C. sapidus* and *Uca*, *P. transversus* settlement was correlated among the coastal site and Shackleford channel, but only among Morehead and Radio Island channels within the estuary (Table 4).

The coastal and estuarine sites differed in the species for which daily settlement was correlated. Settlement at the coast was significantly correlated among *P. transversus* and both *C. sapidus* and *Uca* (Table 5). Within the estuary, *C. sapidus* and *Uca* settlement were significantly correlated at the Morehead channel and Newport River channel sites (Table 5). Additionally, settlement of these two species was significantly correlated at the Radio Island channel site when the September sampling period was considered alone ($r = 0.596$, $p = 0.024$).
Table 5: Comparison of settlement among species at each site. Data presented are correlation coefficient (r) and p-value (in parentheses) for significant comparisons among species.

<table>
<thead>
<tr>
<th></th>
<th>Morehead</th>
<th>Newport River</th>
<th>Radio Island</th>
<th>Shackleford</th>
<th>Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. sapidus vs. Uca</td>
<td>0.511</td>
<td>0.521</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>(0.009)</td>
<td>(0.008)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. sapidus vs. P. transversus</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.490</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.013)</td>
</tr>
<tr>
<td>Uca vs. P. transversus</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.548</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.005)</td>
</tr>
</tbody>
</table>

3.3.3 Diel Sampling at the Coast

Settlement of *C. sapidus* megalopae occurred almost exclusively at night, with no relationship to tidal height. Significantly higher numbers were collected during 2 h deployments at night (268 ± 209 per collector [mean ± 1 SD]) than during the day (13 ± 14 per collector) (Fig. 10; t = 8.136, d.f. = 21, p = 0.001). During the August 28-29, 2005 sampling period, settlement was low early in the night, but increased to very high levels around the time of high tide (Fig. 10b). On the other two nights, however, settlement remained relatively constant throughout the night (Figs. 10a, c). We interpret these data as indicating that settlement at the coast was not related to tide phase and that the dramatic increase on August 28-29, 2005 reflected the arrival of a dense patch of megalopae.
Figure 10: Settlement of *Callinectes sapidus* megalopae over 2 h intervals at Triple S fishing pier during a) July 21–22, 2005 and b) August 28–29, 2005 and at Sportsman’s fishing pier during c) October 4-5, 2006. The y-value of each data point is the mean number of megalopae per collector (n = 3) during the 2 h prior to the sampling time (x-value). Shading indicates the times of day (light) and night (gray), and vertical dashed lines represent the time of slack water after flood on each night.
3.4 Discussion

Megalopae of the blue crab *C. sapidus* were abundant in nearshore coastal areas across the inlet to the Newport River estuary, North Carolina, USA (Beaufort inlet) in nighttime surface plankton samples. During neap tides when flood tide occurred at night, abundance was highest 1-4 km east of the inlet at slack water after ebb tide. Drifter studies indicate the presence of a dynamic eddy in this area during ebb tide that may serve to concentrate megalopae (J. Hench, pers. comm.). Abundance was lowest in the inlet during slack water after ebb tide, reflecting the retention of ingressing megalopae in the estuary. If megalopae followed the paths of drifters and simulated particle tracks during flood tide (Luettich et al. 1998, Churchill et al. 1999), settlement should have occurred throughout the estuary with the highest numbers in eastern channels.

The distribution of estuarine settlers was species-specific and was maintained throughout the spring/neap tidal cycle. Settlement of *C. sapidus* did not match expectations based on the nearshore coastal distribution, but was highest at the coast and in the three westernmost channels of the estuary. In contrast, *Uca* settled in low numbers at the coast, but settled in high abundance throughout the estuary. *P. transversus* settled in high numbers at the coast and in the easternmost channel of the estuary, and was the only species that matched the distribution of ingressing Atlantic
Menhaden larvae *Brevoortia tyrannus* Latrobe (Forward et al. 1999). Physical processes did not appear sufficient to explain the differences between coastal and estuarine distributions of megalopae. Southwestward winds observed during the plankton surveys were relatively light; thus, *C. sapidus* megalopae were not likely impeded from reaching the easternmost channel by winds (Table 5). Transport across the inlet from east to west during flood and ebb tides was also unlikely to occur due to a frontal boundary that formed parallel to the channel near the center of the inlet by opposing pressure gradients and centrifugal forces (Hench and Luettich 2003).

A diel pattern in settlement of *C. sapidus* megalopae was observed at the coastal site, suggesting that they undergo nocturnal diel vertical migration (DVM) in coastal areas. This observation is supported by laboratory studies in which megalopae remained deeper in the water column during the day in the presence of estuarine chemical cues (Forward and Rittschof 1994). Settlement indicative of nocturnal DVM at the coast highlights a clear behavioral difference between megalopae in coastal and estuarine areas, where settlement only occurs during slack water after flood tide at night as megalopae undergo nocturnal Flood Tide Transport (FTT) (Tankersley et al. 2002). This makes comparisons of settlement among coastal and estuarine sites difficult, but could help explain the higher numbers of *C. sapidus* settlers observed at the coast because settlement occurs throughout the night and not just at one phase of the tide.
The estuarine distribution data collected in this study do not allow us to eliminate entirely the possibility that observed settlement patterns resulted from site-specific settlement cues. In brachyuran crabs, settlement can be enhanced or reduced by odors (released from habitats, conspecifics, predators, etc.) present in the environment (Eggleston and Armstrong 1995, Welch et al. 1997). Our collectors were all suspended from wooden docks or piers away from preferred settlement habitats of the species studied. Thus, it is unlikely that the observed patterns were the result of chemical cues associated with nearby settlement habitat. Queiroga et al. (2006) suggested that differences in current speed may affect the distribution of settlers in an estuary. Although not measured directly, we did not observe any relationship between tidal currents and settlement in this study. Conducting plankton sampling in both coastal and estuarine areas would provide a more direct estimate of abundance by removing the potential influence of site-specific settlement cues.

Nevertheless, species-specific differences in the distribution of estuarine settlers among *C. sapidus*, *Uca*, and *P. transversus* suggest that connectivity between coastal and estuarine areas may be affected by larval behavior. Megalopae of *C. sapidus* initiate FTT in response to estuarine cues (Forward et al. 2003), such that gradients in cues across the inlet tidal prism might result in the observed distribution of settlers. Water that exits the Newport River estuary during ebb tide is deflected to the west and primarily re-enters the western channels of the estuary (Hench and Luettich 2003). When flood tide occurs
at night, *C. sapidus* megalopae undergoing nocturnal DVM would swim to the bottom at sunrise near the time of slack after flood when they are distributed across the inlet (Fig. 6b,d). At sunset the following night, megalopae to the west of the inlet would be exposed to estuarine water and would initiate FTT, whereas megalopae to the east of the inlet would be exposed to coastal water, would not initiate FTT, and may not settle at slack water after flood. This gradient in cues could result in the observed estuarine distributions if the appropriate sequence of cues is necessary to stimulate active settlement site selection. Evaluating this hypothesis would require simultaneous sampling of planktonic density and settlement in the estuary during slack after flood.

*Uca* megalopae that enter the tidal prism undergo nocturnal FTT using an endogenous circatidal rhythm characterized by maximum swimming near the end of flood tide (Tankersley and Forward 1994). This behavior would promote up-estuary transport regardless of the presence of environmental cues, and likely resulted in the estuary-wide distribution of *Uca* settlers. Because FTT is under endogenous control, we suspect that ingress of *Uca* most closely follows tidal circulation patterns.

*Pachygrapsus transversus* megalopae are common at the surface during day and night in nearshore areas, although evidence suggest that they may undergo reverse diel vertical migration (Moreira et al. 2007). The coastal distribution of adults suggests that megalopae may not actively move into estuaries (Williams 1984). If megalopae remain in the water column throughout the day and night, relatively few will be mixed from
coastal to estuarine water masses. During flood tide in the absence of winds, coastal water tends to enter the eastern side of Beaufort inlet and flows into Shackleford channel, whereas water entering the western side of the estuary tends to be of estuarine origin (Hench and Luettich 2003). Thus, *P. transversus* megalopae would be more likely to enter the eastern side of the estuary. Larvae of Atlantic menhaden also preferentially entered Shackleford channel, and Forward et al. (1999) hypothesized that this distribution resulted from a source region to the east of the Beaufort Inlet. These data suggest that Atlantic menhaden larvae may have been distributed across the coastal area outside the inlet and preferentially entered Shackleford channel because they remained in coastal water.

This is the first study I am aware of that has attempted to link the nearshore coastal and estuarine distributions of planktonic larvae undergoing estuarine ingress. When nursery habitats are spatially heterogeneous within an estuary, connectivity between coastal sources and estuarine nursery habitats is essential for the successful recruitment of larvae to the juvenile cohort. Submerged aquatic vegetation (SAV) functions as nursery habitat for *C. sapidus* by supporting higher densities of juveniles and promoting increased survival and growth rates versus other estuarine habitats (Thomas et al. 1990, Perkins-Visser et al. 1996), but dramatic declines in SAV have reduced this formerly widespread habitat to isolated areas within estuaries (e.g., Orth and Moore 1983). The remaining SAV can only function as nursery habitat for *C. sapidus*
if it occurs in areas supplied by high numbers of ingressing megalopae. Thus, understanding the spatial patterns of postlarval supply could guide site prioritization for restoration of SAV. Because invasive species also must recruit to suitable habitats, understanding their distributions during estuarine ingress could aid in identifying areas to monitor for their arrival.

