Spring 2010

Aspects of the reproduction of an invasive crab, Hemigrapsus sanguineus, in northern and southern New England

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ASPECTS OF THE REPRODUCTION OF AN INVASIVE CRAB, *Hemigrapsus sanguineus,* IN NORTHERN AND SOUTHERN NEW ENGLAND

BY

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THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

in

Zoology

May, 2010

This thesis has been examined and approved.

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7 May 2010
Date

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Larry Harris for his support and guidance and for sharing his immense knowledge of invertebrates and ecology, and historical perspectives on the Gulf of Maine. Thank you for all of the experiences and opportunities to learn, including many dives and trips to the intertidal over the years. Your knowledge and experience have been truly inspiring and have only deepened my fascination with marine invertebrate ecology.

I thank my other committee members, Dr. James Haney and Dr. Marian Litvaitis. Your ideas, insight and constructive criticism have strengthened my research as well as the presentation of my work.

Special thanks go to Michael Goodison, whose daily counsel was essential to my sanity and success. Thank you for making me laugh when I didn't want to and for believing in me completely.

Thank you to the members of the Harris lab past and present, especially Linda Auker, Helen Day, Megan McCuller, and Jennifer Dijkstra. Thank you for the daily camaraderie and understanding. Thank you Linda for your help reviewing drafts of this manuscript, and for your support during the thesis writing process.

Special thanks go to my amazing team of undergraduate research assistants, who included over the years the following students: Stephen

Boutwell, Paula Teichholtz, Kirsten Ritner, Spencer Skinner, Nicola Groh, Lara Wayman and Angela Piemonte. This project would not have been possible without your help in the field and lab, and I especially appreciate your trustworthiness and ability to work independently. Stephen and Kirsten – thank you for volunteering your time in the summers. Thank you for enduring wet denim, cutting up your hands and getting pinched, and never complaining.

I would also like to acknowledge my family. Thank you to Liz and Jerry Gamelin for supporting me through this and all other endeavors in my life. Thank you for always being proud and confident, especially when I couldn't be myself. Thank you mom for all of your help and enthusiasm in the field, and for not sleeping on the dock, even though you wanted to. I would also like to thank my sister Gillian for being an inspiration as a mom. To my nieces, Audrey and Bailey, thank you for giving me things to look forward to and moments to forget everything but joy and laughter.

This work was funded by the University of New Hampshire Department of Biological Sciences, UNH Marine Program, UNH College of Life Sciences and Agriculture and the UNH Graduate School.

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ABSTRACT

ASPECTS OF REPRODUCTION OF AN INVASIVE CRAB
Hemigrapsus sanguineus IN NORTHERN AND SOUTHERN NEW ENGLAND

by

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University of New Hampshire, May, 2010

Populations of the invasive shore crab, *Hemigrapsus sanguineus*, were studied in northern and southern New England to determine if crabs differ in reproductive behavior or characteristics between these regions. Additionally, effects of temperature on reproductive activity were quantified through laboratory experimentation.

Number of broods per season increased with temperature, but the seasonal total was limited to three broods in laboratory experiments. Broods experienced limited success at the lowest temperature, 10°C. The reproductive season was longer at lower latitudes, and females at this site had smaller average ovigerous size. Patterns of ovigery varied between the regions, suggesting the production of one brood per season in New Hampshire, compared to two to three broods per season in Rhode Island.

Overall, temperature may limit the possibility and degree of reproductive output by females, which may slow the spread or limit establishment of this species in northern latitudes.

INTRODUCTION

***Hemigrapsus sanguineus*: Biology and Ecology**

Hemigrapsus sanguineus, the Asian shore crab, is a brachyuran crab of the family Grapsidae and subfamily Varunidae. Juveniles and adults inhabit rocky intertidal areas on open coasts but may also extend into estuarine habitats. Benthic individuals range in size from approximately 1.6 mm (newly settled juveniles) to 44 mm in carapace width (CW), with males attaining slightly larger sizes than females (Fukui 1988, Epifanio et al. 1998, McDermott 1998). The largest male and female reported to date were collected subtidally, with sizes of 48 mm and 40 mm respectively (Gilman and Grace 2009).

Like other brachyurans, *H. sanguineus* produces planktonic larvae. The first larval form is called a zoea, and *H. sanguineus* larvae pass through five zoeal stages before molting to a megalopal or post-larval stage (Kornienko et al. 2008). The megalopa metamorphoses to the first juvenile crab stage and settles to the benthos where it will spend the remainder of its life cycle.

As a member of the class Crustacea, *H. sanguineus* undergoes a molt cycle, which consists of a number of phases (Charmantier-Daures and Vernet 2004). These stages were initially described for brachyurans by Drach (1939, 1944), and each of these stages can be split further into specific sub-stages. The act of shedding the integument is called ecdysis or molting. The postmolt or postecdysis stage is the stage immediately following molting, during which the

animal is still soft and the cuticle is in the process of hardening. The intermolt period follows, which is the period when the exoskeleton of the animal has reached its normal rigidity and molting is not taking place. The premolt period is when the animal develops a new integument under the exoskeleton in preparation to molt again.

H. sanguineus growth rates are much higher for juveniles than for adults, which is a pattern consistent across the Crustacea (Fukui 1988, Charmantier-Daures and Vernet 2004 and references therein). Fukui (1988) estimated growth rate for immature females as 0.82 to 0.85 mm/month and 0.70 to 0.74 mm/month for immature males in central Japan. Growth rates for mature crabs in the same experiment were 0.16 and 0.23 mm/month for males and females respectively (Fukui 1988).

H. sanguineus is an omnivorous, generalist predator. Though they have been observed to feed primarily on macroalgae in the field (based on gut contents studies), *H. sanguineus* prefer animal prey items in lab choice experiments (Tyrrell and Harris 2000, Bourdeau and O'Connor 2003, Brousseau and Baglivo 2005). *H. sanguineus* feeds on a wide variety of animal prey including bivalves, gastropods, barnacles, and polychaete worms (Tyrrell and Harris 2000, Brousseau et al. 2001, Lohrer and Whitlatch 2002a, Bourdeau and O'Connor 2003). They appear to have a preference for the blue mussel, *Mytilus edulis* over other molluscan prey, and show avoidance of *Littorina littorea* in laboratory feeding trials (Tyrrell and Harris 2000, Brousseau et al. 2001, Bourdeau and O'Connor 2003). Adult crabs forage most actively at night and on

incoming or high tides (Lohrer and Whitlatch 1997, Brousseau and Baglivo 2005).

H. sanguineus is native to the western Pacific, and was first found in the United States in 1988 in Cape May, NJ (McDermott 1988). This species is now distributed from North Carolina to mid-coast Maine (Kraemer et al. 2007, Stephenson et al. 2009). Juvenile and adult crab densities vary inversely with latitude, and dominance also varies with latitude (Delaney et al. 2008). According to Delaney et al. (2008), *H. sanguineus* is currently the dominant intertidal crab south of Boston Harbor, whereas other crabs such as the European green crab, *Carcinus maenas* still dominate in New Hampshire and Maine. *H. sanguineus* is competitively dominant over *C. maenas* for both food and shelter, which has led to the observed shifts in shore crab dominance on the east coast of the United States (Jensen et al. 2002, Lohrer and Whitlatch 2002b).

Little is known about the ecology of *H. sanguineus* in its native range. It inhabits areas from Hong Kong to Russia (approximately 20-50°N latitudinal range), and maximum adult densities in Japan are much lower than those found in southern New England (Fukui 1988, McDermott 1988). Adult populations in the native range of the species are limited by fish predation, competition with other intertidal crab species, and by parasites (Fukui 1988, Takahashi and Matsuura 1994, Pushchina and Panchenko 2002). In its native range, predators of *H. sanguineus* include the sculpins *Myoxocephalus stelleri* and *M. brandti* (Pushchina and Panchenko 2002). There are also at least six other intertidal

crab species co-occurring with *Hemigrapsus* in its native range (Fukui 1988), which may lead to heightened competition, predation or both.

H. sanguineus is parasitized by six known species in its native range, including microsporan, rhizocephalan and trematode parasites (Blakeslee et al. 2009 and references therein). None of these species of parasites have been found in the introduced range of the species, but one nematode parasite has been identified on crabs in CT, NY, DE and MD (Blakeslee et al. 2009). The Japanese rhizocephalan parasite was found to infect up to 70% of the population in some locations, and is known to affect reproduction and growth of *H. sanguineus* (Takahashi and Matsuura 1994).

Brachyuran Reproduction

Female reproduction in crabs entails a variety of energetic costs. The total of these energetic costs is termed reproductive effort (Brante et al. 2003). Reproductive output is a term often used as a proxy for reproductive effort because it is easier to measure. Reproductive output is generally a measure of egg mass (dry or wet) produced by a female per year as a percentage of her body weight. Fecundity is another measure of reproductive output, and may be measured at any of the basic stages in the female reproductive process. Potential fecundity is a measure of the number of oocytes in the ovaries, actual fecundity is the number of extruded eggs, and realized fecundity is the number of larvae produced by a female (Corey 1991, Stechey and Somers 1995, Flores and Paula 2002).

Copulation and Female Receptivity

Reproduction in the Brachyura (true crabs) varies widely with regard to pre- and post-copulatory behavior, timing of copulation, and reproductive seasonality. However, some aspects of reproduction can be generalized across the group. Brachyuran crabs mate with their abdomens extended (Hartnoll 1969). The first and second pairs of pleopods in the male are modified for copulation and are used to transfer sperm into the paired gonopores of the female (Hartnoll 1969). These gonopores are the genital openings of the crab, generally located on the 6th thoracic sternite and connected via the oviduct to the spermatheca and ovary (Hartnoll 1968). The female crab may store sperm in the spermatheca until ovulation (Hartnoll 1969). Females may use sperm from one copulation to fertilize multiple broods of eggs, and may retain sperm through molting, throughout an entire spawning season, or even between spawning seasons (Warner 1977b, Brockerhoff and McLay 2005a, Fischer and Thatje 2008).

One major division in mating patterns of brachyurans is time of copulation, which is determined by female receptivity. Females of some species are morphologically prevented from copulation by the presence of calcified gonopore opercula, which must be temporarily softened for successful transfer of sperm to occur (Hartnoll 1969, Warner 1977a, Zimmerman and Felder 1991). Softening of gonopore opercula, or receptivity, may occur following ecdysis when the entire crab is soft (Hartnoll 1969). Alternatively, females may become receptive during short periods during the intermolt when the crab's shell is otherwise hard

(Hartnoll 1969, Zimmerman and Felder 1991, Jennings et al. 2000, Brockerhoff and McLay 2005a). Conversely, females of some crab species are continuously receptive to mating as adults, such as *Uca vocans* and the grapsid crab *Pachygrapsus transversus* (Abele et al. 1986). In the family Grapsidae the majority of crab species studied to date mate during the intermolt stage (Hartnoll 1969, Brockerhoff and McLay 2005a). This includes four species of the genus *Hemigrapsus*: *H. crenulatus*, *H. sexdentatus*, *H. nudus*, and *H. oregonensis* (Brockerhoff and McLay 2005a). *H. crenulatus* and *H. sexdentatus* have short temporary windows of receptivity (Brockerhoff and McLay 2005b,c), but duration of receptivity has not been studied for other species of *Hemigrapsus* including *H. sanguineus*.

Oviposition and Embryogenesis

Following fertilization as described above, females oviposit by discharging the contents of their ovaries as eggs which are developed externally (Hartnoll 2006). Eggs are carried attached to the female's pleopods, located under the abdomen, and females are referred to as "gravid", "ovigerous" or "berried" while carrying their developing eggs. During brooding, female crabs employ certain behaviors to aerate the eggs, including periodic flapping of the abdomen (Brante et al. 2003, Hartnoll 2006). As eggs develop they change in color due to the maturation of the embryos. Color pattern seen through development varies among groups in the Brachyura, but in the family Grapsidae eggs generally progress from an initial orange or yellowish coloration though a series of

brownish shades to an ultimate brownish-gray color just before hatching (Arshad et al. 2006).

Egg clutch size varies between crab species and may even vary for an individual female throughout one season (Hines 1982, Brante et al. 2003, Bas et al. 2007). This is likely due to the tradeoff in survival chance between many small eggs and few large but higher quality eggs (Hartnoll 2006). Eggs also increase in size as they develop, and this increase is attributed primarily to the uptake of water by the eggs (Zimmerman and Felder 1991, Okamori and Cobo 2003, Figueiredo et al. 2008, Silva et al. 2009). When eggs are fully developed, they hatch and are released from the female as pre-zoeae or stage-one zoeae (Hartnoll 1965, Hartnoll and Paul 1982, Amsler and George 1984). In many crab species, release of larvae is highly synchronous and correlated with certain tidal and light conditions (e.g. nocturnal high tides) (Forward 1987).

Reproductive Strategies

There are a few major reproductive strategies seen in brachyuran crabs, which vary among families as well as geographic areas. Crabs in tropical regions generally produce more clutches per year than those living in temperate areas (Hines 1982). Many species, including temperate species in the Grapsidae and Portunidae, have a spring-summer reproductive season, and may produce multiple consecutive broods (Hines 1982 and references therein). Another reproductive strategy, employed by cancrid crabs in temperate regions involves overwinter egg development (Krouse 1972, Hines 1991). Crabs in this group oviposit in the fall and release their larvae in the spring during the seasonal

plankton bloom (Stone and O'Clair 2002, Park et al. 2007). This strategy is also employed by at least one portunid crab *Geryon fenneri* (Erdman and Blake 1988).

***Hemigrapsus sanguineus* Reproduction**

Female *H. sanguineus* may become reproductively mature at sizes as small as 12.1 mm in carapace width (McDermott 1998). This may occur within one year of metamorphosis to the juvenile stage, based on estimates using growth rate, size at maturity and presumed cessation of growth during winter months (Epifanio et al. 1998). Female crab sexual maturity can be determined in this species by the ratio of the abdomen width (AW) to carapace width (CW), because these measurements have been associated with physiological (gonadal) maturity (Knudsen 1960, Hartnoll 1974, Hartnoll and Paul 1982, Haefner 1990). McDermott (1998) estimated an AW/CW ratio of greater than or equal to 0.60 as indicative of maturity in *H. sanguineus*. McDermott also estimated based on a sample size of 300 *H. sanguineus* from New Jersey, that females are more likely to be mature at sizes greater than 17 mm (McDermott 1998). Number of larvae produced is dependent on female size (McDermott 1998) and ranges from approximately 3,700 to 56,000 eggs per brood (Fukui 1988, McDermott 1998).

Like other brachyurans, *H. sanguineus* females store sperm and are capable of producing at least two broods per season without successive matings (McDermott 1998). Evidence has been reported that female crabs collected in non reproductive times of the year are capable of producing eggs when brought

into warm temperatures; these broods are likely produced with stored sperm from the previous season (McDermott 1998).

Duration of egg brooding and survival of embryos and larvae are affected by temperature and salinity (Epifanio et al. 1998). McDermott (1998) found brooding durations of 22.3 ± 1.8 days (N = 8) at 19 to 20°C, but brooding duration has not been examined under other temperature conditions. Maximum egg diameter in *H. sanguineus* is approximately 0.378 ± 0.021 mm (McDermott 1998). Epifanio et al. (1998) reported egg color to change from bright orange to dark brown to brownish green as the eggs progress through development. Larval development has only been studied in the laboratory at temperatures between 15 and 25°C, but across these temperatures duration decreased with increasing temperature (Epifanio et al. 1998). Crabs in 20°C treatment had a larval duration of 37.5 ± 6.4 days (hatching to crab stage one), and larvae in 25°C had a total duration of 25.4 ± 3.1 days (Epifanio et al. 1998).

Female *H. sanguineus* release their larvae on nocturnal high tides (Saigusa and Kawagoye 1997, Park et al. 2005), and larvae are likely swept offshore by the ebbing tide to develop before returning to nearshore areas as megalopae. A study of larval responses to gravity and pressure supports this prediction (Park et al. 2004). Early stage larvae are negatively geotactic which orients them towards the surface, whereas later stage larvae are positively geotactic, or oriented towards the benthos (Park et al. 2004).

H. sanguineus metamorphosis from the final larval stage (megalopa or post-larva) to the first juvenile stage is stimulated by certain chemical cues.

Molting to the juvenile stage is stimulated in this species by exudates from conspecific crabs, as well as by rocks from natural rocky intertidal habitats (with and without biofilms) and by artificial mesh netting (O'Connor 2007, Steinberg et al. 2007, Steinberg et al. 2008, Anderson and Epifanio 2009). There is also an additive effect of biofilm and substrate texture on metamorphosis (Steinberg et al. 2008, Anderson and Epifanio 2009).

Spawning season in the native range has been reported to vary among three areas studied in Japan. In southern Honshu at Tanabe Bay (~33°N), *H. sanguineus* was found to have an eight month-long breeding season, with a duration of March to November (Fukui 1988). The breeding season was found to have two peaks – one in May to June and another in September to October (Fukui 1988). In the Asaki district of Japan (~35° N) gravid females were found from April to August (Kurata 1968). In Western Hokkaido, Japan (~43° N), gravid females were found June through August in a year-long survey from 1983-1984 (Takahashi et al. 1985).

Two in-depth studies have been published regarding spawning season duration for *H. sanguineus* on the Atlantic coast of the United States (Epifanio et al. 1998, McDermott 1998). In New Jersey, over an eight year period (1990-1997) gravid females were present from late April until late September with a peak in July (McDermott 1998). In a weekly survey from June to October in Delaware Bay, peak percentage of gravid females was found in June, with decreasing percentage through September (Epifanio et al. 1998). No gravid females were found in October in either of these studies. Temperatures during

spawning season ranged from 15 to 26°C in Delaware (Epifanio et al. 1998) and from 11 to 21°C in New Jersey (McDermott 1998). Ledesma and O'Connor (2001) provide additional though limited information about brooding season in Massachusetts. Sites were sampled from early June (Gooseberry Island, MA – south of Cape Cod) or early July (Sandwich, MA – north of Cape Cod) through November, with no sampling in October. Little could be concluded about season start, finish or duration, but percentages greater than 40% were found at Gooseberry Island from early July through late August (Ledesma and O'Connor 2001). One data point in 1997 indicated that ovigerous females were present in Sandwich, MA in late May (22% ovigerous) (Ledesma and O'Connor 2001). A subtidal survey in 2005 and 2006 found ovigerous females from April through September in Clinton Harbor, Connecticut (Gilman and Grace 2009). And lastly, Stephenson et al. (2009) reported the presence of ovigerous females from late May (2004) or early June (2005) in southern Maine. Ovigerous crabs were detected in this region at temperatures greater than 9°C, and percent ovigerous increased markedly when seasonal temperatures surpassed 11.5°C (Stephenson et al. 2009).

The current study was designed to add to the breadth of knowledge of *H. sanguineus* reproduction in its introduced range especially concerning effects of temperature and geographic location on reproductive activities. This species is a fairly recent invader and is still in the expansion phase of its introduction. Data presented in this study will help clarify the potential for spread of this species, as well as exploring how potential future increases in sea surface temperature may

affect the range limits for the species. *H. sanguineus* can survive in a wide range of temperature and salinity conditions; however, conditions required for successful reproduction are likely more narrow and may limit expansion.

CHAPTER I

EFFECTS OF TEMPERATURE ON BROOD PRODUCTION

Introduction

Temperature may be important to crab reproduction in a variety of ways. These include its effects on overall yearly reproductive window, initiation of reproductive season, viability of eggs and larvae, development speed of eggs and larvae, as well as on overall reproductive cost (Giese 1959). A combination of these factors and others, including female size (Hines 1982) likely determine the yearly reproductive output of an individual female crab.

Crab reproductive windows can vary from seasonal to continuous, depending on species and geographic area (Hines 1982). Tropical crab species often produce large numbers of broods per year, reported as high as 14-18 broods a year in *Metopograpsus messor*, a tropical grapsid species (Hines 1982, Sudha and Anilkumar 1996). In species with seasonal reproductive activities, induction of reproductive season is influenced by environmental factors. The most obvious of these include temperature and photoperiod (Giese 1959, Meusey and Payen 1988). Food availability for adult females or larvae may also be factors; however, these may be correlated with photoperiod and temperature (Sastry 1983). Events that need to be triggered before reproduction can begin include ovarian development (Flores and Paula 2002). Additionally, though

many crabs are capable of fertilization of eggs with stored sperm, cues may also be necessary for initiation of seasonal copulatory behavior (Zimmerman and Felder 1991, Brockerhoff and McLay 2005c). In crabs such as *H. sanguineus* that mate during the intermolt period when their exoskeleton is hard, genital opercula must be cued to soften to allow for copulation (Hartnoll 1969).

Egg Survival and Development

Egg rearing, or brooding, occurs on the ventral surface of a female crab's body. Egg survival can be affected by temperature or salinity as well as by biological agents such as egg parasites and predators or microbial infections (Silva et al. 2009 and references therein). Upper thermal limits have been reported for several species, including *Cancer setosus*, which has an upper thermal limit of 22°C for successful egg attachment to pleopods (Fischer and Thatje 2008). Egg size may affect egg survival in extreme temperatures. Smaller less yolky eggs are able to survive higher temperatures and produce viable larvae after higher temperatures than larger ones (Wear 1974).

Low temperatures may slow development of eggs significantly. While this may not lead directly to mortality of the eggs, higher temperatures have been found to be required for successful hatching of eggs and survival of early stage zoeae (Wear 1974). Overall, eggs have been found to tolerate a wider range of temperatures than are supportive of hatching and subsequent development (Wear 1974).

Within the thermal reproductive tolerances for a species, rate of embryogenesis generally increases with increasing temperature, but this is not

necessarily a linear relationship (Wear 1974, Hartnoll and Paul 1982, Amsler and George 1984, Fischer and Thatje 2008). For *Callinectes sapidus*, developmental times were five times longer at 16°C than at 26°C (Amsler and George 1984). This pattern has been observed for a wide variety of brachyurans (Hartnoll and Paul 1982, Amsler and George 1984). Other factors such as female nutrition and salinity may also play a role in the rate of embryogenesis (Sastry 1983).

Yearly Reproductive Output

In groups such as the Grapsidae which are capable of producing multiple broods of eggs per year, total number of broods may be a strong determinant in yearly reproductive output. The time that it takes for each brood to develop likely plays a role in number of broods per year, limiting the seasonal window for reproductive (and sometimes growth) activities.

Another aspect that may play a role in total number of broods produced per season is total energetic cost of each brood. This may be affected by temperature, because oxygen requirements for eggs increase with increasing temperature (Baeza and Fernández 2002, Brante et al. 2003). Female crabs employ specific behaviors to oxygenate their eggs when they are brooding. These behaviors generally include flapping of the abdomen. Frequency of this behavior has been shown to increase with increasing temperature (Brante et al. 2003), and overall oxygen consumption has been found to double or triple in brooding females (Baeza and Fernández 2002, Brante et al. 2003).

Time between successive broods is also a factor in total yearly reproductive output, and may be determined by sperm availability, timing of

ovarian maturation and overall metabolism. Ovarian maturation for subsequent broods may take place while a female is ovigerous, and this is sometimes termed gonad recovery (Flores and Paula 2002).

Potential Thermal Limits to *H. sanguineus* Survival and Reproduction

In the western Pacific, *H. sanguineus* are confined to areas with mean summer temperatures between 12.6 and 29°C. In southern Maine, at the northernmost edge of the US population of *H. sanguineus*, crabs are not yet found in areas with average summer water temperatures below 13°C (Stephenson et al. 2009). Reproduction may be limited for this species by summer temperatures in this region. Stephenson et al. (2009) found ovigerous females at water temperatures above 9°C. However, there was a steep increase in abundance of ovigerous females at temperatures above 11.5°C.

Objectives

H. sanguineus is capable of producing multiple broods of eggs per year. Previous studies on this species indicate that females are capable of producing up to five broods per season (Fukui 1988). An additional study in New Jersey suggested that females produce up to three broods per season and are capable of producing broods with sperm stored from previous copulations (McDermott 1998). The reproductive behaviors of at least two other members of this genus have also been studied. *H. nudus* was found to produce one brood per year and *H. oregonensis* two broods per year in southern California (Hines 1982). However, no study to date has directly quantified number of broods produced by

H. sanguineus in a natural habitat or looked at effects of temperature on brooding.

The objectives of this study were three-fold. First, a lab experiment was conducted to determine the effects of temperature on reproductive activities of *H. sanguineus*. Variables measured included timing of first brood release, last brood release, and first molt of the season. Additionally, brood durations were calculated when possible, as well as total number of broods per season, and total number of molts per season. The second part of this study was conducted to determine the timing and total number of broods produced by female *H. sanguineus* in a natural rocky intertidal habitat in New Hampshire. Lastly, a second lab experiment was conducted to determine if brooding could be induced by elevated temperatures at off-season times of year and if broods could be produced without early season copulation.

Materials and Methods

Lab Experiment – Effects of Temperature on Seasonal Brooding Behavior

A laboratory experiment was conducted with crabs from sites in northern and southern New England to determine the effects of temperature on *H. sanguineus* brood production, brood development time and total number of broods produced per season. Observations were made on crab molting behavior in each temperature treatment, as were observations on overall copulatory behavior for the species. The experiment ran from early June through mid October 2009, though starting dates varied slightly by treatment (Table 1.1). Eight 15 gallon aquaria were used as experimental habitats and were each assigned a temperature and crab origin. Crabs from each of two origins (Jamestown, Rhode Island and Rye, New Hampshire) were exposed to one of four temperature treatments: 10, 16, 20 or 25°C. The 16°C treatment was not constant for the entire duration, due to equipment issues early in the experiment. This treatment ranged from 11.1 to 20.9°C with an average temperature of 16.6°C. Ten female and ten male crabs were placed in each aquarium at the beginning of the experiment. Aquaria were filled with approximately five gallons of seawater and aerated. Rocks were added to provide shelter. Salinity and temperature were monitored daily and salinity was maintained between 28 and 32 psu by the addition of deionized water or artificial sea salts. Tanks were kept under a 14:10 hour light:dark cycle to approximate summer light conditions.

Females were individually marked with small round tags attached to the center of their carapace with cyanoacrylate glue. This allowed for the seasonal activities of each female to be tracked. Females were monitored daily for the presence or absence of eggs and egg color as an indicator of developmental state. Females and males were also monitored daily for molt events. If the shed carapace as well as the live crab were present on the date following molting, both the old carapace and new carapace widths were measured. Measurement technique varied slightly throughout the season, with some measurements taken with digital calipers (0.01 mm), some with dial calipers (0.1 mm) and some with a millimeter ruler. These measurements were used to calculate average molt increment.

Egg presence, absence and color data were used to calculate the total number of broods produced by each crab and brood duration when possible. Average timing of reproductive events were calculated using Julian day. These included first brood release of the season, last brood release of the season and first molt event of the season. Response variables were averaged across individual crabs to provide a value representative of each tank. Tank average values were used as replicates in the statistical analysis. Due to potential tank effects, individual crabs could not be considered as true replicates in this experimental design. All response variables were compared among temperature treatments and between crab origin sites using two-way analysis of variance and post-hoc Tukey's HSD tests when appropriate. In some cases, a linear relationship was predicted between response variable and temperature.

Regression analysis was used to test for correlation. In some cases the 10°C treatments were excluded from analysis. Eggs in the broods of individuals in this treatment did not progress to hatching, and most crabs in this treatment did not molt. Therefore, most response variables could not be calculated for this treatment.

Copulatory Behavior

Limited data on copulatory behavior of *H. sanguineus* were collected while crabs were held in the laboratory for this brooding experiment. When possible, position and size of male and female crabs were recorded and duration of copulation event was noted.

Field Experiment

To determine the number and timing of broods in a natural setting, field mesocosms were installed in the rocky intertidal zone in New Castle, NH. Ten field enclosures (27 cm x 27 cm x 15.5 cm) were constructed from 12 mm^2 wire mesh and filled with rocks from the intertidal zone. Areas of the appropriate size were cleared so that cages could be set down into the habitat at a natural level. Rocks covered with a variety of naturally occurring flora and fauna were placed in each cage to provide habitat and secure cages in place. One female and two male crabs were placed into each enclosure at the beginning of the experiment, and males were replaced periodically if escapes occurred. This experiment was deployed from April 2nd to October 13th, 2009. Female crabs ranged in size from 17-23 mm carapace width and males ranged from 15–23 mm carapace width. Crabs were collected initially in Rye, NH and none of the female crabs were

carrying eggs when the experiment began. Females were monitored for presence or absence of broods and color of eggs once per week (early season and late season) or twice weekly during the middle of the season. During monitoring, food sources within the cage were supplemented with cracked snail tissue (*Littorina littorea*) as well as the occasional addition of green algae (*Ulva lactuca*). A HOBO temperature logger was deployed in one of the cages (starting 4/15/09) to collect data on temperature at 30 minute intervals. Egg presence, absence and color data were used to calculate number of broods produced by each crab as well as brooding duration and time between broods if applicable.

Lab Experiment – Induction of Brood Production at Elevated Temperatures

Two lab experiments were conducted, one in spring and one in the fall of 2009, to determine if female crabs were capable of producing broods at times when they would not be found brooding in the field. This was done to test their potential for expansion of the reproductive season toward the early or late season. Additionally, some information was gathered concerning the females' ability to oviposit without early season copulation. This ability to utilize stored sperm was initially suggested by McDermott (1998).

Spring Experiment

Male and female *H. sanguineus* were collected in the rocky intertidal zone in Rye, NH in early March 2009. At this time, wild crabs at this site showed no signs of reproductive activity in the field (Fig. 2.1). Males and females were separated and maintained in 10°C seawater under a 24 hour light regime for three weeks. Crabs were then moved to a 20-22°C (room temperature) room

and assigned to one of two treatments. Half of the female crabs were exposed to males and half were held in female only tanks. Crabs were maintained in warm temperatures for four weeks, and checked daily for oviposition or molting. Water was aerated and changed weekly, and crabs were fed blue mussel tissue (*Mytilus edulis*) twice a week. Females that produced eggs were removed from the group tank and held separately in boxes (15 cm x 16 cm) of seawater. Ovigerous females were checked daily for egg condition and to determine when spawning of larvae occurred. Water in ovigerous female containers was changed every two days.

Fall Experiment

Female *H. sanguineus* were collected in the rocky intertidal zone in Rye, NH on October 27, 2009. At this time, the reproductive season for *H. sanguineus* was completed for the year and no ovigerous females were detectable at this site (Fig 2.2). Female crabs were brought to the lab and 15 crabs were placed into each of five treatments: 10, 14, 16, 20 or 25°C. Tanks were filled with eawater and placed into one of three constant temperature rooms at 20°C, 10°C or 16°C and brought to temperature with aquarium heaters if necessary. Tanks contained rocks for shelter and water was aerated and changed once a week. Two to three times per week crabs were monitored for presence of eggs. Crabs were also fed *Mytilus edulis* tissue at this time, and temperature and salinity were recorded.

Results

Lab Experiment

Brooding

Dates of First Brood Release. Dates of first brood release differed significantly among temperature treatments (two-way ANOVA, $p = 0.03$) (Table 1.2). The 16°C treatment differed significantly from the other two temperature treatments according to post-hoc Tukey's HSD test (Table 1.2). Date of first brood release decreased with increasing temperature indicating a shift towards the early part of the season for crabs at higher temperatures (Fig. 1.1).

Dates of first brood release also differed significantly between crab origin sites (two-way ANOVA, $p = 0.04$) (Table 1.3). Average date of first brood release was earlier in the year for Rhode Island crabs versus New Hampshire crabs (Figure 1.2).

