ABSTRACT

Title of Thesis: DEVELOPMENT OF A NUCLEIC ACID-
                                        BASED SPECIFIC GROWTH MODEL FOR
                                        JUVENILE BLUE CRAB, *CALLINECTES
                                        SAPIDUS*

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                                        Estuarine Environmental Sciences

The evaluation and identification of habitats that function as nurseries for marine species has the potential to improve conservation and management. A key assessment of nursery habitat is estimating individual growth. However, the discrete growth of crustaceans presents a challenge for many traditional *in situ* techniques to accurately estimate growth over a short temporal scale. To evaluate the use of nucleic acid ratios (*R:D*) for juvenile blue crab (*Callinectes sapidus*), I developed and validated an *R:D*-based index of growth in the laboratory. *R:D* based growth estimates of crabs collected in the Patuxent River, MD indicated growth ranged from 0.8-25.9 (mg·g^{-1}·d^{-1}). Overall, there was no effect of size on growth, whereas there was a weak, but significant effect of date. These data provide insight into patterns of habitat-specific growth. These results highlight the complexity of the biological and physical factors which regulate growth of juvenile blue crabs in the field.

DEVELOPMENT OF A NUCLEIC ACID-BASED SPECIFIC GROWTH
MODEL FOR JUVENILE BLUE CRAB, *CALLINECTES SAPIDUS*

by

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Dedication

This is dedicated work is dedicated to Jill Hutchison and Dave Morrell, who have changed my life and will be greatly missed.

Acknowledgements

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

The Habitat Selection Concept

Aquatic environments are comprised of a diverse range of habitat types from open pelagic zones to highly structured reefs and soft bottom estuaries. This immense diversity has provided opportunity for species to become uniquely adapted to specific habitats and conditions. Given this inextricable association of species to specific habitats, it is often assumed that protecting and maintaining specific habitats should be included in species conservation and management plans. As a result, special habitat protection has been integrated into the management of federally regulated fisheries through the Magnusson Stevens Fishery Conservation and Management Reauthorization Act (MSFCMRA 2007). Within the Magnusson Stevens Act, Essential Fish Habitat (EFH) is defined as "those waters and substrate necessary for fish spawning, breeding, feeding or growth to maturity" and the Act requires both the identification and conservation of these essential habitats (NMFS 2007). Since its adoption, approximately 1,000 EFH areas have been described. Implicit within these regulatory measures is the presumed relationship between species' population dynamics and the amount or quality of available suitable habitat. However, for many species this relationship remains poorly understood (Hayes et al. 1996).

For many marine fishes and invertebrates with complex life histories, juveniles and adults utilize markedly different habitats. Often, juvenile development occurs in nearshore, shallow systems (e.g., estuaries) and shifts to pelagic or benthic habitats as adults. Shallow coastal

1

estuarine systems are particularly conducive to juvenile development as they support high rates

of primary and secondary production and provide favorable environmental conditions, and

refuge habitats which enhances growth and survival (Beck et al. 2001, Dahlgren et al. 2006).

Due to these characteristics, estuaries have been nicknamed "nurseries of the sea", and have long

been identified as important ecosystems for species' development (Boesch and Turner 1984,

Butler and Jernakoff 1999).

Creating management strategies that focus on essential habitat for juveniles over a large

spatial scale such as an entire estuary however presents a considerable challenge (Levin and

Stunz 2005). To aid in developing management strategies, Beck et al. (2001) framed an approach

to rank juvenile habitats to refine this broad description of juvenile habitat and identify prime

conservation targets. In doing so, Beck et al. define a nursery as a habitat which contributes

disproportionally more juvenile production per area on average to the adult stock. Under this

"nursery- role hypothesis", expansive juvenile habitats can be broken into categories and

evaluated.

This simple definition of juvenile nursery habitat however can be difficult to measure

using traditional methods. In order to quantifiably rank juvenile habitat, Beck et al. (2001)

proposed four evaluation metrics consisting of: 1) habitat associated abundance, 2) habitat

associated growth, 3) individual survival rates, and 4) successful recruitment to adult habitats.

The use of any metric independently may be insufficient and integrating multiple metrics may be

needed for a more realistic and comprehensive habitat review. For example, traditional

approaches that rely solely upon habitat-specific abundance estimates, have been found to be

misleading indicator of habitat quality (Van Horne 1983, Figueria and Crowder 2006).

Moreover, population models tend to indicate that patch specific abundance can be a poor

predictor of the contribution of the patch to the overall population (Pulliam 1988, Figueria and Crowder 2006). Similarly, the use of growth as a single indicator is not always a reliable indicator of habitat quality for various finfish species, (e.g., juvenile Atlantic croaker, *Micropogonias undulatus*, Seacry et al. 2007). Even transport dynamics alone do not necessarily provide a link to adult abundance (see Lipcius et al. (1997) for spiny lobster, *Panulirus argus* and Figueria and Crowder (2006) for reef fish). In light of the unreliability associated with the single metric approach, an effort to provide a better estimate of habitat quality through the use of integrated metrics can provide a greater holistic approach to habitat assessment.

Production, the measure of total biomass elaboration- both individual growth and population biomass, is one potential integrated metric that can be used to estimate habitat quality. Incorporating individual growth into a habitat assessment captures key aspects that shape many early life stage processes which affect overall population dynamics. Juvenile growth has strong influence on survival and recruitment (Houde et al. 1987) with variation in juvenile growth leading to subsequent recruitment variation. For example, increased growth is advantageous for juvenile survival by shortening the duration of vulnerability to size-based predator fields (Rice et al. 1990, Hare and Cowen 1997) and increasing overwintering capacity (Sogard 1997, Post and Parkinson 2001). The ability to out-grow many of these dangers can increase the probability for successful adult recruitment. In turn, including density estimations within habitat is important for understanding habitat selection and utilization. Thus by tying individual growth measurements to spatially explicitly population measurements gives a clearer picture of population size, growth rate, and habitat use.

When estimating habitat production it is important to understand how a species settles into a habitat, specifically, the mechanisms that guide habitat selection. The underpinning of

many habitat selection theories focus on optimality rules where individuals behave to optimize their fitness (e.g., Fretwell and Lucas 1970, Rosenzweig 1981, Gilliam and Frazer 1987, Morris 2003). Under this premise, new settlers select available habitats that are associated with the highest fitness. Habitat quality is assumed to be density dependent where increased densities will lower habitat fitness. The ideal free distribution (IFD – Fretwell and Lucas 1970) predicts that individuals will distribute themselves according to the expected fitness in each habitat (Figure 1.1). Each additional individual settling in a habitat consequentially lowers the habitat's quality either by resource use or increased negative conspecific interaction. The settlement pattern that emerges has the "best" habitat attracting settlers until the perceived habitat quality is reduced to the next "best" habitat. At this point, incoming settlers will use both habitats. Over time, IFD equilibrium creates an equal per capita fitness where inherently high quality habitat supports greater densities and low quality supporting fewer individuals. Subsequently, MacCall (1990) expanded the IFD to a landscape approach, termed a basin model (Figure 1.2). The equilibrium under the basin model predicts differences in habitat-specific population growth potential across habitats. Thus, high quality sites will have better growing individuals. Both the IFD and the basin model can be readily adapted to Beck et al.'s nursery habitat framework and examined by observing individual growth and abundance.