In conclusion, larval behavior during estuarine ingress may alter the connectivity of coastal and estuarine areas predicted by particle tracking models (Luettich et al. 1999, Brown et al. 2004). *C. sapidus* megalopae were abundant in nearshore coastal plankton samples across Beaufort Inlet, but settlement within the estuary was restricted to the three westernmost channels. *C. sapidus* megalopae appear to undergo nocturnal DVM in coastal areas, and may only switch to nocturnal FTT in areas where they are exposed to the appropriate environmental cues. Species-specific differences in the distribution of settlers suggest that larval behavior is a critical component of estuarine ingress, and can restrict larvae to specific areas within an estuary.
4. Shoreward Transport and Estuarine Ingress of Brachyuran Crab Postlarvae Inferred from Daily Coastal and Estuarine Abundance in North Carolina, USA

4.1 Introduction

Estuarine species such as the blue crab *Callinectes sapidus* commonly undergo a phase of planktonic larval development in the coastal ocean, returning to estuaries at late larval or postlarval stages. Because they are relatively weak horizontal swimmers, postlarvae may return to the coast by taking advantage of oceanographic processes that promote shoreward transport and estuarine ingress. Postlarval recruitment to coastal and estuarine areas has alternately been associated with wind and tidal transport processes during various segments of the return migration (e.g., Mense et al. 1995, Forward et al. 2004). Because postlarval abundance has only been monitored at coastal or estuarine locations separately, it has not been possible to distinguish between mechanisms of shoreward transport and estuarine ingress (Shanks 1998). We addressed this problem by examining mechanisms of shoreward transport and estuarine ingress of *C. sapidus* postlarvae to the Newport River estuary, North Carolina, USA using daily abundance data collected simultaneously at nearby coastal and estuarine locations. Data are included from additional brachyuran crab taxa for comparison.

*Callinectes sapidus* inhabit estuaries of the western Atlantic from Massachusetts to Argentina (Williams 1984) and support important commercial fisheries throughout
much of this range. Following mating, ovigerous females migrate to coastal areas where they release several clutches of larvae (Carr et al. 2005, Dickenson et al. 2006). Larval development consists of seven zoeal stages and one postlarval (megalopal) stage, lasting about 30-50 days in laboratory studies (Costlow and Bookhout 1959). Return to estuaries occurs at the megalopal stage, which remains in surface waters offshore (Johnson 1985) but apparently begins a nocturnal diel vertical migration in nearshore areas (Forward and Rittschof 1994, Chapter 3). Megalopae return to estuaries primarily in summer and fall where they settle in submerged aquatic vegetation or other structured benthic habitats and metamorphose to the first juvenile instar (Orth and van Montfrans 1987).

In the South Atlantic Bight (SAB), shoreward transport of *C. sapidus* megalopae is thought to occur primarily during southwestward winds that force downwelling due to coastal Ekman circulation (Epifanio and Garvine 2001). Under this scenario, megalopal abundance at the coast should increase one to two days after the onset of southwestward winds. Transport also occurs in shoreward-moving frontal zones during relaxation of upwelling events (Shanks et al. 2000), supplying larvae to the coast shortly after dissipation of northeastward winds. Shanks (1988, 1998) has suggested that megalopae may be transported in convergence zones associated with tidally generated internal waves, which would result in a semilunar or lunar periodicity in abundance,
but abundance would not be associated with a particular phase of the tidal cycle (Miller and Shanks 2004).

Estuarine ingress could result from either wind or tide forced mechanisms. Downwelling events are associated with subtidal increases in coastal sea level that drive barotropic flow into estuaries (Blanton et al. 1995). As at the coastal site, downwelling would result in ingress one to two days after the onset of southwestward winds. Tropical cyclones also increase coastal sea level to the east of the storm center due to storm surge resulting in barotropic inflow to estuaries. This mechanism should result in increased estuarine abundance during passage of a cyclone to the west of the collection site.

Megalopae of *C. sapidus* that reach an inlet tidal prism move up the estuary by nocturnal Flood Tide Transport (FTT) (e.g., DeVries et al. 1994). During nocturnal FTT, megalopae move up into the water column in response to increasing salinity during flood tide, remain swimming in turbulent flood currents, and settle to the bottom as turbulence declines at the end of flood tide (Forward and Tankersley 2001). Megalopae remain on the bottom during ebb tide and during the day. Abundance in the estuary should increase during the longest flood tides at night when maximum up-estuary transport is possible, which occur during neap tides at the quarter phases of the moon in the SAB (Queiroga and Blanton 2004).
In the Newport River estuary, about 30-70 % of annual *C. sapidus* megalopal settlement occurs in distinct episodic settlement events, with the remainder settling on intervening days in low numbers (Forward et al. 2004). We focused this study on identifying the biophysical mechanisms resulting in high settlement events. Our objectives were: 1) to compare coastal and estuarine abundances of *C. sapidus* and other brachyuran crab postlarvae, 2) to determine the biophysical mechanisms underlying coastal arrival and estuarine ingress of *C. sapidus*, and 3) to construct a conceptual model describing the likelihood of estuarine ingress events under various wind and tide conditions. Coastal abundance of *C. sapidus* megalopae was highest during weak onshore winds and was correlated with maximum daily tidal range, but settlement on passive collectors did not appear to be an effective method for estimating abundance in turbulent coastal environments. Estuarine ingress events were associated with wind-driven subtidal inflow or nighttime flood tides, with most events occurring when these mechanisms coincided.

### 4.2 Materials and Methods

#### 4.2.1 Sample Collection

Nightly abundance of crab megalopae was determined for three years (2004-2006) at a coastal site near Beaufort inlet and a site in the Newport River estuary, North Carolina, USA. Beaufort inlet is a relatively narrow (~1 km wide), south-facing inlet that
Figure 11: Map of study regions highlighting collection sites at the Newport River estuary and Oregon Inlet (black dots).

connects the Atlantic Ocean with the partially-mixed Newport River estuary and a system of lagoonal back-barrier island sounds (Fig. 11). The coastal site was located about 3 km west of the inlet in 2004-2005 at Triple S fishing pier (34°41.60’N, 76°38.62’W), but was moved about 1.5 km further west in 2006 to Sportsman’s fishing pier (34°41.68’N, 76°43.81’W) following the demolition of Triple S fishing pier. The estuarine site was located at the Duke University Marine Laboratory dock (34°43.01’N, 76°40.23’W), the site of eight previous years of blue crab megalopal collections (Forward et al. 2004).

Nightly settlement of megalopae on passive collectors was used to estimate abundance. Megalopae settled on substrates made of a ‘hog’s hair’ sleeve surrounding
an inner PVC cylinder that was partially filled with concrete to help maintain a vertical position in the water column (Metcalf et al. 1995). Three replicate collectors were used at each site and were suspended approximately 1 m below the surface at mean low water, which roughly corresponded to the middle of the water column. At the coastal site, megalopae settled on collectors throughout the night (Chapter 3), whereas settlement primarily occurred at slack water after flood tide in the estuary (Tankersley et al. 2002). Shanks (1998) observed higher settlement on surface vs. bottom collectors at a coastal site near Duck, NC, but given that our coastal site was located in a shallow water column (2-4 m) and we collected very large numbers of megalopae, it is unlikely that depth was an important factor at our coastal site. There were no differences in surface and bottom numbers of *C. sapidus* megalopae at our estuarine site (De Vries et al. 1994).

The period of collector deployment differed slightly among sites and years. At the coastal site, samples were collected from September 16 to November 23, 2004, June 8 to November 9, 2005 and June 1 to November 5, 2006. Samples were collected at the estuarine site from June 2 to November 23, 2004, June 2 to November 15, 2005 and June 1 to November 15, 2006. Collectors were deployed on the morning prior to the first sampling date and were retrieved daily (usually in the morning). Megalopae were removed from the collectors using standard techniques (Metcalf et al. 1995). To compare settlement at the south-facing Beaufort inlet with an east-facing inlet, megalopae were also collected at Oregon Inlet fishing pier from August 4 to October 29, 2005. The
collectors used at this site were suspended at the surface using floats and held in a vertical position by a weight attached at the bottom. Because many collectors were lost due to rougher sea conditions and lines cut by fishermen, data gaps limited our use of these data to comparisons of relative monthly settlement and relative species abundance.

### 4.2.2 Physical Data

Predicted tide height and time data were obtained from the software Tides and Currents (Nobeltec). For the coastal site, maximum daily tidal range was calculated as the maximum difference between a low tide and subsequent high tide each day. For the estuarine site, the duration of nighttime flood tide was calculated as the number of minutes of flood tide between sunset and sunrise during the night of collector deployment. When flood tides occurred in both evening and morning, only the tide of longer duration was used. The duration of nighttime flood tide was used for the estuarine site rather than tidal amplitude because it more closely corresponds to predicted abundance patterns based on larval behavior.

Observed water level and temperature data were obtained from NOAA’s National Data Buoy Center Station BFTN7 located at the Duke University Marine Laboratory dock. A daily water level anomaly value was generated by subtracting the predicted level of slack high water after nighttime flood tide from observed values. This
anomaly provided a relative measure of the effects of winds and rain on water inflow into the estuary. Daily water temperature at slack water after flood was calculated by averaging the hourly temperature recorded nearest slack water for the two tides each day. The seasonal trend in temperature was removed for time series analysis by calculating residuals from a 21-day moving average.