Overall, individual New Hampshire crabs in 16, 20 and 25°C treatments produced their first brood of eggs between June 17th and July 19th (Table 1.4). Individual Rhode Island crabs released their first broods of eggs between June 10th and July 12th (Table 1.4).

Number of Broods per Season. New Hampshire and Rhode Island crabs produced between zero and three broods per season and there was a pattern of increase in number of broods per season with increasing temperature. When analyzed with regression analysis, there was a significant positive correlation between temperature and average number of broods per season for New Hampshire crabs ($p = 0.04$) (Fig. 1.3). There was also a high correlation

coefficient between these variables (r^2 = 0.93) (Fig. 1.3). No significant correlation was found between temperature and number of broods per season for Rhode Island origin crabs using regression analysis (p = 0.08) (Fig. 1.4).

There was no significant effect of temperature treatment on average number of broods per season (two-way ANOVA, p = 0.06) (Fig. 1.5). There was also no significant effect of crab origin site on average number of broods per season (two-way ANOVA, p = 0.84) (Fig. 1.6).

Brood Duration. Average brood duration could not be analyzed with two-way analysis of variance, because there were too few Rhode Island origin crabs with known first brood durations. A pattern of decrease in average brood duration with increasing temperature was observed for New Hampshire crabs (Fig. 1.7). However, there was no significant correlation between temperature and average first brood duration using regression analysis (p = 0.16) (Table 1.2). Average first brood duration was approximately 33 days in the 16°C treatment, 19 days in the 20°C treatment and 13 days in the 25°C treatment (Fig. 1.7).

Some crabs in the 16, 20 and 25°C treatments produced a second brood of eggs. Average second brood duration appeared to decrease with increasing temperature (Fig. 1.8), but there was no significant effect of temperature on average brood duration (p = 0.49). There was also no significant effect of crab origin site on average brood duration (p = 0.82) (Fig. 1.9).

Dates of Last Brood Release. Last brood release of the season appeared to occur earlier in the year for crabs in higher temperature treatments (Fig. 1.10), but there was no significant effect of temperature on last brood

release of the season. There was also no significant effect of crab origin site on average day of last brood release of the season ($p = 0.32$), but the pattern observed suggested earlier last release of the year for Rhode Island crabs (Fig. 1.11).

Brooding season for individual New Hampshire crabs in the 16°C treatment extended at maximum to August 3rd, whereas the latest dates New Hampshire crabs released their final broods of the season in the 20 and 25°C treatments were July 22nd and 23rd respectively (Table 1.5). Brooding season for individual Rhode Island crabs in the 16°C treatment extended at maximum to August 5th, whereas the latest dates that crabs released their final broods of the season in the 20 and 25°C treatments were July 6th and 7th respectively (Table 1.5).

Molting

Number of Molts per Season. There was a significant effect of temperature treatment on number of molts per year ($p = 0.006$) (Fig. 1.12). The pattern observed indicated increase in average number of molts per season for the 10, 16, and 20°C temperature treatments and a slight decrease at the highest temperature (25°C) (Fig. 1.12). The 10°C treatment was found to differ significantly from all other treatments in average number of molts per season, according to post-hoc Tukey's HSD test (16°C: $p = 0.023$, 20°C: $p = 0.006$, 25°C: $p = 0.009$). There was no significant effect of crab origin site on average number of molts per season ($p = 0.11$), but the pattern observed indicated higher number

of molts per season for Rhode Island crabs versus New Hampshire crabs (Fig. 1.13).

Overall, New Hampshire and Rhode Island origin individuals molted from zero to two times per season under the temperatures tested.

First Molt of Season. There was a significant effect of temperature treatment on average date of first molt of the season ($p = 0.05$) (Fig. 1.14). The 10°C treatments were excluded from this analysis, because no crabs in the New Hampshire tanks molted during the course of this experiment and only one individual in the Rhode Island tanks molted. The 16°C treatment was found to differ significantly from the 20°C treatment ($p = 0.04$) but the 25°C treatment did not differ significantly from either of the other two temperature treatments (16°C: $p = 0.124$, 25°C: $p = 0.185$) (Fig. 1.14). There was no significant effect of crab origin on average date of first molt of the season (Fig. 1.15)

Average, minimum and maximum days of first molt event were calculated for individuals of each crab origin in each temperature treatment. New Hampshire female crabs in the 16, 20 and 25°C treatments molted for the first time between July 12th and August 31st (Table 1.6). For Rhode Island individual crabs all first molts of the season occurred between July 5th and Sept 8th across the temperature treatments (Table 1.6).

Molt Increment. There was no significant difference in average molt increment among temperature treatments ($p = 0.35$) or between crab origin sites ($p = 0.52$) for female crabs (Table 1.7). There was also no significant difference in average molt increment among temperature treatments ($p = 0.35$) or between

crab origin sites ($p = 0.38$) when male and female crabs were combined (Table 1.7).

When all measurements were included, molt increment varied from 1-4 mm for individual New Hampshire crabs and from 1.5-6 mm for individual Rhode Island crabs (Tables 1.7 and 1.8). Overall average molt increment for New Hampshire crabs was approximately 2.5 mm (Table 1.7), and overall average molt increment for Rhode Island crabs was approximately 2.7 mm (Table 1.8).

Growth-Reproduction Partitioning

Female molting and brooding were seasonally distinct. Brooding occurred in the early to mid summer. New Hampshire crab brooding extended to early August in the 16°C treatment or late July in 20 and 25°C treatments (Table1.9). Rhode Island brooding season extended until early August in the 16°C treatment and until early July for 20 and 25°C treatments (Table 1.9). Growth via ecdysis occurred in the mid to late summer. When all New Hampshire crabs were included, first molt of the season was observed in early August in the 16°C treatment and in mid July in the 20 and 25°C treatments (Table 1.9). When all Rhode Island crabs were included first molt of the season was observed in the 16°C treatment in early August and in early July in the 20 and 25°C treatments (Table 1.9). Though timing of brooding and molting overlap slightly when looking at the population of crabs in this experiment, in general individual crabs did not produce a subsequent brood once they had molted. However, there was one anomaly from this pattern observed amongst the Rhode Island female crabs in the 25°C treatment. One crab produced a brood on October 15th (the date of

experiment termination) after having brooded one clutch of eggs early in the season and molting twice previously.

Average time between last brood release and first molt of the season did not differ significantly among temperature treatments ($p = 0.56$) or between crab origin sites ($p = 0.12$) when analyzed with two-way analysis of variance (Tables 1.2 & 1.3). However, time between last brood release of season and first molt event of the season showed a decreasing pattern with increasing temperature among the 16, 20 and 25°C treatments (Table 1.9). Overall, average time between last brood release and first molt event ranged from approximately 19-32 days for individual New Hampshire crabs across temperature treatments and from approximately 33-42 days across temperature treatments for individual Rhode Island crabs (Table 1.9).

Mortality

Substantial mortality occurred across the duration of this experiment. Higher temperature treatments experienced the most mortality, as high as 60% in the 25°C treatment (Fig. 1.16). Only the lowest temperature treatment experienced 90–100% survival (Fig 1.16). Mortality was due primarily to conspecific aggression at the time of ecdysis. A few mortalities occurred due to crab escape from tanks or unknown causes, wherein the crab was present but dead.

10°C Treatment

One significant observation made during the course of this experiment was the difference in behavior and brood development between crabs in 10°C

treatments and all other temperature treatments. Lower feeding rates were observed but not quantified. When approximately equal amounts of food were placed into experimental tanks at the different temperatures, crabs in 10°C tanks failed to finish the provided food even though crabs in other tanks did.

Crabs were capable of extruding eggs at this temperature, with 90% of New Hampshire crabs ovipositing in 10°C water. Ninety percent of Rhode Island crabs used in the experiment were ovigerous when initially placed into 10°C water, so it is unknown what percentage of these crabs would have extruded eggs under these conditions. Though oviposition occurred in 10°C water, brood development was slow and likely unsuccessful. Two observations point toward the failure of the majority of broods in this treatment. Eggs on crabs in 10°C tanks did not progress in color in the way that generally indicates egg development (Epifanio et al. 1998). If ovigerous at the start of the experiment, as many of the Rhode Island crabs were, eggs were maintained at the color stage they appeared when collected. Additionally, most broods appeared to slowly decrease in size over time, whereas healthy broods generally increase in size through development and are expelled in their entirety when embryogenesis is complete (Zimmerman and Felder 1991, Okamori and Cobo 2003, Figueiredo et al. 2008, Silva et al. 2009).

Copulation Observations

Several copulation events were observed in experimental treatments in June. All observed copulations were in Rhode Island crab tanks at 20 or 25°C. Two out of three mating events occurred with a female currently carrying a brood

of eggs. Matings were observed in both the morning and late evening. One copulation was observed for the entire duration; this event lasted for approximately 16 minutes. Observed matings all involved the same positioning and general behavior of male and female crabs. The male crab was positioned on its dorsal side below the female. Both male and female abdomens were extended, and the male abdomen was positioned under the female abdomen. Following copulation, the male moved immediately away from the female; no post-copulatory guarding was observed. A summary of available data on crab size, copulation date and event for all observed events is listed in Table 1.10.

Field Experiment

Because field enclosures were not checked daily, data on timing of oviposition, brood release, brood durations, and time between broods could not be pinpointed to specific dates or number of days. Date ranges during which these events may have occurred are provided in Table 1.11. In this summary of results, these variables will be expressed as the median of each of these ranges. All crabs in this experiment produced at least one brood of eggs, and one crab (out of ten) produced two broods of eggs. Median day of first oviposition ranged from May 26th to July 31st, with an average median date of first ovipositon of June 16th (Table 1.11). Median first brood release of the season ranged from July 19th to August 28th with an average median date of August 3rd (Table 1.11). Median brood duration ranged from 28-54 days, with an average median value of 48 days in duration (Table 1.11). Brood duration windows fit well into the period of

time in which high percentages of ovigerous females were found in field surveys in 2009 (Figs. 1.17 & 2.5).

One crab produced two broods of eggs during the 2009 season. The second brood was oviposited between July 24th and August 6th (Median date of July 31st). This brood was released between August 23rd and September 1st (median date of August 28th). Median brood duration was therefore 28 days for this brood.

Lab experiment – Induction of Brood Production at Elevated Temperatures

In the spring experiment, 50% of female crabs in both the male and female tank and female only tank produced broods within four weeks of being exposed to warm temperatures (20-22°C). Six crabs oviposited within the first week of exposure to warm (20-22°C) temperatures. Two of these crabs were from the female only tank and four were from the male and female tank. Four crabs oviposited during the second week of the experiment. Three of the crabs were from the female only tank and one was from the male and female tank. Brood duration was determined for two of the crabs in this experiment; these crabs brooded for 16 or 17 days.

In addition to crabs used in this experiment, another group of female crabs were observed to produce early season broods at elevated temperatures. A group of crabs was brought into the laboratory on Feb 20th, 2009 and kept in room temperature tanks for an undergraduate lab course. Ovigerous females were observed in this batch of crabs on March 13th, 2009 and at least one individual successfully spawned on March 28th.

Four female crabs molted during the spring experiment. All were females that did not produce broods, and all molted in the fourth and final week of the experiment. Molt increment was measured for two of these crabs that were not damaged post-molt. These two crabs were initially 16.16 and 15.6 mm and gained 3.21 and 3.54 mm in carapace width respectively.

No crabs collected in the fall produced a brood of eggs within the four week period in any of the temperature treatments.

Table 1.1: Laboratory brooding experiment start dates (2009) and indication of number of crabs in each treatment with eggs at the start of the experiment.

	Treatment	Starting Date	# With eggs at start
New Hampshire	10 °C	6/4	2
	16 °C	6/10	4
	20 °C	6/4	6
	25 °C	6/4	5
Rhode Island	10 °C	6/8	9
	16 °C	6/10	10
	20 °C	6/8	6
	25 °C	6/4	8

Table 1.2: Summary of the effects of increase in temperature on brooding and molting behavior of crabs in 2009 laboratory experiment. Effects of temperature were tested for using two-way analysis of variance and post-hoc Tukey's HSD test where applicable. Regression analysis was conducted to test for correlation between temperature and average number of broods per season and average brood duration.

Response Variable	Effect of ↑ in Temp	Significant Difference	P-value
First brood release	Earlier	ANOVA: 16° ≠ 20 & 25°	p = 0.03
Last brood release	Earlier	ANOVA: ns	p = 0.32
# of Broods	Increase	ANOVA: ns NH Regression: significant RI Regression: ns	p = 0.06 NH: p = 0.04 RI: p = 0.079
First brood duration	Decrease	NH Regression: ns	p = 0.16
Second brood duration	Decrease	ANOVA: ns	p = 0.49
# of molts	Increase and decrease	ANOVA: 10°C ≠ 16, 20 & 25°	p = 0.006
First molt of season	Earlier	ANOVA: 16≠ 20	p = 0.05
Last brood to 1st molt	No pattern	ANOVA: ns	P = 0.56

Table 1.3: Effects of crab origin on brooding and molting behavior of crabs in 2009 laboratory experiment. Significant effects of crab origin were tested for using two-way analysis of variance.

Response Variable	Effect of ↓ in Latitude	Significant Difference	P-value
First brood release	Earlier	significant	p = 0.04
Last brood release	Earlier	ns	p = 0.32
# of Broods	No pattern	ns	p = 0.84
Second brood duration	Increase	ns	p = 0.82
# of molts	Increase	ns	p = 0.11
First molt of season	Decrease	ns	p = 0.36
Last brood to 1st molt	Increase	ns	p = 0.12

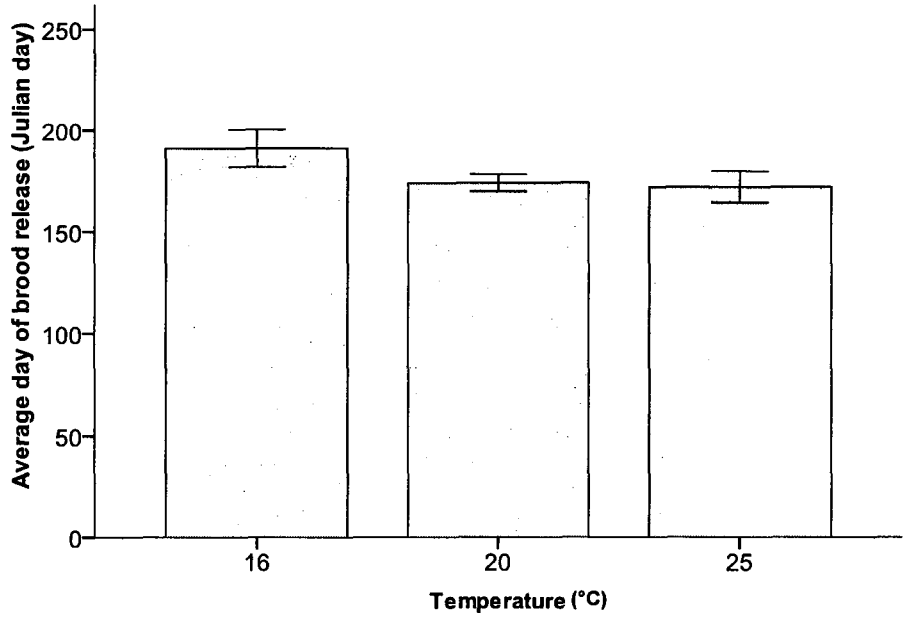

Figure 1.1: Average Julian day of first brood release of the season for temperature treatments in 2009 laboratory experiment. Averages were calculated across crab origin sites (NH and RI, N = 2). There was a significant difference in average day of first brood release among temperatures ($p = 0.03$). Error bars indicate ± one standard deviation from the mean.

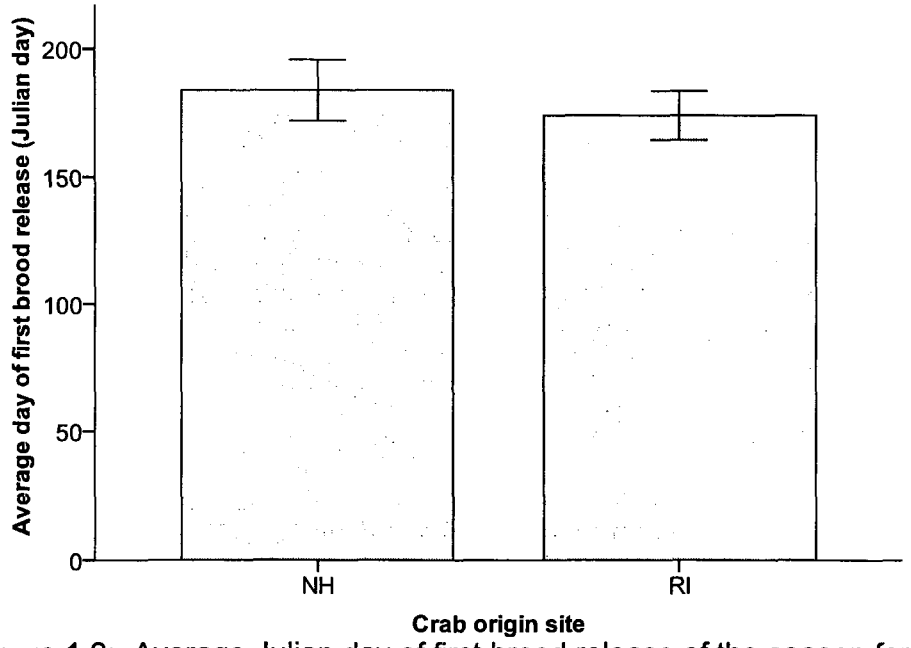

Figure 1.2: Average Julian day of first brood release of the season for New Hampshire and Rhode Island origin crabs. Averages were calculated across 16, 20 and 25°C temperature treatments (N = 3). There was a significant difference in average day of first brood release between crab origin sites ($p = 0.04$). Error bars indicate ± one standard deviation from the mean.

Table 1.4: Average, minimum and maximum days of first brood release for female crabs from New Hampshire and Rhode Island in four temperature treatments. Averages are given ± one standard deviation from the mean.

		New Hampshire		Rhode Island	
		Julian Day	**Date**	**Julian Day**	**Date**
16 °C	Average	197.6±3.5	7/17	184.5±8.0	7/4
	Min	191	7/10	166	6/15
	Max	200	7/19	193	7/12
20 °C	Average	176.8±7.1	6/26	170.8±4.0	6/20
	Min	168	6/17	163	6/12
	Max	195	7/14	174	6/23
25 °C	Average	177.1±4.7	6/26	166.3±5.6	
	Min	170	6/19	161	6/10
	Max	182	7/1	178	6/27

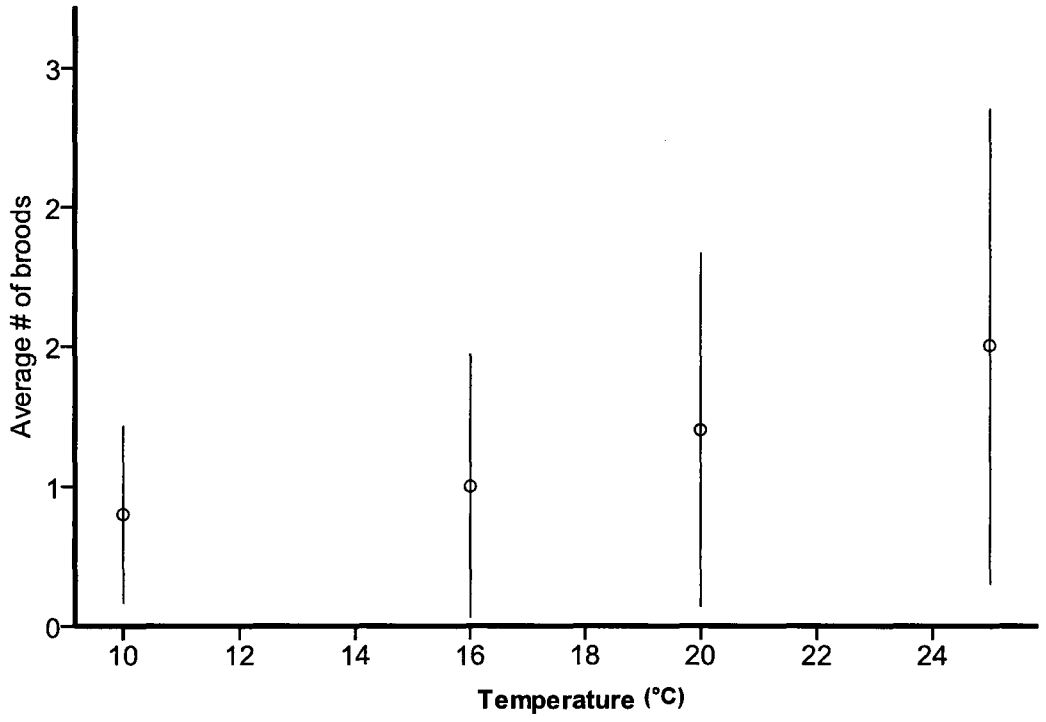

Figure 1.3: Average number of broods per season for New Hampshire crabs in four temperature treatments. Regression analysis indicates significant correlation between the variables ($p = 0.037$), and $r^2 = 0.928$.

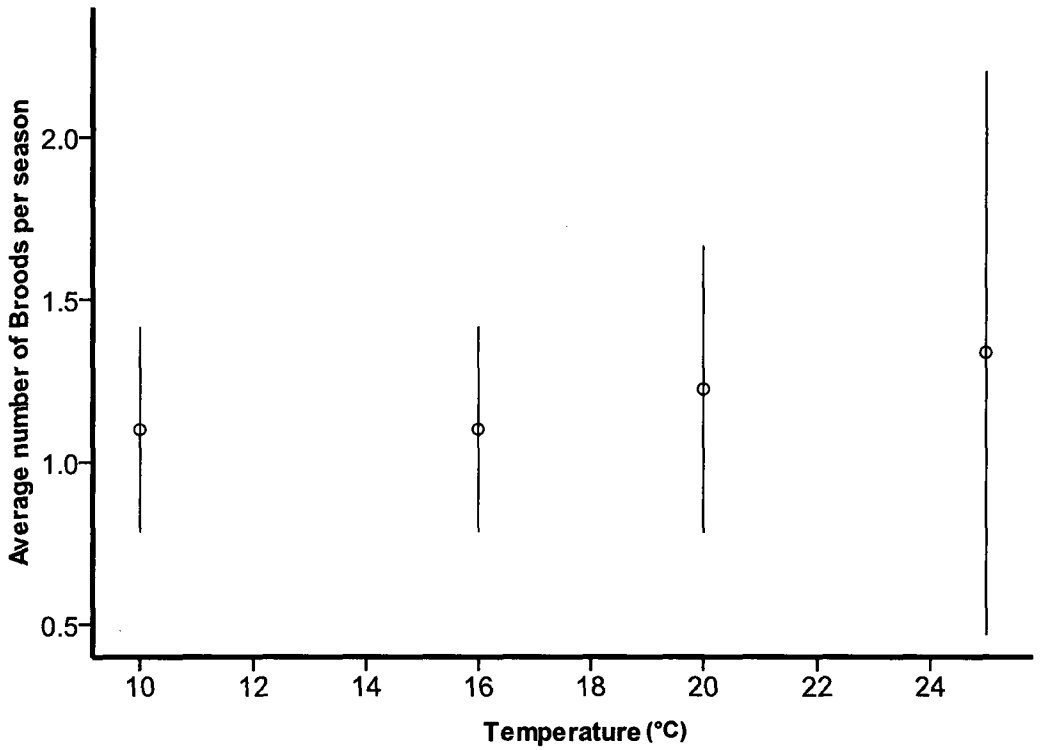

Figure 1.4: Correlation of average number of broods per season and temperature for Rhode Island crabs. Correlation between the two variables is non-significant when analyzed with regression analysis ($p = 0.079$).

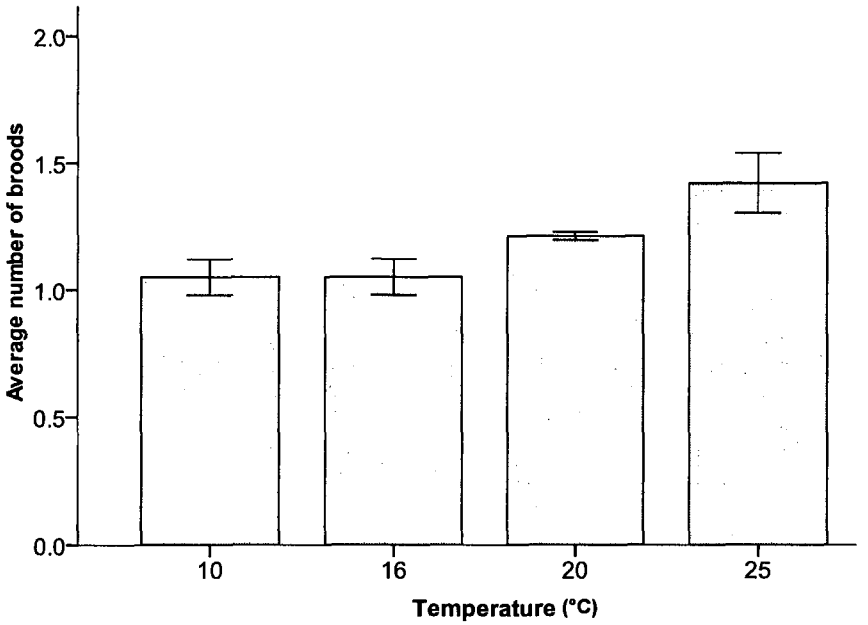

Figure 1.5: Average number of broods per season in three temperature treatments. Averages were calculated across New Hampshire and Rhode Island crabs for each temperature (N = 2). Error bars indicate ± one standard deviation from the mean. There was no significant effect of temperature treatment on the response variable (two-way ANOVA, p = 0.06).

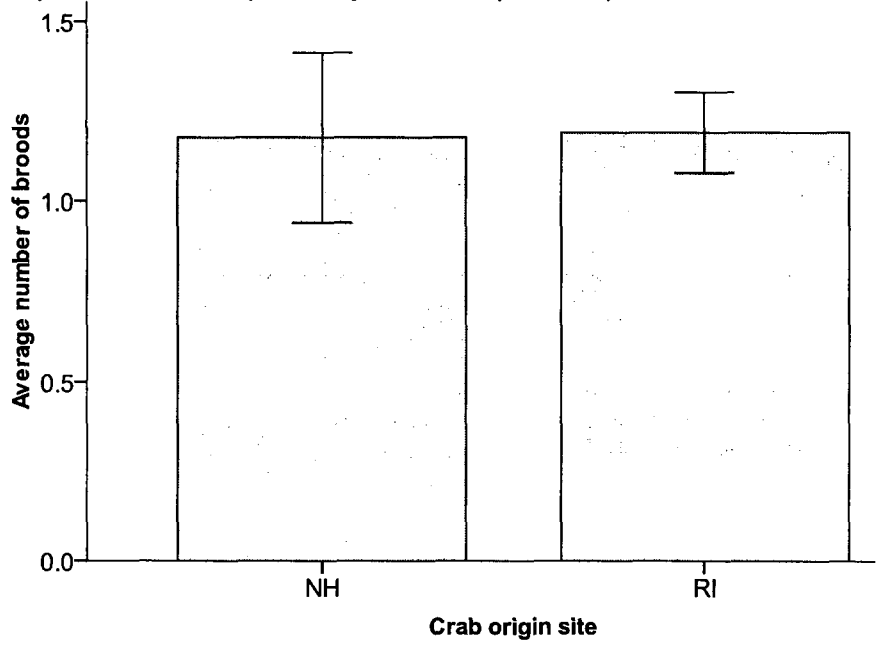

Figure 1.6: Average number of broods per season in New Hampshire and Rhode Island. Averages were calculated across 10, 16, 20 and 25°C temperature treatments (N = 4). Error bars indicate ± one standard deviation from the mean. There was no significant effect of temperature treatment on the response variable (two-way ANOVA, p = 0.84).

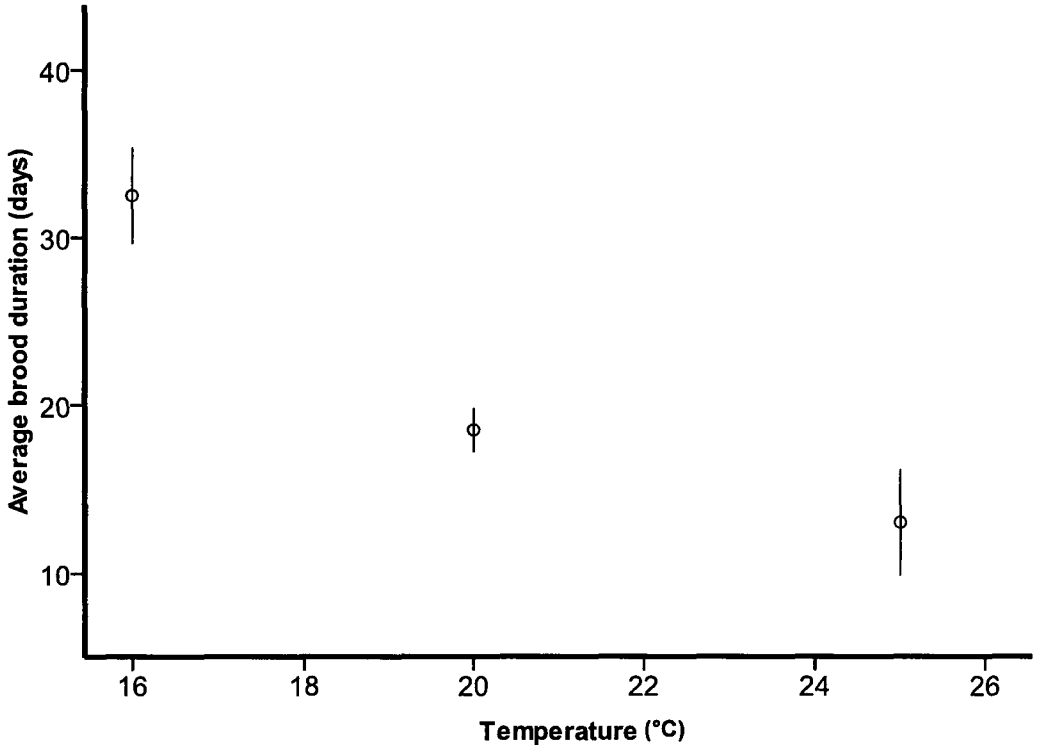

Figure 1.7: Average duration of first brood of the season for New Hampshire crabs in three temperature treatments. There was no significant correlation between variables with regression analysis ($p = 0.157$).