In this thesis, I seek to apply concepts from nursery habitat identification and habitat selection theory to a model system – the blue crab in Chesapeake Bay – to develop and validate a methodology of measuring habitat-specific production.

The Blue Crab

The blue crab, *Callinectes sapidus* (Rathbun 1896, Portunidae), is both an important ecological and economic species which relies on shallow estuary and coastal habitats for its

juvenile development. Characterized by flattened fifth perepods, these agile portunid crabs are key predators of nearshore communities. Endemic to the eastern coast of North America, blue crab inhabit a wide latitudinal range extending from Massachusetts to Uruguay (Williams 1974). As dominant, opportunistic foragers, they feed on a variety of bivalves, crustaceans, fish, plant, and detrital material (Hines and Wolcott 1990). This broad diet elevates them to keystone species status within estuaries, capable of direct top down effects on community diversity, density and structure (Hines 2007).

Economically, blue crab support substantial commercial and recreational fisheries. Due to their near shore accessibility and market appeal, the blue crab fishery has become the most valuable fishery within the Chesapeake region, with recent annual vessel landings valued near $84 million dollars[1]. Despite supporting a historic fishery for over a century, this iconic Chesapeake Bay species has suffered substantial stock declines in the last 25 years. As indicated by recent winter dredge surveys, the abundance of adult blue crab in Chesapeake Bay has declined by two thirds from 1990 to 2000 (Jensen and Miller 2005, Miller et al. 2005). Following the implementation of measures aimed at conserving mature females in 2008, overall abundance increased during 2008-2011 to levels seen in the early 1990s (CBSAC 2014). However, recent abundance estimates (2013-2014) have shown sharp decline (CBSAC 2014). This disconnect between spawner abundance and subsequent recruitment, highlights the need to understand factors which regulate recruitment of juvenile blue crab (Colton et al. 2011).

Blue crab have a complex life history (Figure 1.3). For crabs within the Chesapeake Bay, during late summer and fall, females mate and migrate south toward higher salinities to

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[1] Data from NOAA's Fishery Statistics and Economics Division, available online at http://www.st.nmfs.noaa.gov/commerical-fisheries

overwinter and brood their eggs. After overwintering, females release their zoea larvae at the bay mouth and larvae are advected offshore to develop along the Atlantic continental shelf (Epifanio 2007). During their final larval stage, megalopae ingress into the estuary, undergo primary dispersal and settle into complex habitats along the lower portion of the bay (Lipcius et al. 2007). After molting to approximately 20mm in size, juveniles often undertake a secondary dispersal (Etherington and Eggleston 2000, 2003) to shallow, low salinity portions of the tributaries of the Chesapeake Bay (Hines et al. 1987). Once settled into their secondary dispersal habitats they continue to molt until reaching maturity and migrate to deeper adult habitats.

In an attempt to tease out the influence of habitat quality on juvenile blue crab development, previous work has predominantly focused on the primary dispersal stage. Numerous studies highlight the importance of habitat complexity for successful development. Specifically, structurally complex habitats such as seagrass beds and marsh edges have been shown to support relatively high crab abundances and afford lower mortality rates (Orth and van Montfrans 1987, Etherington and Eggleston 2000, 2003).

Unlike primary dispersal, where a specific set of habitats are settled, secondary dispersal habitats encompass a boarder array of habitat types including coarse woody debris (Everett and Ruiz 1993), marsh edges (Etherington and Eggleston 2000, Minellow et al. 2003), and mud flats (King et al. 2005). This redistribution of juveniles from primary habitats often involves movement from high density primary settlement habitats to lower density habitats (Etherington and Eggleston 2000). This expansion in habitat diversity raises the question of how juvenile blue crab select these secondary habitats, and if any of these serve as nursery habitats (*sensu* Beck et al. 2001). Of the few investigations that focus on secondary dispersal, only single metrics are used as a proxies for habitat quality such as crab growth or abundance. For example, Seitz et al.

6

(2003) reported positive correlations between the abundances of juvenile blue crab and their potential prey in sand and mud habitats in the York River, VA, a tributary of the Chesapeake Bay. Subsequently, Seitz et al. (2005) used *in situ* caged juvenile blue crab and measured growth over 3-6 months. As a result, there were able to infer long term growth rates and report increased crab growth at sites with greater abundances of the Baltic clam*, Macoma balthica*, a preferred prey.

Although these previous studies give insight into long term trends in blue crab growth following secondary dispersal, they overlook short term growth that results from habitat selection. Active habitat selection has been observed in blue crab throughout their life cycle including megalopae, early juvenile (Moksnes and Heck 2006), and adults (Shirely et al. 1990). Selection behavior is thought to be important to secondary dispersal juveniles, as dispersal appears to be largely behaviorally driven with cues for habitat degradation and conspecific density (Pardieck et al. 1999, Etherington and Eggleston 2000, Reyns and Eggleston 2004, Moksnes and Heck 2006). However, the extent to which habitat selection reflects behavioral selection based on perceptions of habitat quality remains unknown.

Objectives

The estimation of short term growth in blue crab presents as central challenge due to the nature of growth in crustaceans. Blue crab grow by molting in which rapid increases in shell size on molting (growth per molt) alternate with long periods of stasis (intermolt period – Brylawski and Miller 2006; Smith and Chang 2007). Laboratory studies show that the growth per molt is relatively canalized in blue crab, averaging about 119.5%, over a wide range of environmental conditions (Brylawski and Miller 2006). In contrast, the intermolt period is more plastic (Brylawski and Miller 2006). Thus, to measure changes in size, a crab has to be monitored for at

least one entire intermolt period. As a result, the intermolt period defines the period of residency in a habitat of an individual crab that is required to attribute all growth to the result of habitat selection. After settling into tributaries, juvenile blue crab slowly meander (2m· h^{-1}; Hines et al. 1995) and so it thought sampling crabs within habitats during their intermolt may give insight to habitat effects on growth.

RNA:DNA ratios have been used to measure the health of cells in culture (Traganos et al. 1982) and the short term growth of larval and juvenile fish (e.g., Bergeron 1997; Heyer et al. 2001; Stierhoff et al. 2009). RNA:DNA-based indices of growth are known to have fast response times (Heyer et al. 2001) and thus if they can be validated for blue crab, offer a way of developing short term estimates of habitat-specific growth which can reveal mechanisms underlying habitat selection in blue crab, and can provide a way to quantify the quality of habitats used by juvenile blue crab.

This thesis had two objectives:

1. *Develop an RNA:DNA based index of growth for juvenile blue crab.*

To address this objective I conducted laboratory experiments in which the growth of juvenile blue crab was varied by both an environmental factor (temperature) and by varying ration. Growth of crabs was monitored over 36 days. Size and weight was measured at three time points within the 36 day period. At the end of the experiment, individual crabs were killed and the RNA:DNA ratio of muscle tissue was quantified. Subsequently, statistical approaches were used to assess whether RNA:DNA ratios could be used as an indicator of growth in blue crab.