Wind data were obtained from NOAA’s National Data Buoy Center Station CLKN7 at Cape Lookout, NC for 2004-2006. Instrument outages in 2006 prevented the use of these data for time series analysis. Instead, data from station 41035 located near New River inlet, NC were used. This station was chosen because the data were the most closely correlated ($r^2 = 0.63$ for wind direction; $r^2 = 0.80$ for wind velocity) of several nearby sites with the 55 days of data from Cape Lookout that were available for 2006. Original hourly data were averaged over the 24 h (diel) period starting at 07:00 h the day before collection and ending at 06:00 h on the morning of collection. This ensured that wind data corresponded to the approximate times of collector deployment. Wind stress was calculated according to Large and Pond (1981) at 15° intervals from 0° (southward directed winds) to 165°, and seasonal trends in wind direction, although small relative to short term variability, were removed for time-series analysis by calculating residuals from a 21-day moving average. Because of the relative orientations of the shoreline and continental shelf (Fig. 11), the ‘alongshore’ (90° axis) and ‘across-shore’ (0° axis) directions are distinguished from the ‘alongshelf’ (15°-60° axes) and across-shelf (105°-
165° axes) for interpretation of the effects of wind-forcing. Water temperature and water level anomaly data were cross-correlated with maximum daily tidal range and alongshelf (15°) and across-shelf (105°) wind stress.

4.2.3 Biological Data

In 2004, only *Callinectes* spp. megalopae were counted and individuals were not identified beyond the genus level. In 2005 and 2006, crab megalopae were initially sorted visually into four easily identifiable taxa including *Callinectes* spp. (Costlow and Bookhout 1959, Bookhout and Costlow 1977), *Pachygrapsus transversus* (Flores et al. 1998), *Menippe mercenaria* (Martin et al. 1988) and *Arenaeus cribrarius* (Stuck and Truesdale 1988). These taxa were chosen for ease of identification and because the primary goal of this research was to study the dynamics of *C. sapidus* immigration to estuaries. First instar juvenile *C. sapidus* were also collected regularly. Because larger juveniles were rarely collected, we assumed that these individuals had settled on the collectors at night as megalopae and had undergone metamorphosis prior to removal from the collectors.

During much of 2004 and on six days in 2005, large numbers of *Callinectes* spp. megalopae were collected at the coastal site (>1000 per collector). On these days, three (in 2004) or five (in 2005) sub-samples of 10 ml were taken from each sample using a large mouth pipette from a vigorously stirred 500 ml mixture of sample and seawater.
Counts were multiplied by a factor of 50 and averaged to determine the daily number for each collector.

*Callinectes* spp. megalopae were identified to species (*C. sapidus* or *C. similis*) in 2005 and 2006 under a dissecting microscope. All individuals were identified on days when the total number collected was 60 or less. For daily totals greater than 60, the three replicate samples were pooled and 60 individuals were randomly chosen using a large mouth pipette during vigorous stirring. Identifications were made for the representative sub-sample and the ratio of each species was applied to the daily total to determine the count of each species on that day. The statistic \( \sqrt{(P(1-P)/N)} \), where \( P \) equals the proportion of *C. sapidus* and \( N \) equals the number of individuals, was used to determine that sub-sampling had a standard error of < 7% (Zar 1999). Cross-correlation analysis was used to test for relationships between settlement at the coast and within the estuary for each species.

To look for relationships among species, settlement time series (megalopae collector\(^{-1}\) over time) were compared by calculating a non-parametric Spearman Rank Order correlation for each possible species pair (Zar 1999). This test was chosen in favor of parametric correlations because data did not meet the assumptions of normality and equal variance and these issues were not corrected using transformations.

Short gaps in each settlement time series were filled using linear interpolation. In 2006 at the coastal site, a 10-day gap from October 7-16 was filled by adding in a
representative eight day period of low settlement (September 15-22) and using linear interpolation to connect it with the observed settlement data. This was necessary because settlement was high at each end of the gap and linear interpolation would have resulted in a two week period of constant high settlement and prevented meaningful time-series analysis. This manipulation made it necessary to exercise caution when interpreting these data, but we felt that it was important to have data from two full seasons for comparisons between coastal and estuarine settlement. A 21-day moving average was calculated and subtracted from the raw settlement data to remove the seasonal trend (Shanks 2006). This method is different from that of previous studies that removed autocorrelation due to tide forcing prior to cross-correlation with wind stress (e.g. Shanks 1998, Forward et al. 2004). This was necessary to preserve the non-linear signal of settlement events that we were attempting to investigate and which were also apparent in the wind data.

Megalopal settlement was related to environmental variables using time series analysis techniques. Cross-correlation analysis was used: 1) to compare nightly settlement with daily wind stress (0° to 165° directions), 2) to compare the seasonal trend in settlement with the seasonal trend in wind stress (21-day moving averages, 0° to 165° directions), and 3) to compare nightly settlement with the maximum daily tidal range (coastal site) or the duration of nighttime flood tide (estuarine site). Due to significant autocorrelations for both settlement and tide time series in each year, 95 % confidence
intervals for these comparisons were calculated both assuming independent points and autocorrelation. The most conservative value was used to determine significance (Wing et al. 1995). Cross-Fourier analysis was used to determine the dominant period of covariance between tide and settlement data and the percent of variation in settlement that could be explained by tides (Roegner et al. 2007). For periods with large cross-amplitudes, squared coherence can be interpreted as the percent of variation in settlement that is explained by variation in the duration of nighttime flood tide.

4.3 Results

4.3.1 Physical Data

Winds were typically weak and northward during summer and increased and shifted to predominantly southwestward in fall (Figs. 12-14). Southwestward directed (alongshelf) winds resulted in increased water level and decreased water temperature at slack water after flood, with the strongest correlations at lags of 0 and -1 days (Table 6). Northwestward (across-shelf) winds were also positively correlated with water level and negatively correlated with water temperature at lags of +1 to -1 days (Table 6).

Tides were semidiurnal with amplitude ranging from 0.33 m to 1.49 m at the estuarine site (Figs. 12-14). In 2004 and 2005, there were significant positive correlations between maximum daily tidal range and water temperature at slack water after flood at
Table 6: Time series analyses between physical data. Lags represent the number of days temperature or water level data follow (negative values) or precede (positive value) wind and tide data. Values in parentheses are correlation coefficients for significant comparisons ($p < 0.05$). Data analyzed are alongshelf (‘Along’, 15° axis) and across-shelf (‘Across’, 105° axis) wind stress, maximum daily tide range (‘Tide’), water level anomaly at slack water after flood (‘Level’), and water temperature at slack water after flood (‘Temp’).

<table>
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<th>2005</th>
<th>2006</th>
</tr>
</thead>
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<td>NS</td>
</tr>
<tr>
<td></td>
<td>-1 (-0.242)</td>
<td></td>
<td></td>
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<td>Along vs. Temp</td>
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<td>0 (-0.365)</td>
<td>0 (-0.297)</td>
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<td>-1 (-0.345)</td>
<td>-1 (-0.391)</td>
<td>-1 (-0.377)</td>
</tr>
<tr>
<td></td>
<td>-2 (-0.188)</td>
<td>-2 (-0.356)</td>
<td>-2 (-0.215)</td>
</tr>
<tr>
<td></td>
<td>-3 (-0.299)</td>
<td>-3 (-0.299)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-4 (-0.234)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tide vs. Temp</td>
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<td>-1 (0.229)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>-2 (0.298)</td>
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<tr>
<td></td>
<td>-3 (0.327)</td>
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</tr>
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<td>-4 (0.272)</td>
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<tr>
<td>Across vs. Level</td>
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<td>0 (0.331)</td>
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<tr>
<td>Along vs. Level</td>
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<td>-1 (0.256)</td>
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<tr>
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<td>-2 (0.178)</td>
<td>-2 (0.309)</td>
<td>-2 (0.256)</td>
</tr>
<tr>
<td></td>
<td>-3 (0.179)</td>
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</tr>
<tr>
<td>Level vs. Temp</td>
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<td>0 (-0.179)</td>
<td>NS</td>
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<td>-1 (-0.307)</td>
<td>-1 (-0.258)</td>
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<tr>
<td></td>
<td>-2 (-0.235)</td>
<td>-2 (-0.202)</td>
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Figure 12: Physical and biological data for 2004. Physical data include: A) N-S (across-shore, solid line) and E-W (alongshore, dotted line) wind stress, B) daily change in water temperature at slack water after flood, C) duration of nighttime flood tide (solid line) and maximum daily tidal range (dotted line), and D) water level anomaly at slack water after flood. Biological data are daily settlement of *Callinectes sapidus* megalopae on collectors at: E) the estuarine site, and F) the coastal site.
Figure 13: Physical and biological data for 2005. Physical data include: A) N-S (across-shore, solid line) and E-W (alongshore, dotted line) wind stress, B) daily change in water temperature at slack water after flood, C) duration of nighttime flood tide (solid line) and maximum daily tidal range (dotted line), and D) water level anomaly at slack water after flood. Biological data are daily settlement of *Callinectes sapidus* megalopae on collectors at: E) the estuarine site, and F) the coastal site.
Figure 14: Physical and biological data for 2006. Physical data include: A) N-S (across-shore, solid line) and E-W (alongshore, dotted line) wind stress, B) daily change in water temperature at slack water after flood, C) duration of nighttime flood tide (solid line) and maximum daily tidal range (dotted line), and D) water level anomaly at slack water after flood. Biological data are daily settlement of *Callinectes sapidus* megalopae on collectors at: E) the estuarine site, and F) the coastal site.
Table 7: Mean monthly settlement (megalopae collector\(^{-1}\) day\(^{-1}\)) of *Callinectes sapidus, Callinectes similis, Pachygrapsus transversus, Menippe mercenaria,* and *Arenaeus cribrarius* at the Newport River estuary coastal and estuarine sites. Months not sampled denoted as n/s.