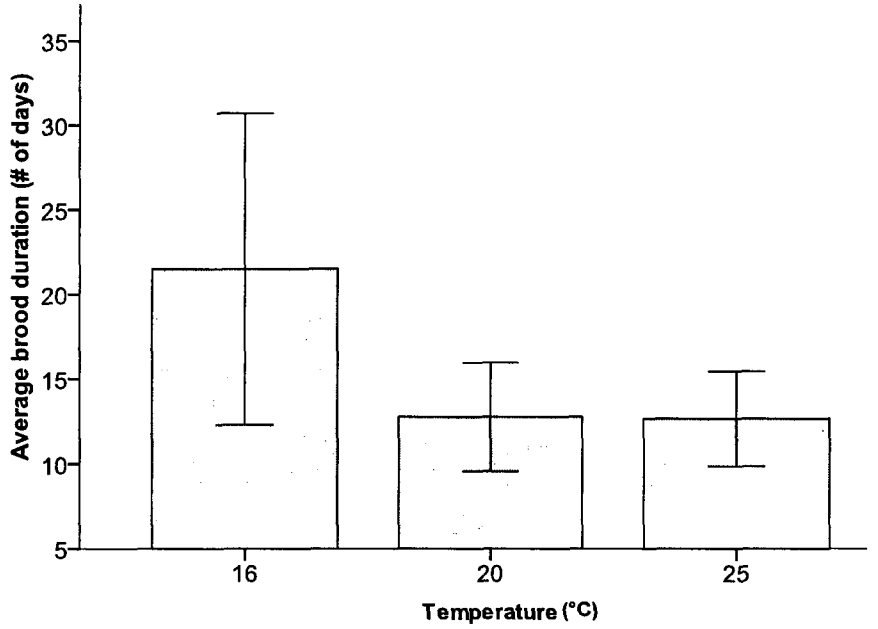

Figure 1.8: Average duration of second brood of the season in three temperature treatments. Averages were calculated across crab origin sites for each temperature (N = 2). Error bars indicate ± one standard deviation from the mean. There was no significant effect of temperature treatment on the response variable (two-way ANOVA, p = 0.49).

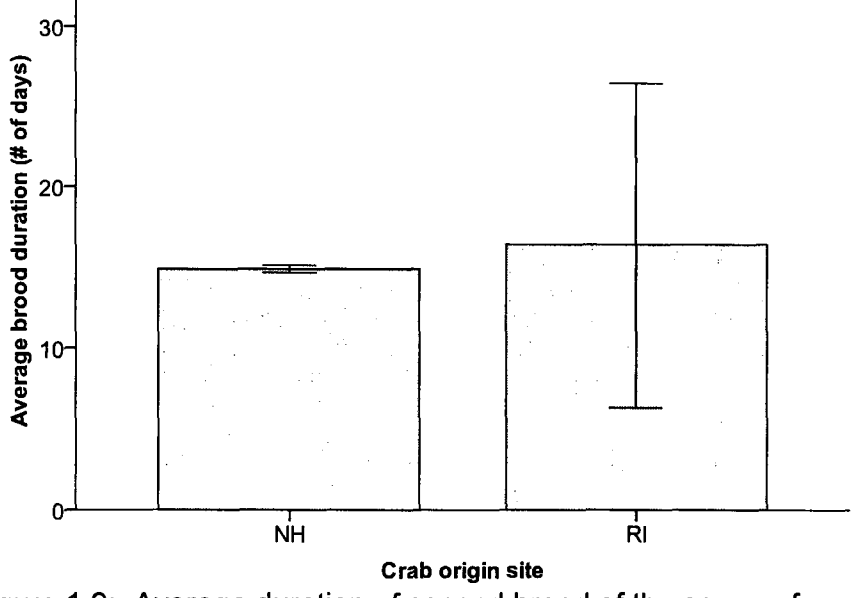

Figure 1.9: Average duration of second brood of the season for crabs from New Hampshire and Rhode Island. Averages were calculated across 16, 20 and 25°C temperature treatments (N = 3). Error bars indicate ± one standard deviation from the mean. There was no significant effect of crab origin on the response variable (two-way ANOVA, p = 0.82).

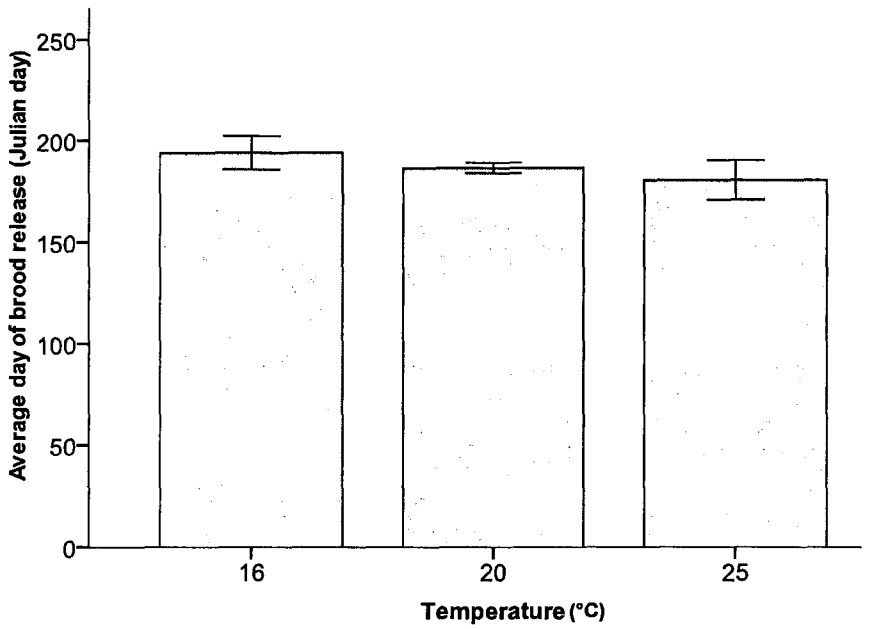

Figure 1.10: Average day of last brood release of the season for temperature treatments in 2009 laboratory experiment. Averages were calculated across crab origins (N = 2). Error bars indicate ± one standard deviation from the mean. There was no significant effect of temperature treatment on the response variable (two-way ANOVA, p = 0.32).

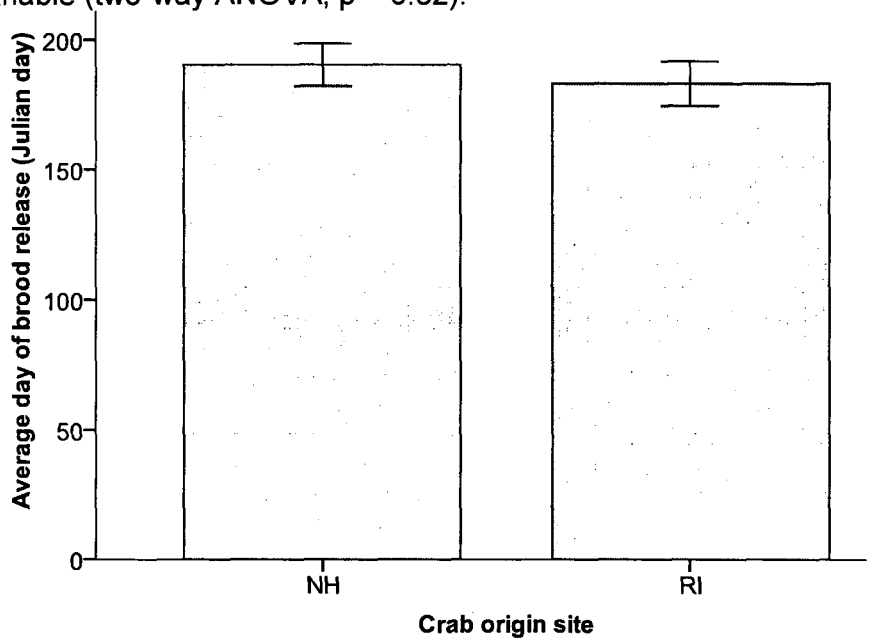

Figure 1.11: Average day of last brood release of the season for New Hampshire and Rhode Island crabs in 2009 laboratory experiment. Averages were calculated across 16, 20 and 25°C temperature treatments (N = 3). Error bars indicate ± one standard deviation from the mean. There was no significant effect of crab origin on the response variable (two-way ANOVA, p = 0.32).

Table 1.5: Average (± one standard deviation from the mean), minimum and maximum days of last brood release for New Hampshire and Rhode Island crabs in three temperature treatments. Data is shown for analysis including all crabs (including those that died during the course of the experiment), as well as average (± one standard deviation from the mean) for the subset of crabs that survived three months of the experiment.

		New Hampshire		Rhode Island	
		Julian Day	**Date**	**Julian Day**	**Date**
16 °C	Average (all crabs)	199.7 ±7.5	7/19	188.1 ±13.5	7/7
	Min (all crabs)	191	7/10	166	6/15
	Max (all crabs)	215	8/3	217	8/5
	Average (3 month survivors)	200.3±8.0	7/19	191±10.6	7/10
20 °C	Average (all crabs)	184.5±12.4	7/4	175.1±5.2	6/24
	Min (all crabs)	171	6/20	170	6/19
	Max (all crabs)	203	7/22	187	7/6
	Average (3 month survivors)	194.2±10.3	7/13	174.8±6.1	6/24
25 °C	Average (all crabs)	187±11.4	7/6	173.3±9.5	6/22
	Min (all crabs)	170	6/19	162	6/11
	Max (all crabs)	204	7/23	188	7/7
	Average (3 month survivors)	190.4±14.1	7/9	175.7±7.8	6/25

Table 1.6: Range in days of first molting for female crabs from New Hampshire and Rhode Island in three temperature treatments. Julian day and date are given for average, minimum and maximum days of first molting. First molt date was assigned as the day after the termination of the experiment for any crab that produced successful broods but did not molt during the course of the experiment.

		New Hampshire		Rhode Island	
		Julian Day	**Date**	**Julian Day**	**Date**
16 °C	Average	223	8/11	224.67	8/13
	Min	214	8/2	214	8/2
	Max	243	8/31	251	9/8
20 °C	Average	216	8/4	208.63	7/28
	Min	193	7/12	186	7/5
	Max	229	8/17	247	9/4
25 °C	Average	205	7/24	201.43	7/20
	Min	195	7/14	186	7/5
	Max	223	8/11	224	8/12

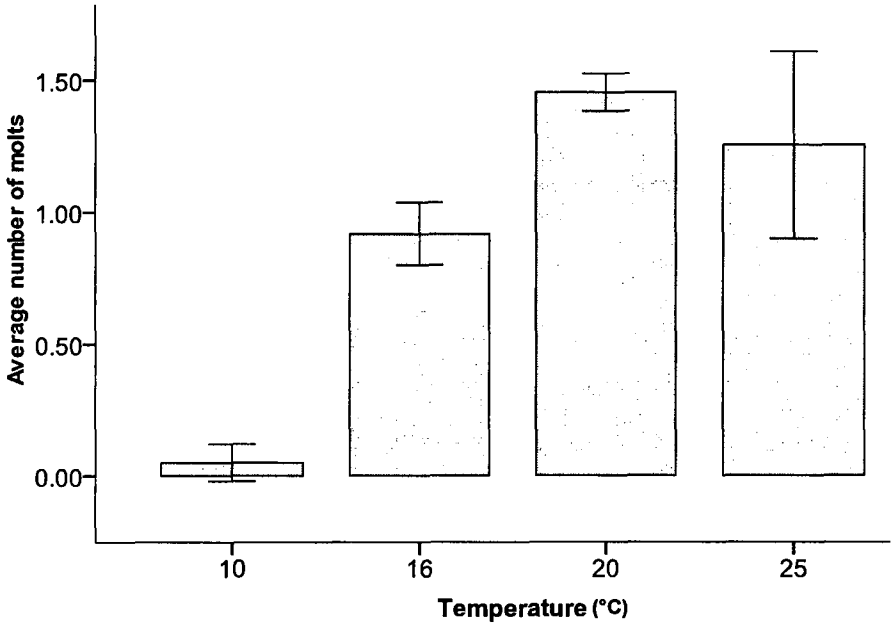

Figure 1.12: Average number of molts per season for temperature treatments in 2009 laboratory experiment. . Averages were calculated across New Hampshire and Rhode Island crabs (N = 2). Error bars indicate ± one standard deviation from the mean. There was a significant effect of temperature on average number of molts per season(two-way ANOVA, $p = 0.006$), and the 10°C treatment varied significantly from all other temperature treatments according to post-hoc Tukey's HSD test (16°C: $p = 0.023$, 20°C: $p = 0.006$, 25°C: $p = 0.009$).

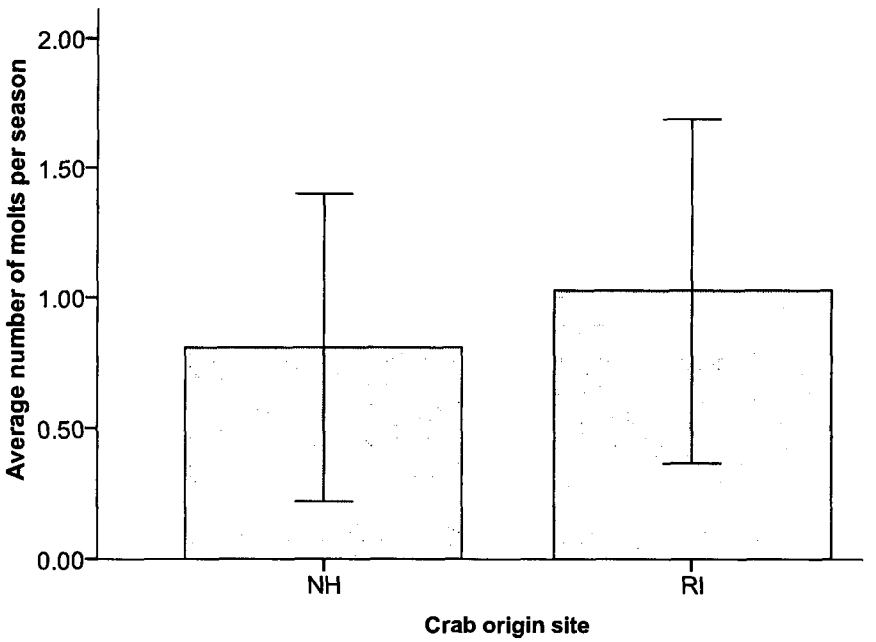

Figure 1.13: Average number of molts per season for New Hampshire and Rhode Island crabs. Averages were calculated across the 10, 16, 20 and 25°C temperature treatments (N = 4). Error bars indicate ± one standard deviation from the mean. There was no significant effect of crab origin on average number of molts per season (two-way ANOVA, $p = 0.11$).

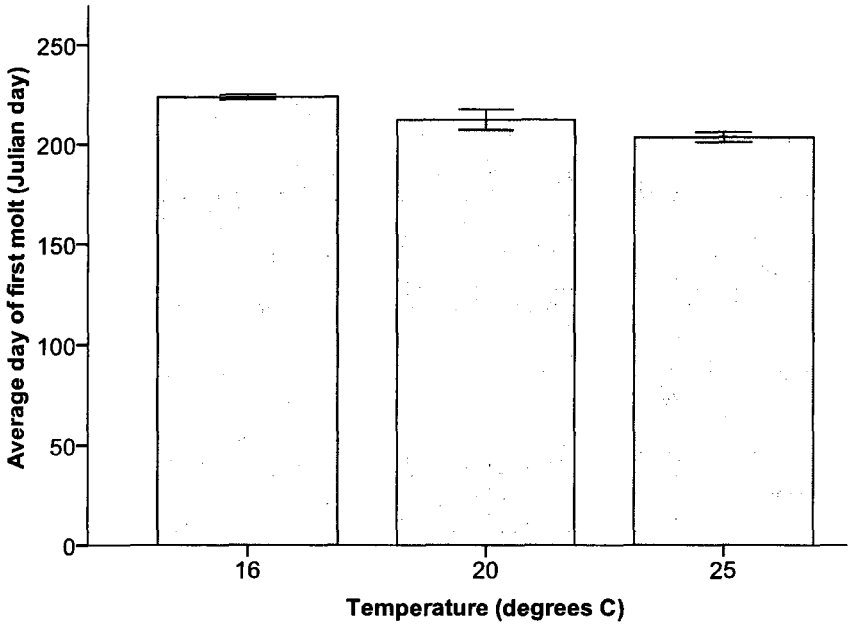

Figure 1.14: Average day of first molt event of the season for three temperature treatments. Averages were calculated across the crab origin sites (N = 2). Error bars indicate ± one standard deviation from the mean. There was a significant effect of temperature treatment on average date of first molt (two-way ANOVA, p = 0.05).

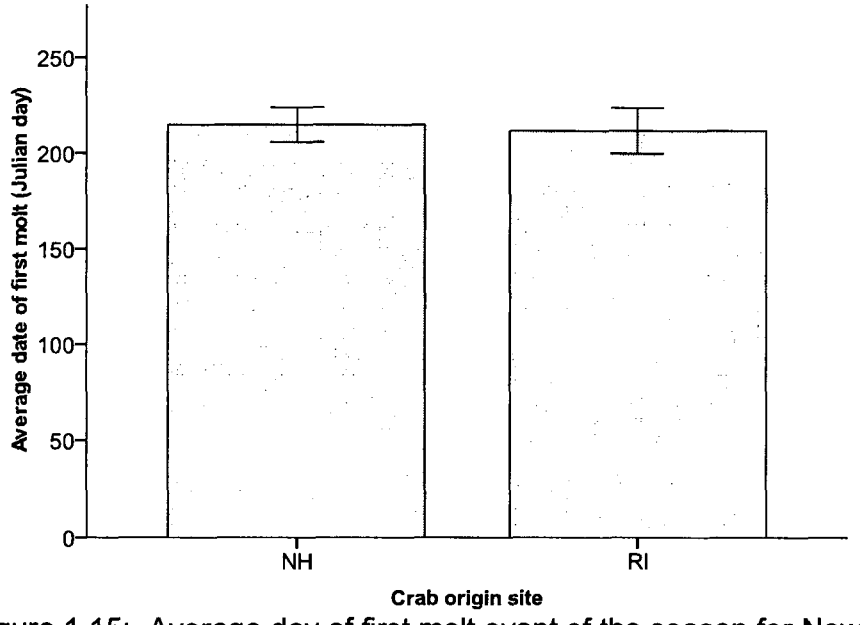

Figure 1.15: Average day of first molt event of the season for New Hampshire and Rhode Island crabs. Averages were calculated across the 16, 20 and 25°C temperature treatments (N = 4). Error bars indicate ± one standard deviation from the mean. There was no significant effect of crab origin on average date of first molt (two-way ANOVA, p = 0.36).

Table 1.7 Average, minimum and maximum molt increment for New Hampshire male and female crabs in each of three temperature treatments.

		Molt Increment (mm)		
Temperature	**Sex**	**Average ± St. Dev**	**Minimum**	**Max**
16°C	F	2.44 ± 0.42	1.97	3
	M	2.56 ± 0.81	1.42	3.27
	Both	2.49 ±0.58	1.42	3.27
20°C	F	2.45 ± 0.33	1.9	2.74
	M	2.41 ± 1.14	1	4
	Both	2.43±0.79	1	4
25°C	F	2.6 ± 0.22	2.43	2.85
	M	2.52 ± 0.38	2.05	3
	Both	2.54 ± 0.34	2.05	3
All temperatures combined	F	2.48 ± 0.34	1.9	3
	M	2.50± 0.72	1	4
All temperatures	Both combined	2.49 ±0.57	1	4

Table 1.8: Average, minimum and maximum molt increment for Rhode Island male and female crabs in each of three temperature treatments

		Molt Increment (mm)		
Temperature	**Sex**	**Average ± St. Dev**	**Minimum**	**Max**
16°C	F	2.76±0.34	2.14	3
	M	2.95 ±0.53	2.44	3.67
	Both	2.49 ±0.58	1.42	3.27
20°C	F	2.23 ±0.46	1.85	3
	M	3	3	3
	Both	2.43±0.79	1	4
25°C	F	2.91±1.60	1.5	6
	M	2.46 ±0.41	2	3
	Both	2.54 ±0.34	2.05	3
All temperatures combined	F	2.62±1.05	1.5	6
	M	2.67±0.49	2	3.67
All temperatures	Both combined	2.66± 0.81	1.5	6

Table 1.9: Average day of last brood release, first molt, and average number of days between the last brood release and first molt of the season for New Hampshire and Rhode Island crabs in three temperature treatments. Averages are shown ± one standard deviation from the mean.

		New Hampshire		Rhode Island	
	Averages	Julian Day	Date	Julian Day	Date
16 °C	Last brood release	199.7 ±7.5	7/19	188.1 ±13.5	7/7
	First Molt	223	8/11	224.67	8/13
	Time between last brood & first molt (# days)	32.4 ± 23.1		40.7 ±23	
20 °C	Last brood release	184.5±12.4	7/4	175.1±5.2	6/24
	First Molt	216	8/4	208.63	7/28
	Time between last brood & first molt (# days)	25.3 ± 8.1		33.4±18.7	
25 °C	Last brood release	187±11.4	7/6	173.3±9.5	6/22
	First Molt	205	7/24	201.43	7/20
	Time between last brood & first molt (# days)	19.6 ± 7.1		42.4 ± 28.9	

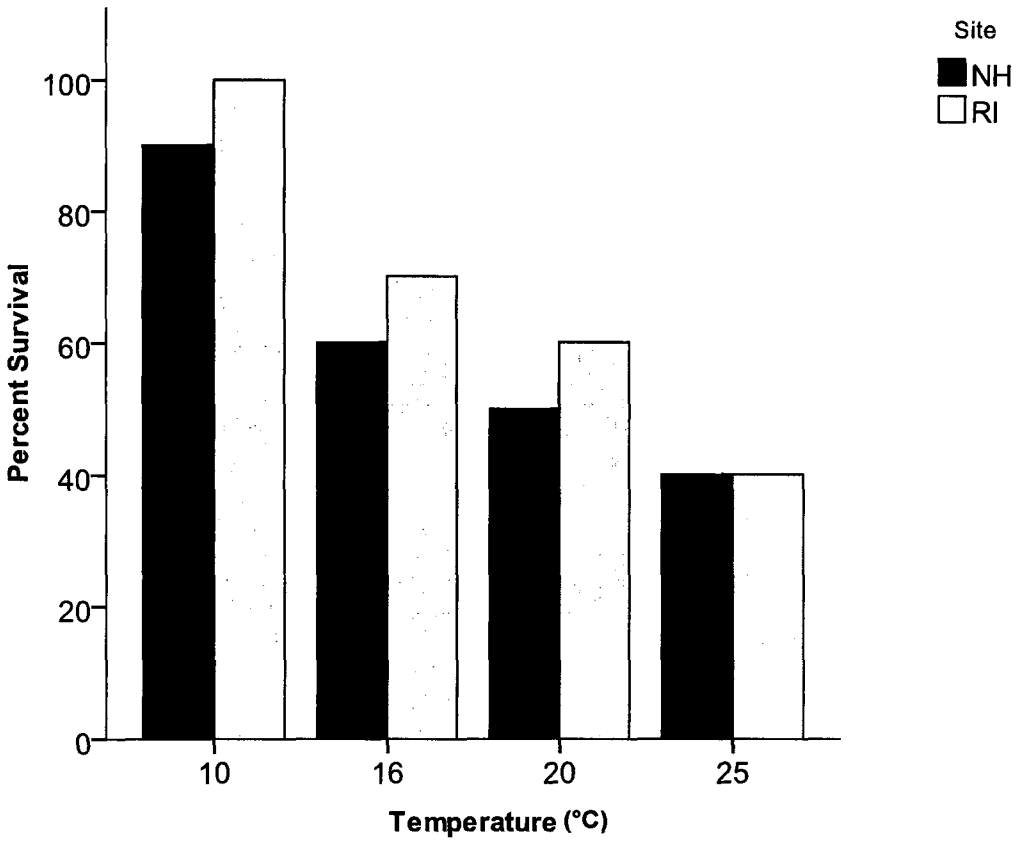

Figure 1.16: Percent survival for female crabs in three month long brooding experiment in each of four temperature treatments. Black and gray bars indicate crabs of New Hampshire and Rhode Island origin respectively. N = 10 crabs for each temperature treatment and crab origin site.

Table 1.10: Summary of copulation observations which occurred in 2009 lab brooding experiment.

Date	6/18/09	6/19/09	6/22/09
Time	9:45am	10:32-10:48am	8:13-8:19pm
Temperature (°C)	25	25	20
Crab origin	RI	RI	RI
Female CW (mm)	Not measured	25	Not measured
Male CW (mm)	Not measured	25	25
Ovigerous at time of Copulation?	Yes	Yes	No
Duration (min)	Unknown	16	Unknown
Days to next brood	Unknown	Unknown	1

Table 1.11: Possible ranges for all individual female crab ovipositions, brood releases and brood durations from New Hampshire field experiment. Variables expressed in date and Julian day.

	First brood Oviposition		First brood release		Duration
Crab	**Date range**	**Julian day range**	**Date range**	**Julian day range**	**Range (# days)**
1	6/5 - 6/11	156-162	7/24 - 8/6	205-218	43-62
2	7/7 - 7/13	188-194	8/18 - 8/22	230-234	36-46
3	5/20 - 5/31	140-151	7/16 - 7/20	198-201	47-61
4	6/5 - 6/11	156-162	7/24 - 8/6	205-218	43-62
5	6/5 - 6/11	156-162	7/24 - 8/6	205-218	43-62
6	6/5 - 6/11	156-162	7/24 - 8/6	205-218	43-62
7	5/20 - 5/31	140-151	7/16 - 7/20	198-201	47-61
8	5/20 - 5/31	140-151	7/16 - 7/20	198-201	47-61
9	7/24 - 8/6	205-218	8/23 - 9/1	235-244	17-39
10	7/7 - 7/13	188-194	8/18 - 8/22	230-234	36-46

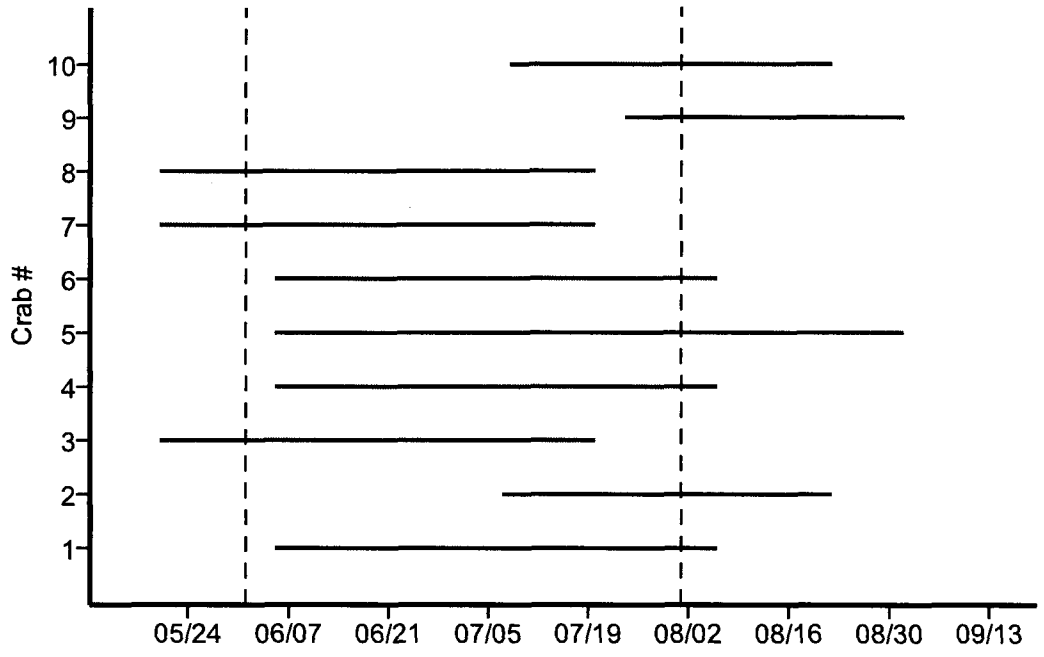

Figure 1.17: Brood duration for crabs deployed in field cages in New Castle, New Hampshire from March through October, 2009. Lines may be overestimates of the true brood durations, because crabs were not monitored daily. Vertical dashed lines mark the time period in which highest seasonal percentages of ovigerous females were found in field surveys in New Hampshire. The line for crab number five indicates two separate broods, produced in quick succession.

Discussion

Laboratory Experiment – Effects of Temperature on Seasonal Brooding Behavior

Overall, patterns observed in the lab experiment conform to expected results of faster brood development and higher metabolism at higher temperatures (Wear 1974, Hartnoll and Paul 1982, Amsler and George 1984, Fischer and Thatje 2008). Though in most response variables differences were only significant between the lowest temperature and all others, or not significant at all, this may be due to low survival and therefore low sample sizes in higher temperature treatments. Additionally, more replication in the experimental design in the form of more tanks, or truly independent individual crabs could lead to greater confidence in the patterns observed in this study.

The general pattern of brooding and molting activity for individual crabs conformed to expectations for temperate brachyurans, with single or multiple broods produced within one intermolt period and molting occurring most frequently after the breeding season (Knudsen 1964, Flores and Paula 2002, Brockerhoff and McLay 2005b).

Pre-copulatory behavior, positioning of male and female and copulation duration behaviors observed in this experiment were similar to known behaviors of other species of *Hemigrapsus* (Lindberg 1980, Brockerhoff and McLay 2005c). During copulation, males in the genus *Hemigrapsus* are generally positioned with their dorsal side resting on the substrate, and females are positioned above the

male (Lindberg 1980, Brockerhoff and McLay 2005c). Courtship behaviors were not observed for *H. sanguineus* in this study, and have not been observed in other species of this genus to date (Lindberg 1980, Brockerhoff and McLay 2005c). The one copulation observed in its entirely lasted 16 minutes, which is comparable to the 1-10 minutes seen for *H. oregonensis* (Lindberg 1980), especially when compared to other species of brachyurans which copulate on the order of hours (Knudsen 1960, Brockerhoff and McLay 2005a). However, there seems to be some diversion within the genus in regards to post-copulatory guarding, which was observed in *H. crenulatus* and *H. sexdentatus* but not in *H. oregonensis* or *H. sanguineus* (Lindberg 1980, Brockerhoff and McLay 2005c , this study).

Total Number of Broods

An increasing pattern in average number of broods per season was observed with increasing temperature between 10 and 25°C when all crabs were included in the analysis (Figs. 1.3 & 1.4). This correlation was significant for New Hampshire crabs but not for Rhode Island crabs using regression analysis (Figs. 1.3 & 1.4).

Interestingly, no individual crab produced more than three broods over the course of the experiment, and many crabs produced their last broods of the season earlier in the year than expected based on field brooding activity (Table 1.9 & Fig. 2.5). In a previous publication from the native range of this species, Fukui (1988) suggested that *H. sanguineus* are capable of producing five broods of eggs per season. However, no evidence of this potential was observed in

crabs from either origin tested in this experiment. In some previous studies of crab reproduction, estimates of number of broods produced per season were simply calculated by dividing reproductive window by development time for one brood of eggs (e.g. Fukui and Wada 1986). Based on the results of this study, showing decreased brooding activity toward the end of the reproductive window, this does not seem like an effective means of estimation.

The data on number of broods per season suggest that there is some factor other than temperature limiting reproductive activity at the end of the reproductive season. Cessation of brood production may occur in order to provide females with enough time and energy to devote to somatic growth, or alternatively, to provide larvae with the conditions they need for development. This cessation of brooding after one or two broods may also have to do with temperature acclimatization. The females may be capable of producing more broods, but may have conformed in this experiment to the pattern of brooding and molting that they had been entrained to in their natural habitat. Keeping crabs in elevated temperatures for longer durations, and allowing them to acclimate to these conditions may be the only way to test their true reproductive potential in heightened temperature environments. Ideally, a seasonal cycle of elevated temperatures could be established in conjunction with seasonal light conditions.

One crab with anomalous behavior was observed in this experiment. This crab from the 25°C Rhode Island treatment reared and released one brood of eggs early in the season (released June 19th), molted twice, and produced a

second brood of eggs on October 15th, the last day of the experiment. This suggests that after a certain amount of energy was allocated toward reproduction, or after a certain amount of time passed, and temperatures were still high, energy was once again allocated to reproduction. This is the type of pattern seen in tropical species, where temperatures are fairly stable year round, though oviposition will generally follow molting more quickly than was observed for this individual (Sudha and Anilkumar 1996). This individual seems to have adapted somewhat to the elevated and constant temperature condition of the experiment, rather than following the reproductive pattern that she would have in her previous, natural environment.