2. *Field validation of the RNA-DNA based index of juvenile blue crab growth for measuring habitat-specific growth of blue crabs in the Patuxent River, MD*

For the field validation, I selected various juvenile blue habitats in the Patuxent River and sampled individuals on 5 sampling events. Growth rate was estimated for each individual sampled using the aforementioned RNA:DNA-based index of growth. Collective habitat growth rate was compared by habitat type as well as spatially and temporally. The growth rate estimates from the field sampling of juvenile blue crabs were then used to explore hypotheses on habitat selection and nursery habitat potential.
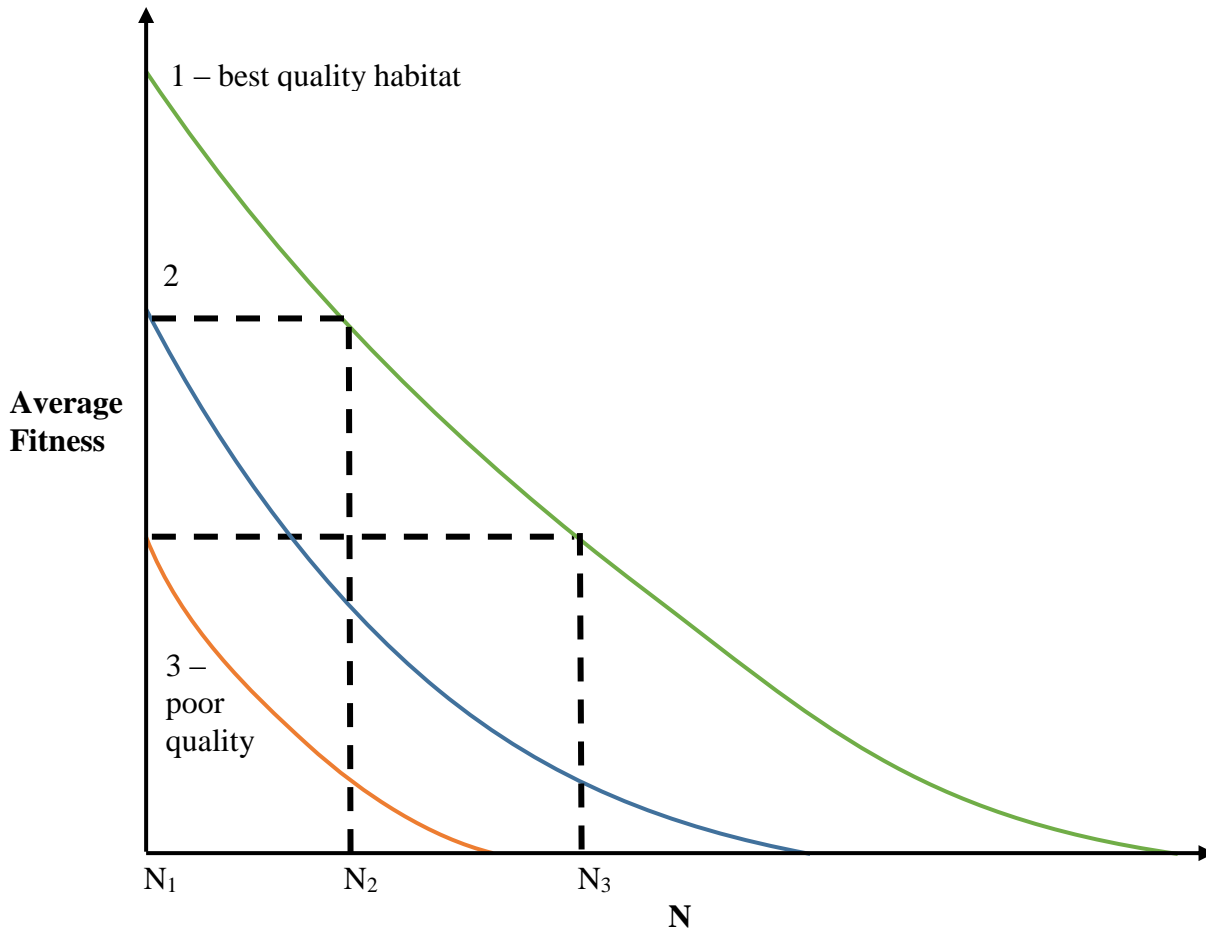
Figure 1.1 An illustration of how changes in population size of a habitat-selecting species alter the average fitness within the habitat when compared across different habitat qualities. Horizontal dashed lines show as the numbers of competitors increase (N) in the best quality habitat, 1, habitats 2 and 3 become more favorable at low densities (N_2 and N_3 respectively) (an ideal free distribution, Fretwell and Lucas 1970).
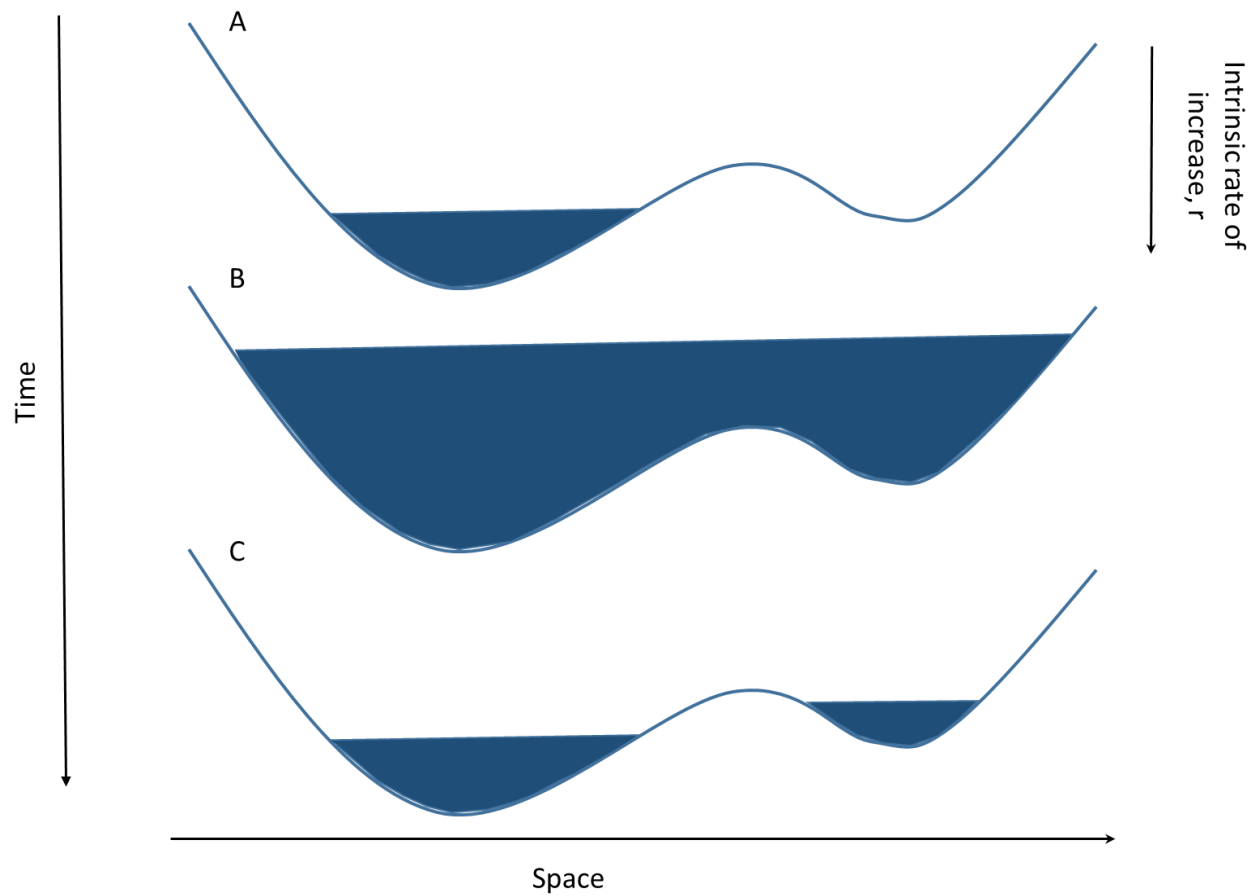
Figure 1.2 Schematic of MacCall's Basin Model. In panel A, individuals begin filling the landscape, selecting the habitats that support the highest intrinsic rate of population increase, r (y-axis). As the population abundance increases, the population expands the spatial range (x-axis). The expanding population (Panel B) continues to expand spatially. However should the population contract (Panel C), some individuals can become spatially isolated forming a new population.
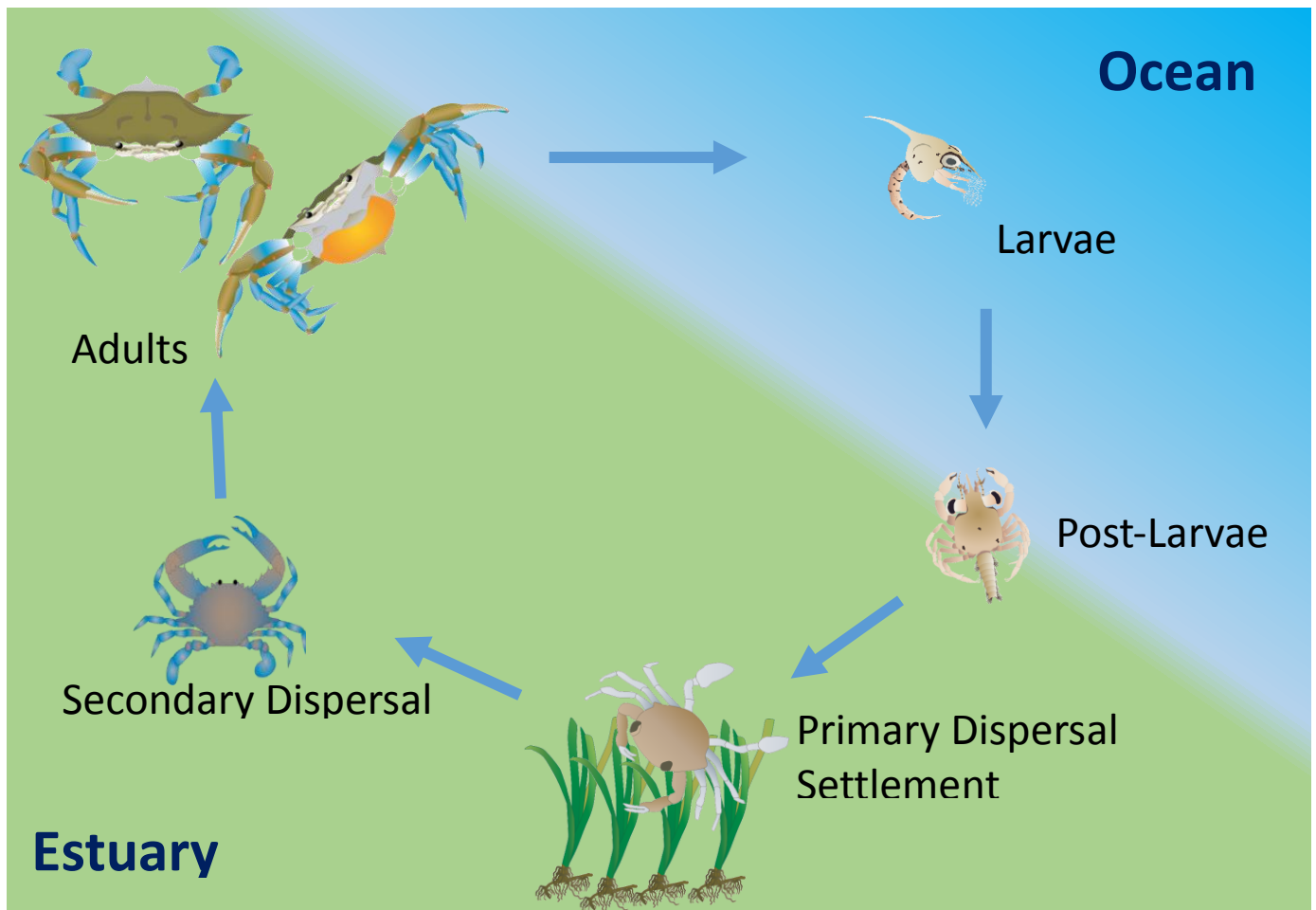
Figure 1.3 Schematic of the life cycle of blue crab showing the stages resident in high salinity water, and the lower salinity estuarine waters. (Icon credits: Chip Chenery, Jane Thomas, Tracey Saxby; Integration and Application, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/))