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<td><em>C. sapidus</em></td>
<td>2004</td>
<td>n/s</td>
<td>n/s</td>
<td>n/s</td>
<td>1221</td>
<td>738</td>
<td>410</td>
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<tr>
<td>(coast)</td>
<td>2005</td>
<td>223</td>
<td>1039</td>
<td>323</td>
<td>811</td>
<td>132</td>
<td>296</td>
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<td></td>
<td>2006</td>
<td>8</td>
<td>39</td>
<td>46</td>
<td>67</td>
<td>483</td>
<td>226</td>
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<td><em>C. sapidus</em></td>
<td>2004</td>
<td>1</td>
<td>29</td>
<td>47</td>
<td>198</td>
<td>47</td>
<td>35</td>
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<tr>
<td>(estuary)</td>
<td>2005</td>
<td>26</td>
<td>37</td>
<td>88</td>
<td>194</td>
<td>13</td>
<td>20</td>
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<tr>
<td></td>
<td>2006</td>
<td>1</td>
<td>17</td>
<td>46</td>
<td>102</td>
<td>106</td>
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<td></td>
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<tr>
<td><em>C. similis</em></td>
<td>2005</td>
<td>88</td>
<td>177</td>
<td>106</td>
<td>236</td>
<td>23</td>
<td>68</td>
</tr>
<tr>
<td>(coast)</td>
<td>2006</td>
<td>0.4</td>
<td>1.7</td>
<td>1.5</td>
<td>0.6</td>
<td>21.2</td>
<td>68</td>
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<tr>
<td><em>C. similis</em></td>
<td>2005</td>
<td>0.7</td>
<td>0.1</td>
<td>1.3</td>
<td>3.7</td>
<td>0.1</td>
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<td>(estuary)</td>
<td>2006</td>
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<td>0.6</td>
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<tr>
<td><em>P. transversus</em></td>
<td>2005</td>
<td>0.2</td>
<td>1.4</td>
<td>1.2</td>
<td>3.4</td>
<td>6.6</td>
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<td>2006</td>
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<tr>
<td><em>M. mercenaria</em></td>
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<td>0.43</td>
<td>0.42</td>
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<td>0.15</td>
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<tr>
<td><em>A. cribrarius</em></td>
<td>2005</td>
<td>0.00</td>
<td>0.00</td>
<td>0.18</td>
<td>0.12</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.04</td>
</tr>
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</table>

lags of -2 to -4 days suggesting that positive temperature anomalies occurred between spring and neap tides (Table 6).

### 4.3.2 Arrival at the Coast

Arrival of megalopae at the coast was episodic and characterized by a common broad period of peak settlement from July to October (Table 7, Figs. 12-14). At Atlantic
Beach, NC, *C. sapidus*, *C. similis*, and *M. mercenaria* were most abundant from July to October, whereas *P. transversus* and *A. cribrarius* were abundant later in the fall from late August to November (Table 7). A similar seasonal pattern of settlement was observed at Oregon inlet, NC (Table 8). *M. mercenaria* were not collected at this site. Nightly megalopal settlement at the coast ranged from a low of zero to a maximum of 6637 collector$^{-1}$ for *C. sapidus*. Maximum settlement was lower for *C. similis* (568 collector$^{-1}$). *P. transversus* (59 collector$^{-1}$), *M. mercenaria* (6 collector$^{-1}$), and *A. cribrarius* (2 collector$^{-1}$), and all species settled in lower numbers in 2006 (Table 7). Settlement of *C. sapidus* was significantly correlated (p < 0.05) with most other species in both 2005 (except *P. transversus*) and 2006 (*A. cribrarius* too rare for analysis) (Table 9). Because of these correlations, comparisons with physical data are only included for *C. sapidus* except where noted.

**Table 8: Mean monthly settlement (megalopae collector$^{-1}$ day$^{-1}$) of Callinectes sapidus, Callinectes similis, Pachygrapsus transversus and Arenaeus cribrarius at the Oregon Inlet fishing pier in 2005.**

<table>
<thead>
<tr>
<th></th>
<th>August</th>
<th>September</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. sapidus</em></td>
<td>522</td>
<td>42</td>
<td>68</td>
</tr>
<tr>
<td><em>C. similis</em></td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td><em>P. transversus</em></td>
<td>3</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td><em>A. cribrarius</em></td>
<td>0.2</td>
<td>0</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Table 9: Correlations among species collected at the coast in 2005 (upper right) and 2006 (lower left) at the coast. Values shown are correlation coefficient (top) and p-value (bottom). Asterisks denote statistically significant relationships. Species include *Callinectes sapidus*, *Callinectes similis*, *Pachygrapsus transversus*, *Menippe mercenaria*, and *Arenaeus cribrarius*.

<table>
<thead>
<tr>
<th></th>
<th><em>C. sapidus</em></th>
<th><em>C. similis</em></th>
<th><em>P. transversus</em></th>
<th><em>M. mercenaria</em></th>
<th><em>A. cribrarius</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. sapidus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r = 0.841</td>
<td>0.148</td>
<td>0.212</td>
<td>0.166</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>p &lt; 0.001*</td>
<td>0.066</td>
<td>0.027*</td>
<td>0.040*</td>
</tr>
<tr>
<td><em>C. similis</em></td>
<td>0.529</td>
<td></td>
<td>0.131</td>
<td>0.245</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.001*</td>
<td>X</td>
<td>0.105</td>
<td>0.010*</td>
<td>0.059</td>
</tr>
<tr>
<td><em>P. transversus</em></td>
<td>0.431</td>
<td>0.338</td>
<td></td>
<td>0.0655</td>
<td>0.223</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>X</td>
<td>0.498</td>
<td>0.005*</td>
</tr>
<tr>
<td><em>M. mercenaria</em></td>
<td>0.178</td>
<td>0.248</td>
<td>0.296</td>
<td></td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td>0.038*</td>
<td>0.004*</td>
<td>&lt; 0.001*</td>
<td>X</td>
<td>0.131</td>
</tr>
</tbody>
</table>

First stage juvenile (J1) *C. sapidus* were often collected at the coast, where they were 1% (2006) to 3% (2005) of megalopal abundance. Juvenile settlement was significantly correlated with megalopal settlement at lags of 0 to -5 days, but correlations were strongest when the data were compared directly (Table 10). There was no correlation between megalopal settlement and the proportion of juveniles in the total daily catch (Table 10).

Settlement at the coast was weakly associated with wind stress and tides. In 2004-2006, settlement was negatively correlated with southward (across-shore) and southwestward (alongshelf) directed winds (Fig. 15), suggesting a potential association between onshore winds and high settlement. An inverse relationship between settlement and the strength of the wind velocity along the 0° (across-shore) axis (Fig. 16)
Table 10: Time series analyses between *Callinectes sapidus* megalopal and first instar juvenile abundances on collectors. Lags represent the number of days that the number of juveniles collector\(^{-1}\) (JUV) and the percent of juveniles in the total catch (% JUV) followed the number of megalopae collector\(^{-1}\). Values in parentheses are correlation coefficients for significant comparisons (p < 0.05).

<table>
<thead>
<tr>
<th>Year</th>
<th>Data</th>
<th>Coast</th>
<th>Estuary</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>JUV</td>
<td>0 (0.687)</td>
<td>0 (0.579)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1 (0.501)</td>
<td>-1 (0.489)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-2 (0.305)</td>
<td>-2 (0.403)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3 (0.195)</td>
<td>-3 (0.630)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-4 (0.216)</td>
<td>-4 (0.601)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-5 (0.169)</td>
<td>-5 (0.594)</td>
</tr>
<tr>
<td></td>
<td>% JUV</td>
<td>NS</td>
<td>-2 (0.368)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-3 (0.261)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-4 (0.249)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-5 (0.230)</td>
</tr>
<tr>
<td>2006</td>
<td>JUV</td>
<td>0 (0.691)</td>
<td>0 (0.268)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1 (0.349)</td>
<td>-1 (0.210)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-2 (0.252)</td>
<td>-2 (0.184)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-4 (0.229)</td>
<td>-4 (0.156)</td>
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<tr>
<td></td>
<td></td>
<td>-5 (0.336)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% JUV</td>
<td>NS</td>
<td>-4 (0.151)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-5 (0.242)</td>
</tr>
</tbody>
</table>
Figure 15: Time series analyses between wind stress (at 15° intervals) and *Callinectes sapidus* settlement at the coastal (A) and estuarine sites (B) in 2004, 2005 (C, D), and 2006 (E, F). Lags indicate the number of days that changes in the settlement time series follow changes in wind stress. Correlation coefficients greater or less than the first marked interval (e.g., ±0.280 in A) are significant (p < 0.05). Positive (negative) correlation coefficients represent a positive (negative) relationship between settlement and winds from the indicated direction.
may have contributed to low correlation coefficients. Settlement of *C. sapidus* was significantly cross-correlated with maximum daily tide range in 2006 only, with high settlement occurring between spring and neap tides (Fig. 17). Periodicities around the semilunar and lunar cycles explained 29-39% of the variability in settlement (Fig. 17).