Brood Duration

A pattern of negative correlation was observed between temperature and average brood duration for New Hampshire crabs, but this difference was not significant according to regression analysis (Figure 1.7). Average brooding duration (± standard deviation) was 32.5 ± 2.9 days in the 16°C treatment, 18.5 ± 1.3 days in the 20°C treatment, and 13.0 ± 3.2 days in the 25°C treatment. 20°C brood duration can be compared to a previous study by McDermott (1998), which found brood duration of 22.3 ± 1.8 days at 19-20°C, with a sample size of eight females. Sample sizes in both studies were fairly low, so additional replicates would be necessary to pinpoint the duration at this temperature. However, even with limited data, the two studies show fairly similar results, within approximately four days of each other.

Applying this temperature induced difference in egg development rate to field conditions in New Hampshire suggests that brooding durations likely become shorter in the late part of the summer when temperatures are warmest (Figs. 2.14-2.17). In 2009, average daily intertidal temperatures in New Hampshire exceeded 17°C for 2.5 months from early July through early September (Table 2.4). They exceeded 18°C from late July through late August (1.5 months) and exceeded 19°C only on 12% of days in late August (Table 2.4). Average daily intertidal temperatures never exceeded 20°C in New Hampshire in 2009 (Table 2.4 & Fig 2.15). Interestingly, peak brooding activity was observed in field surveys before average daily intertidal temperatures started to reach 18°C and above (Figs. 2.5 & 2.15). This may indicate that timing of brooding is not determined to optimize number of broods per season or for shortest brooding duration, but instead to optimize conditions for larval development and/or adult female growth.

Thermal Reproductive Limits

Maximum thermal limit for reproduction was not reached in this experiment, though the maximum temperature treatment was higher than maximum summer temperatures seen historically in New England (http://www.nodc.noaa.gov). The southern limit of this species' current distribution is Oregon Inlet, NC (McDermott 1998, Delaney et al. 2008). According to historical bi-monthly averages from the NOAA's National Oceanographic Data Center (NODC), temperatures exceed 25°C from mid July through mid September in Cape Hatteras, NC, which is slightly south of Oregon

Inlet. Summer maximums in this location reach 26.7°C (NODC). Maximum thermal limit to reproduction for *H. sanguineus* is therefore likely 27°C or higher. Lower reproductive thermal limits are likely more important in potentially limiting the expansion of this species, due to large areas of favorable habitat available in Maine and Atlantic Canada where seasonal temperatures are generally lower.

A lower thermal limit for this species was not determined through this study, but 10° temperature treatments did have an observable effect on crab behavior. Crabs in 10°C treatments appeared to have lower feeding rates, did not molt during the course of the experiment, and did not successfully complete brooding. However, crabs were able to oviposit in 10°C water, and eggs seemed to survive to a certain extent, even if they were not developing normally. Overall, these observations suggest that very slow development likely took place for broods in 10°C water, and additionally, that some eggs likely died and were picked off and discarded by the mother, accounting for the gradual loss of brood mass over time.

If this experiment were to be repeated, measures could be taken to test this theory. These might include filtering tank water through a fine mesh sieve to collect and examine microscopically any discarded eggs. Eggs could also be plucked from egg masses at intervals during development and examined microscopically to determine developmental stage and health. To determine if embryogenesis and hatching could be completed successfully at this temperature, crabs could also be kept in individual containers where eggs and larvae could be more carefully observed for each female.

Females may simply need more time than was provided in this experiment to brood successfully at 10°C. However, even if embryogenesis is only slowed, hatching and survival of larvae may be inhibited by 10°C water, or even by temperatures in between the lowest tested in this experiment. In a study of 14 species of brachyurans, Wear (1974) found that temperature tolerances were broader for egg survival and development than for successful release of larvae and larval development. Therefore, even if eggs can be oviposited and develop under cold temperatures, reproduction may not be completely successful. Further study on the temperature requirements for larval hatching and development are needed to more fully understand the temperature limits for reproduction of this species. Indeed, this limitation on hatching and larval development, if it exists for *H. sanguineus*, may explain why brooding is shifted earlier in the season, rather than occurring during times of peak yearly temperature.

Thermal Reproductive Tolerances - Implications

To predict the potential for spread of this species, based on predicted thermal limits to reproduction, temperature data for Atlantic Canada was obtained from the Department of Fisheries and Oceans Canada. Data used has been historically averaged by monthly intervals from data collected since the1960s (http://www.mar.dfo-mpo.gc.ca). Summer temperatures reportedly reach or exceed 10°C in the majority of areas sampled in Atlantic Canada, with the exception of areas on the north coast of the Gulf of St. Lawrence and the northern tip of Newfoundland (Table 1.10). However, simply reaching a minimum

threshold temperature during summer months is probably not enough for successful reproduction. Temperatures must be sustained at high enough levels to be sufficient for completion of brooding, successful larval release and development. Temperatures are sustained above 10°C in some areas of Atlantic Canada for one to six months. However, average monthly temperatures only exceed 15°C near the Cabot Strait and in the southwest Gulf of St. Lawrence, and then only for approximately two to three months in late summer (Table 1.12). Data from areas representative of a sampling of regions in Atlantic Canada are summarized in Table 1.12.

Further information is needed regarding egg and larval development rates to concretely determine whether successful reproduction could take place in this region. Crabs would be capable of oviposition in those areas that reach temperatures of 10°C and above. However, the completion of the reproductive process (oviposition through larval development) would likely take too long to fit into the short summers in some areas in this region. Data have not yet been collected for brood duration and larval duration at temperatures below 15°C, but this study indicates that brooding takes approximately 33 days at 16°C, and development at lower temperatures can be assumed to be significantly slower. Larval duration (hatching to megalopal stage) at 15°C was found in laboratory study to be approximately 53 days (Epifanio et al. 1998). This suggests a period of approximately 86 days from oviposition to the megalopal stage at 15-16°C, which would not fit into the summer season in many locations in Atlantic Canada. Additionally, Epifanio et al. (1998) found decreased survival of megalopae

compared with early stage larvae, though survival was best at warmest temperatures. This could indicate even narrower environmental tolerances for the megalopal stage, though it could also be an artifact of larval rearing. Overall, the expansion of *H. sanguineus* into some areas of northern Maine and Atlantic Canada would likely require warmer temperatures than currently seen for reproductive success, in addition to the supply of recruits to northern latitudes and the survival and growth of juveniles to reproductive size. Other areas, such as Prince Edward Island, New Brunswick, Eastern Nova Scotia, Cape Breton Island have stronger potential for supporting reproductive populations of *H. sanguineus* (Table 1.12).

Experimental Design: Issues and Future Research

If a follow-up study to this research were to be conducted, more individuals should be included in the laboratory brooding study. Increased replication could be key in strengthening the statistical power of this experiment. One possibility would be to use more tanks for each temperature treatment and crab origin, perhaps five tanks with five male and female crabs for each temperature and crab origin combination. However, this would require a substantial amount of maintenance as well as a large increase in required equipment. Experiments could also be conducted in a facility with flow-through seawater. Effects of natural seasonal temperatures on reproductive behavior could then be tracked.

Crabs should be collected earlier in the season, to ensure that first ovipositions would occur within the experimental period. Additional temperature

treatments could be used to further test the thermal limits to reproduction for this species. Ideally, temperature treatments could be controlled enough to replicate the increases and decreases seen during the normal breeding season.

Most mortality seen in this experiment seemed to be due to conspecific aggression at the time of ecdysis. In order to decrease this behavior, crabs could be kept at lower density in future experiments and provided with additional food. A few instances of mortality were due to escape from tanks. This issue was corrected for by using more secure lids after the initial escapes occurred.

It is important to note that laboratory conditions were different from field conditions in a number of ways, most notably the absence of a tidal cycle. Because brachyuran reproductive behaviors are often closely linked to tidal cycles or entrained by tidal rhythms (Williams 1969), this may have had significant effects on crab behavior in this experiment.

Field Experiment

New Hampshire Brooding Season – Synthesis

Ninety percent of the crabs in the New Hampshire field experiment produced only one brood of eggs and one crab out of ten produced two broods. However, date of first oviposition varied over a long period of time from May 26th to July 31st (Fig. 1.17). Brood duration windows in this experiment fit well into the period of time during which high percentages of ovigerous females were found in field surveys (Figs. 1.17 & 2.5). The peak in ovigerous females observed from early June through early August in New Hampshire likely represents the timing of the first or only broods of a large percentage of *H. sanguineus* females (Fig. 2.5).

The field experiment indicates that approximately 30% of crabs produced broods slightly earlier, which would account for the initial, low frequency, appearance of ovigerous females in field surveys in late May (Fig. 1.17 crabs 3,7, and 8; Fig. 2.5). Ovigerous crabs observed from late August through late September (Fig. 2.5) are likely a combination of crabs that oviposited their sole broods later in the season and a small proportion of crabs producing a second brood of the season.

A similar experiment could be deployed in Rhode Island in the future to better understand the seasonal brooding pattern observed in this region (Fig. 2.5).

This field experiment also provided some data on brood duration under seasonal field conditions. Brood durations ranged from 28-54 days across the season, with shorter longer durations seen in crabs ovipositing earlier in the season (Fig. 1.17).

Lab Experiment - Induction of Brood Production at Elevated Temperatures

A simple conclusion can be drawn from the spring experiment, which is that crabs can be induced to oviposit earlier in the season than brooding generally occurs in nature. This was observed in crabs taken from ambient temperatures likely less than 3°C (NODC) and kept at 10°C for three weeks prior to being exposed to 20-22°C. However, little can be said based on this experiment about the exact temperatures required for induction of oviposition or ovarian maturation. The three weeks that crabs were kept in 10°C water may have allowed for the maturation of ovaries. It is also possible that light cycle, and not solely temperature, may have played a role in stimulation of brood production

for these females. Crabs were exposed to constant light conditions for three weeks prior to being transferred to a 20°C room with a natural light cycle. Both light and temperature have been found to stimulate vitellogenesis in brachyurans (Meusey and Payen 1988 and references therein). Additionally, photoperiod as long as 24 hours would never occur naturally in the known range of this species, so this treatment may have elicited unnatural behavior.

Overall, this experiment suggests that females are not inhibited physically or physiologically on the early side of the season from producing eggs. If exposed to warm enough temperatures earlier in the year, it can be predicted that the season would lengthen in this direction. Based on the inability of warm temperatures to induce brooding after the reproductive season had ended in late October, it does not seem likely that brooding season will lengthen at this end within a temperate geographic range. There is likely some other factor or combination of factors contributing to the end of the reproductive season. To strengthen these conclusions, more precisely designed experiments with a wider range of temperature treatments and carefully controlled light conditions should be conducted.

Assuming that crabs did not copulate before being brought into the lab, this experiment also affirms that females are capable of storing sperm between reproductive seasons (McDermott 1998). Because sea temperatures were approximately (3°C) at time of collection, it is highly unlikely that copulations took place prior to collection, and females were isolated from males in the laboratory.

Conclusions

Some potential limits to the reproduction of this species have been suggested by this work. While number of broods per season increases with temperature, the seasonal total seems to be limited to three broods. However, thermal acclimation may play a role in this apparent limitation, and further study is necessary to support this conclusion. Brooding success seems to be limited at low temperatures of 10°C, and other reproductive processes such as hatching and larval development may further narrow the temperature tolerances of the species. I would strongly suggest further study in the areas of thermal limits to these aspects of reproduction. Some work has addressed larval development rates at various temperatures (Epifanio et al. 1998), but these experiments had low survival rates at low temperatures. These may either be an artifact of laboratory rearing or may be true thermal limits to development.

Aside from absolutely limiting reproduction of *H. sanguineus* in latitudes with low seasonal temperatures, temperature may limit the degree of reproductive output by females, which may slow the spread or limit establishment of the species. The majority of crabs in New Hampshire populations likely produce one brood of eggs per season. This may be an important difference from populations in southern New England, where a higher proportion of crabs may produce two or three broods of eggs per season.

Table 1.12: Summary of timing and number of months with average daily temperatures exceeding 10°C and 15°C in various sample areas in Atlantic Canada and northern Maine. Data provided by the Department of Fisheries and Oceans Canada and NOAA's NOCD (US Data).

Location	# months > 10	months>10	# months >15	months >15
North coast Gulf of St. Lawrence	0	NA	0	
N tip of Newfoundland	0	NA	0	
SW tip of Nova Scotia	5	Jun-Oct	0	
NE tip of Nova Scotia	4	July-Oct	2	Aug-Sept
SW Newfoundland	4	Jun-Sept	2	Jul-Aug
N Bay of Fundy	3	Aug-Oct	0	
St. Lawrence river	3	Jun-Aug	0	
Bay of Fundy	4	July-Oct	0	
East side of Nova Scotia	3	Aug-Oct	0	
NW Strait of Belle Isle	1	Aug	0	
NE tip of Newfoundland	3	Aug-Oct	0	
New Brunswick N of PEI	4	Jun-Sept	3	Jul-Sep
New Brunswick	3	Jul-Sept	0	
PEI	6	May-Oct	2	Jul-Aug
East side of Newfoundland	2	Aug-Sep	0	
East side of Newfoundland	2	Aug-Sep	0	
Portland, ME	5.5	Late May - Oct	2.5	Jul - Sept
Bar Harbor, ME	6.5	Late May - Nov	1.5	Late Jul – Aug
Eastport, ME	3.5	Jul - Oct	0	

CHAPTER II

REPRODUCTIVE SEASONALITY AT TWO LATITUDES IN NEW ENGLAND

Introduction

The spread, establishment, and population growth of invasive species are influenced by their reproductive activities. For this reason, it is important to study the reproductive behaviors of invasive species, such as *H. sanguineus*. For species such as *H. sanguineus* with fairly wide latitudinal ranges, populations may experience a wide range of environmental conditions (Delaney et al. 2008, Stephenson et al. 2009). If reproductive output varies spatially due to environmental differences associated with latitude, the effects of invasive species on the communities they inhabit may vary as well (Hines 1989). Few reliable predictions regarding spread and establishment can be made without basic information on the biology, and specifically reproduction of *H. sanguineus*.

Crab reproductive seasons vary widely between the various groups of brachyuran crabs (Hines 1989 and references therein). Examples of reproductive strategies within the Brachyura include spring-summer reproduction, overwinter egg development with spring spawning, or year round reproductive activity (Knudsen 1964, Krouse 1972, Hines 1982, Erdman and Blake 1988, Hines 1991, Stone and O'Clair 2002, Park et al. 2007). Seasonal patterns also vary within genera. In the case of the genus *Hemigrapsus*, *H. nudus* in

Washington brood from January through June producing a total of one to two broods during this five month period (Knudsen 1964). Another study of *H. nudus* in California reported breeding season extending from October to May (Boolootian et al. 1959). *H. oregonensis* shows a similar though extended pattern in Washington, brooding from mid February though September, with a higher proportion of females producing two broods per season (Knudsen 1964). *H. sexdentatus* in New Zealand brood from late March through mid July (austral fall-winter), with only one brood produced per year (Brockerhoff and McLay 2005b). *H. sanguineus* in contrast, has a summer reproductive season, and likely produces one to three broods per season on the Atlantic coast of the United States (McDermott 1998).

Latitudinal Patterns in Brachyuran Reproduction

Reproductive behavior may also vary on a finer scale, between populations of the same species (Table 2.1). Because environmental characteristics tend to vary with latitude, many studies have been done to compare crab reproductive traits within a species across latitude (Table 2.1). In the Brachyura, intraspecific differences have been detected in ovarian development, size at maturity, egg size and number, total number of broods per season, as well as season duration (Table 2.1). These differences may be influenced by biogeographic barriers, which often lead to substantial differences in environmental characteristics, as well as separating populations spatially (Hines 1989). This introduction will focus on the reproductive parameters

measured in this study. Additional effects of latitude on crab reproduction are summarized in Table 2.1.

Size at Maturity

Size at maturity is critical in determining the overall reproductive output of a species, as earlier maturation may lead to increased lifetime reproductive output (Hines 1989, Flores and Paula 2002). Female size at maturity has been found to vary with latitude for several species of crabs, including the families Grapsidae, Xanthidae and Majidae and two species of the genus *Hemigrapsus*: *H. nudus* and *H. oregonensis* (Jones and Simmons 1983, Hines 1989). Observed latitudinal patterns in size at maturity include increase and decrease in size with latitude, as well as initial increase and subsequent decrease (Jones and Simons 1983, Hines 1989, Dugan et al. 1991, Ituarte et al. 2006). *Pachygrapsus crassipes*, *Helice crassa* and the anomuran crab *Emerita analoga* attain maturity at larger sizes in carapace width (CW) at higher latitudes (Hines 1982, Jones and Simons 1983, Dugan et al. 1991). *H. oregonensis* showed an increase in size at maturity at mid latitudes, but decreases in size at maturity at the edges of the latitudinal ranges tested (Hines 1989). A contrasting pattern, smaller size at higher latitude, was found for *Panopeus herbestii* , a xanthid crab studied on the Atlantic coast of the US (Hines 1989). Ituarte et al. (2006) also found smaller average carapace widths for female *Chasmagnathus granulatus* at higher latitude; however this was explained by differences in productivity between their study sites.

Hines (1989) determined that observed shifts in female size at maturity were often associated with the presence of a biogeographic barrier, such at Cape Hatteras or Point Conception (Hines 1989). Some theories suggest that latitudinal differences in size at maturity may be caused by slower metabolic rates and growth leading to delayed maturity at higher latitudes due to colder temperatures (Hines 1989 and references therein). Predation has also been proposed as a cause for this pattern, due to increased predation at lower latitudes (Wallerstein and Brusca 1982). Individuals producing offspring at a younger age might be more likely to pass on genetic material in environments with high predation pressure (Wallerstein and Brusca 1982).

Number of Broods per Season

Number of broods produced per season may also vary with latitude. This is generally thought to be related to increased rates of egg development at higher temperatures (Wear 1974, Hartnoll and Paul 1982, Amsler and George 1984, Fischer and Thatje 2008). Intraspecific differences in number of broods per year were found for *Cancer setosus* populations in Chile and Peru (Fischer and Thatje 2008). Crabs in the central latitudes studied were estimated to produce two to three egg masses per year, whereas, crabs at either extreme of their latitudinal range produced only one brood per season (Fischer and Thatje 2008).

In addition to or conjunction with changes in number of broods per season, crabs may also exhibit changes in overall reproductive season duration with changing latitude (Boolootian et al. 1959, Knudsen 1964, McDermott 1998, Ituarte et al. 2006). This was observed for *C. granulatus* populations in

Argentina, which had longer season durations at lower latitude (Ituarte et al. 2006). Changes in season length with latitude have also been observed for species in the genus *Hemigrapsus* (Boolootian et al. 1959, Knudsen 1964, Kurata 1968, Takahashi et al. 1985, Fukui 1988, McDermott 1998).

Effects of Latitude on *H. sanguineus*

H. sanguineus brooding season has been found to vary in both the native and introduced ranges of this species. In the Western Pacific, *H. sanguineus* ranges from Southern China to Russia. Season durations have been reported in Japan from approximately 33 to 43°N (Kurata 1968, Takahashi et al. 1985, Fukui 1988, McDermott 1998). Within this range, brooding season varies from three to eight months, with shorter season durations at higher latitudes (Kurata 1968, Takahashi et al. 1985, Fukui 1988, McDermott 1998). On the Atlantic coast of the United States a similar pattern may exist, but research has been somewhat limited and has not to date shown clear patterns of season duration with latitude. Brooding extends from late April through late September (five months) in New Jersey, and from at least June through late September in Delaware Bay (> four months) (Epifanio et al. 1998, McDermott 1998). The Delaware Bay survey was not conducted early enough in the season to establish the beginning of the season (Epifanio et al. 1998). Additionally, a subtidal survey in Connecticut found ovigerous females from April through September (Gilman and Grace 2009). No studies have been conducted on effects of latitude on other reproductive traits for this species.

Objectives

The primary objective of this study was to characterize the reproductive seasonality of *H. sanguineus* at sites in northern and southern New England. Surveys were conducted to determine seasonality of brooding, spawning and molting. These data are used to make comparisons of reproductive timing and traits between the two regions studied and to look for potential correlations between temperature and reproductive events. Additionally, data from plankton surveys were used to make predictions about larval behavior.

Table 2.1: Latitudinal patterns in reproductive traits for brachyuran crabs.

Reproductive Trait	Effect of increase in latitude	Species Studied	Study location	References
Ovarian Development	Earlier maturation	*Chasmagnathus granulatus*	Argentina	Ituarte 2006
Size at maturity	Smaller	*Panopeus herbestii*	US - Atlantic	Hines 1989
		Chasmagnathus granulatus	Argentina	Ituarte 2006
	Larger	*Pachygrapsus crassipes*	California	Hines 1989
		Helice crassa	New Zealand	Jones and Simons 1983
		Emerita analoga (Anomuran)	California	Dugan et al. 1991
	No Effect	*H. nudus*	California	Hines 1989
	Larger at intermediate latitudes	*H. oregonensis*	California	Hines 1989
Egg size	No change	*Helice crassa*	New Zealand	Jones and Simons 1983
	Larger	*Cancer setosus*	Chile	Brante 2003
		Pinnaxodse chilensis	Chile	Lardies and Castilla 2001
Number of eggs	Higher	*Helice crassa*	New Zealand	Jones and Simons 1983
		Cancer setosus	Chile	Brante et al. 2003
	Fewer	*Pinnaxodes chilensis*	Chile	Lardies and Castilla 2001
Number of broods per season	Higher at intermediate latitudes	*Cancer setosus*	Chile and Peru	Fischer and Thatje 2008
Season duration	Shorter	*Chasmagnathus granulatus*	Argentina	Ituarte et al. 2006
		Hemigrapsus sanguineus	Japan	McDermott 1998
		Hemigrapsus nudus	US – Pacific	Knudsen 1964, Boolootian et al. 1959

Materials and Methods

Study Sites

H. sanguineus populations were studied at two sites in New England. Sites were chosen based on the presence of a floating dock from which to sample plankton and to include habitat suitable for *H. sanguineus* adults. These two locations were chosen to represent regions with different levels of *H. sanguineus* density. Since its invasion, *H. sanguineus* has colonized areas from south to north in New England, and is not yet as dominant and abundant in northern New England as in southern New England (Delaney et al. 2008).

Northern New England sites were on the coast of New Hampshire (~43°N) (Fig. 2.1). Plankton samples were collected from a floating dock at the Rye Harbor commercial dock. In 2008 adult crabs were sampled in a rocky area just north of Rye Beach. In 2009, three local sites in New Hampshire were surveyed: Odiorne Point, Rye Harbor State Park (outside the breakwater) and a rocky site just south of Jenness Beach in Hampton, NH. Southern New England sites were located on the southern portion of Conanicut Island in Jamestown, RI (~41°N) (Fig. 2.1). In 2008 and 2009 plankton samples were collected from a floating dock at Conanicut Marina, and adults were sampled in a rocky area just south of the marina. In 2009 two additional sites on Conanicut Island were surveyed: Fort Wetherill, and the western shore of Mackerel Cove.

Adult Crab Surveys

In 2008, rocky intertidal areas were sampled approximately weekly (New Hampshire) or biweekly (Rhode Island) from June to October to determine the

duration of breeding season and timing of peak reproductive output of *H. sanguineus*. To find crabs, rocks were turned haphazardly in the mid to low intertidal areas for a period of 30 minutes to an hour on each sampling date. Crabs of potentially reproductive size (≥12 mm) were targeted, and only females were collected. Following the search, all mature and immature female crabs were counted, and percent of ovigerous mature crabs was calculated.

Methods were expanded in 2009 to include three local sites in each region. Surveys were conducted twice monthly from March to November 2009. Rye Harbor State Park was sampled with greater frequency, approximately weekly from March to November. Male and female crabs of potentially reproductive size were collected in the mid to low intertidal zone at each site. Searches were conducted for 45 minutes to an hour at New Hampshire sites, and 20-30 minutes at Rhode Island sites. The shorter searches were conducted when more than one individual was searching. Carapace width was measured for all crabs and abdomen width was measured for all female crabs. All measurements were made with a ruler to the nearest millimeter. Brood presence or absence was noted for each female crab, and if ovigerous, egg color was noted. Number of females with eggs was divided by total number of females collected at each site on each sampling date to determine percent ovigerous.

Seasonal patterns for percent ovigerous females were examined for local sites in each region. Because the local sites showed similar seasonal patterns (Figs. 2.2 & 2.4), values for proportion ovigerous were compared between the two regions (New Hampshire and Rhode Island) using one-way analysis of

variance with the three local sites designated as replicates for each sampling date. Egg color was used to categorize broods as early (orange-brown) or late stage (all other colors) in development. This was the simplest designation that could be made quickly in the field, without examining the eggs microscopically. This information was used to determine when in the season crabs produced new clutches of eggs, though it could not be determined if broods were the first of the season or not.

Female Size

Female size data, measured in carapace width for each individual collected, were used to look for patterns in size distribution of ovigerous females over time. Data were prepared in the form of histograms, with crabs binned into 1 mm increments by carapace width. Percentages of non-ovigerous females, ovigerous females with late stage eggs and ovigerous females with early stage eggs were presented for each size class on each sample date. Average ovigerous crab carapace width was also calculated for each sampling date for each region. For this analysis, all crabs from the three local sites in each region were pooled. In addition, carapace widths for all ovigerous females collected across the season and in all local sites were pooled for each region. A one-way analysis of variance was used to test for an effect of region on average ovigerous carapace width.

Temperature Data Collection

Historical temperature data were obtained from the National Oceanographic Data Center coastal water temperature guide

(http://www.nodc.noaa.gov/dsdt/cwtg/natl.html). Locations used as representative of sample regions were Portsmouth Harbor, NH and Newport, RI.

Seasonal temperature data were collected in 2009 using HOBO temperature loggers in New Castle, NH and Jamestown, RI. A temperature logger was deployed in the mid intertidal zone in New Castle, NH from April 15th to October 5th, 2009. A temperature logger was deployed from a floating dock at Conanicut Marina in Jamestown, RI from May 27th to October 15th, 2009. Both loggers collected temperature data at 30 minute intervals for the duration of their deployment. Minimum, maximum and average daily temperatures were calculated from these temperature measurements. Averages of these parameters were taken over semimonthly intervals. Month days 1-15 were averaged as "early-month" and days 16 to the end of the month were considered "late-month".

In addition, because the beginning of the reproductive season of *H. sanguineus* is likely cued by temperature or light availability (Meusey and Payen 1988), temperatures during the time of year when ovigerous females were first observed were analyzed closely. This analysis was only conducted for New Hampshire temperature data because daily temperature measurements began before the initiation of the brooding season, whereas Rhode Island temperature measurements did not. Number of days with minimum, maximum and average temperatures above certain, potential threshold temperatures were quantified within each bi-monthly time period, to look for initial increases in number of degree days that correlated with the initial increase in brooding activity (early to

late May). Data for percent of days within each time interval with temperatures above these threshold temperatures (9-22°C) were summarized in table form (Table 2.5). Degree day patterns for temperatures that correlated with increase in brooding activity were presented graphically (Figs. 2.20-2.23).

Plankton Surveys

Surface waters were sampled twice monthly in 2008 and 2009 at each site using a 50 µm mesh size plankton tow. Tow location varied slightly between sampling periods due to changes in available space at the docks. Tow duration was four to five minutes, and volume of water sampled ranged from 10 to 12.5 m^3. Tow volume was estimated using the net diameter (50 cm) and distance covered during each tow. Efficiency of 100% was assumed for the plankton tow, which likely led to underestimates of density for each sample. Three tow samples were collected on each sampling date within a 30 minute window around a nocturnal high tide. Nocturnal high tide sampling was chosen because *H. sanguineus* females release their larvae on nocturnal high tides (Park et al. 2005). Contents of tows were filtered through a 300 µm sieve and preserved in ethanol. Any large items such as macroalgae or small fish were rinsed with seawater to collect any larvae attached to these organisms and removed from the sample. Samples were collected twice monthly from May to October in 2008 and 2009. In addition to horizontal surface tows, vertical tows were collected in 2009 from the seafloor to the surface of the water. Three replicate vertical tows were conducted on each sampling date. Tow volumes were estimated in the same manner as for horizontal tow samples. Average volume sampled was 0.92

± 0.06 m^3 in New Hampshire and 0.50 ±0.05 m^3 in Rhode Island. Differences in tow volume were due to differences in depth at the two sites

In 2008, as an alternate method of collecting larvae, traps were constructed utilizing light as an attractant for zooplankton. Light traps were deployed twice monthly concurrently with the collection of plankton tow samples. Traps were constructed from 38 cm x 25 cm x 24 cm white translucent jugs with four white translucent funnels (85 mm outside diameter, 8 mm inside diameter) inserted into the center of each of four sides. A collection cup (8 cm inside diameter) with 300 µm mesh was attached to the bottom of each trap using a bulkhead fitting. Trap construction was modeled after Porter et al. (2008). Traps were outfitted with green submersible lights powered by a 120 volt marine battery. Traps and the lights within each trap were weighted to assure upright positioning in the water. Traps were secured to cleats on the docks, and suspended approximately 30 cm below the surface. Three traps were deployed overnight (eight or nine hours) twice monthly at each site on dates with nocturnal high tides. The contents of each trap were preserved in ethanol.

Megalopae Behavior: Field Surveys

In an effort to target *H. sanguineus* megalopae moving back into coastal areas following development offshore, submersible light traps were deployed on nocturnal flood and ebb tides in Rhode Island and New Hampshire in September and October 2009. Two replicate traps were suspended just below the surface, and three replicate traps suspended near the seafloor on each sampling date. It was hypothesized that megalopae would be found on nocturnal flood tides, and

that significantly more megalopae would be caught in traps at the bottom versus traps at the surface. Deployment dates and conditions are summarized in Table 2.2.

Sample Processing

Samples collected using each of these methods were later sorted, and all crab zoeae and megalopae were quantified. *H. sanguineus* larvae were identified to stage and counted (zoeal stages I–V and megalopae). Stages I and II were grouped as "early-stage" larvae, due to similarities in morphology that could not be distinguished readily under a dissecting microscope. All other species of brachyuran zoeae were counted as a group as were all other brachyuran megalopae.

Larval identification was based on specimens cataloged from laboratory rearing of *H. sanguineus* from egg through the 1st juvenile stage. Additionally, a study by Kornienko et al. (2008) provided detailed descriptions and drawings of larval morphology for *H. sanguineus*. *Zooplankton of the Atlantic and Gulf coasts* by Johnson and Allen (2005) was also used as a reference regarding other species of brachyuran larvae. The text provides a comprehensive list of crab larvae in the regions sampled as well as morphological characteristics of each.

H. sanguineus zoeae were distinguishable from other species of larvae in New Hampshire and Rhode Island by the presence of several morphological characteristics (Sastry 1977, Johnson and Allen 2005, Kornienko et al. 2008). The first identifying features used were the lateral carapace spines, found on all stages of *H. sanguineus* zoeae but not on many other species of crab larvae,

including *C. maenas*. Stage I and II *H. sanguineus* zoeae are very similar in characteristics that could be examined under a stereomicroscope (Kornienko et al. 2008). Characteristics of the telson of early stage zoeae were also important in distinguishing *H. sanguineus*. Early larvae of this species have a laterally smooth, forked telson with a number of medial setae, and quantity of setae increases with zoeal stage (Kornienko et al. 2008). Many other species of crab zoeae have projections of some sort on the outside edge of the telson (Johnson and Allen 2005). Lastly, the abdominal somites of the zoeae were used. *H. sanguineus* has smooth edged abdominal somites, with the exception of the second and third abdominal somites (Kornienko et al. 2008). The second abdominal somite has obvious paired dorsolateral projections, and the third has very small paired dorsolateral projections (Kornienko et al. 2008). Later stage *H. sanguineus* zoeae adhere to this basic pattern with the addition of medial setae on the telson and abdominal somites (Kornienko et al. 2008). In stage IV and V pleopodal buds are present ventrally on the abdomen (Kornienko et al. 2008).