Chapter 2

Development of an RNA:DNA based index of growth in juvenile blue crab (*Callinectes sapidus*)

Abstract

The evaluation and identification of habitats that function as nurseries for marine species has the potential to improve conservation and management. A key assessment of nursery habitat is estimating individual growth. The discrete growth of crustaceans presents a challenge for many traditional *in situ* techniques to accurately estimate growth over a short temporal scale. To evaluate the use of a nucleic acid ratio-based index of growth (*R:D*) for juvenile blue crab (*Callinectes sapidus*)*,* I developed and validated an *R:D*-based index of growth in the laboratory from a calibration experiment with temperature and ration treatments. Only ration in the calibration experiment significantly affected juvenile growth in the laboratory. The growth model developed from the calibration explaining 70% of the variability in juvenile blue crab growth. *R:D* based growth estimates of blue crabs collected in the Patuxent River, MD indicated growth ranged from 0.8-25.9 ($mg \cdot g^{-1} \cdot d^{-1}$). Overall, there was no effect of site on growth, whereas there was a weak, but significant effect of sampling month. These data provide insight into patterns of habitat-specific growth. These results highlight the complexity of the biological and physical factors which regulate growth of juvenile blue crabs in the field.

<u>Introduction</u>

To assess nursery habitat quality and to understand habitat selection requires the quantification of the growth of individuals. For crustaceans, growth is often estimated as a rate using the difference in carapace size between successive molts and the duration of the intermolt period (Brylawski and Miller 2006). This technique however, presents a challenge for measuring habitat-specific growth *in situ,* due to the need to track individuals over time to derive growth estimates. Despite a variety of creative approaches that have been developed to monitor free ranging blue crab in the field (e.g., Wolcott and Hines 1989, Hines et al. 1990), if the intermolt period is longer than residence time in a habitat, assigning a growth-based measurement of habitat quality becomes problematic. A common approach to overcoming the challenge of measuring discrete growth is use of enclosures (e.g., Chenery 2002, Seitz et al. 2005), thereby constraining growth expression to a single habitat. However, enclosure use often raises concerns over cage artifacts making comparisons of cage-based growth rates problematic. Therefore the ability to reliably estimate growth at time scales that reflect current habitat use requires measuring growth near an instantaneous time scale.