Figure 16: Relationship between *Callinectes sapidus* megalopal settlement and wind velocity along the 0° axis (northward or southward) at the coastal site in 2004-2006.
Figure 17: Statistical comparison between maximum daily tidal range and Callinectes sapidus megalopal settlement at the coastal site in 2004 (A, B), 2005 (C, D), and 2006 (E, F). Panels in the left hand column are results of cross-correlation analyses. Lags represent the number of days that changes in settlement follow changes in tidal range. Values outside the 95% confidence interval (dashed lines) are significant. Panels in the right hand column, except for 2004 and 2005 when no significant correlations were observed (NS), are results of cross-Fourier analyses. Values for cross-amplitude, a measure of covariance of tidal range and settlement, are plotted and for squared coherence are presented in text. Squared coherence is an estimate of the percentage of variation in settlement at each period due to tidal range.
4.3.3 Entry into the Estuary

Entry of megalopae into the Newport River estuary was restricted to *C. sapidus* and *C. similis*, and was significantly but weakly correlated with arrival at the coast in 2005 and 2006 at lags of -1 to -4 days ($r < 0.25$). The number of megalopae collected in the estuary as a percent of megalopae collected at the coast was higher for *C. sapidus* (8.9 %) than for *C. similis* (1.5 %). The time of peak abundance in the estuary was less variable than at the coast, occurring in September or October each year (Table 7).

Nightly megalopal settlement was much lower than at the coast, ranging from zero to 495 collector$^{-1}$ for *C. sapidus* and 55 collector$^{-1}$ for *C. similis*. Settlement was significantly correlated among species for both 2005 ($r = 0.275$, $p < 0.001$) and 2006 ($r = 0.494$, $p < 0.001$) at lags of 0 days.

The mean percentage of J1 crabs was similar to the coastal site, but the percent of juveniles in the catch increased following high settlement events. Juvenile settlement was 1 % (2006) to 3 % (2005) of megalopal settlement, with significant cross-correlations at lags of 0 to -5 days (Table 10). Unlike at the coast, however, significant correlations between megalopal settlement and the percent of juveniles in the total daily catch were observed at lags of -2 to -5 days (Table 10). During this period, juveniles made up as much as 50 % of the catch.
The relationship between estuarine abundance and wind stress differed each year depending on the yearly pattern of winds. In 2004, positive correlations were observed between settlement and southwestward (alongshelf) directed winds at lags of -2 to -4 days, northwestward directed (across-shelf) winds at a lag of -1 days and northward directed (onshore) winds at 0 days lag (Fig. 15), reflecting a shift from downwelling to onshore winds that occurred just prior to the day of maximum settlement during each of the four settlement peaks in September (Fig. 12). Settlement in 2005 was positively correlated with southwestward directed (alongshelf) winds at lags of -1 to -2 days, again suggesting a relationship between coastal downwelling and high estuarine settlement (Fig. 15). In 2006, however, there were positive correlations between northwestward wind stress and settlement at lags of -1 to -2 days (Fig. 15). In this year, settlement events were associated with two tropical cyclones and two continental low pressure systems with strong westward (alongshore) or northward (onshore) directed winds that, based on changes in water levels and temperatures (Table 6), also resulted in inflow of coastal water to the estuary and may have promoted shoreward transport of surface water. The seasonal trend in NE-SW wind stress was also significantly correlated with the seasonal trend in estuarine settlement, explaining 53-89 % of the seasonal settlement pattern (Fig. 18).
Figure 18: Seasonal trends in wind stress (along the 60° axis; dotted lines) and *Callinectes sapidus* megalopal settlement (solid lines) at the estuarine site in 2004-2006. Trends were calculated from daily wind stress and settlement using a 21-day moving average. Squared correlation coefficients can be interpreted as the percent of variation in settlement that can be explained by variation in wind stress.
Figure 19: Statistical comparison between duration of nighttime flood tide and *Callinectes sapidus* megalopal settlement at the estuarine site in 2004 (A, B), 2005 (C, D), and 2006 (E, F). Panels in the left hand column are results of cross-correlation analyses. Lags represent the number of days that changes in settlement follow changes in the duration of nighttime flood tide. Values outside the 95 % confidence interval (dashed lines) are significant. Panels in the right hand column are results of cross-Fourier analyses. Values for cross-amplitude, a measure of covariance of duration of nighttime flood tide and settlement, are plotted and for squared coherence are presented in text. Squared coherence is an estimate of the percentage of variation in settlement at each period due to duration of nighttime flood tide.
The duration of nighttime flood tide was positively cross-correlated with estuarine settlement in each year, with periods around the semilunar cycle explaining 44-80% of the variability (Fig. 19). In 2005 and 2006, winds favoring shoreward transport and estuarine ingress usually co-occurred with nighttime flood tides, leading to close associations between high settlement events and the longest duration nighttime flood tides (Figs. 13, 14). In September 2004, however, wind-driven settlement events occurred at shorter intervals than the semilunar tidal cycle, shifting the phase relationship between settlement and the longest duration nighttime flood tides by a few days (Fig. 12).

4.4 Discussion

This study was designed to identify the biophysical mechanisms underlying coastal arrival and estuarine ingress of blue crab *C. sapidus* postlarvae (megalopae). As in other *C. sapidus* megalopal settlement studies (e.g., van Montfrans et al. 1995, Rabalais et al. 1995), there were persistent low levels of daily settlement punctuated by episodic high settlement events. We observed simultaneous megalopal settlement of five species at the coastal site. These included *C. sapidus, C. similis, P. transversus, M. mercenaria*, and *A. cribrarius*. Monthly settlement was usually highest in September and October, but peak settlement of *C. sapidus* and *M mercenaria* in 2005 occurred during July.
Predicted mechanisms of shoreward transport were not supported by observed patterns of coastal settlement. Coastal downwelling should have resulted in increased settlement one to two days following the onset of southwestward directed (alongshelf) winds (Epifanio and Garvine 2001). Shoreward moving frontal zones due to relaxation of upwelling should have resulted in high settlement as winds shifted from northeastward (alongshelf, upwelling favorable) to southwestward (downwelling favorable) (Shanks et al. 2000). Instead, coastal settlement was significantly but weakly associated with northeastward to northwestward directed (onshore) winds at lags of 0 to -2 days. One explanation for the lack of strong correlation between wind stress and coastal settlement may be that shoreward transport occurs due to other mechanisms such as tide driven processes (Shanks 1998). Maximum daily tidal range was correlated with settlement in 2006, when two high settlement events occurred between spring and neap tides (Fig. 14), but tides were insufficient to explain the variability in settlement in other years.

An inverse relationship between coastal settlement and across-shore wind velocity (Fig. 16) suggests a more likely explanation for the lack of association between settlement and wind stress observed in this study and by Shanks (1998). *C. sapidus* megalopae respond to turbulence in the water column by sustaining swimming activity (Welch et al. 1999). During strong wind events, when wind driven shoreward transport is most likely, megalopae may remain swimming in response to wind forced turbulence.
and would not likely settle on passive collectors. A similar effect occurs in the estuary, as megalopae only settle on passive collectors during slack water, but not during flood or ebb tide (Tankersley et al. 2002). Because of this problem, settlement on passive collectors may not accurately estimate the nightly abundance of *C. sapidus* megalopae at sites exposed to turbulence from wind waves. Increasing the accuracy of abundance estimates at exposed sites will require a volumetric sampling technique that avoids problems associated with turbulence.

High abundances of megalopae in the estuary were most often associated with tropical cyclones and downwelling favorable winds, with the exact relationships between abundance and wind stress changing each year due to differences in yearly wind patterns. Tropical cyclones that passed directly over the study area resulted in elevated estuarine water levels and decreased estuarine water temperatures that were consistent with barotropic inflow of coastal water to the estuary due to storm surge. Southwestward directed (alongshelf) wind stress, often associated with tropical cyclones passing to the southeast of the study area, also resulted in elevated estuarine water levels and decreased estuarine water temperatures. Changes in water level and temperature often occurred one to two days after the onset of southwestward directed (alongshelf) winds, consistent with shoreward transport of continental shelf water and barotropic inflow of this water to the estuary during coastal downwelling. These results are consistent with observations of *C. sapidus* settlement in Delaware Bay (Jones and
Epifanio 1995), Chesapeake Bay (Goodrich et al. 1989, Olmi 1995), North Carolina (Mense et al. 1995, but not Forward et al. 2004), South Carolina (Boylan and Wenner 1993), and the Gulf of Mexico (Rabalais et al. 1995, Morgan et al. 1996). Because coastal settlement likely did not accurately reflect coastal abundance, it was not possible to separate the effects of wind driven shoreward transport from wind driven estuarine ingress on estuarine abundance. However, simultaneous sampling of the nearshore coastal abundance of megalopae using plankton tows and settlement in the Newport River estuary suggested a strong positive relationship between coastal abundance and estuarine settlement (Chapter 3).