Whenever possible, the entirety of a sample was sorted through for crab larvae. However, when larvae were extremely numerous one of two sub-sampling techniques was used. For New Hampshire samples or Rhode Island tow samples, the sample in question was split into two halves with the use of a plankton splitter. One of the halves was randomly selected for analysis. Counts from this sample were used to estimate the number of larvae in the whole sample by multiplying by two. The second sub-sampling technique employed was used on Rhode Island trap samples, which were very dense with crab larvae, as well

as large in total biomass. Five three mL subsamples were extracted from each total sample. Total sample and subsamples were massed to the nearest 0.01 g using a digital balance. Volume was estimated by using the density of water (1 g = 1 mL). Subsamples ranged from 6.6-11.7% of total sample, with an average of 8.5%. The ratio of subsample volume to total sample mass was used to estimate number of larvae per total trap sample.

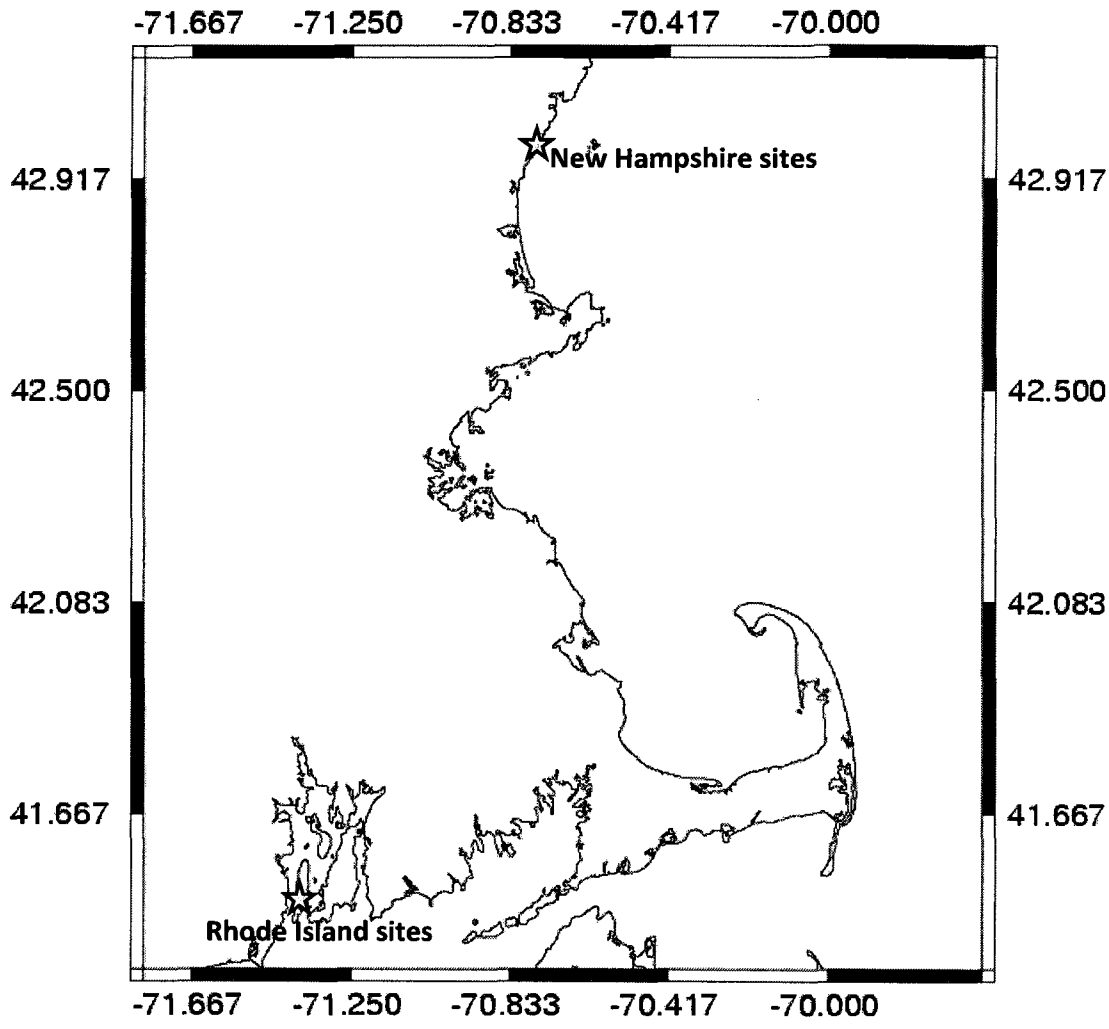

Figure 2.1: Study site locations in New Hampshire and Rhode Island. Ovigerous surveys and plankton collections were conducted in both regions.

Table 2.2: Dates and details for targeted sampling for *H. sanguineus* megalopae using light traps deployed at surface and depth under various tidal conditions in 2009. No *H. sanguineus* megalopae were found in any of these samples.

Date	Site	Tide	Depth	Duration (hours)	# of replicates
9/22/09	NH	Ebb/flood	Deep	9	3
9/22/09	NH	Ebb/flood	Surface	9	2
9/19/09	RI	Flood	Deep	0.5	3
9/19/09	RI	Flood	Surface	0.5	2
9/19/09	RI	Ebb	Deep	1	1
9/19/09	RI	Ebb	Surface	1	2
10/6/09	RI	Ebb	Deep	2.5	3
10/6/09	RI	Ebb	Surface	2.5	2

Results

Adult Crab Surveys

New Hampshire

H. sanguineus reproductive season extended from late May to late September at three sites in New Hampshire in 2009 (Fig. 2.2). All three sites showed similar patterns of seasonality, with the exception of one data point at Odiorne in late June. Percent ovigerous increased quickly from late May to early June and remained at levels from 60-70% until early August when percent ovigerous began declining at all three sites. Percent ovigerous continued to decrease through September, and the last ovigerous females of the season were observed in late September. Data from 2008, collected in Rye, NH, varied somewhat from the 2009 data (Fig. 2.2). A peak in percent ovigerous greater than 80% was seen in early July 2008, which agrees with the peak seen in 2009. However, percent ovigerous was higher later in the year in 2008 than in 2009, and the season extended into late October 2008 (Fig. 2.2).

Weekly ovigerous surveys in Rye, NH showed a similar pattern to the bi-weekly surveys at multiple sites (Fig. 2.3). First ovigerous females of the season were collected on May 21st, and the last ovigerous crab of the season was collected on September 23rd, 2009. Percent ovigerous increased quickly in late May and early June, and levels were sustained above 50% from June 11th through July 21st (Fig 2.3). There was a general pattern of decrease starting July 16th, with two small increases on August 17th and September 10th (Fig. 2.3).

Rhode Island

H. sanguineus reproductive season extended from early May to early October at three local sites in Rhode Island in 2009 (Fig. 2.4). Survey data from a single site in Rhode Island in 2008 also show the season ending in early October, though surveys were not conducted early enough in 2008 to establish the beginning of the season (Fig. 2.4). The three local sites surveyed in 2009 showed similar seasonal patterns with regard to ovigerous females. Percent ovigerous increased quickly from early May to late May and these levels were maintained above 50% until early August when a marked decline in percent ovigerous was seen at two sites (Mackerel cove was not sampled on this date). Percent ovigerous dropped to a lower level at Jamestown Marina than at Fort Wetherill. These two sites saw rebounds in percent of ovigerous crabs in late August, but to levels lower than their previous peaks seen in late July. One of the sites, Mackerel Cove, showed a slightly different pattern, with a peak in percent ovigerous in late August. Data from 2008 agree in general with patterns seen in 2009 (Fig. 2.4). Low levels of ovigerous crabs were observed in late July 2008, and then peak levels in late August to early September. This 2008 peak coincides with the second peak in percent ovigerous recorded in 2009 (Fig. 2.4).

Regional Comparisons

Data for the three local sites in each region were used to examine seasonal differences in reproduction between New Hampshire and Rhode Island. There were several distinct differences observed between these regions. First, reproductive season began earlier in Rhode Island in 2009 than in New

Hampshire (Fig. 2.5). The first ovigerous females of the season were found on May 9th in Rhode Island, whereas the first ovigerous females were found on May 21st in New Hampshire. Percent ovigerous varied significantly between the two regions in late May, when Rhode Island had an average of approximately 58% ovigerous, and New Hampshire had only approximately 15% (Fig. 2.5). In addition to this difference in start of the season, there was a difference between the sites toward the end of the season. Percent of ovigerous females varied significantly between the two regions in late August, when Rhode Island again had a much larger percent of ovigerous females than New Hampshire (Fig. 2.5). There were, however, periods of similarity for the two regions during the 2009 season. From early June through early August, similar percentages of ovigerous crabs were observed at both sites. The very end of the season was also similar between regions. Both sites had very low percentages of ovigerous females in late September and this dropped to zero in early October, with the exception of two ovigerous crabs found at Mackerel Cove in Rhode Island.

Initiation of Clutches – Presence of Early Stage Eggs

Crabs carrying early stage eggs, which are orange-brown in coloration for *H. sanguineus,* were found during every time period in New Hampshire when ovigerous females were present (late May – late September) (Fig. 2.6). Percentages of crabs with early stages eggs detected in New Hampshire were fairly low across the season, never exceeding 30% (Fig. 2.6). The highest percentages of early stage eggs were found in New Hampshire in early June and late September (Fig. 2.6).

In Rhode Island, crabs with early stage eggs were found on all dates sampled when ovigerous females were present, with the exception of early October (Fig 2.6). Percentages of early stage eggs detected in Rhode Island were higher than those seen in New Hampshire overall (Fig. 2.6). The highest percentage of early stage eggs detected was in early May, with approximately 80% of ovigerous females carrying orange-brown eggs. This timing coincides with the first observed presence of ovigerous females in this region (Figs. 2.5 and 2.6).

Ovigerous Female Size

Ovigerous female size was measured using carapace width. There was no clear seasonal change in ovigerous crab carapace width in either region (Figs. 2.7 & 2.8). When all ovigerous females were combined for each region across sample dates, New Hampshire ovigerous crabs ranged from 12 to 36 mm in carapace width (Table 2.3), and Rhode Island crabs ranged from 12 to 33 mm in carapace width (Table 2.3). 1086 ovigerous crabs were collected in New Hampshire in 2009, and the overall average in carapace width for these crabs was 20.6 ±3.6 mm (Table 2.3). 1344 ovigerous crabs were collected in Rhode Island in 2009, and the overall average carapace width for these crabs was 19.7± 3.5 mm (Table 2.3). There was a significant of difference in ovigerous female carapace width between these two regions when tested using one-way analysis of variance ($p < 0.001$).

Results for size distribution of female crabs by site and sample date are represented in Figure 2.9. Females were also pooled across sample dates for

each site, and overall size distributions for each site are presented in Figure 2.10 and 2.11. The majority of ovigerous females were between 15 and 25mm for both sites (Figs. 2.10 & 2.11). However, the Rhode Island population had more ovigerous females in the 15-20 mm size class than in the 20-25 mm category (Figs. 2.10 & 2.11).

Seasonality of Molting

In New Hampshire, recently molted *H. sanguineus* were observed from early June through the end of the sampling season in early October (Figure 2.12). Higher percentages of recently molted males than females were observed on the majority of sample dates. For males, average percentage recently molted was highest in early October, with an average of 17.1% recently molted (Fig. 2.12). There was also a smaller peak in percent of recently molted males in late July, with 10.9% of males having recently molted (Fig. 2.12). Peak percentage of recently molted females was observed in late September, with an average of 8.9% recently molted (Fig. 2.12).

In Rhode Island, recently molted *H. sanguineus* were observed from late May through the end of the sampling season in early October (Fig. 2.13). Peak percentages were seen for both males and females in early October (Fig. 2.13). Smaller peaks were seen for males in late June and for females in early June (Fig. 2.13).

Temperature Seasonality

Based on historically averaged data dating to the 1980s (NOAA – NODC), New Hampshire temperatures range from approximately 7.2-17.2°C between late

April and early October (Fig. 2.14). Peak temperatures have historically been seen in late August, with the fastest rates of increase in temperature between early May and late May and between late June and early July (Fig. 2.14).

Intertidal data collected in 2009 via HOBO temperature logger showed a similar pattern to historical averages, though with slightly lower values than the historical sea surface temperatures (Fig. 2.15). Average daily temperatures were calculated from HOBO measurements taken every 30 minutes. These average daily temperatures were averaged over bi-monthly time periods to compare with historical data and to match up with ovigerous survey data. These bi-monthly averages ranged from approximately 5°C (late April) to approximately 14.6°C in late July (Fig. 2.15). However there was very little difference in average bi-monthly temperatures in the time period between late July and early August (Fig. 2.15). The time of fastest temperature increases according to compiled HOBO data was between late May and early June when average bi-monthly temperature jumped from 7.8-11.1°C (Fig. 2.15).

Based on historically averaged data dating to the 1980s (NOAA – NODC), Rhode Island temperatures near study sites for this project range from approximately 8.9-21.7°C between late April and early October, with peak temperatures in early August (Fig. 2.16). The fastest rates of temperature increase based on this data set were from early May to late May and from late June to early July (Fig. 2.16). Data from the temperature logger deployed at the sea surface in Jamestown, RI were very similar in pattern and values to the historical temperature data set (Figs. 2.16 & 2.17).

For comparison with seasonality of *H. sanguineus* egg brooding, seasonal temperatures averaged across bi-monthly time intervals were combined with a color code to indicate level of brooding activity at each time point (Fig. 2.18). Ovigerous crabs were first seen in New Hampshire when sea surface temperatures were above 10°C, and peak levels of brooding took place before peak summer temperatures were reached (Fig. 2.18). In Rhode Island ovigerous crabs were also first seen when sea surface temperatures were above 10°C (Fig. 2.18). Peak levels of brooding activity were seen both before (1st peak) and after (2nd peak) the time of peak summer temperatures (Fig. 2.18).

Potential Temperature Cues for Initiation of Reproductive Season

New Hampshire temperatures recorded by the HOBO temperature logger in the mid-intertidal zone in New Castle, NH were examined for correlation with reproductive events. When number of days at temperatures between 9 and 22°C were quantified, some potential threshold temperatures were found in minimum, maximum and average daily temperature. In New Hampshire, brooding activity was initiated between early and late May in 2009. During this time period, there was an initial increase in number of days with minimum temperatures above 9°C and 10°C (Fig. 2.20). Prior to late May, minimum intertidal temperatures did not exceed 11°C in 2009. There was also an initial increase of the season in number of days with average daily temperatures greater than 11°C and 12°C (Figs. 2.21 and 2.22). Maximum daily temperatures of 22°C and above were also first seen in late May (Fig. 2.23). Initial increases in number of degree days for other

temperatures tested did not correlate with initiation of brooding activity at this site (Table 2.5).

Seasonal Temperatures, Brooding Activity and Predictions from Laboratory Studies – a Synthesis

Experimental data about brood duration from Chapter I were combined with seasonal temperature data to make predictions about brood duration and total number of broods per season in each region studied. Temperature data from 2009 HOBO loggers as well as historical bi-monthly averages from NOAA (NODC) were used to calculate average monthly temperatures and average reproductive season temperatures for each region (Table 2.4). Observations regarding brood duration for New Hampshire crabs in the laboratory experiment described in Chapter I were used to make predictions about brood duration throughout the reproductive season in each region. Brood durations observed in Chapter I were approximately 13 days at 25°C, 19 days at 20°C and 33 days at 16°C (Fig. 1.7).

New Hampshire

In New Hampshire, peak ovigery was observed from early June to late July (Fig. 2.5). This is an approximately 40 day period, across which average historic sea surface temperatures are 14.2°C (Fig. 2.19). Average daily intertidal temperatures, averaged across this period give a slightly higher estimate of temperature, 15.1°C (Fig. 2.19). Brood durations within this period are estimated to be greater than 33 days (Fig. 1.7). This suggests that only one brood of eggs may be produced by an individual female during this peak ovigery window.

The entire reproductive window for this region lasted from late May to late September, or approximately 126 days (Fig. 2.6). Average intertidal temperature across the reproductive season was approximately 16°C (Table 2.4). Therefore, brood durations in New Hampshire can be estimated as approximately 33 days across the season. Historical average temperatures indicate a slightly lower seasonal average of 14.9°C (Table 2.4), which suggests brood durations greater than 33 days. Overall, longest brood durations likely take place in the early season, and shortest in August when temperatures reach the seasonal peak (Table 2.4).

In New Hampshire, early stage *H. sanguineus* zoeae were first detected on July 5th, 2009, 46 days after the first ovigerous females were collected in Rye on May 21st (Figs. 2.3, 2.19, and 2.26) and 26 days after ovigery above 50% was detected in this region. Average temperature from first ovigery to first zoeae was 13.1-14°C, using historical average temperature data or intertidal data from 2009, respectively (Fig. 2.19). Because temperatures this low were not tested in Chapter I, brood duration cannot be estimated for this time period. However, it can be suggested that brood durations would exceed 33 days during this period.

Rhode Island

In Rhode Island peak ovigery was observed from late May through late July. During this approximately 58 day period, average daily temperature was approximately 17.3°C using HOBO temperature logger data from 2009 (Fig. 2.19). Based on laboratory brood duration observations, brood duration during this period is likely between 19 and 33 days (Fig. 1.7). Average daily

temperature during the second peak in ovigery (early August – early September) was approximately 22°C (Fig. 2.19). Brood durations were likely between 13 and 19 days during this time period in Rhode Island (Fig. 1.17 & Table 2.4).

Average monthly temperatures were calculated based on historical average temperatures and HOBO logger data. These temperatures were used to estimate brood duration throughout the brooding season. Brood duration estimates ranged from 13 to longer than 33 days in Rhode Island (Table 2.4). Average daily temperature across the season in Rhode Island is approximately 17.9°C (Table 2.4), which indicates brood durations of 19-33 days.

In Rhode Island, early stage *H. sanguineus* zoeae were first detected on June 24th, 47 days after the first ovigerous females were found at this site and 30 days after ovigery was detected above 50% (May 26th) (Figs. 2.5, 2.19, & 2.29). Average temperature from the time of first ovigery to first zoeae was 14.6°C, based on historical average data (NODC) (Fig. 2.19). Because temperatures this low were not tested in Chapter I, brood duration cannot be estimated for this time period. However, it can be suggested that brood durations would exceed 33 days during this period (Fig. 1.7).

Plankton Surveys

Early H. sanguineus Zoeae

Season Duration. Seasonal patterns of *H. sanguineus* early zoeae in the water column varied somewhat between years and sampling techniques. By combining methods, spawning season duration was estimated for 2008 and 2009. New Hampshire 2008 spawning season likely extended from early July

through late October (Figs. 2.24 and 2.25). Both trap and tow samples collected early-stage *H. sanguineus* zoeae on all dates from July 2nd through October 24th, the last date sampled in 2008 (Figs. 2.24 and 2.25). New Hampshire 2009 spawning season likely extended from early June through late September (Fig. 2.26). Horizontal surface tows detected early-stage *H. sanguineus* zoeae at very low levels in early June through late September. Vertical tows collected early-stage zoeae from late July through early September (Fig. 2.26). Zero early-stage *H. sanguineus* zoeae were collected via either method in early October, 2009 (Fig. 2.26)

In 2008 Rhode Island spawning season likely extended from early June through late October and beyond (Figs. 2.27 and 2.28). Sampling dates in Rhode Island in 2008 did not seem to bracket the entire season. Early *H. sanguineus* zoeae were detected with traps in early June 2008 at very low levels, but this was the first date sampled this season (Fig. 2.27). Early *H. sanguineus* zoeae were not detected until early July in tow samples, but tow samples were not collected in late June (Fig. 2.28). Early zoeae were still present in the last tow and trap samples of the season in 2008, collected on October 18th (Figs. 2.27 and 2.28). Spawning season in Rhode Island in 2009 was more clearly defined within the sampled dates. The season likely extended from late June through early October (Fig. 2.29). Vertical tows captured *H. sanguineus* zoeae during this entire time frame, whereas zoeae were only detected in horizontal tows from late June through late September (Fig.2.29). No *H. sanguineus* larvae were found on sampling dates at the beginning and end of the season (Fig. 2.29).

Seasonal Patterns of Spawning Activity. Overall, similar seasonal spawning patterns were observed in New Hampshire amongst sampling methods and across the two years studied. In 2008, relatively low densities of early *H. sanguineus* larvae were observed in tow samples, with a seasonal range from 0-5.7 zoeae per m^3 (Fig. 2.24). The first pulse of zoeae was detected in early to mid July, with average densities in this time period of approximately 1.4 zoeae/m^3 (Fig. 1.24). The seasonal peak in density of early stage zoeae was seen on August 13th in 2008 tow samples (Fig. 2.24). 2008 trap samples averaged from zero to approximately 1700 zoeae per trap across the season (Fig. 2.25). There were two peaks in early zoeae abundance; these occurred in mid July and early August, with the larger of the peaks in August (August 13th) (Fig. 2.25). In 2009, both vertical and horizontal tows detected a single peak in early-stage zoeae density in late August (August 17th) (Fig. 2.26).

2008 Rhode Island trap samples showed a broad time period with high abundances of early *H. sanguineus* zoeae which extended from late June through late August (Fig. 2.27). Averages during this period ranged from approximately 1400-1800 zoeae per trap, with a dip in zoeae densities in early July (Fig. 2.27). In early September, number of early zoeae per trap dipped to about one third of the previous levels, down to an average of 531 zoeae per trap (Fig. 2.27). Peak abundances of early zoeae were collected in late September (Sept 17th) at an average of approximately 3397 zoeae per trap (Fig. 2.27). Zoeal abundances in traps declined from this point to the end of the sample season in late October (Fig. 2.27). Rhode Island tow data had two gaps in

sampling in 2008, occurring in late June and late July (Fig. 2.28). Tow samples showed a single peak in early zoeal density in late September (Sept 17th); early zoeal densities then decreased through the remainder of the sample dates (Fig. 2.28).

Rhode Island samples collected in 2009 were somewhat variable between horizontal and vertical tows. Both tow methods detected an initial pulse in early-stage *H. sanguineus* zoeae in late June and both also showed dips in density in early July (Fig.2.29). However, after this point, observed patterns differed between the two sampling methods. Horizontal surface tows detected highest seasonal density of early zoeae in late July and very low densities for the remainder of the season (Fig. 2.29). Notably, horizontal tows were not possible in early September due to high ctenophore densities, and tows may have been less efficient due to clogging by ctenophores from late August to late September, leading to underestimates of zoeal density. In contrast, vertical tows showed a broad period of time with relatively high densities of early zoeae, lasting from late July through early August (Fig. 2.29). There was a slight dip in density in late August, and the peak in density in vertical tows was in early September (Fig. 2.29).

Interestingly, Rhode Island 2008 trap samples and 2009 vertical tow samples showed similar seasonal patterns. Both showed peaks in early *H. sanguineus* zoeae in September. This peak occurred on September 17th in 2008 and on September 3rd in 2009 (Figs. 2.27 and 2.29). They also both showed broad periods with relatively high abundances of early zoeae from late June

through late September (2008) or early September (2009) (Figs. 2.27 and 2.29). Both methods also showed a dip in early zoeae approximately two weeks prior to their peaks (Figs. 2.27 and 2.29). If 2009 horizontal tows are excluded from the analysis because of inability to sample effectively with this method during late August and September, 2008 and 2009 peaks in spawning are were within two weeks of one another, having occurred on September 17th 2008 and September 3rd 2009 (Figs. 2.27 and 2.29).

H. sanguineus Late-Stage Larvae

Very few late stage *H. sanguineus* zoeae were found in samples in 2008 or 2009. In 2008, late-stage *H. sanguineus* zoeae were found in Rhode Island in all traps on one sample date, October 18th through the 19th. All stages of *H. sanguineus* were found on this date, though early zoeae abundances were very low (Fig. 2.27). Stage four *H. sanguineus* zoeae were also found in Rhode Island on two sample dates in 2009. One was collected in a horizontal trap sample on July 22nd and another was collected in a trap deployed near the seafloor on October 6th, 2009. One stage four *H. sanguineus* was found in New Hampshire across 2008 and 2009. This larva was collected on August 17th, 2009 in a horizontal tow sample.

Five *H. sanguineus* megalopae were collected in tows in 2008 and 6 megalopae were collected in plankton traps in 2008 (Table 2.6). No *H. sanguineus* megalopae were collected in 2009 tows.

Other Zoeae

Non-*Hemigrapsus* zoeae were detected in New Hampshire from late June through early August in 2008 (Figs. 2.30 and 2.31) and from early June through late August in 2009 (Fig. 2.31). Late June was the first sampling date in 2008. Peak densities of non-*Hemigrapsus* zoeae were found in late June in 2008 tows (Fig. 2.31). Two peaks (late June and mid July) were seen in total number of zoeae per trap in 2008 samples (Fig. 2.30). In 2009, peak densities were seen in horizontal tow samples in early June, and from late June through late July in vertical tow samples (Fig. 2.31).

Non-*Hemigrapsus* zoeae were detected In Rhode Island during the whole sampling period, from late May through late October (Figs. 2.31 and 2.32). In 2008, peak number of non-*Hemigrapsus* zoeae were found in traps in late August, and in tows in early June (Figs. 2.32 and 2.33). In 2009 peak densities were seen in vertical tows in early June and early August and in horizontal tows in early June and late July (Fig. 2.33).

Overall, non-*Hemigrapsus* zoeae seem limited to the beginning part of the sampled season in New Hampshire, with zoeae not found in September or October (Figs. 2.30 and 2.31). In contrast, In Rhode Island non-*Hemigrapsus* zoeae were found throughout the sampled season (Figs. 2.32 and 2.33).

Other Megalopae

Non-*Hemigrapsus* megalopae were detected in New Hampshire from late July through late October in 2008, and from late July though early October in 2009 (Figs. 2.34 and 2.35). Peak numbers of non-*Hemigrapsus* megalopae were

found in traps in early August 2008 with a smaller pulse in early to late September (Fig. 2.34). In 2008 tow samples a first pulse of non-*Hemigrapsus* megalopae was seen in early August, with peak densities observed in early to late September (Fig. 2.35). In 2009 peak densities of non-*Hemigrapsus* megalopae were found in both horizontal and vertical tows in late September (Fig. 2.35).

Non-*Hemigrapsus* megalopae were detected in 2008 in Rhode Island from early June through late October (Figs. 2.36 and 2.37). In 2008, peak densities were seen in early September in both traps and tows (Fig. 2.36 and 2.37). In 2009, horizontal tows showed low densities of non-*Hemigrapsus* zoeae across the season, but horizontal samples were not collected in early September (Fig. 2.37). In 2009, vertical tows showed peak densities in early June and an additional, lower peak in late August through early September (Fig. 2.37).

Non-*Hemigrapsus* megalopae showed up earlier in Rhode Island than in New Hampshire (Figs. 2.34 – 2.37). They were first detected in Rhode Island tows in early June and were observed through the remainder of the sample season at this site (Fig. 2.37). In New Hampshire, non-*Hemigrapsus* megalopae were first detected in early or late July and were also observed through the end of the sampled season in late October (Figs. 2.34 and 2.35).

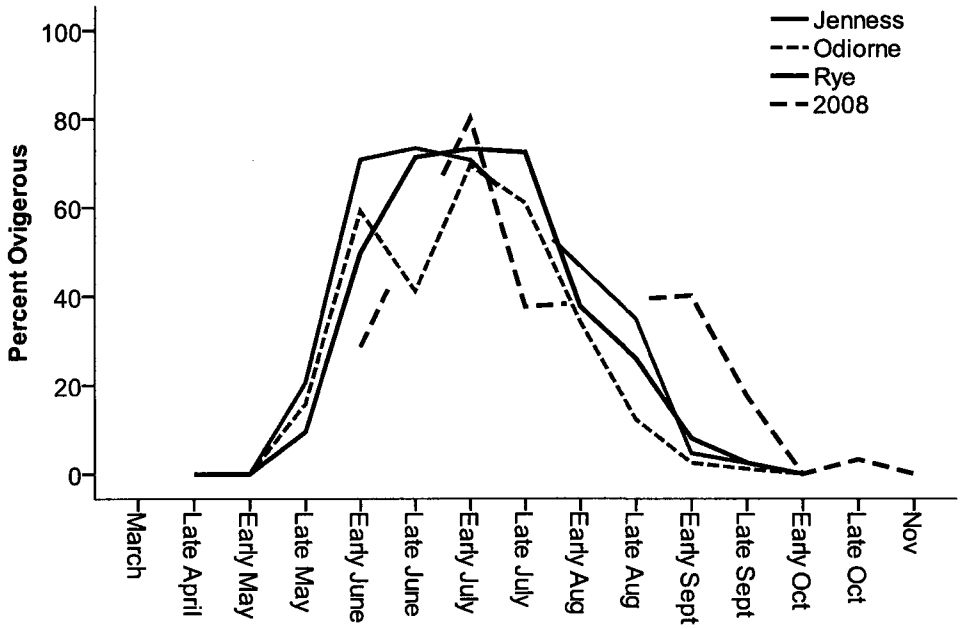

Figure 2.2: Seasonal proportion of ovigerous crabs at three sites in New Hampshire in 2009 and one site in 2008. Data was collected only from early June to November with gaps in sampling late June and all of August. In 2009, Jenness was not sampled in late July.

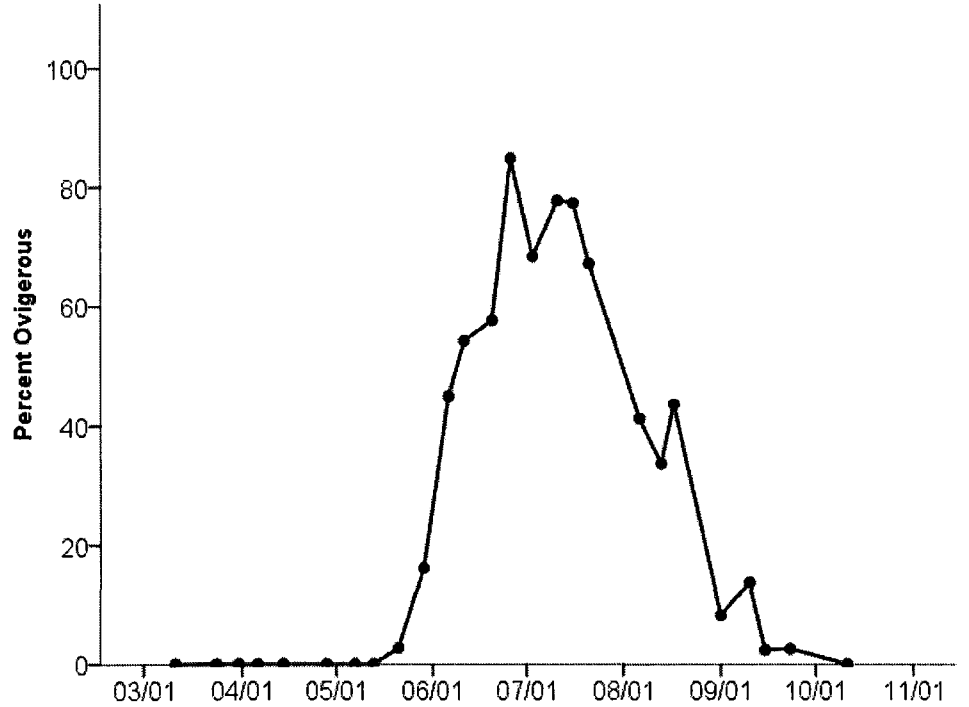

Figure 2.3: Seasonal percentage of ovigerous crabs at Rye State Park in 2009 collected at approximately weekly intervals.