Bioindicators such as nucleic acid-based growth indices, provide a useful tool for the estimation of physiological condition and growth over very short time intervals (Sutcliffe 1970, Bergeron 1997, Buckely et al. 1999). Nucleic acid growth indices measure tissue nucleic acid concentrations and are thought to be closely tied to physiological condition, reacting to change in condition on the orders of hours to days (Bergeron 1997, Ciotti et al. 2010). These indices rely on ribonucleic acid (RNA) concentration in muscle tissue correlating to the level of protein biosynthesis within tissue. Changes in tissue RNA levels are often in response to ribosomal protein synthesis (Buckley 1984). Hence the quantity of ribonucleic acid then should positively

14

vary with the level of expressed organismal growth. The use of nucleic acid indices to measure growth however, requires the conversion of the relative index of growth to empirical growth (Ciotti et al. 2010). Often this relationship can differ by species and life stage and may involve a variety of standardization techniques such as the ratio of RNA to DNA and additional variables (Buckley 1984). Once calibrated, a direct relationship between tissue RNA concentrations correlating to overall organismal growth has been demonstrated across a spectrum of taxa including marine bacteria (e.g., Kerkhof and Ward 1993), fish (e.g. Buckley 1984, Caldarone et al. 2003), and crustaceans (e.g., Moss 1994, Parslow-Williams et al. 2001, Grimm et al. 2015). However this correlation is not always consistent for every species (*e.g.,* the great spider crab *Hyas araneus,* Anger and Hirche 1990).

The blue crab (*Callinectes sapidus*) is both an important ecological and economic species which relies on estuary and coastal habitats for its juvenile development. Endemic to the eastern coast of North America, blue crab inhabit a wide latitudinal range extending from Massachusetts to Uruguay (Williams 1974). As dominant, opportunistic foragers, they feed on a variety of bivalves, crustaceans, fish, plant, and detrital material (Hines et al. 1990). Blue crab play an important ecological role and can have direct top down effects on community diversity, density and structure (Hines 2007) and serve as important energy linkages coupling benthic and pelagic food webs (Baird and Ulanowicz 1989).

Blue crab exhibits a complex life history (Figure 1.3), in which larval stages occur in the ocean and juvenile and adult stages occur in estuaries and coastal embayments. During their final larval stage, megalopae ingress into the estuary and undergo primary dispersal and settle into complex habitats along the lower portion of the Chesapeake Bay (Lipcius et al. 2007). Research has suggested that primary dispersal sites provide a refuge from predation (Pile et al. 1996). In

these primary dispersal sites, crabs undergo several initial molts. Upon reaching approximately 20mm carapace width (CW) in size, juveniles redistribute with a secondary dispersal (Etherington and Eggleston 2000, 2003) to shallow, low salinity portions of the upper Chesapeake Bay. At these secondary dispersal habitats they continue to molt until reaching maturity and then migrate to deeper adult habitats.

Active habitat selection has been observed in blue crab throughout their life cycle including megalopae, early juveniles (Moksnes and Heck 2006), and adults (Shirely et al. 1990). For secondary dispersal juveniles, habitat selection appears to be largely behaviorally driven with crabs migrating on cues for habitat degradation and conspecific density (Pardieck et al. 1999, Etherington and Eggleston 2000, Reyns and Eggleston 2004, Moksnes and Heck 2006). However, the extent to which habitat selection reflects behavioral selection based on perceptions of habitat quality remains unknown.

Here I attempt to develop and evaluate a nucleic acid-based growth index to estimate juvenile blue crab growth in situ over a range of habitats to assess nursery condition and habitat selection. By using a nucleic acid index, I can estimate very recent growth which may lead to insights on habitat-associated growth and subsequently habitat selection. To estimate absolute growth, a nucleic acid-based growth model will be tailored to secondary dispersal-sized crabs, through a laboratory-based calibration experiment. Using the Patuxent River as a study site, the aim of this study is twofold, to identify potential juvenile blue crab nursery habitat within a tributary and to examine juvenile habitat selection.

Growth calibration experiment

Nucleic acid-based growth indices were developed from a laboratory experiment conducted from 23 July–17 August 2013 that quantified juvenile blue crab growth. The experiment was designed as a 2-factor, randomized complete block design employing 3 levels of temperature (20, 24 and 28°C) and ration (starvation, mid ration, and *ad libitum* ration). Levels of the factors were selected to generate a wide range of individual crab growth rates while keeping the temperature realistic to spring-summer conditions in the Chesapeake Bay. Each treatment combination was replicated twice for a total of three blocks.

Juvenile blue crab used in the calibration experiment were collected during the nighttime flood tide at the Chesapeake Biological Laboratory pier in Solomons, Maryland USA on 22 July 2013 (29°C and salinity of 11.4). Crabs were collected with dip nets and transferred to circular 511-L fiberglass holding tanks, supplied with flow-through, filtered (approx. 10µm) water from the Patuxent River. During a 36h acclimation period, crabs were fed *ad libitum* pelleted food (Zeigler Finfish Starter Meal with Vpack #2 crumble, Gardners, PA) and light controls were set to emulate the ambient 14h:10h light-dark cycle conditions. Each holding tank was filled with netting to provide structure to reduce cannibalism and tanks were cleaned daily to remove uneaten food and feces.

The growth calibration experiment was conducted in a single constant environmental chamber maintained at 20°C. Each experimental block of treatment combinations was assigned to a separate wire metal rack. Blocks comprised nine 5.7-L aquaria randomly assigned to a treatment. The 20°C temperature treatment temperature was regulated by the environmental chamber and the 24°C and 28°C treatments were maintained by individual submersible heaters

(Hydor Theo Heater 25W) placed in each aquarium. Temperatures were set 2d prior to the start

of the trial and were checked and adjusted daily to ensure temperature remained within +/-

1.0°C. Minor temperature fluxes in the 24°C and 28°C aquaria resulted from evaporative cooling

within the environmental chamber. Aquaria were outfitted with air stones and partitioned with a

perforated sheet of Plexiglas to accommodate to 2 crabs. Water was changed frequently to

maintain water quality. Ration treatments consisted of a starvation, 30% daily maximum ration

and *ad libitum* ration treatments. The starvation treatment was maintained by using 10µm filtered

seawater in each aquaria to limit detrital food sources. Daily maximum ration was calculated

using the bioenergetics model developed by Brylawski and Miller (2003). *Ad libitum* fed crabs

were offered food until they appeared satiated and uneaten food remained the following day.

The growth calibration experiment involved 54 fully intact juvenile blue crab between

35–47mm carapace width (*CW*, the distance between lateral spines). Upon random assignment to

treatment aquaria, each individual's initial size and weight was recorded. On days 16, 23, and 30

each crab was removed from the aquarium wet-weighed, measured, and its condition (i.e., limb

loss) recorded. On day 36, crabs were removed from aquaria, individually marked, measured,

wet weighted, euthanized and stored at -80°C until nucleic acid quantification.

Nucleic acid quantification

Nucleic acid concentrations within muscle tissue were quantified using a one dye, two

enzyme fluorometric assay based on the methods described in Caladrone et al. (2001). Minor

modifications were made to the protocol, including expanding the range of standards and the

dilution ratios for tissue samples to adjust for crab tissues. Prior to all analyses, the stability of

the nucleic acid concentrations within tissues was tested and found to remain constant for up to 6

months while stored at -80°C. No single sample was stored for more than 6 months prior to analysis.