High abundances of megalopae in the estuary occurred during nighttime flood tides (neap tides at the quarter phases of the moon), when movement up the estuary by nocturnal FTT is maximized. This result is also consistent with many previous C. sapidus settlement studies in Chesapeake Bay (van Montfrans et al. 1990, Olmi 1995), North Carolina (Forward et al. 2004), South Carolina (Boylan and Wenner 1993) and the Gulf of Mexico (Rabalais et al. 1995, Morgan et al. 1996). The exceptions are studies by Jones and Epifanio (1995) and Mense et al. (1995). Jones and Epifanio (1995) probably did not observe a tidal signal because their division of the lunar cycle was out of phase with the duration of nighttime flood tide. Mense et al. (1995) observed high settlement when flood tide occurred in the morning and evening, but this relationship may have resulted from strong associations between settlement and downwelling winds. We observed a
similar change in the phase relationship between tides and settlement in 2004, due to a series of upwelling/downwelling wind reversals. In 2005 and 2006, downwelling events and nighttime flood tides generally coincided. Reanalysis of the data collected by Jones and Epifanio (1995) and Mense et al. (1995) using additional time series analyses might resolve these differences.

Our results are also consistent with observations of estuarine ingress of other crabs that export larvae to the continental shelf for development. Queiroga et al. (2006) observed maximum supply of *Carcinus maenas* megalopae to estuaries in Portugal during nighttime flood tides. On the US west coast, *Cancer magister* megalopae recruit to estuaries 0-7 days after spring tides (Miller and Shanks 2004, Roegner et al 2007), which corresponds to the time in the semilunar cycle when flood tide occurs at night. Roegner et al. (2007) attributed the semilunar settlement pattern to transport in convergence zones over internal waves, arguing that crab abundance during tidal transport should change linearly, not episodically, with tide range. However, if *C. magister* megalopae undergo nocturnal FTT, there is about a 5-7 day window of optimal conditions for FTT immediately following spring tides that would result in pulsed ingress because flow at night is ebb tide dominated at other times.

The proportion of juveniles collected at the estuarine site changed over time, and probably resulted from changes in the molt stage of megalopae entering the estuary. We assumed that juveniles settled as megalopae and underwent metamorphosis prior to
collection. Because molt stage is not related to the likelihood of settlement (Hasek and Rabalais 2001), the proportion of juveniles in the catch each day can be considered a relative measure of the proportion of premolt megalopae in the total catch. Most megalopae entering the estuary are in intermolt, but the proportion of premolt megalopae varies during the reproductive season (Forward et al. 1996). Although the number of juveniles collected was highest on the day of megalopal settlement events, a higher proportion of juveniles was collected at the estuarine site 2-5 days after settlement events. This result suggests that some megalopae were retained near the inlet for several days following major settlement events and entered the estuary as premolt megalopae since the collection site was within a single tidal excursion of the inlet (Churchill et al. 1999).

We observed a strong association between the seasonal pattern of wind forcing and estuarine ingress. Peak estuarine settlement and southwestward directed wind stress co-occurred in September and October in all years. Atmospheric forcing has also been shown to influence recruitment of Dungeness crab *Cancer magister* megalopae to estuaries along the US West Coast and western rock lobster *Panulirus cygnus* postlarvae to the west coast of Australia, explaining much of the variability in fishery landings (Pearce and Phillips 1988, Shanks and Roegner 2007). Taken together with observations that estuarine settlement may predict commercial landings in North Carolina (Forward et al. 2004) and that wind patterns may predict landings in the Chesapeake Bay (Johnson
and Hester 1989), our data suggest that inter-annual variation in wind forced estuarine ingress in conjunction with post settlement processes could potentially regulate \textit{C. sapidus} populations on the US East Coast.

### 4.5 Conceptual Model

We developed a conceptual model for estuarine ingress of \textit{Callinectes sapidus} postlarvae that describes the likelihood of high estuarine settlement events based on the results of this study and on previous studies (Fig. 20). In general, estuarine ingress

![Figure 20: Conceptual diagram depicting the relative likelihood of \textit{Callinectes sapidus} estuarine ingress events as a function of wind conditions and the phase of tide occurring at night.](image-url)
events are driven by wind events that promote both shoreward transport and ingress of megalopae to estuaries. Tides interact with wind driven mechanisms by increasing (during nighttime flood tides) or decreasing (during nighttime ebb tides) the chances of entering an estuary during a given wind event. Wind-driven events, the more important factor between wind and tides, are most likely to result in shoreward transport and ingress during storm surges on the eastern side of tropical cyclones and by barotropic inflow during downwelling events. Onshore winds not associated with tropical cyclones may promote shoreward transport and ingress, but this mechanism appears less important than the previous two. Ingress rarely occurs during periods of light winds, upwelling events or offshore winds.

Estuarine ingress events are promoted during nighttime flood tides and are hindered during nighttime ebb tides due to the nocturnal FTT behaviors of *C. sapidus* megalopae. The impact of tides on wind driven mechanisms of ingress at a particular location likely depends on tidal amplitude, such that ingress events along the Gulf Coast are probably wind dominated and ingress events near the center of the South Atlantic Bight are probably limited to periods of nighttime flood tides. Ingress events can also occur when winds are light or unfavorable if megalopae are present along the coast during nighttime flood tides. Low levels of nightly settlement throughout the
recruitment season suggest that some megalopae are able to undergo ingress even during the least favorable conditions (offshore winds and nighttime ebb tides).
5. Effects of Sampling Interval on Estimates of Annual Blue Crab Postlarval Abundance

5.1 Introduction

Estimating the annual abundance of larval shellfish and finfish entering estuarine nursery habitats can provide important information on inter-annual variability in year-class strength of commercially important species. Collecting larval abundance data is costly and time consuming, because the accuracy of recruitment estimates depends largely on sampling interval. For example, based on sub-sampling from a data set of daily abundance, Hettler et al. (1997) determined that variability in estimates of annual Atlantic menhaden larval abundance was acceptable only at sampling intervals of seven days or less. Intervals of 14 and 30 days were deemed to be too variable to be useful for estimating annual larval abundance. Because their analysis was derived from a single year of daily abundance data, it did not account for inter-annual variations in the pattern of daily abundance. We addressed this problem by assessing the effect of inter-annual variation on estimates of annual blue crab postlarval abundance derived from sub-sampling an 11-year survey of nightly settlement at intervals of 2-7 days.

Planktonic larvae develop in 30-50 days in continental shelf surface waters (Costlow and Bookhout 1959), and return to estuarine nursery habitats as postlarvae (Orth and van Montfrans 1987). Postlarvae enter the estuary in higher abundance during nighttime flood tides (DeVries et al. 1994), but the annual pattern of postlarval settlement depends on the interaction between atmospheric forcing and nighttime flood tides (Chapter 4). Since Forward et al. (2004) found that maximum settlement consistently occurred at the time of neap tides, restricting sampling effort to neap tides could potentially reduce variability in estimates of annual abundance. Although this method would result in overestimates of annual abundance, it is possible that a correction factor could be derived to estimate annual abundance.

The limited data available suggest that inter-annual variability in *C. sapidus* postlarval settlement may explain some of the variability in annual fishery landings (Forward et al. 2004). For future determinations of this relationship, it will be useful to monitor settlement in the most cost effective and efficient manner while minimizing sampling error. Thus, the objectives of this study were to: 1) determine the variation in annual abundance estimates at sampling intervals when sampling occurs at intervals of 2-7 days, 2) determine whether directed sampling during neap tides can be used to decrease the variability in annual abundance estimates, and 3) compare the usefulness of observed and estimated annual abundances for predicting fishery landings.
5.2 Materials and Methods

Nightly postlarval settlement data were collected at the Duke University Marine Laboratory dock in the Newport River estuary, North Carolina, USA. Data were available from September to November in eight years between 1993 and 2003 (Forward et al. 2004). In 2004-2006, data were collected from June to November (Chapter 4). Prior to analysis, gaps in settlement time series were filled using linear interpolation.

To investigate the effect of sampling interval on abundance estimates, the data were sub-sampled in two ways. First, data were sampled at set intervals of two, three, four, five, and seven days because these intervals were analyzed in Hettler et al. (1997). All possible data sets were generated at each interval so that at an interval of two days, there were two sub-sampled data sets, three at three days, and so on. To determine the interactions between inter-annual variability and sampling interval, we calculated the coefficient of variation of the estimated annual means for each sampling interval.

To determine whether restricting sampling to neap tides reduces variability in abundance estimates, data were sub-sampled for seven, five, and three consecutive days surrounding the day of neap tide. Data were also sub-sampled on the first, middle, and last days of the seven day neap tide period to decrease the chance of missing a settlement pulse when only sampling three days each neap tide period and on the first and seventh days to approximate weekly sampling. Because only one data set was
generated each year for these intervals, different techniques for assessing variability were necessary. The percent difference from the daily mean was calculated to enable inter-annual comparisons. Comparisons of variability and the degree of overestimation among sampling intervals were made using the mean and standard deviation of the percent difference from annual means.