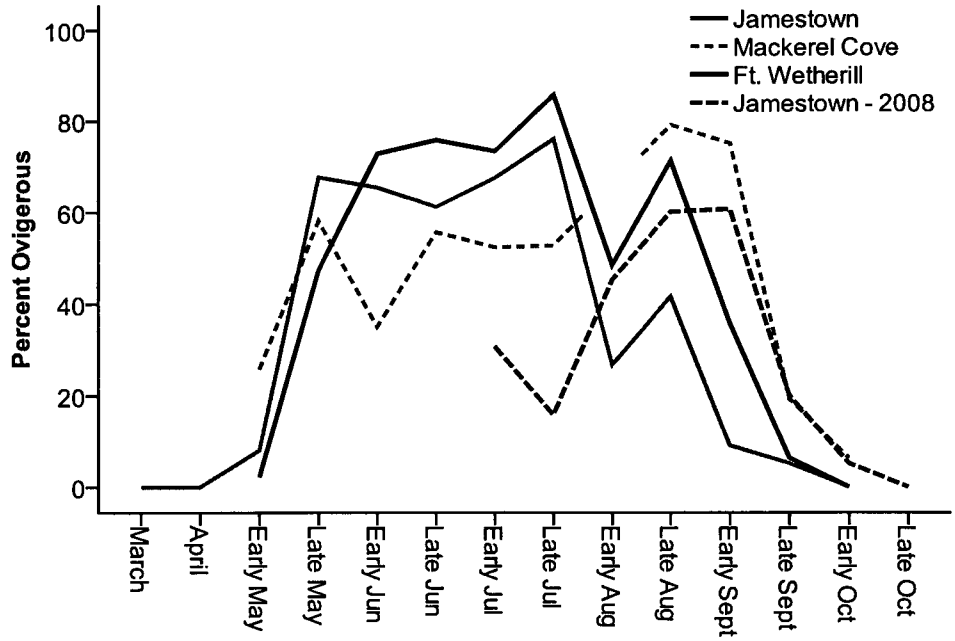

Figure 2.4: Seasonal percent of ovigerous crabs at three sites in Rhode Island in 2009 and one site in 2008. Data was collected only from early July to late October in 2008. In 2009, the gap seen in Mackerel Cove data was due to an inability to sample on this date.

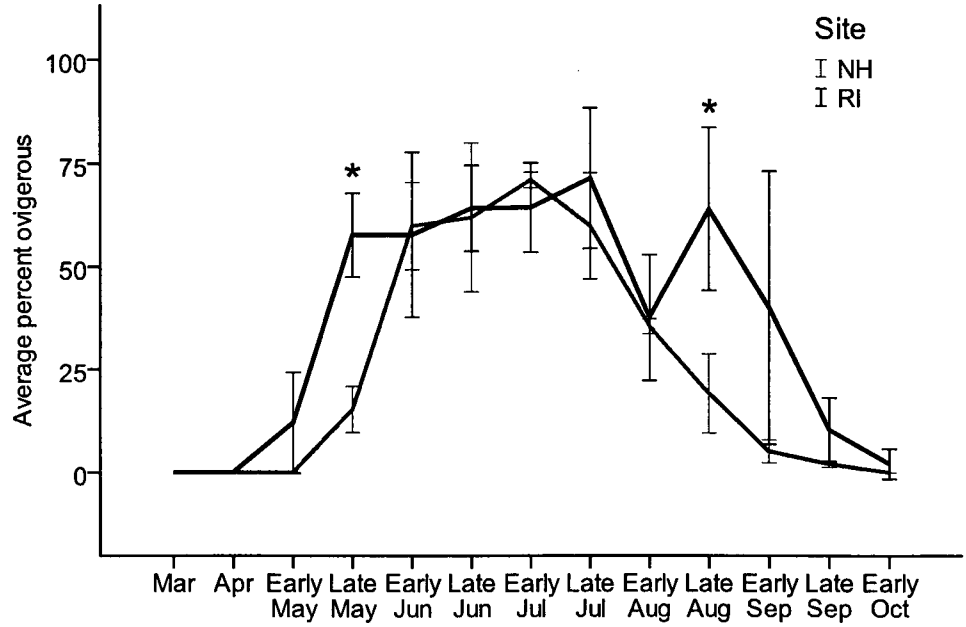

Figure 2.5: Average percent ovigerous across three local sites in New Hampshire and Rhode Island from April to October 2009. Error bars indicate standard deviation. Stars indicate dates with significant differences in percent ovigerous between regions.

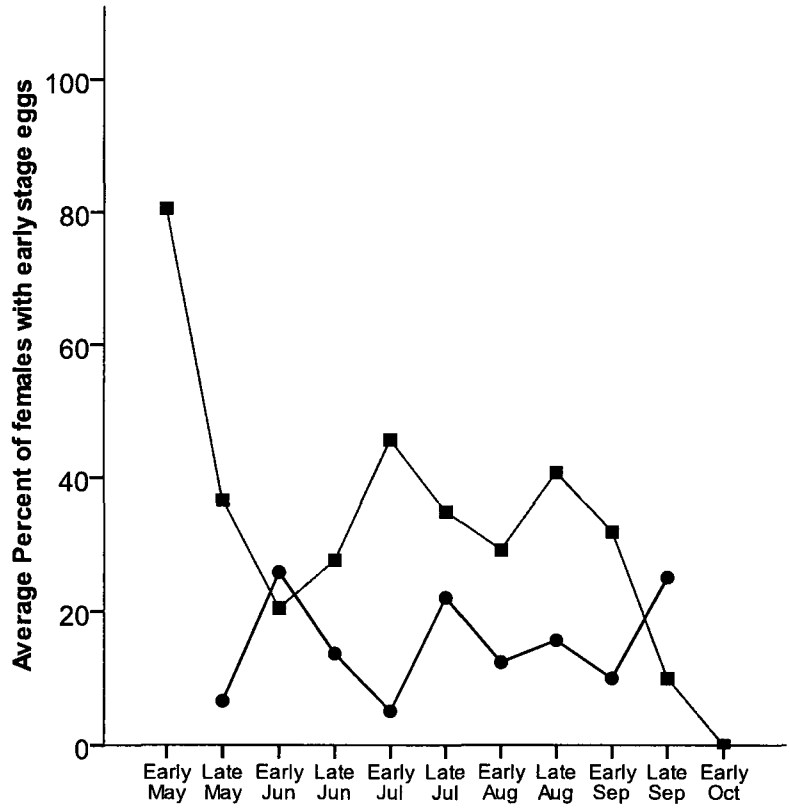

Figure 2.6: Average percent of ovigerous females carrying early stage (orange-brown) eggs. Crabs found in New Hampshire (black line, black circles), and Rhode Island adult crab surveys (gray line, gray squares).

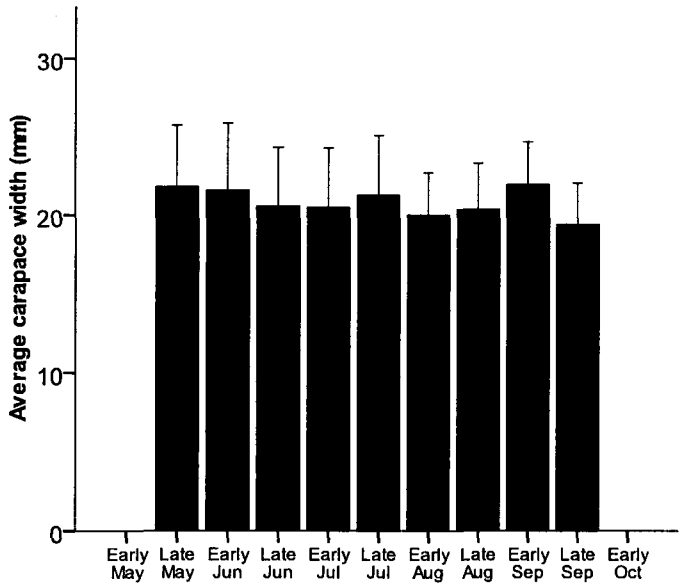

Figure 2.7: New Hampshire – average carapace width of ovigerous females by sample date. Data represent combination of three local sites in each region for each sample date. Error bars indicate ± one standard deviation from the mean. No ovigerous females were collected in early May or early October.

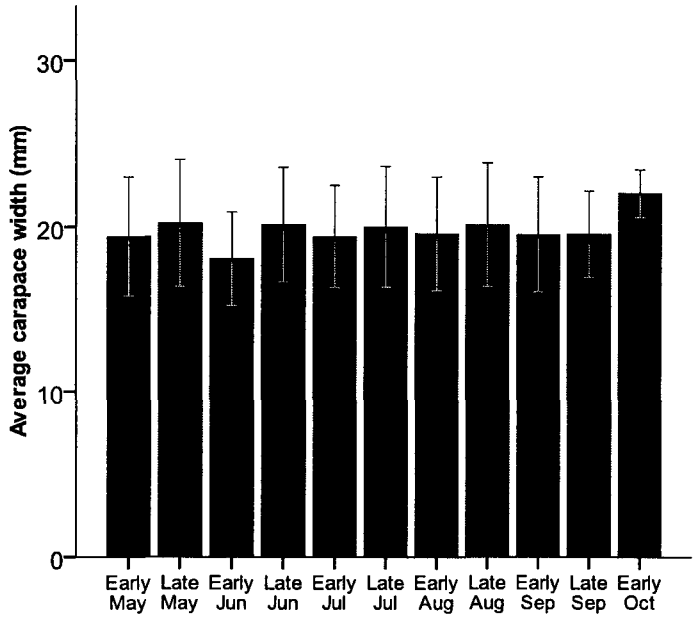

Figure 2.8: Rhode Island - average carapace width of ovigerous females by sample date. Data represent combination of three local sites in each region for each sample date. Error bars indicate ± one standard deviation from the mean.

Table 2.3: Number of ovigerous females, minimum, maximum and average (± one standard deviation) carapace width of ovigerous females from each region on each sampling date. Data for each region is a composite of data from three local sites in that region.

			Carapace width (mm)			
Site	Date	N	Minimum	Maximum	Average	Standard Deviation
New Hampshire	Early May	0	N/A	N/A	N/A	N/A
	Late May	26	17	32	21.8	3.9
	Early Jun	148	13	33	20.8	3.8
	Late Jun	199	13	35	20.6	3.7
	Early Jul	267	12	33	20.5	3.8
	Late Jul	174	15	36	21.2	3.8
	Early Aug	151	14	31	19.9	2.7
	Late Aug	99	15	30	20.3	3.0
	Early Sep	16	17	26	21.9	2.8
	Late Sep	6	15	23	19.3	2.7
	Early Oct	0	N/A	N/A	N/A	N/A
	Overall	1086	12	36	20.6	3.6
Rhode Island	Early May	22	15	26	19.4	3.6
	Late May	171	14	32	20.2	3.8
	Early Jun	170	13	27	18.1	2.8
	Late Jun	123	13	28	20.1	3.4
	Early Jul	149	13	27	19.4	3.1
	Late Jul	334	13	33	20.0	3.6
	Early Aug	92	12	31	19.6	3.4
	Late Aug	182	12	33	20.1	3.7
	Early Sep	77	13	29	19.5	3.5
	Late Sep	22	16	24	19.5	2.6
	Early Oct	2	21	23	22.0	1.4
	Overall	1344	12	33	19.7	3.5

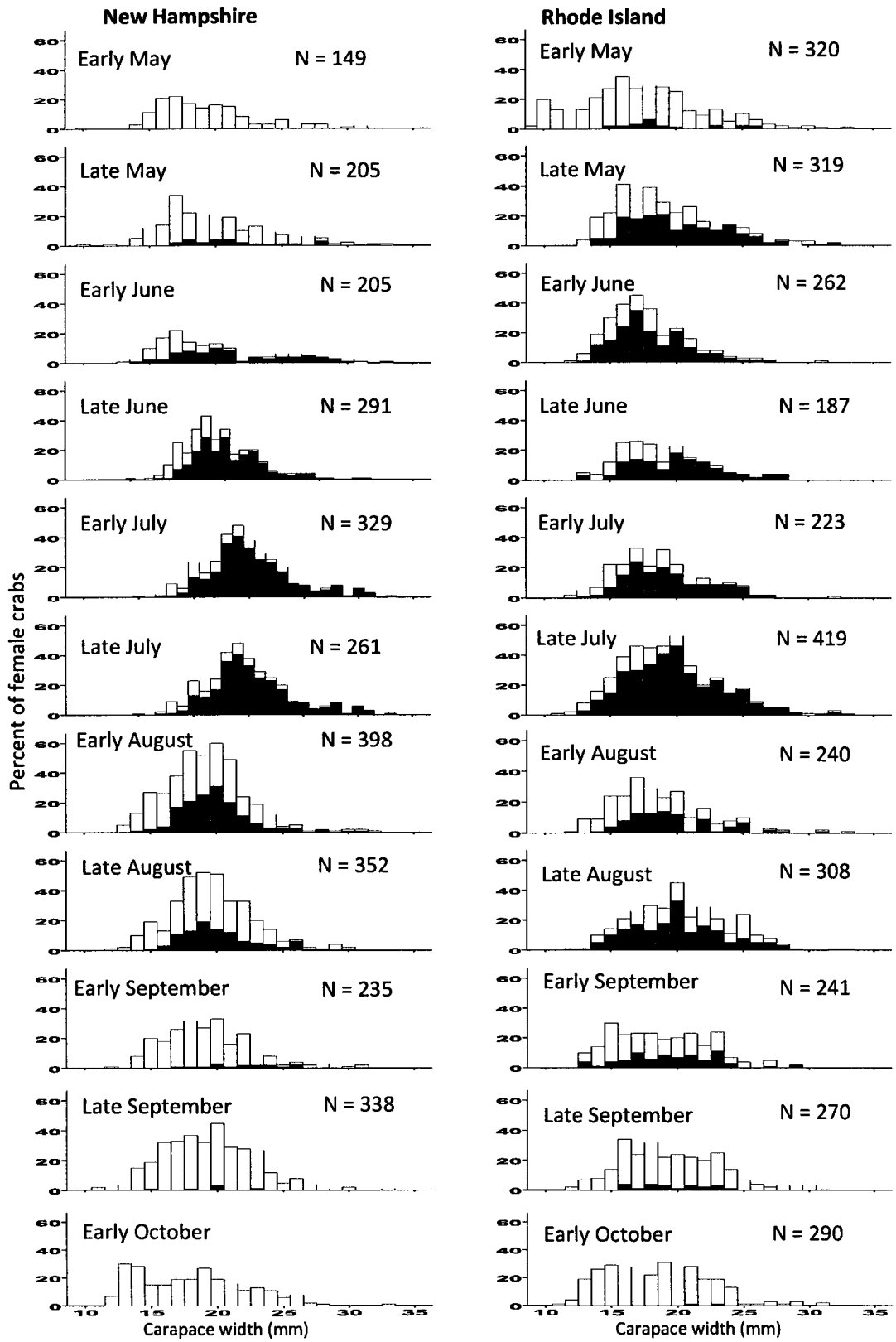
New Hampshire
Rhode Island
Early May N = 149
Early May N = 320
Late May N = 205
Late May N = 319
Early June N = 205
Early June N = 262
Late June N = 291
Late June N = 187
Early July N = 329
Early July N = 223
Late July N = 261
Late July N = 419
Early August N = 398
Early August N = 240
Late August N = 352
Late August N = 308
Early September N = 235
Early September N = 241
Late September N = 338
Late September N = 270
Early October
Early October N = 290
Percent of female crabs
0 20 40 60
10 15 20 25 30 35
Carapace width (mm)
Carapace width (mm)

Figure 2.9: Size frequency distribution for non-ovigerous (white bars), ovigerous with late stage eggs (gray bars), and ovigerous crabs with early stage eggs (black bars).

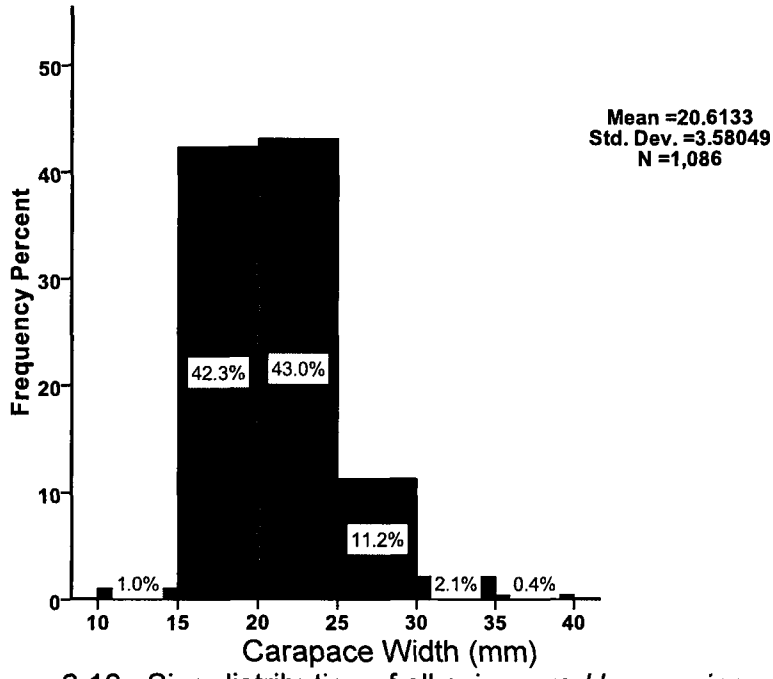

Figure 2.10: Size distribution of all ovigerous *H. sanguineus* collected in New Hampshire in 2009 surveys.

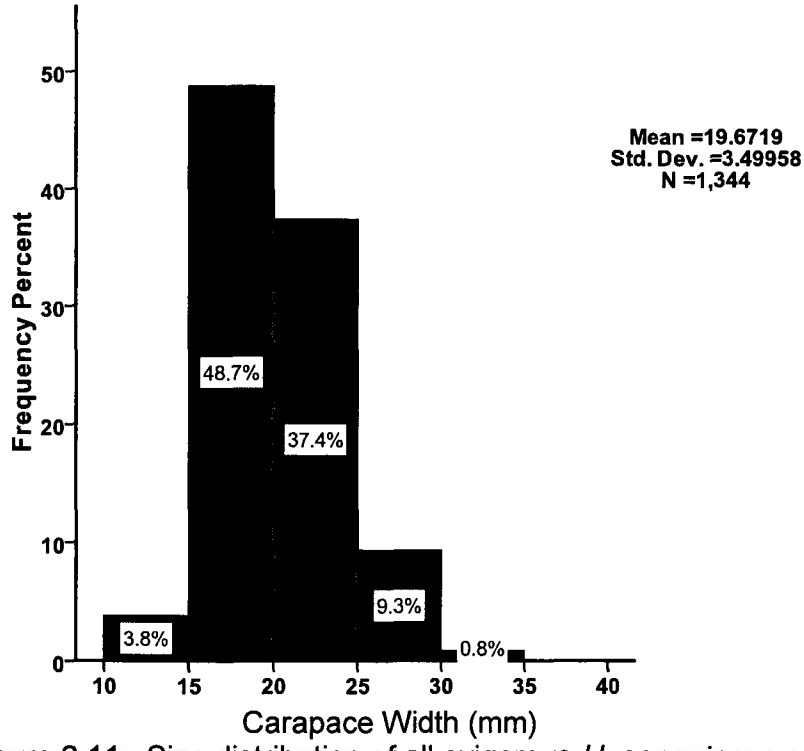

Figure 2.11: Size distribution of all ovigerous *H. sanguineus* collected in Rhode Island in 2009 surveys.

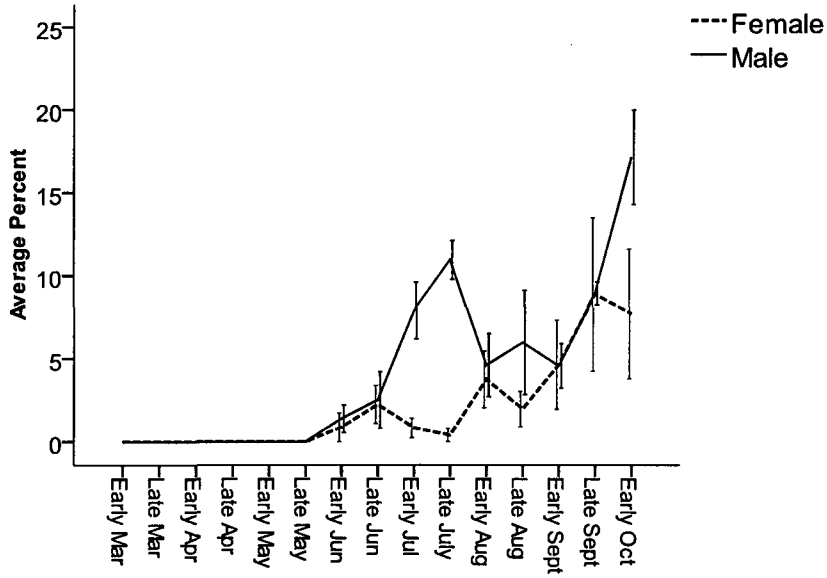

Figure 2.12: Average percent recently molted male (solid line) and female (dotted line) *H. sanguineus*. Values are averages across three local sites in New Hampshire at each time point. From early March through early April, only one site was monitored (Rye). Error bars indicate standard error.

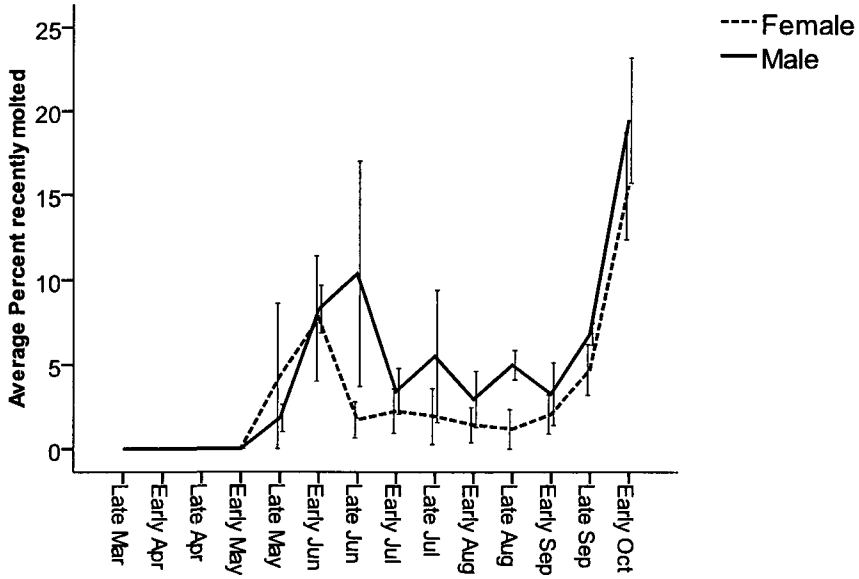

Figure 2.13: Average percent recently molted male (solid line) and female (dotted line) *H. sanguineus*. Values are averages across three local sites in Rhode Island at each time point. Only one site (Jamestown Marina) was monitored in late March through late April. Error bars indicate standard error.

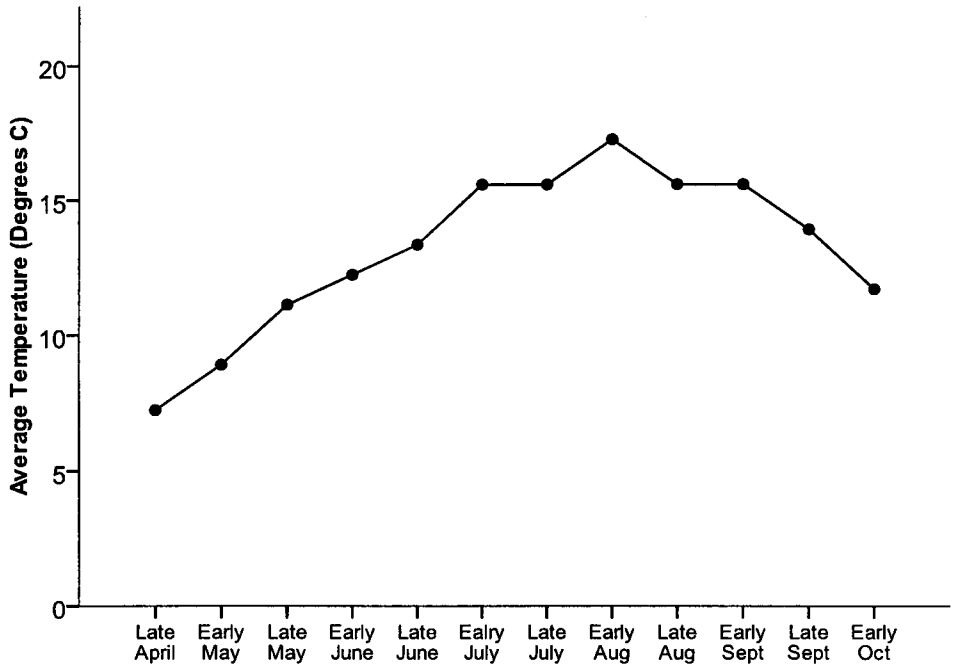

Figure 2.14: Historical average sea surface temperatures for Portsmouth Harbor, NH as compiled by NOAA's National Oceanographic Data Center.

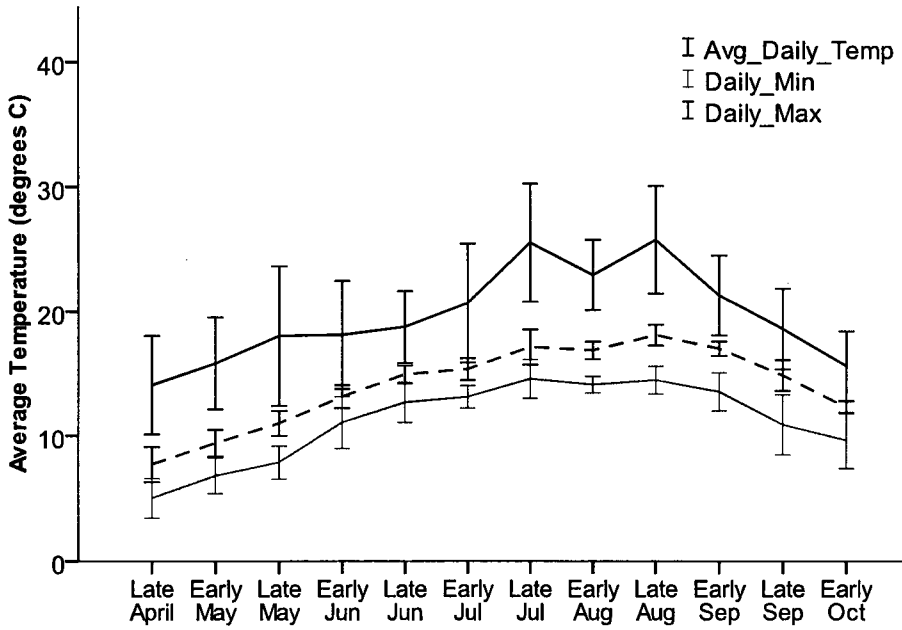

Figure 2.15: New Hampshire – daily minimum (gray), maximum (black), and average (dashed black) temperatures averaged over bi-weekly time intervals. Data collected in the mid-intertidal zone in New Castle, NH using a HOBO temperature logger collecting at 30 minute intervals.

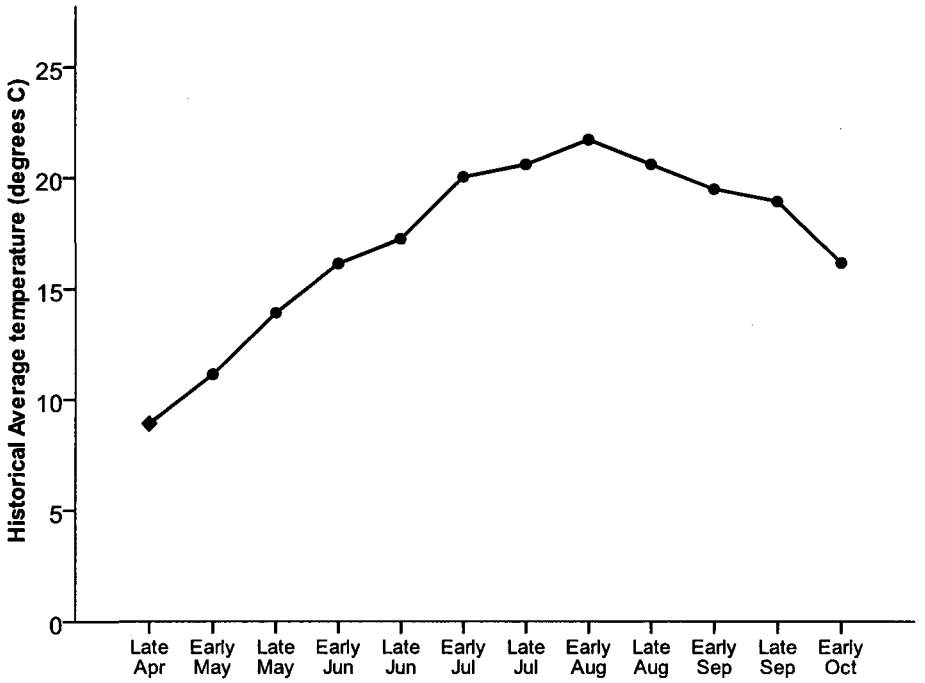

Figure 2.16: Rhode Island – historical average sea surface temperatures, averaged across bi-monthly time intervals. Data from NOAA's National Oceanographic Data Center.

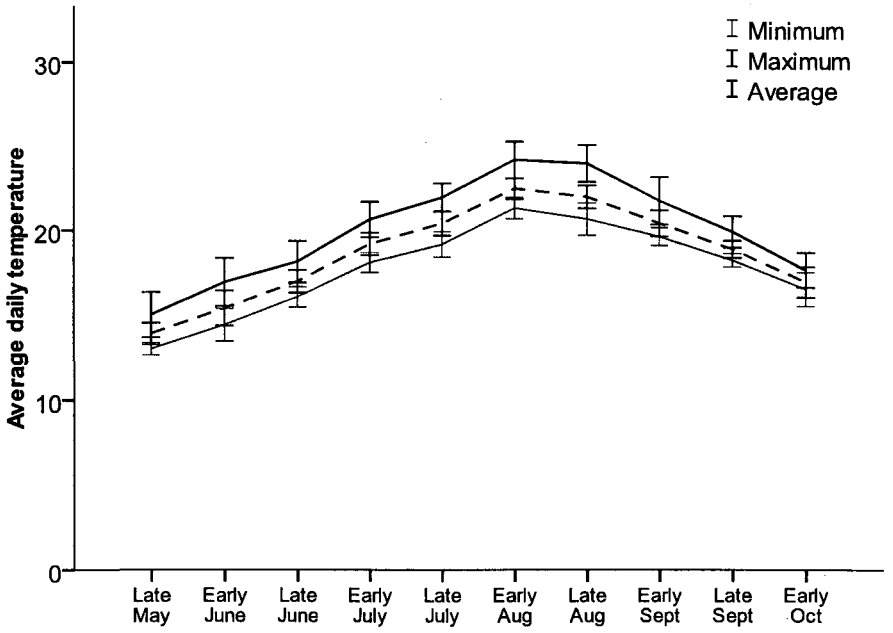

Figure 2.17: Rhode Island – daily minimum (gray), maximum (black), and average (dashed black) temperatures averaged across bi-weekly intervals. Surface temperature data collected with HOBO temperature logger deployed in Jamestown, RI. Error bars indicate ± one standard deviation from the mean.