To quantify tissue RNA (*R*) and DNA (*D*) concentrations, each crab was dissected over ice and approximately 7-20mg (wet weight) of muscle was extracted from the abdominal cavity at the 5th thoracic sternite. Individual muscle samples were placed in 2mL centrifuge tubes with 150μL of 1% n-lauroylsarcosine sodium salt solution and vortexed for 90 minutes at the highest setting or until fully dissolved. Once the tissue was degraded, 1.5mL of TRIS EDTA buffer was added to each sample and the resultant suspension was centrifuged for 15 min at 14,000 x G to separate nucleic acids from cellular debris. Following centrifugation, 150μL of the nucleic acid supernatant was pipetted into a 2mL centrifuge tube and diluted with a 1:1 mix of 0.1% n-lauroylsarcosine to form a sample solution that was subject to further analysis.

Triplicate 75μL aliquots of each diluted sample supernatant were pipetted into 96 well microplates. Each microplate was loaded with serial dilutions of both an *R* standard (16S- and 23S- RNA from E. coli MRE600, Roche Applied Science) and a *D* standard (purified calf thymus DNA, Sigma D4764) which were used to generate plate-specific fluorescence concentration curves. Two additional 75μL samples from a control homogenate were added to each plate to verify the integrity of fluorescence integrity readings over time.

A total of 75μL of 2.0μg/mL ethidium bromide solution (Sigma E7637) was added to each well, binding a florescent tag to all nucleic acids present. Following ethidium bromide addition the plate was shaken gently on a benchtop vortexor. Fluorescence was quantified using a spectroflourometer (SPECTRAmax® GEMINI XPS Microplate Spectrofluorometer, Molecular Devices, Sunnyvale, CA.). The excitation wavelength was fixed to 525nm and the emission wavelength was read at 600nm. Total nucleic acid (TNA) fluorescence was separated into

specific components after the sequential additions of RNA and DNA nucleases (Sigma R6513 and D4263 respectively). RNA fluorescence was determined as the difference between the TNA fluorescence and DNA fluorescence. DNA fluorescence was separated from background fluorescence following the addition of DNAse.

Fluorescence values from the spectroflourometer were converted to concentration values using the nucleic acid standard curves developed for each microplate. All standard curves used to estimate nucleic acid concentration were linear throughout their range and had regression coefficients $r^2 > 0.995$. The nucleic acid concentration for each tissue sample was determined as the mean of the triplicate aliquots. Nucleic acid concentration values with a difference greater than 5% from the triplicate mean were removed and the remaining subsamples were used to calculate the sample mean. A total of 5 values of the 156 readings from the analyses were discarded based on this criterion.

Statistical analysis of the growth calibration experiment

Specific growth by weight, G, was calculated from the percent weight change from measurements recorded on day 0 (W_0) and day 36 (W_t) over time using the following equation:

Equation 1

$$G = \frac{W_t - W_0}{W_0} \times t^{-1}$$

Growth was determined as a change in mass rather than change in body size due to the logistical constraints of the growth calibration experiment. Growth measurements based on size or carapace widths, would require an experimental treatment exposure for two consecutive molting

20

events to estimate both growth per molt and the intermolt period (Brylawski and Miller 2006). Over the course of the calibration experiment, none of the 20°C starving treatments had molted by day 36. It was decided that additional treatment time could potentially bias the nucleic acid-based measurements by over-stressing the animals.

Two-way analysis of variance (ANOVA) with type III sums-of-squares was used to determine if individuals from the calibration experiment could be pooled together to develop a nucleic acid-based growth index. Specifically, the presence of a block effect on G was examined with the ANOVA. Further analyses of the calibration experiment data examined the interactive and independent effects of the fixed treatment factors on individual G and nucleic acid concentrations. Subsequent significant effects were further explored using *post hoc* Tukey Honest Significant Difference (HSD) tests which were used to compare different levels of treatments.

Analyses of the growth calibration experiment, nucleic acid-based growth index development, and habitat comparisons were performed in R 3.0.0 (www.R-project.org) with significance determined at $p < 0.05$.

Nucleic acid growth index development

The development of a growth model based on nucleic acids depends on the assumptions of an underlying mechanistic framework linking nucleic acid concentration to growth. The rate of protein synthesis, and consequentially overall organismal growth, is governed by the number of active ribosomes within tissue cells. RNA concentration (R, $g_{RNA} * g_{wetmass}^{-1}$) serves as a reliable proxy for ribosomal activity (Millward et al. 1973). Assuming the protein proportion

within muscle tissue remains constant over time, the daily rate of protein synthesis can then be

equated to instantaneous growth rate (G) (Ciotti *et al.* 2010).

The use of R as an indicator of protein synthetic capacity and assuming protein synthesis

reflective of instantaneous growth, R can be linked to overall body growth using the following

equation:

Equation 2.

$$G = \beta_0 + \beta_R R$$

where the intercept β_0 denotes protein degradation and β_R represents the ratio of muscle to body

mass and ribosomal potential.

I expanded this mechanistic framework (Equation 2) and adopted an information

theoretic approach (Anderson and Burnham 2002) to determine the best statistical model to

predict G using a suite of potential independent predictor variables. Independent variables

included the following: R, D, $R{:}D$, final weight (W), final carapace width (CW), and temperature

(T), and sex (S) together with all possible interactions. Using a general linear modeling

framework, the global model and all possible combinations of reduced models of the form:

Equation 3.

$$G = \beta_0 + \beta_R R + \beta_{R:D} R{:}D + \beta_D D + \beta_M W + \beta_{CW} CW + \beta_T T + \beta_S S + possible\ interactions\ ...$$

where β_0 denotes protein degradation and the individual β_i's represent slope parameters.

The global and subsequent candidate models were evaluated using Akaike information criterion adjusted for small sample sizes (AIC_c). AIC_c values were used to rank models based on fit and performance. Smaller AIC_c values within the set of candidate models indicate better performance. AIC_c values were compared using Akaike differences (Δ_{model}), which determines the relative difference in AIC_c value for each model from the model with the lowest AIC_c value. Potential parsimonious models were selected using Burnham and Anderson's (1998) criteria for model confidence, where Δ_{model} values < 2, indicates substantial support.

The linearity of the nucleic acid G relationship was evaluated using a Box-Cox power transformation analysis. This analysis was used to evaluate the potential for an improvement of the nucleic acid G relationship through the transformation of the model variables. Box-Cox transformation analyses search for the most appropriate power transformation of the dependent variable through computing log-likelihood fits to a normal curve (Box and Cox 1964). The most appropriate power transformation values those transformations that fall within the 95% maximum likelihood confidence interval. To compare the transformed and non-transformed models using AICc, the inverse of the dependent variable transformation was applied to the independent variables.

Field application

Habitat-associated specific growth dynamics of juvenile blue crabs was estimated using the nucleic acid-based growth index developed and validated in the laboratory experiment. A total of 10 sites were surveyed within the Patuxent River, MD, a tributary on the western shore of the Chesapeake Bay (Figure 2.1). Field sampling was conducted bi-weekly from 20 June-27 August 2013. Site selection was based on habitat type, distance between neighboring sites, and

river position. Nominal habitat types were based on presence of vegetation, substrate type, and presence of structural complexity. Habitat types consisted of vegetated marsh edges, unstructured sandy bottom, mudflats, and hardened shoreline (mix of rock and bulkhead). The distance between adjacent sites ranged from 1–9.5km to limit the influence of site proximity.