To compare the usefulness of observed and estimated mean annual postlarval abundances, we correlated observed and estimated abundance with fishery landings. Estimated annual postlarval abundance data were calculated by generating all possible combinations of annual abundance estimates for the two day sampling interval (n = 152). At longer sampling intervals, the number of possible combinations was too high to calculate within a reasonable time period. Fishery landings (kg) and effort (trips) data were obtained from the North Carolina Division of Marine Fisheries for hard, soft, and peeler crabs landed statewide in the crab pot fishery and catch per unit effort (CPUE) was calculated (kg/trip). CPUE was correlated with postlarval abundance at a lag of two years, the approximate age at which crabs enter the fishery (Forward et al. 2004). One outlier (1994 postlarval abundance and 1996 landings) was removed so that the observed postlarval abundance data underlying the analysis were significantly correlated with CPUE (p = 0.003).
Figure 21: Estimated mean daily abundance (number of postlarvae collector\(^{-1}\)) of *Callinectes sapidus* postlarvae at sampling intervals of 2-7 days (triangles) compared to observed abundance (dotted lines). Estimates were generated from data sets of daily abundance from September to November in 11 years.
5.3 Results

Sub-sampling the nightly settlement data collected from September to November resulted in a wider range of annual abundance estimates as sampling interval increased (Fig. 21). The range in annual abundance estimates was lowest at the two day interval in 1998 (10.8-11.1 postlarvae day\(^{-1}\)) and widest at the seven day interval in 1996 (29-167 postlarvae day\(^{-1}\)). The coefficient of variation increased 2-16 times from the two day to

Table 11: Coefficient of variation of the September to November and June to November nightly postlarval settlement data sets. Data presented are for sub-sampling of the nightly data set at intervals of 2-7 days. Means and standard deviations (in parentheses) are included for comparison.

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<td>Mean</td>
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seven day intervals (Table 11). Extending the sampling period to six months in 2004-2006 only (June to November) resulted in a 20 % decrease (0.27 to 0.22) in the average coefficient of variation at the seven day interval (Table 11).

Inter-annual differences in the pattern of settlement strongly influenced the variability of annual postlarval abundance estimates. The range in annual abundance estimates derived from sub-sampling the nightly settlement data set was typically large in years in which settlement was dominated by a single settlement event as in 2005, but was small when settlement was spread out over many days as in 2006 (Fig. 22). Determining the level of variation that is appropriate for a particular sampling effort is not the subject of this paper. However, if we set the maximum acceptable coefficient of variation at 0.25, equivalent to 25 % of the annual mean, the two day sampling interval performed within this threshold in 9 of 11 years, but the seven day interval only provided a sufficient estimate of abundance in 2 of 11 years (Table 11). Applying the 20 % decrease in coefficient of variation that resulted from extending the sampling period from three to six months in 2004-2006 to the 1993-2003 data would not have reduced the number of years for which the coefficient of variation exceeded 0.25.

Directed sub-sampling of the nightly settlement data set during neap tides tended to result in over-estimates of annual abundance without reducing variability (Fig. 23). On average, estimates of annual abundance ranged from a low of 2 ± 35 % (mean ± standard deviation) higher than the mean for the three days surrounding neap
tides to a high of 34 ± 70 % for the first and seventh days of neap tides (Table 12). Neap tide sampling tended to over-estimate abundance in years such as 2005 when wind-driven increases in postlarval abundance coincided with neap tides (Chapter 4). In no case were estimates of the annual abundance within 25 % of the mean

Table 12: Percent difference from the mean annual abundance of the September to November and June to November nightly postlarval settlement data sets. Data are presented for sub-sampling of the nightly data set for: A) seven days surrounding neap tide, B) five days surrounding neap tide, C) three days surrounding neap tide, D) the first, middle and last days of the seven day neap tide period, and E) the first and last days of the seven day neap tide sampling period. Means and standard deviations (in parentheses) are included for comparison.

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Figure 22: Daily abundance (number of postlarvae collector$^{-1}$) of Callinectes sapidus postlarvae in 2005, when most settlement occurred in a single high settlement event, and 2006, when settlement was spread over several high settlement events.

Figure 23: Percent difference between the observed mean annual abundance and estimates derived from sub-sampling of the nightly data set for: A) seven days surrounding neap tide, B) five days surrounding neap tide, C) three days surrounding neap tide, D) the first, middle and last days of the seven day neap tide sampling period, and E) the first and last days of the seven day neap tide sampling period. Each triangle represents the estimate from one year of data collected from September to November (n = 11). Estimates that fall on the zero line were equal to the observed annual mean.
Correlating each possible combination of annual abundance estimates at a sampling interval of two days with CPUE in the North Carolina pot fishery resulted in a wide range of correlation coefficients. The correlation coefficient for observed mean daily abundance and CPUE was 0.88, whereas the correlation coefficients for estimated abundances ranged from a low of 0.64 to a high of 0.96 (Fig. 24). Correlations between estimated abundance data and CPUE exceeded the 95 % confidence interval for 82 % of possible combinations.
Monitoring the annual recruitment of larval shellfish and finfish is a costly and time-consuming endeavor. Researchers often use sampling intervals of a week or more to reduce the cost of data collection (e.g., Warlen 1994), but it is important to understand the consequences of increasing the sampling interval. Sampling at intervals longer in duration than the wind or tide-driven processes that underlie fluctuations in abundance could result in high variability in annual abundance estimates. Hettler et al. (1997) suggested that a weekly sampling interval was sufficient for estimating the annual recruitment of larval Atlantic menhaden in the Newport River estuary, NC, but their analysis did not account for inter-annual variability in the pattern of abundance because it was based on a single year of data. If their study was conducted in a year of unusually episodic abundance, they may have overestimated variability in yearly abundance estimates at each sampling interval. More importantly, if their study was conducted using data from a year in which abundance was relatively constant over time, they may have underestimated variability. In our study, inter-annual variability in the pattern of blue crab *C. sapidus* postlarval settlement resulted in estimates of annual abundance that varied by more than 25% of the mean in two of eleven years at a sampling interval of two days and nine of eleven years at a sampling interval of seven days.
Sub-sampling the nightly data sets during neap tides, when *C. sapidus* megalopae were most likely to be abundant (Forward et al. 2004), did not reduce variability in annual abundance estimates. Neap tide sampling also did not consistently result in overestimates of annual abundance. This was probably because atmospheric forcing can obscure the relationship between *C. sapidus* postlarval abundance and the semilunar tidal cycle (Chapter 4). Thus, directed sampling during periods of predicted high abundance does not appear to be an effective method for reducing variability in annual abundance estimates.

Increasing the sampling interval from daily to two days decreased the chances of detecting a significant correlation between annual *C. sapidus* postlarval abundance and CPUE in the North Carolina pot fishery by nearly 20%. Longer sampling intervals would lead to further declines in the likelihood of detecting significant correlations. Thus, scientists or managers must carefully consider the goals of multi-year larval surveys prior to establishing the appropriate sampling interval. For instance, weekly surveys are probably sufficient for estimating seasonal trends in abundance, size, or age of larvae (Hettler et al. 1997). If the goal of a survey is to determine annual year class strength, however, weekly sampling can result in widely ranging estimates of annual abundance. Factors that affect variability in annual abundance estimates include the duration of sampling or recruitment period, the magnitude of differences between days of high and low abundance, and the number and duration of high abundance events.
Setting the acceptable level of variability in annual larval abundance estimates is the prerogative of the scientist or manager conducting a particular survey. Increasing the sampling interval may reduce costs, but can result in highly inaccurate estimates of abundance in some years. Restricting sampling to known periods of high abundance such as during neap tides for blue crab postlarvae in North Carolina, can not be used to reduce variability in estimates and does not result in consistent overestimates of abundance.
6. Summary and Conclusions

This dissertation investigated physiological changes and biophysical transport mechanisms during estuarine ingress of the blue crab *Callinectes sapidus* to the Newport River estuary, North Carolina, USA. Comparisons with additional species were often included to yield insights into *C. sapidus* physiology and behavior. The effect of sampling interval on estimates of annual postlarval (megalopal) abundance was also assessed. The major research findings of each chapter are summarized in the following sections.

6.1 Chapter 2: Comparison of Low Salinity Tolerance in *Callinectes sapidus* Rathbun and *Callinectes similis* Williams Postlarvae upon Entry into an Estuary

I investigated the tolerance of blue crab *C. sapidus* and lesser blue crab *C. similis* megalopae collected in the field to a range of salinities. Juvenile and adult *C. sapidus* live at lower salinities and have stronger osmoregulatory capabilities in brackish water than *C. similis*, thus I hypothesized that their megalopae would also be more tolerant of brackish water. Megalopae were collected at a coastal site (salinity 33-34) and an estuarine site (salinity 27-32) and exposed to salinities of 5, 10, 15, 20, and 30 for six hours. They were transferred back to water from the collection site for 24 h after which percent survival was determined. For *C. sapidus*, survival was >95% at salinities of 15 or
higher but declined at lower salinities. For *C. similis* collected at the coast (*C. similis* megalopae were rare at the estuarine site), few megalopae survived at salinities of 15 or less.