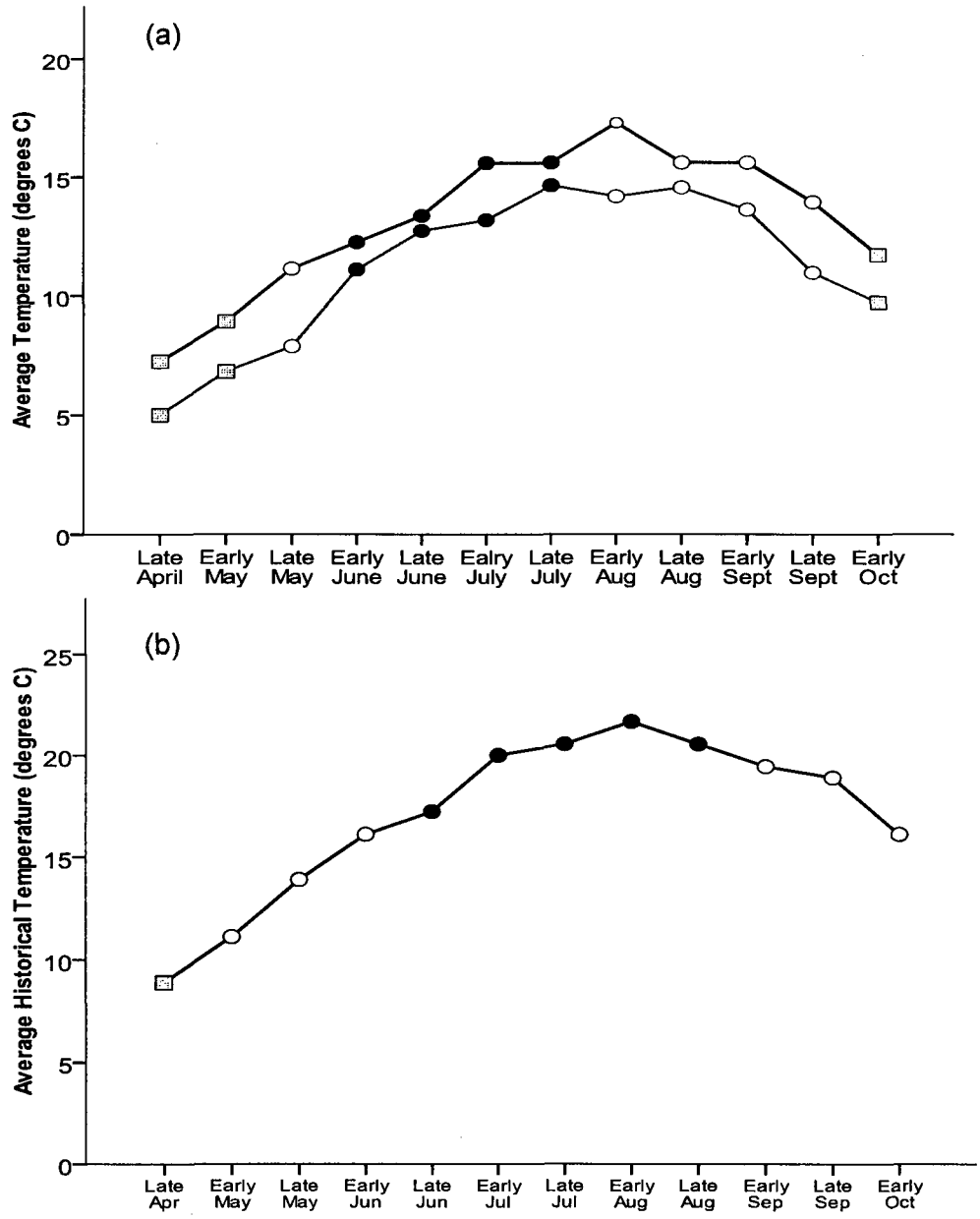

Figure 2.18: Correlation of seasonal temperature with observations of ovigerous females in New Hampshire (a) and Rhode Island (b) field surveys. The upper (black) line in figure (a) represents historical average bi-monthly temperature data for Portsmouth Harbor, NH from NOAA's National Oceanographic Data Center. The bottom (gray) line in figure (a) represents 2009 intertidal temperature data collected via HOBO temperature logger in New Castle, NH. Dates with no ovigerous females (gray squares), less than 50% ovigerous (white circles), and greater than 50% ovigerous (black circles) are indicated by shapes for each sample date.

Table 2.4: Average monthly temperature calculated using bi-monthly historical data (NODC) or temperature data collected by in each region with HOBO temperature loggers. New Hampshire temperatures were calculated from intertidal daily averages and Rhode Island temperatures were calculated with average daily sea surface temperatures. Brood duration estimates based on brood durations observed for New Hampshire crabs in Chapter I laboratory experiment.

Site	Month	Historical Avg. temp (°C)	Avg. temp (°C)	Brood duration estimate (# days)
New Hampshire	June	12.8	14.1	> 33 days
	July	15.6	16.4	~ 33 days
	August	16.4	17.6	19-33 days
	September	14.7	15.9	~ 33 days
	June-September	14.9	16.0	≥ 33 days
Rhode Island	May	12.5	No data	>> 33 days
	June	16.7	16.2	~ 33 days
	July	20.3	19.8	~19 days
	August	21.1	22.2	13-19 days
	September	19.2	19.6	~ 19 days
	May - September	17.9	Incomplete data	19-33 days

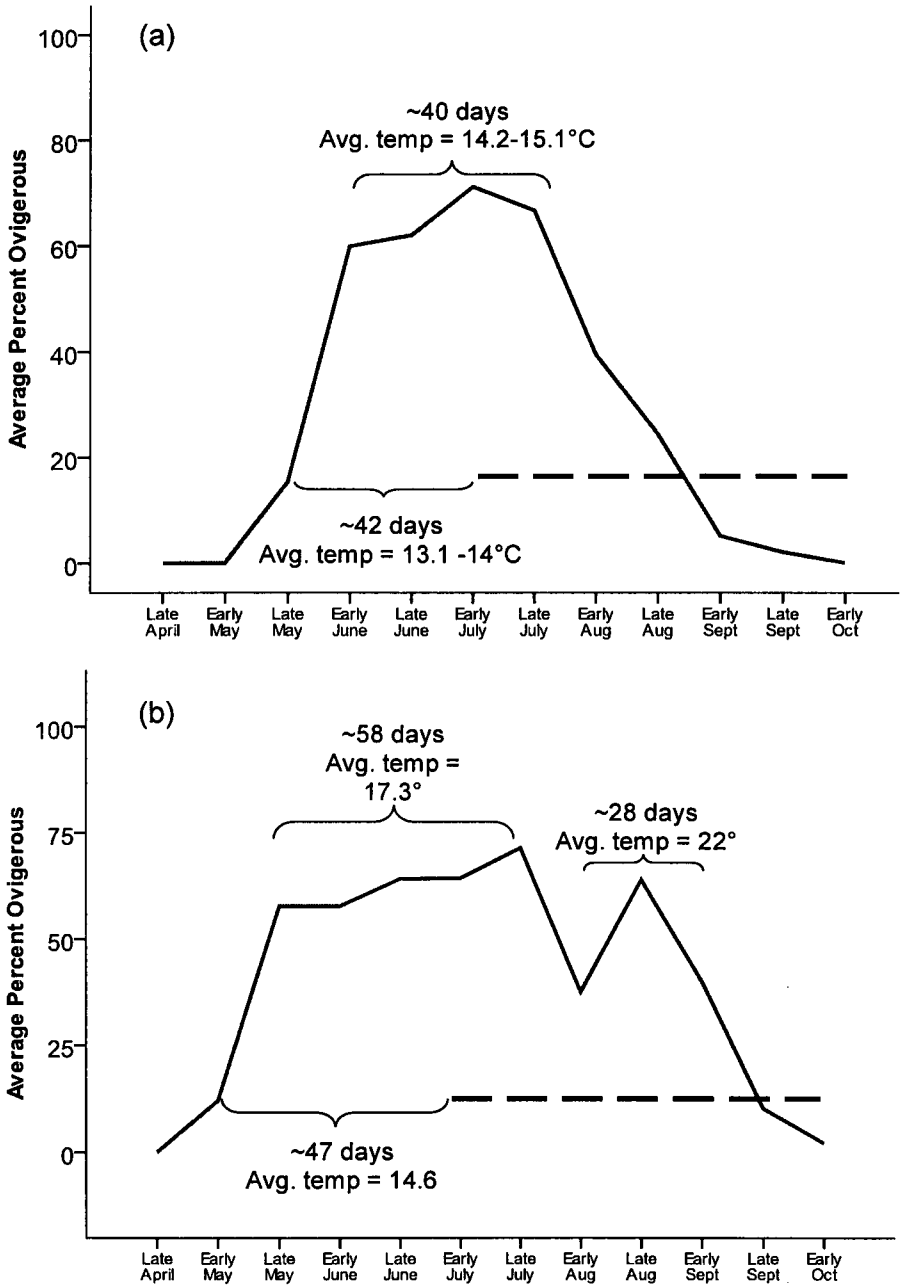

Figure 2.19: Graphical representation of percentage of ovigerous crabs and the timing of early zoeae presence in plankton samples (indicated by dashed line) across the 2009 season. Average temperatures are given for both historical data (NODC) and average daily intertidal temperatures from HOBO temperature logger during certain time periods. Figure (a) represents New Hampshire data and figure (b) represents Rhode Island data.

Table 2.5: New Hampshire - percentage of days within given bi-monthly intervals with average daily temperatures greater than certain temperatures from 9 to 20°C. Temperature data collected at 30 minute intervals using a HOBO temperature logger in the mid-intertidal zone in New Castle, NH were averaged over daily time intervals.

Date Range	9°C	10°C	11°C	12°C	13°C	14°C	15°C	17°C	19°C	20°C
Late April	18.8	6.3	0	0	0	0	0	0	0	0
Early May	57.1	40	0	0	0	0	0	0	0	0
Late May	100	81.3	50	25	0	0	0	0	0	0
Early June	100	100	100	93.3	46.7	20	6.7	0	0	0
Late June	100	100	100	100	100	93.3	66.7	0	0	0
Early July	100	100	100	100	100	100	46.7	7.1	0	0
Late July	100	100	100	100	100	100	93.8	58.8	0	0
Early August	100	100	100	100	100	100	100	35.7	0	0
Late August	100	100	100	100	100	100	100	94.1	11.8	0
Early Sept	100	100	100	100	100	100	100	50	0	0
Late Sept	100	100	100	6.7	93.3	93.3	40	0	0	0
Early Oct	100	100	100	40	0	0	0	0	0	0

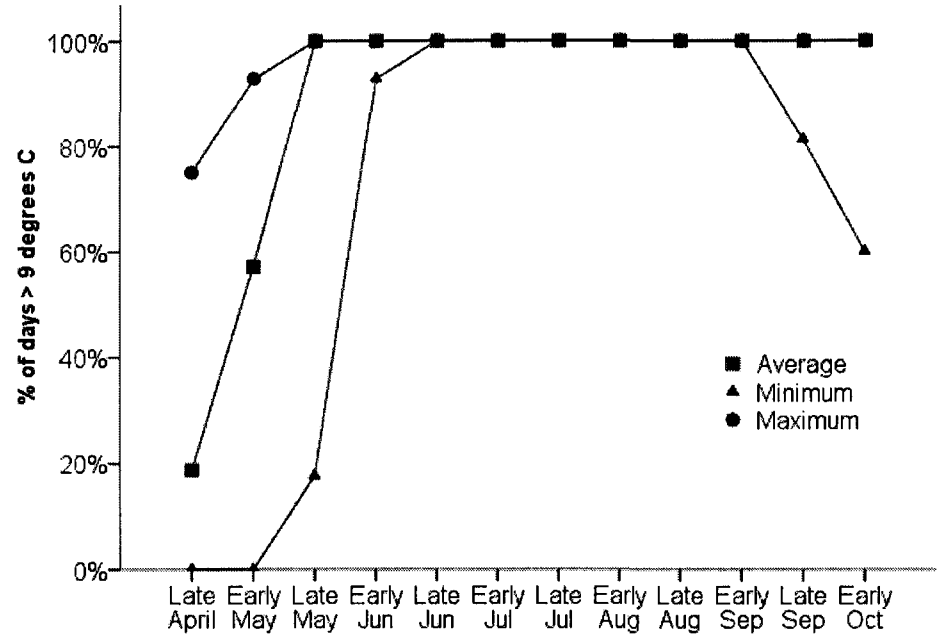

Figure 2.20: New Hampshire – percent of days within a given bi-monthly interval with maximum (circles), minimum (triangles) and average (squares) daily intertidal temperatures greater than 9°C. Analysis of degree days >10° shows approximately the same pattern.

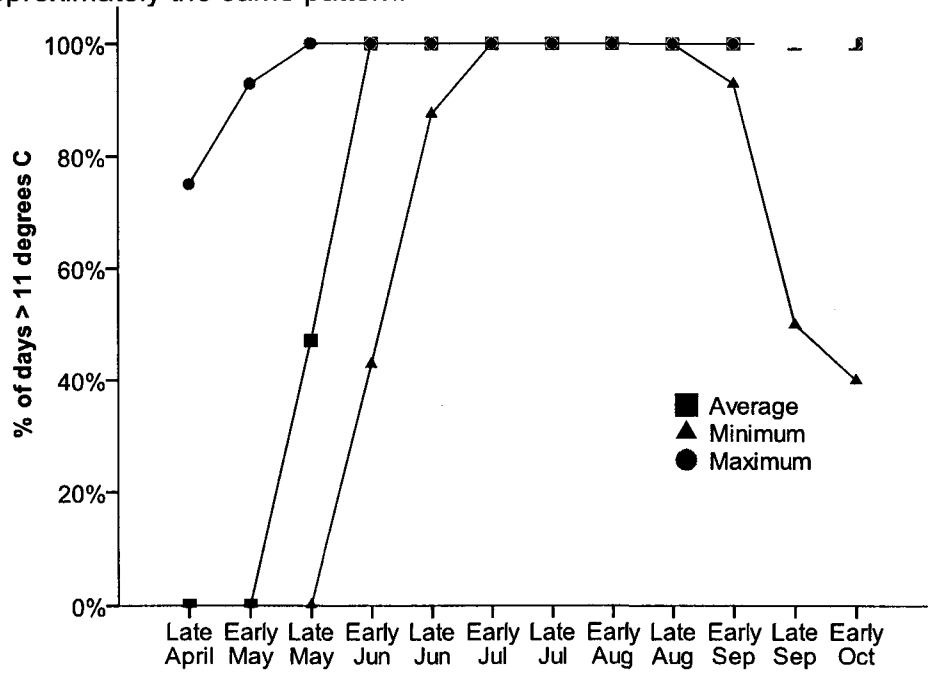

Figure 2.21: New Hampshire – percent of days within a given bi-monthly interval with maximum (black line), minimum (gray line) and average (dashed line) daily temperatures greater than 11°C. Temperature data was collected at 30 minute intervals in the min-intertidal zone in New Castle, NH by a HOBO temperature logger.

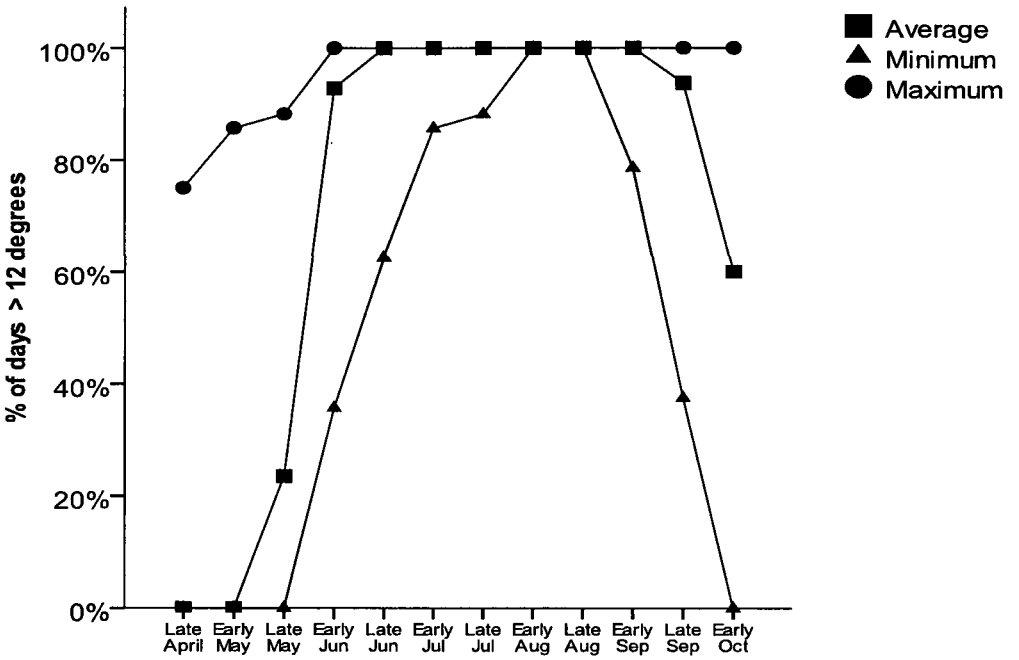

Figure 2:22: New Hampshire - percent of days within a given bi-monthly interval with maximum (circles), minimum (triangles) and average (squares) daily intertidal temperatures greater than 12°C.

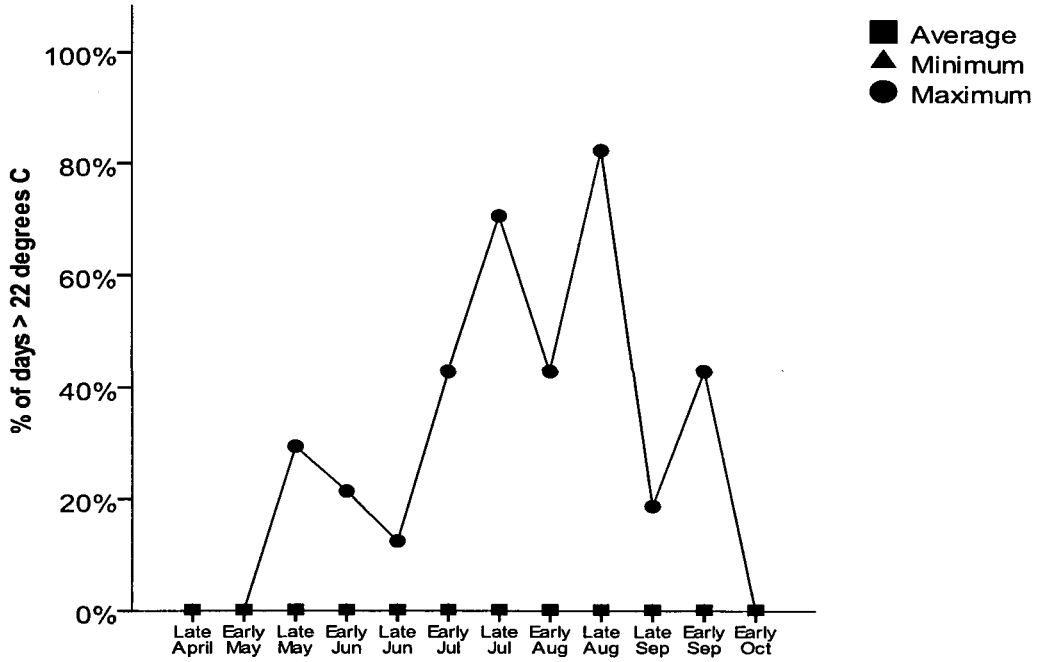

Figure 2.23: New Hampshire – percent of days within a given bi-monthly interval with maximum (circles), minimum (triangles) and average (squares) daily intertidal temperatures greater than 22°C.

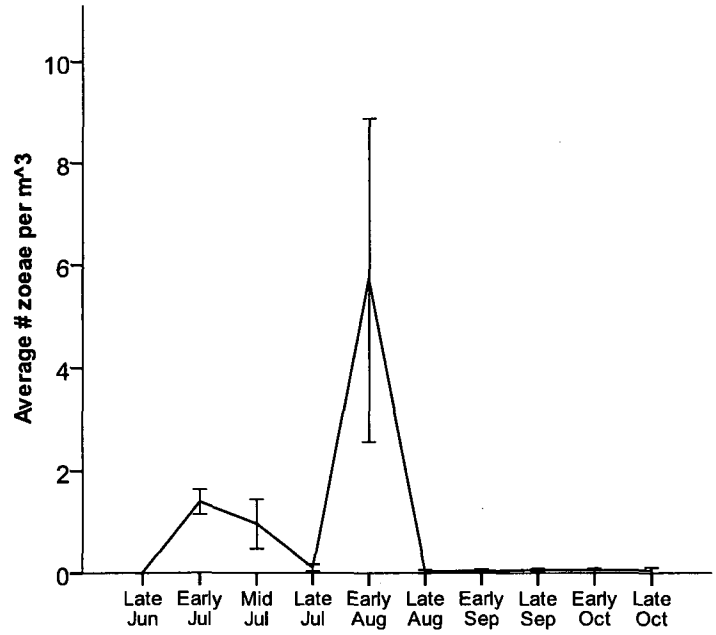

Figure 2.24: Average density of early stage *H. sanguineus* larvae from New Hampshire horizontal surface tows collected in 2008. Error bars indicate standard error.

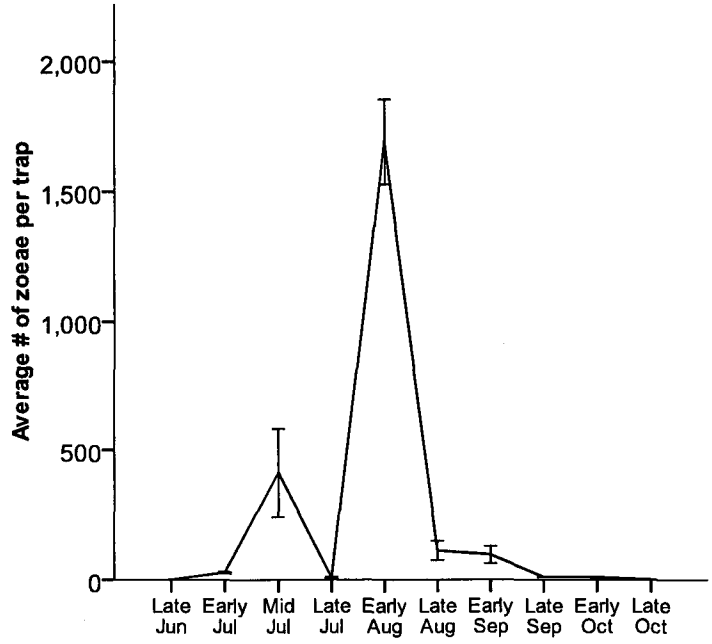

Figure 2.25. New Hampshire plankton traps deployed in 2008 – average number of early stage zoeae per trap. Error bars indicate standard error.

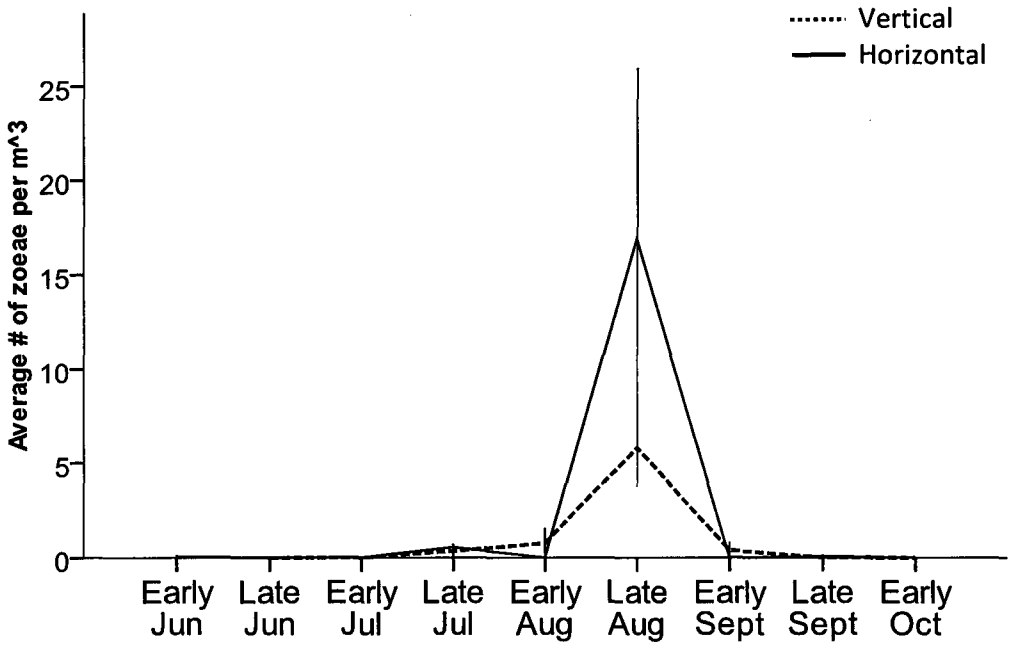

Figure 2.26: New Hampshire tow samples collected in 2009 – average number of early stage *H. sanguineus* zoeae per cubic meter of water sampled in vertical (dotted line) and horizontal (solid line) tows. Error bars indicate standard error.

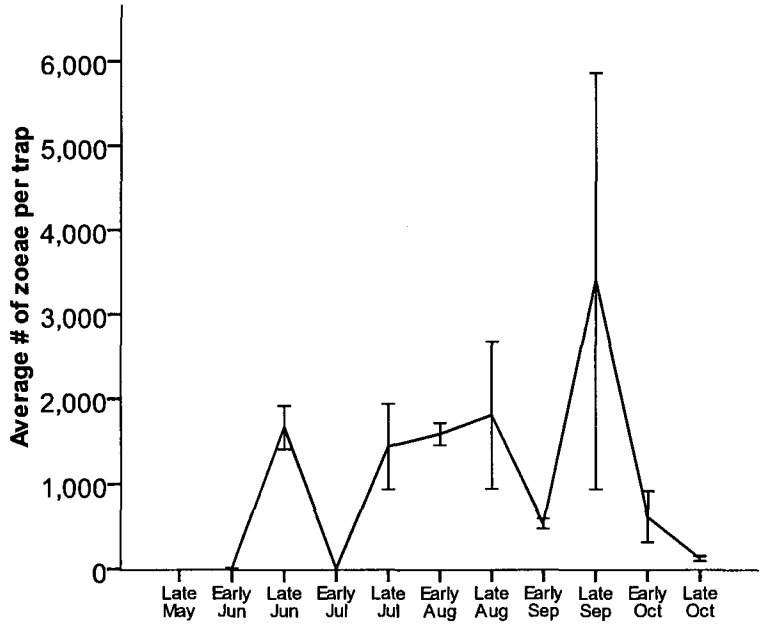

Figure 2.27: Average number of early stage *H. sanguine*us zoeae in Rhode Island trap samples collected in 2008. Error bars indicate standard error.

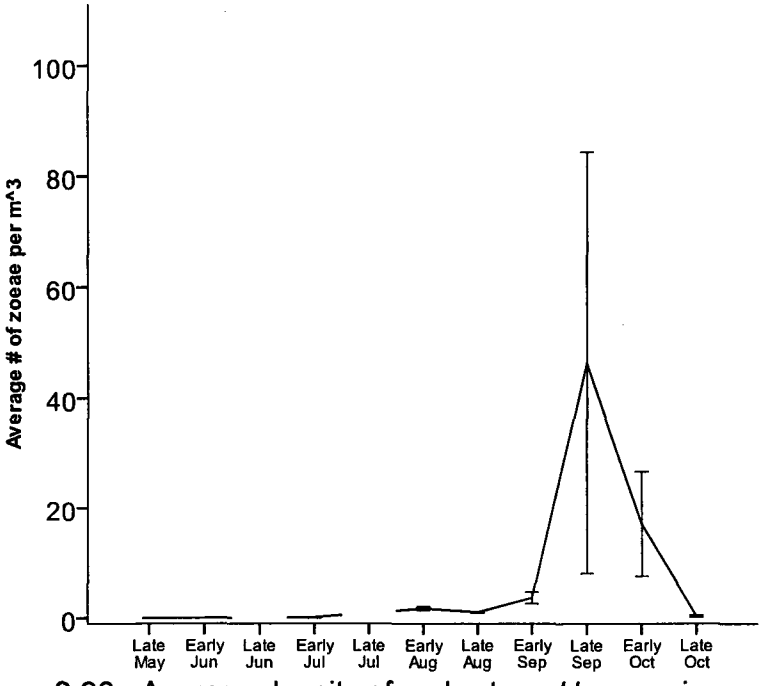

Figure 2.28: Average density of early stage *H. sanguineus* zoeae in Rhode Island surface tows collected in 2008. Error bars indicate standard error. Gaps in line represent dates when sampling was not conducted.

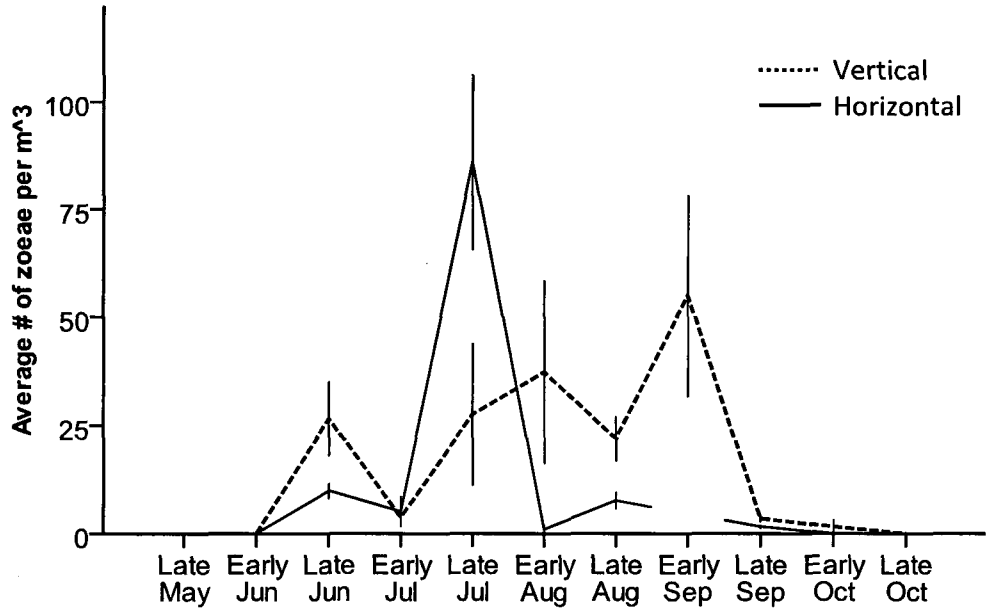

Figure 2.29: Rhode Island vertical (dotted line) and horizontal (solid line) tow samples collected in 2009. Lines indicate average number of early stage *H. sanguineus* zoeae per m^3. Error bars indicate standard error. Gap in black line (Horizontal tows) was due to an inability to collect horizontal samples on this date because of high densities of ctenophores.