Sites with limited structure were sampled using a 30.5m long, bag-less beach seine with 6.4mm mesh, pulled parallel to the shore at a depth of 1m. Crabs were counted and those between 25-65mm CW were immediately preserved on dry ice or in liquid nitrogen in the field. At sites where seining was ineffective, crabs were collected with dip nets. For each sampling event, water temperature, salinity and dissolved oxygen were measured using YSI Professional Plus (Yellow Springs, OH) multisensory probe.

Crabs collected in the field were transferred and stored in a -80°C freezer at the Chesapeake Biological Laboratory prior to determination of nucleic acid concentrations. Whole crabs were preserved for 2-5 months prior to biochemical analysis. Nucleic acids were quantified using the previously described methods above.

Statistical analysis of the field sampling

The estimation of G in the field was restricted to the analysis of intermolt juvenile crabs between 30-55mm CW, a size range similar to that used in the laboratory calibration experiment. Intermolt animals were used to limit the potential influence of ecdysteroids affecting nucleic acid synthesis in blue crab (Chang 1995) and the effect of molt is known to affect nucleic acid concentrations in other crustaceans (Gorokhova and Kyle 2002). Molt stage was coarsely defined as the presence of a white line on the swimmeret and shell hardness, with the assumption that crabs with soft carapaces were still in the post molt phase. For each sampling event, the

24

instantaneous growth was estimated for each individual using the best fitting model determined from the laboratory calibration experiment. Site specific growth was examined using a one way ANOVA based on type III sum-of-squares. However, due to unavoidable constraints in collecting crabs at different sites; the number of crabs sampled during each sampling event was unbalanced. Subsequent analyses pooled sites by sampling month for more balanced comparisons and increased statistical power. Additional variables including were habitat type, site location in the river, sampling date, and measured environmental conditions were evaluated as well using multi-factor linear models.

Results

Growth calibration experiment

There was no difference in the initial sizes of crabs among treatments (Table 2.1; 1-factor ANOVA, p = 0.937). Two of the 54 individuals died during the laboratory calibration experiment. The overall mean increase in size over 36d was 6mm CW (range from 0-20mm) and 2.90g increase in weight (ranged from -0.9-13.39g). Muscle tissue RNA concentration (*R*) ranged from 1.13-8.56µg·mL^{-1} (Figure 2.2). In comparison, DNA concentration (*D*) varied less with a range of 0.56-1.96µg·mL^{-1} (Figure 2.2). The resulting RNA:DNA ratios (*R:D*) for individuals ranged from 1.45-6.62 (Figure 2.2). Estimated individual specific growth, *G* (Equation 1), varied from -6.1-19.3mg·g^{-1}·d^{-1} (Table 2.1).

ANOVA indicated no significant block effects allowing each block group to be combined for subsequent analyses. Further exploration of the calibration data examining treatment effects on *G* and nucleic acids in the experiment were explored with a multi-way ANOVA where ration had significant positive effects for *G* ($F_{(2,47)} = 24.14$, $p < 0.001$), *R* ($F_{(2,47)} = 29.50$, $p <$

0.001), D (F(2,47) = 2.70, p < 0.1), and $R:D$ (F(2,47) = 38.92, p < 0.001). Lack of significant interaction between temperature and ration treatments allowed for independent analyses of each factor's influence on G and nucleic acids. Ration had a significant effect on G (F(2,49) = 13.71, p < 0.001) and both R and $R:D$ (F(2,49) = 29.503, p < 0.001; F(2,49) = 38.922, p < 0.001 respectively). There was a marginally significant effect of ration on D (F(2,49) = 2.703, p = 0.077). Surprisingly, there was no evidence for a significant effect of temperature on G, R, D, or $R:D$ (p > 0.1) over the range of temperature teste. Linear contrasts indicated the potential for nucleic acids to serve as indicators of food and energetic availability. Ration and R comparisons were significantly different (p < 0.05) among each treatment level, and $R:D$ ratios were only significantly different between fed and unfed treatments groups (p < 0.05). Overall, higher ration individuals had greater $R:D$ and higher observed G (Figure 2.3).

Growth model development

Multiple linear regression was used to synthesize a nucleic acid-based predictive model for juvenile blue crab growth (Table 2.2). Based on AIC$_c$ values, one model outperformed the others. Model (E) was indicated as the most parsimonious candidate model (AIC$_c$ value of 417.11 and $w_i' = 0.63$). This model explained 67.3% of the variation in G from $R:D$ and W alone. All other models exhibited ΔAIC$_{model}$ values > 2. For example, the global model (A) explained almost the same amount of variability (66.5%) in juvenile G but required 12 estimated parameters and hence had a ΔAIC$_{model}$ = 15.25 and $w_i' = 0$.

A plot of $R:D$ and observed G (Figure 2.3) indicated the potential for a linear or asymptotic function to describe the relationship. To linearize the relationship, a Box-Cox power transformation of independent variables was used. Probable transformations were evaluated with

26

a plot of the log-likelihood and power transformation (λ) from -2 to 2. The log-likelihood peak 95% interval encompassed λ values 1 and 2. Model (E) was adapted using a second degree power transformation ($\lambda = 2$), forming model (H). Although models (E) and (H) were not significantly different (paired t test, df = 49, p > 0.1), AIC$_c$ comparisons indicated model (H) as the most appropriate candidate model (Table 2.3) explaining 70.3% of variance in growth with 3 estimated parameters (Table 2.4).

Model performance was visually validated from a plot of observed G from the calibration experiment and predicted G derived from model H (Figure 2.4). The model's low estimator bias was seen through a lack of significant difference between the linear regression of the observed and predicted G and a 1:1 line. The low estimation bias was demonstrated by the lack of observed pattern of residuals from the predicted values (Figure 2.5).

Field application

A total of 10 sites were sampled on 5 occasions within the Patuxent River from June to August 2013 (Figure 2.1, Table 2.5). Nucleic acid concentrations and body weight were measured, and G was estimated for a total of 169 crabs (Table 2.5). Individual $R{:}D$ ratios ranged from 2.40-10.67 with resulting G estimates ranging from 0.80-26.0mg·g^{-1}·d^{-1}. Field estimates of $R{:}D$ ratios were generally higher than those observed in the laboratory (Figure 2.6), which was also reflected in estimates of G (F(1,150) = 59.44, p < 0.001; Figure 2.7).

Comparisons across sites including location and habitat characteristics did not explain estimated growth patterns. Site-specific estimates of G varied slightly with G ranging from 0.03 to 4.57 mg·g^{-1}·d^{-1}. The inclusion of environmental conditions such as salinity, temperature and dissolved oxygen did not explain the underlying patterns in growth. Site mean G varied from 6.6

27

-16.7mg·g^{-1}·d^{-1}. General linear models fitted to the estimated individual G values provided no evidence for a site effect on estimated G ($F_{(10,99)} = 1.165$, $p = 0.32$).