At salinities of 5 and 10, percent survival of *C. sapidus* megalopae collected in the estuary was significantly higher than that of megalopae collected at the coast. This difference might have resulted from advanced development of individuals collected in the estuary, or could have resulted from physiological acclimation in response to decreasing salinity or estuarine chemical cues. I tested these hypotheses by investigating: 1) whether reduced salinity or estuarine chemical cues resulted in increased low salinity tolerance, 2) the time to acclimation, and 3) the salinity necessary for acclimation. *C. sapidus* megalopae collected at the coast showed significantly increased survival at a salinity of 5 after acclimation to salinities of 27 and 23 for 12 h, but did not show any additional response to estuarine chemical cues. *C. similis* megalopae were unable to survive at a salinity of 5 after acclimation to a salinity of 23. These results suggest that *C. sapidus* megalopae rapidly increase their tolerance to low salinity upon entering estuaries, and that this increase occurs in response to reduced salinity.
6.2 Chapter 3: Ingress of Brachyuran Crab Postlarvae to the Newport River Estuary

The distribution of *C. sapidus* megalopae in nearshore coastal and estuarine areas was compared to: 1) investigate spatial patterns in abundance during estuarine ingress, 2) determine whether these patterns persisted over the spring/neap tidal cycle, and 3) determine the diel timing of postlarval settlement at the coast for comparison with estuarine settlement. The coastal distribution was determined using nighttime surface plankton tows on four nights, two during spring tides when ebb tide occurred at night, and two during neap tides, when flood tide occurred at night. Samples were collected at thirteen stations at 1 km intervals from 6 km west of Beaufort inlet to 6 km east of Beaufort Inlet and 0.5 km from shore. This transect was sampled from west to east twice each night around the times of slack water after flood and ebb tides. Megalopae were distributed across the transect each night. Megalopal density was usually highest east of the inlet at slack water after ebb tide, possibly due to an eddy that forms in this area during ebb tide. Few megalopae were collected in the estuarine plume at slack water after ebb, indicating retention in the estuary during ebb tide. Based on hydrodynamic particle tracking models, this distribution would result in high abundances of settlers in eastern areas of the Newport River estuary.

The estuarine distribution of megalopae was determined using passive ‘hog’s hair’ settlement collectors on the four nights of plankton sampling and during two
spring/neap tidal cycles. Three replicate collectors were deployed at sites in each of the four major channels of the Newport River estuary which were, from west to east: Morehead channel, Newport River channel, Radio Island channel, and Shackleford channel. During the spring/neap sampling, three additional collectors were suspended from a coastal fishing pier approximately 3 km west of Beaufort inlet. Collectors were deployed in the morning and retrieved the following day. Megalopae of the fiddler crabs *Uca* species (*U. minax*, *U. Pugilator*, and *U. pugnax*) and *Pachygrapsus transversus* were collected in addition to *C. sapidus* for purposes of comparison.

In contrast to expectations based on the coastal distribution, *C. sapidus* megalopae settled in higher numbers at the coast and in the three western channels of the estuary than in the easternmost channel. This pattern was maintained throughout the spring/neap tidal cycle. Settlement patterns of *Uca* and *P. transversus* differed from that of *C. sapidus*. *Uca* settled in low numbers at the coast, but were common throughout the estuary, whereas *P. transversus* only settled in high numbers at the coastal site and in the easternmost channel of the estuary. These results suggest that larval behaviors may alter the spatial patterns of estuarine ingress from expectations based on hydrodynamic particle tracking models in species-specific ways.

The diel timing of *C. sapidus* megalopal settlement at the coast was determined using ‘hog’s hair’ collectors sampled at two hour intervals during the day and night. Megalopae settled in low numbers during the day and high numbers during the night,
with no apparent relationship to the phase of the tide. This was different from
settlement in the Newport River estuary which occurred primarily during slack water
after flood tide (Tankersley et al., 2002), and suggests that C. sapidus megalopae undergo
nocturnal diel vertical migration in coastal areas. Differences in the diel timing of
settlement may help explain why C. sapidus megalopae settled in higher abundance at
the coastal site.

6.3 Chapter 4: Shoreward Transport and Estuarine Ingress of
Brachyuran Crab Postlarvae Inferred from Daily Coastal and
Estuarine Abundance in North Carolina, USA

I investigated biophysical mechanisms of estuarine ingress by comparing nightly
abundance of C. sapidus at coastal and estuarine sites with environmental data. C.
sapidus megalopae were collected nightly from June to November 2004-2006 on ‘hog’s
hair’ settlement collectors deployed at a coastal fishing pier located about 3 km west of
Beaufort inlet and at the Duke University Marine Laboratory dock in the Newport River
estuary. Megalopae of C. similis, P. transversus, Menippe mercenaria, and Areneaus
cribrarius were also collected for comparison. Settlement followed a seasonal pattern,
with peak abundances of each species usually occurring from August to October, and
was highly episodic. For C. sapidus and C. similis, the only two species collected with
regularity in the estuary, higher numbers were collected at the coast. Settlement was
usually correlated among species at the coastal and estuarine sites, but correlations
between the coastal and estuarine sites for *C. sapidus* and *C. similis* were weak. This may have been due to differences in the diel timing of settlement at the two sites (Chapter 4).

Nightly settlement of *C. sapidus* megalopae was compared to wind stress using cross-correlation analysis and to tides using cross-correlation and cross-Fourier analyses. Prior to analysis, gaps in the settlement time series were filled using linear interpolation and the seasonal trend was removed by calculating the residuals from a 21-day moving average. Wind stress was calculated at 15° intervals from 0° (N-S) to 165° and the seasonal trend was removed as described above. Because *C. sapidus* megalopae move up-estuary during nighttime flood tides, settlement was compared to the duration of nighttime flood tide instead of tidal amplitude. Wind stress and tidal amplitude were also compared to daily water temperature and water level anomalies. Settlement was usually correlated among species, thus results were reported for *C. sapidus* except when relationships differed.

High settlement events in the estuary were associated with wind-driven estuarine inflow events and nighttime flood tides, and were most likely when these conditions co-occurred. Both tropical cyclones and downwelling favorable winds resulted in increases in estuarine sea-level and decreases in estuarine water temperature that were associated with high settlement events. High settlement events also occurred when wind stress was weak, but only during nighttime flood tides when *C. sapidus* megalopae move into the estuary by nocturnal Flood Tide Transport. At longer time
scales, the seasonal pattern of estuarine settlement was strongly associated with the seasonal pattern of alongshore wind stress. This result suggests that inter-annual variations in atmospheric forcing may play an important role in determining the abundance of *C. sapidus* megalopae recruiting to estuarine nursery habitats each year.

**6.4 Chapter 5: Effects of Sampling Interval on Estimates of Annual Blue Crab Postlarval Abundance**

Monitoring the daily abundance of *C. sapidus* postlarvae in estuaries during the recruitment season may be an effective measure of annual year class strength, but these data are costly and time consuming to collect. I investigated the effects of increasing the sampling interval and decreasing the sampling duration on variability in estimates of annual postlarval abundance. Daily abundance of *C. sapidus* postlarvae was determined using nightly settlement on artificial ‘hog’s hair’ collectors from September to November for eight years between 1993 and 2003. Similar data were collected from June to November in 2004-2006.

To generate estimates of annual abundance at sampling intervals longer than daily, the daily data sets were sub-sampled in two ways. First, daily data were sub-sampled at intervals of two, three, four, five, and seven days, and yearly means were calculated for these new data sets. Variability in yearly means at each sampling interval was compared using the coefficient of variation. Second, because *C. sapidus* megalopae
enter estuaries in higher numbers during nighttime flood tides (during neap tides at the quarter phases of lunar cycle), data were sub-sampled 1) on seven, five, and three days surrounding the day of neap tides, 2) on the first, middle, and last day of the seven day neap tide period, and 3) on the first and last days of the seven day neap tide period. The percent difference from the observed yearly mean was calculated for each sampling interval, and variability in these values was used to compare different sampling intervals. For both methods of sub-sampling, the effect of sampling duration was investigated by comparing data from June to November and September to November in 2004-2006. The usefulness of data at a sampling interval of two days was compared to daily data by correlating all possible combinations of two day annual abundance estimates and daily data with catch per unit effort (CPUE) of the C. sapidus pot fishery in North Carolina.

Increasing the sampling interval resulted in higher variability in estimates of annual postlarval abundance. Estimates of annual abundance varied by more than 25% in two of eleven years at a two day sampling interval and nine of eleven years at a seven day sampling interval. Sampling around the time of neap tides did not reduce variability in annual abundance estimates, nor did it consistently overestimate abundance. Increasing the sampling interval from daily to every two days decreased the chances of detecting a significant correlation between annual postlarval abundance and
CPUE by nearly 20%. These results suggest that daily sampling may be necessary to accurately predict annual year class strength from postlarval abundance.

6.5 Significance

The blue crab *C. sapidus* and many other estuarine species share a common life history strategy of exporting larvae to the continental shelf during development. This dissertation adds to our understanding of physiological changes, spatial patterns, and biophysical transport mechanisms of *C. sapidus* postlarvae as they move from nearshore coastal areas to estuarine nursery habitats. Recognizing that permanent spatial patterns in postlarval abundance may exist during estuarine ingress highlights the importance of determining connectivity between nursery habitats and larval sources when sites are chosen for restoration of submerged aquatic vegetation. This result is broadly applicable to commercial species that use estuaries as nursery habitat. Understanding how atmospheric forcing and tides shape the seasonal and event scale patterns of *C. sapidus* postlarval recruitment may lead to the development of models that help explain variability in population size and fishery landings. Finally, documenting the effect of sampling interval on estimates of annual postlarval abundance will provide important information for scientists and managers who wish to sample larvae for the purpose of determining the year class strength of species that recruit to estuaries for juvenile development.
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Biography

Matthew Bryan Ogburn

Birthplace: Blacksburg, Virginia
April 25, 1979

Education

2008  Doctoral Degree, Duke University, Nicholas School of the Environment and Earth Sciences.
2004  Master of Science, The University of Georgia, Department of Marine Sciences
2001  Bachelor of Science, Duke University, Biology

Honors and Awards

2007  Fellow, Preparing Future Faculty Program, Duke University
2005  Graduate Fellowship, Chi Psi Fraternity
2003  Graduate Fellowship, Chi Psi Fraternity
2002  Graduate School Fellowship, The University of Georgia

Publications