Table 2.6: Dates of *H. sanguineus* megalopae collection in plankton tows and traps and number collected.

Date Collected	Collection method	Number collected
8/29/08	Horizontal surface tow	2
	Plankton trap	3
9/11/08	Plankton trap	1
9/25/08	Horizontal surface tow	3
	Plankton Trap	2

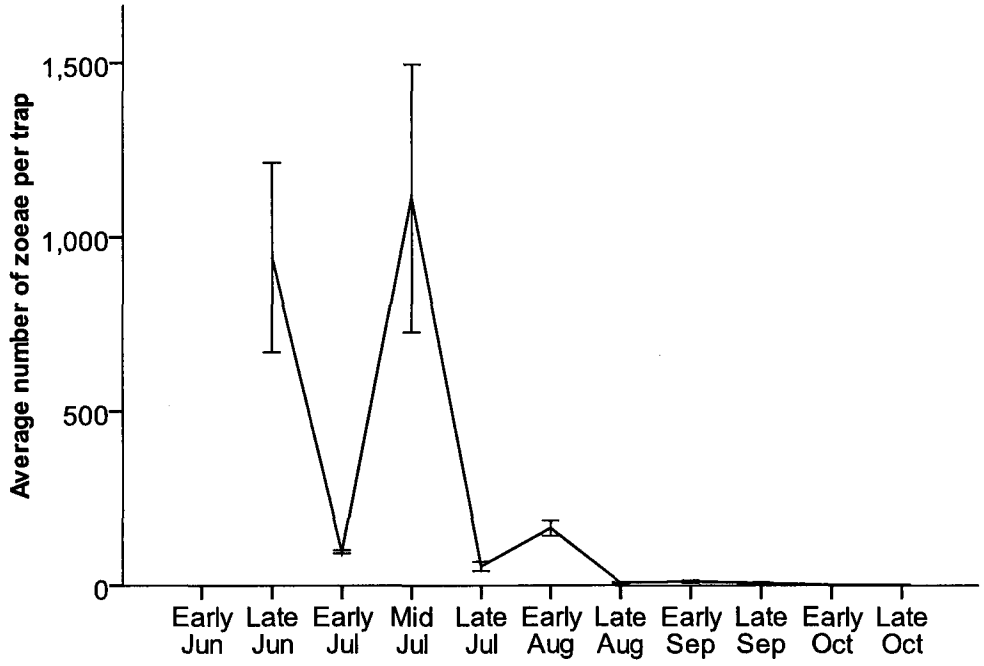

Figure 2.30: New Hampshire – average number of non-*Hemigrapsus* zoeae per trap collected in plankton traps deployed in 2008. Error bars indicate standard error.

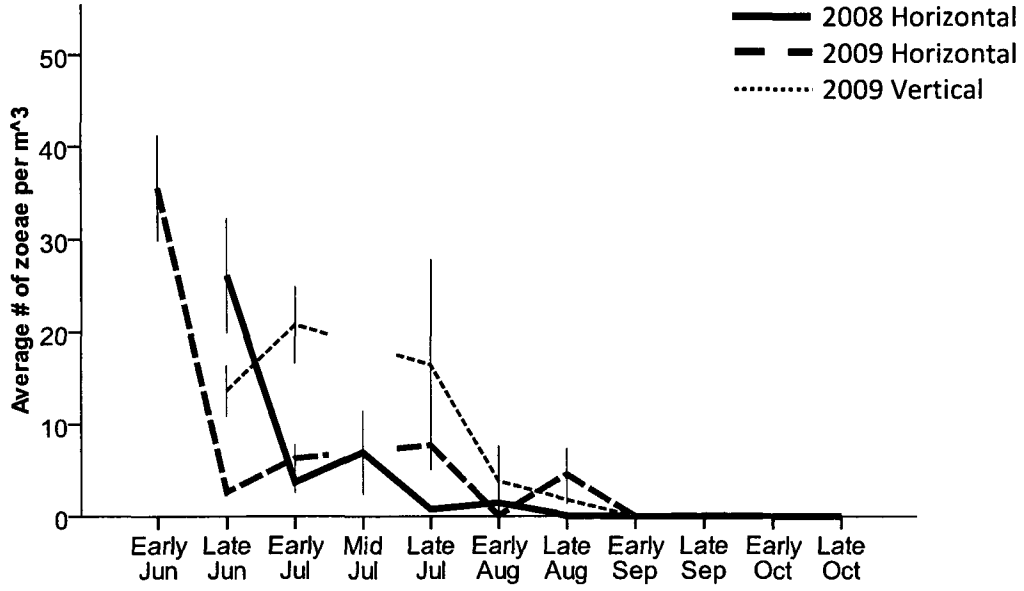

Figure 2.31: New Hampshire – average number of non-*Hemigrapsus* zoeae per m^3 from horizontal tow samples collected in 2008 and vertical and horizontal tow samples collected in 2009. Error bars indicate standard error. Gaps in lines indicate time periods when samples were not collected.

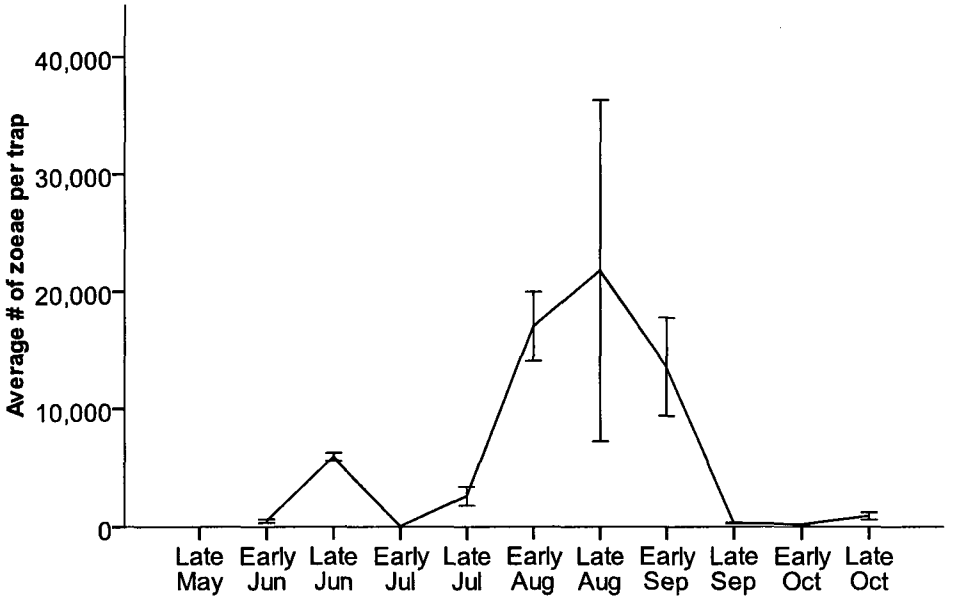

Figure 2.32: Rhode Island – average number of non-*Hemigrapsus* zoeae found in traps deployed in 2008. Error bars indicate standard error.

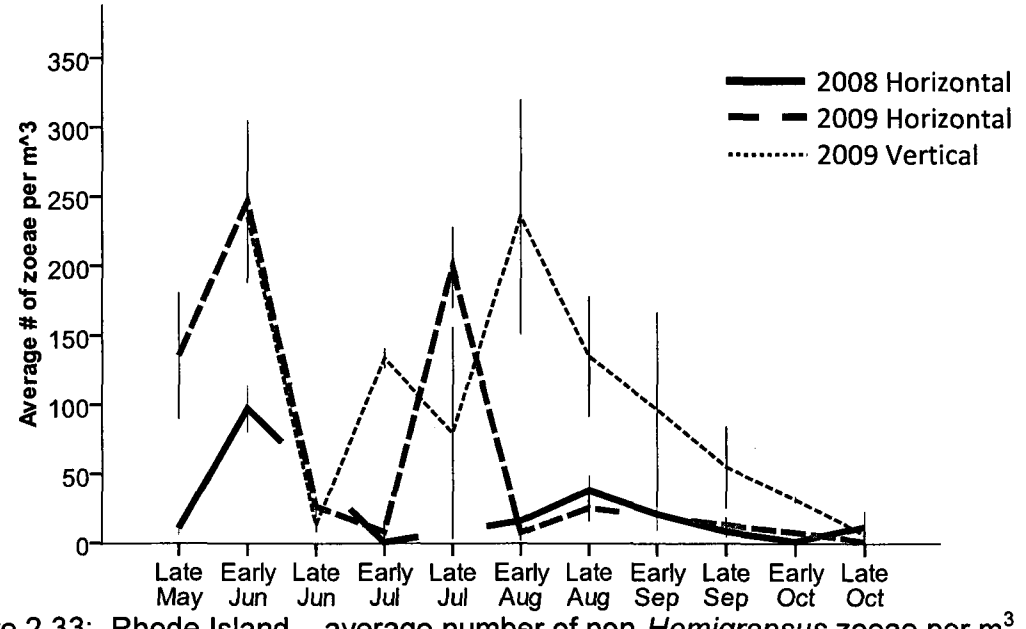

Figure 2.33: Rhode Island – average number of non-*Hemigrapsus* zoeae per m^3 found in 2008 horizontal and 2009 horizontal and vertical tows. Error bars indicate standard error.

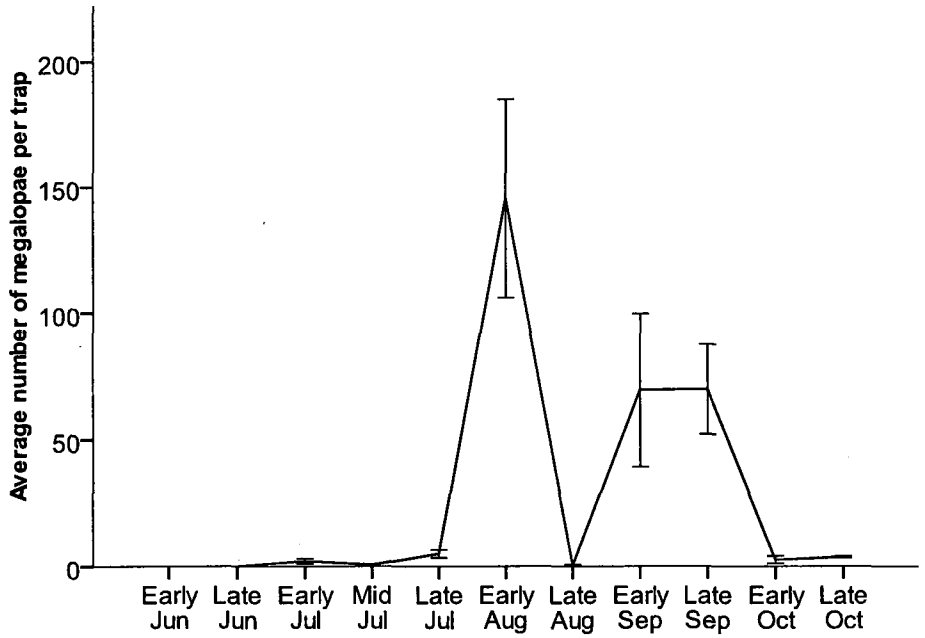

Figure 2.34: New Hampshire – average number of non-*Hemigrapsus* megalopae per trap from traps deployed in 2008. Error bars indicate standard error.

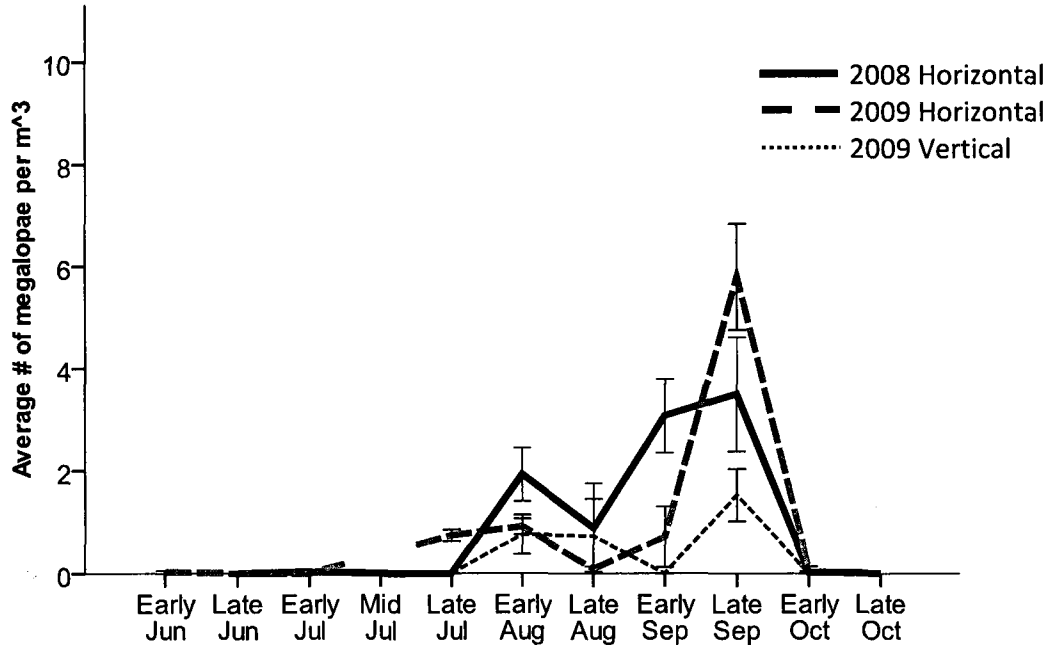

Figure 2.35: New Hampshire – average number of non-*Hemigrapsus* megalopae per m^3. Data shown for horizontal tows collected in 2008 and 2009 and vertical tows collected in 2009. Error bars indicate standard error.

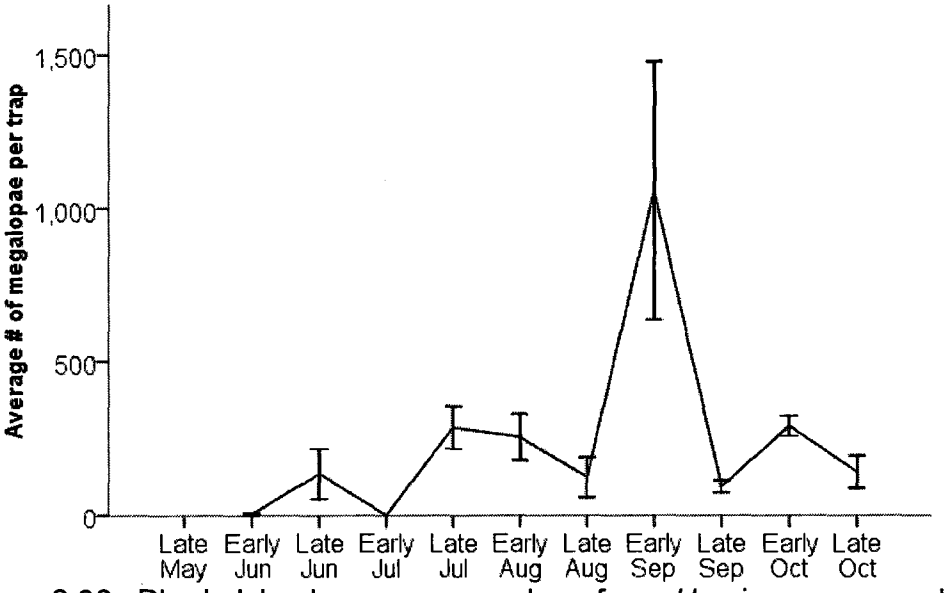

Figure 2.36: Rhode Island – average number of non-*Hemigrapsus* megalopae found in plankton traps deployed in 2008. Error bars indicate standard error.

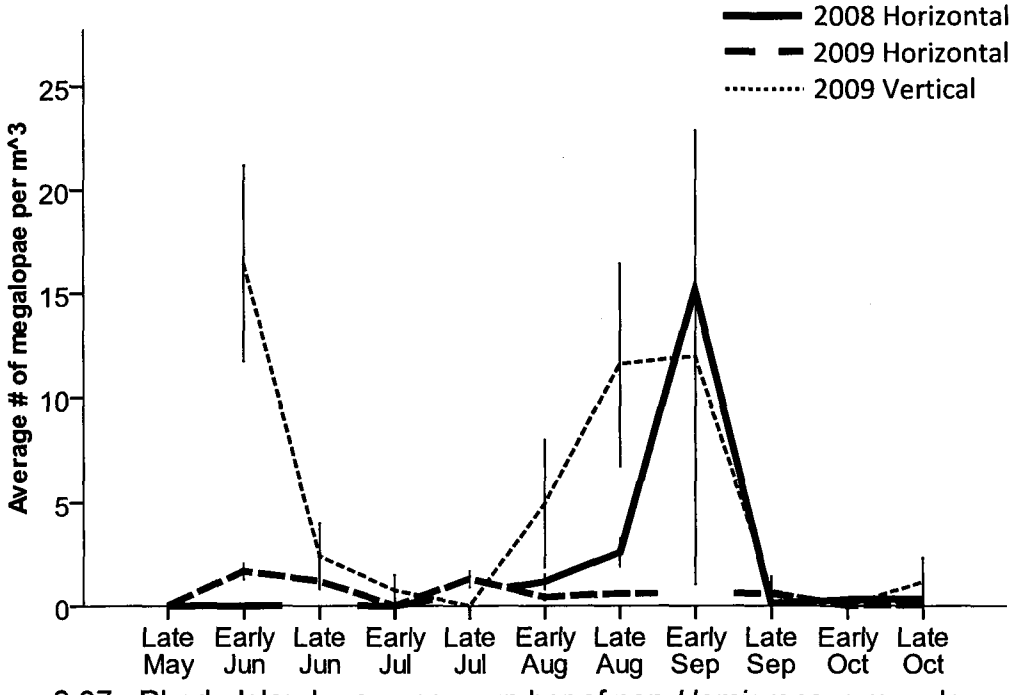

Figure 2.37: Rhode Island – average number of non-*Hemigrapsus* megalopae per m^3 found in horizontal tow samples collected in 2008 and horizontal and vertical tow samples collected in 2009. Error bars indicate standard error.

Discussion

Seasonal Patterns of Brooding Activity

Field surveys in New Hampshire and Rhode Island allowed for the characterization of reproductive cycles in the two regions studied. Brooding season lasted for approximately 4 months in New Hampshire and 5.5 months in Rhode Island in 2009. These season durations as well as seasonal patterns were upheld across three local sites in each region (Figs. 2.2, 2.4 and 2.5). Spawning season started approximately 42 or 47days (NH and RI respectively) after the initiation of brooding season, and extended into October at both sites (Figs.2.5 and 2.19). Larval development times could not be estimated through this study, as *H. sanguineus* megalopae were captured very infrequently with plankton sampling methods used in this study.

The observed difference in season length conforms to expectations given the change in latitude between the sites. The pattern of shorter reproductive seasons at higher latitudes has been reported for a number of brachyuran crabs, as well as for *H. sanguineus* in its native range (Boolootian et al. 1959, Knudsen 1964, McDermott 1998, Ituarte et al. 2006). Although the New Hampshire site is very close in latitude to the northernmost site studied in Japan (43°N), season was not as restricted in the US (Takahashi et al. 1985a, McDermott 1998). At 43°N in Japan, reproductive season for *H. sanguineus* was reported as three months in duration, from June through August (Takahashi et al. 1985a, McDermott 1998) versus four months observed in coastal New Hampshire.

Differences in season length between the regions were due to earlier season start in Rhode Island. Surveys were conducted in both regions beginning in March 2009, so estimates of season initiation should be accurate. Surveys were conducted weekly in New Hampshire, so start of season can be estimated with a higher degree of certainty that in Rhode Island where surveys were conducted bi-weekly. However, credence is leant to the estimation of season start in Rhode Island by the fact that approximately 80% of ovigerous females were carrying early-stage eggs on this date. A small proportion of these initial brooders may have oviposited more than a few days prior to the early May sample date. But, according to the laboratory study in Chapter I, 72% of recently extruded eggs lose their orange hues within 1-2 days of oviposition, which suggests that the majority of ovigers on May 9th oviposited within a few days of this survey.

Though the timing of season start is offset between the two regions (Fig. 2.5), the pattern of season initiation is similar. The first appearance of ovigerous females marked an 8-15% increase in two weeks (RI and NH respectively). Percent ovigerous females then rose steeply at both sites in the second two-week period with an average increase of 45 -60% (NH and RI respectively) (Figure 2.5). This beginning of the season pattern is likely due to a close synchrony in season initiation for the majority of females, with some outliers (approximately 10%) beginning to brood a few days or weeks earlier. This synchrony is further evinced by the presence of 80% of females carrying early-

stage eggs on the first sample date in May in Rhode Island (across local sites) (Fig 2.6).

The duration and pattern of ovigery in the two regions studied can be used to make predictions about the number of broods females in each population produce per year. Though New Hampshire and Rhode Island brooding seasons ended at approximately the same time in 2009, levels of ovigery differed during the latter part of the season (Fig. 2.5). In August, Rhode Island females showed a dip and subsequent peak in percent ovigery, whereas New Hampshire populations showed a slow decline in ovigery (Fig. 2.5). The most obvious explanation for the bimodal pattern seen in Rhode Island is the production of an additional brood of eggs for a percentage of the population. Because the New Hampshire population shows a narrower, unimodal pattern of ovigery (Fig. 2.5), most *H. sanguineus* females in this region are likely producing only one brood of eggs per season in this region.

Seasonality and Predicted Brood Duration

New Hampshire

Seasonal temperatures combined with implications for brood duration from lab experiments indicate that brood durations in New Hampshire likely range from 19 to greater than 33 days (Table 2.4). During all months but August, temperatures indicate that brood durations will be around 33 days or greater (Table 2.4). Based on this prediction, some crabs may produce two or three broods within the approximately 126 day season (Fig. 2.3). However, the majority of brooding activity was observed during an approximately 40 day period

(Fig. 2.19), which suggests that the majority of crabs are producing one brood of eggs. The broadness of the window (slightly wider than required for one brood) is most likely due to slightly off-set brood timing within the population. The tapering off of ovigery from early August through September is likely a combination of crabs producing initial broods late in the season, and a small number of crabs producing second clutches late in the season. This conclusion is strengthened by the field experiment conducted in Chapter I of this work (Fig. 1.17)

Rhode Island

A broad period of ovigery, interrupted by a dip and subsequent increase, was observed in Rhode Island in 2009 (Fig. 2.5). The initial period of ovigery lasted approximately 58 days (Fig. 2.19). Based on the prediction of brood durations of 19 to 33 days during this period (see Results), it is possible that some females may have produced two broods within this window. Levels above 50% during this entire window further support this conclusion. This window is also likely shaped by off-set brood timing within the population.

Because the proportion ovigerous increases approximately 15% between the dip seen in early August and the second peak seen in late August, it can be predicted that at least 15% of females produced an additional brood of eggs at this time. Brood durations would be quite short during this time of year (between 13 and 19 days), which may explain why high levels of ovigery were only observed on one sample date following the dip in early August. This dip and

subsequent increase were observed across two local sites in Rhode Island in 2009 (Fig. 2.4) which lends credence to the observation.

In Rhode Island, early stage *H. sanguineus* zoeae were first detected on June 24th, 47 days after the first ovigerous females were found at this site (Fig. 2.19). Zoeae were detected slightly earlier in Rhode Island than in New Hampshire, as would be predicted based on the earlier presence of brooding females. Zoeae were detected even earlier (early and mid June) in a study in Delaware Bay in 2001 (Park et al. 2005) suggesting that spawning season may shift earlier in the season as latitude decreases.

Further understanding of field brooding activity could be gained by repeating laboratory brood duration experiments with larger sample sizes and greater ranges of temperatures. Though a negative correlation between temperature and brood duration was suggested by Chapter I experiments (Fig. 1.7), this correlation was not significant. If a significant correlation is determined in future studies, this could be used to predict brood durations more closely at temperatures between those tested directly in the experiment.

The average monthly temperatures calculated from HOBO temperature logger data in the New Hampshire intertidal zone were consistently higher than historical average temperature data (Table 2.4). However, average monthly temperature data from HOBO loggers deployed subtidally in Rhode Island varied between higher and lower than historical average temperatures. This suggests that intertidal temperatures are generally warmer than sea surface temperatures, which makes sense based on their exposure to air and sun for a portion of each

day. This difference in temperature may translate to effects on reproduction for *H. sanguineus*. Therefore, to truly understand the relationship between temperature and reproduction in these regions and others, intertidal temperatures should be monitored in future studies.

Temperature and Initiation of Reproductive Season

It has been suggested for several species of brachyurans that the beginning of the reproductive season is triggered by temperature or photoperiod (Meusey and Payen 1988). Initiation of the season for *H. sanguineus* in New England shows correlation with several temperature events. Reproductive season begins when temperatures are in a period of increase, and after average daily temperatures reach 10°C. Stephenson (2009) reports the presence of ovigerous females of temperatures of 9°C and above. This agrees with the finding that timing of minimum daily temperatures rising above 9 or 10°C for the first time of the season coincided with first presence of ovigerous females in New Hampshire in 2009 (Fig. 2.20).

Latitudinal Variation in Size at Maturity

Average ovigerous size was used as a proxy for size at maturity in the two populations studied. The connection between average ovigerous female size and size at maturity was made by observing that the lower average ovigerous size observed in Rhode Island was due primarily to higher percentages of mature females in the lower size classes (Figs. 2.10 and 2.11). Though crabs have similar minimum sizes at maturity at the two sites (Table 2.3), size at 50% maturity likely varies between the two regions studied. To concretely determine

size at 50% maturity for these two regions, crabs would have to be dissected to examine their reproductive tissues. Unlike some other species of crabs, there is no discrete change in external abdomen morphology between the juvenile, pre-pubetal and mature stages for *H. sanguineus*. Abdomen shape simply widens as the crabs develop, and it is hard to determine if small, non-ovigerous crabs are mature or immature.

The smaller size at 50% maturity suggested by this study agrees with general theory which predicts slower growth and delayed maturity in higher latitudes (Hines 1989 and references therein). Previous study by Hines (1989) along the coast of California found significantly larger size at 50% maturity at higher latitudes for two species of grapsid crabs, *Pachygrapsus crassipes* and *Hemigrapsus oregonensis*. This pattern has also been observed in the anomuran crab *Emerita analoga* on the California coast (Dugan et al. 1991). Larger size at maturity may lead to decreased lifetime reproductive output for *H. sanguineus* in higher latitudes, and this could limit population growth of the species in these areas.

Hines (1989) also suggests the potential effect of geographic barriers on crab reproductive traits. In his study, the location of Point Conception was found to correlate with the shifts seen in size at 50% maturity for *P. crassipes* and *H. oregonensis*. Another species studied, *H. nudus*, is not found south of point conception, and no significant differences was seen between latitudes between 35 and 41°N in latitude (Hines 1989). In the present study, Cape Cod may be a similar biogeographic barrier, or smaller scale physical processes may be

contributing to the differences in reproductive behavior and size seen between the two regions.

Plankton Surveys

It was difficult to explain seasonal patterns observed in early zoeal densities. Spawning seasons started at times of the season that correlated well with seasonal brooding patterns and estimates of brood duration based on laboratory study (Figs. 1.7 and 2.5). The end of the spawning season must of course correlate with the last incidence of ovigerous females in a population, in the case of regions in this study, by the end of October. Very little can be concluded based on the seasonal patterns of early stage zoeae density found in this study. However, the data from this study could be combined with future studies of recruitment in these regions. This type of synthesis could potentially lead to some conclusions about larval development speed and success for the species under variable environmental conditions.

Several plankton sampling methods were employed over the course of this study, and each method had some strengths and weaknesses. Plankton traps were initially employed in order to ensure detection of larvae, even if they were at levels too low to be detected by tow samples. However, densities of larvae could not be calculated using this method. Horizontal surface tows could not provide comparable density estimates across an entire season in Rhode Island, because large blooms of gelatinous zooplankton occur at this site in the late summer, which made surface tows impossible. Vertical tow samples

seemed to be the most reliable. They were able to collect *H. sanguineus* larvae at both sites, but the method was not limited by net clogging as in surface tows.

One of the most interesting observations from plankton sampling was the paucity of late stage *H. sanguineus* zoeae in samples. A very small number of late stage zoeae and megalopae were found across the two years of sampling (Table 2.6). This result supports the prediction that *H. sanguineus* larvae are swept offshore for development and return to coastal areas as megalopae (Park et al. 2004). However, it is strange that more than a few megalopae were not observed in samples. Limited targeting sampling for megalopae was conducted by setting traps on the seafloor on nocturnal flood tides (Table 2.2). No megalopae were detected through these efforts. It was predicted that megalopae migrate demersally, and it seems most likely that they do this on flood tides. One potential explanation is that late stage megalopae have been suggested to be negatively phototactic (Park et al. 2005), and may have been avoiding the traps used in this study. Further sampling on all combinations of light and tidal cycle would be necessary to determine the migratory behaviors of *H. sanguineus* megalopae. Alternative sampling methods for sampling near the benthos may also be necessary to detect these larvae. Potential sampling methods include sediment grabs or Niskin bottles deployed near the seafloor.

Conclusions and Implications

Both reproductive seasonality and reproductive traits were found to vary between the two regions studied. These regional differences are likely affecting the spread and population growth of *H. sanguineus* on the northern edge of its

range in the US. Temperature seems to play a strong role in reproduction of this species. Increased reproductive output at lower latitudes is likely related to temperature differences between the regions. This difference has likely, in part, led to disparity in abundance and dominance of this species between northern and southern New England. Though *H. sanguineus* densities are rising in New Hampshire (L. Harris, University of New Hampshire, personal communication), differences in reproductive output between these regions suggest that northern New England populations of this species will not likely reach densities currently observed in southern New England.

However, this crab will likely continue to move northward, potentially establishing brooding populations in areas of the Canadian Maritimes that have sustained warm summer temperatures. These areas include Prince Edward Island, New Brunswick, Eastern Nova Scotia, and Cape Breton Island. There is also the potential for the establishment of non-reproductive sink populations in northern areas with colder summers, as adults seem to have wider temperature tolerances than are required for successful reproduction.

Data from this research project may be useful not only in predicting the potential for expansion of the latitudinal range of *H. sanguineus* on the east coast of the US, but also for predicting its potential for establishment and spread in other areas of the world. Additionally, these data provide some baseline information regarding the phenology of *H. sanguineus* in the current climactic conditions. The seasonal behaviors of this species may be modified by increased sea surface temperatures associated with climate change. This study

also provides data regarding the behavior of this species as a fairly "new" invader. It is possible that the behaviors of the species could change through acclimation to conditions in this region, or over time, evolve to thrive and reproduce in a wider or shifted range of temperatures.

Though it seems logical that the effect of increase in sea surface temperatures on this species may be higher reproductive output, there could also be negative effects from increasing temperature. These may include unforeseen interactions within communities, due to differing effects of temperature change on various functional groups (Edwards and Richardson 2004). These effects could include mismatch of larval release with food availability. Temperature has strong effects on the seasonal cycles of some organisms, such as *H. sanguineus.* Reproductive season of these types of organisms may shift forward in the year with increases in sea surface temperature. However, the pelagic seasonal cycle may be determined by other factors, such as day length and light intensity, and therefore fail to shift seasonally with changes in temperature (Edwards and Richardson 2004). This cycle is integral to seasonal food availability for primary consumers, including invertebrate larvae including those of *H. sanguineus.*

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