Subsequent analyses were conducted on 4 sites with sufficiently large samples sizes (sample $n > 12$). General linear models indicated an effect of sampling month on G estimates ($F_{(1,75)} = 19.12$ $p < 001$). Growth was typically slower in August compared to July (Figure 2.9; mean difference -3.2mg·g^{-1}·d^{-1}).

Discussion

The results demonstrate the successful development of a nucleic acid-based growth index for juvenile blue crab. By developing and calibrating a growth index, I was then able to assess the growth distribution for free ranging juveniles from the Patuxent River, MD, a tributary of the Chesapeake Bay. Using this index, I found significant declines in specific growth rates (*G)* from July to August at several sites. Comparisons of G estimates across all sampled sites indicated a lack of inter-site differences, regardless of habitat type, location, or abiotic conditions Although this study failed to detect differences in specific growth across the sampled habitats in the Patuxent River, it remains a strong possibility that growth may vary across larger spatial scales, particularly in large, complex estuary such as the Chesapeake Bay. Alternatively, juvenile blue crabs may distribute themselves across space so that density and productivity are balanced under an ideal free distribution (Fretwell and Lucas 1970). Previous research has clearly identified the potential for active habitat selection in blue crabs (e.g., Moksnes and Heck 2006). For example, food availability (Seitz et al. 2005), conspecific density (Reyns and Eggleston 2004) and predation risk (Williams et al. 1990, Shirely et al. 1990, Moksnes and Heck 2006, Hovel and Fonseca 2005) have been demonstrated to influence habitat preferences. Early life history studies for blue crab have emphasized a nursery role for seagrass beds in supporting lower mortality

rates and higher growth rates for primary dispersal blue crab (Perkins-Visser et al. 1996).

However, few studies have examined the later secondary dispersal stage juvenile habitat. These

previous studies have chiefly used tethering and enclosure-based approaches to assess habitat

quality, in part, because of the lack of a tool to measure *in situ* growth rates of free ranging

individuals.

Nucleic acid-based growth index

The nucleic acid-based growth index developed here for juvenile blue crab relies on

several mechanistic assumptions linking nucleic acid tissue concentrations to recent organismal

growth (Buckley et al. 1999, Chicharo and Chicharo 2008, Ciotti et al. 2010). Violation of these

assumptions may limit the reliability of this and other indices derived from nucleic acid

concentrations. A core assumption of the technique is a constant proportionality between overall

body growth and tissue growth from which nucleic acids are quantified. This is likely realistic

assumption for organisms with continuous growth, such as fishes (e.g., Buckley 1984, Caldarone

et al. 2003, Heyer et al. 2001), but uncertainty exists over this assumption's validity for

organisms with discontinuous growth, such as crustaceans. For crustaceans, the formation of a

new carapace may represent a considerable energetic investment that is not reflected in the

biomass of muscle. For example, blue crabs rapidly absorb water to expand their newly formed

carapace. This uptake of water leads to an approximate doubling in overall body mass (Neufeld

and Cameron 1994), without any associated change in muscle volume. This change in weight

could potentially confound our G estimates due to water intake. To reduce the potential for

molting effects on nucleic acid ratios such as seen in work with daphnia (Gorokhova and Kyle

2002), only intermolt crabs individuals were compared. To compensate for discontinuous

changes in body mass, Moya-Larano et al. (2008) recommends standardizing by animal density

rather than mass or volume, however measurements of density in marine animals can be imprecise and thus were not used in this study.

In addition, this index assumes RNA concentration (R) is exclusively reflective of active ribosomal units. The concentration of ribonucleic acid, R, was measured as total RNA, which include ribosomal RNA (rRNA), messenger RNA (mRNA), and transfer RNA (tRNA) each with different attributed functions and half-lives. In some species such as rainbow trout (*Oncorhynchus mykiss*), rRNA dominates the RNA pool, constituting between 85-95% (McMillian and Houlihan 1988) making total RNA a viable proxy for rRNA. The relative dominance for rRNA in blue crab is still unknown, however, levels of mRNA and tRNA may be upregulated during periods of physiological stress (Chung and Zmora 2008, Lovette et al. 2006) and thus differences in $R{:}D$ concentrations could reflect differences in exposure histories (Wang and Stickle 1988) as well as differences in rates of biomass elaboration. Therefore it may be important in future to target specific species of RNA to improve accuracy in growth estimation. Specific targeting of RNA species has been successful in larval fish growth where McNamara et al. (1999) used actin- and myosin-specific tRNAs in developing their $R{:}D$ ratio. McNamara et al. found minimal differences between estimates derived from $R{:}D$ and $tRNA{:}D$, however this may not always be the case depending on the species.

Despite concerns over the inherent assumptions in nucleic acid-based indices, several previous crustacean growth studies have demonstrated positive relationships between nucleic acid concentrations and individual growth. Examples of positive relationships include post larval American lobster *Homarus americanus* (Junio and Cobb 1994), European green crab *Carcinus maenas* (Houlihann 1990), whiteleg shrimp *Penaeus vannamei* (Moss 1999), signal crayfish *Pacifastacus leniusculu* (Edsman 1994), and various species of copepods (Wagner et al. 2001,

Vrede et al. 2002). However, this relationship is not ubiquitous. For example nucleic acid

concentration has no significant correlation with leg muscle growth in the great spider crab *Hyas*

araneus (Anger and Hirche 1990) or with growth in terminal molt snow crab *Chionoecetes opilio*

(Mayrand 2000).

The results reported herein from the laboratory experiment indicate that a nucleic acid-

based growth model can reliably estimate specific growth rates (*G*) in juvenile blue crab. In

developing a nucleic acid-based growth model from the calibration data, the most parsimonious

model incorporated the square root transformation of the ratio of RNA to DNA (*R:D*) and final

body mass as independent terms. Our model explained approximately 71% of variation in

observed juvenile *G*. The percent variation explained here is similar to other crustacean and fish

nucleic acid-based growth models (e.g., Buckley 1984, Moss 1994).

In developing the model nucleic acids were fit individually and as a ratio of R:D.

Although the use of issue *R* independently did serve as an indicator for juvenile growth, by itself

explained less variation than *R:D* ratio model. A similar response with a direct relationship to *R*

and feeding conditions has been demonstrated for blue crab using homogenized animals (Wang

and Stickle 1987) and also in white legged shrimp (*Litopenaeus vannamei*) (Moss 1999, Mente

et al. 2002). Overall, the performance of the candidate models using *R* independently was not

nearly as accurate as candidate models standardizing *R:D*, as it explained only 61% of the

observed variance and was improved by incorporating body mass.

Included with *R:D* in the model, body weight was a significant model term. Although the

addition of body mass or size is less common in nucleic acid-based growth models (Mathers

1992 and Ciotti et al. 2010), it is thought to be important in accounting for size-dependent

processes. These dependent processes can be integrated explicitly or implicit in *G* models and

include protein accumulation (Houlihann 1991) and energetics (Fry 1971). Unlike Buckley (1984) which indicates tissue *R:D* relationships may be affected by size, we report a lack of significant interaction between tissue nucleic acid concentration with body size or mass.

Surprisingly, temperature was not a significant model term for predicting *G*, either in isolation or in combination with other factors. Yet, Brylawski and Miller (2006) demonstrated a strong role for temperature regulating growth of juvenile blue crabs. As with many biological processes, temperature is often a controlling factor, governing reactions for metabolism and growth (Fry 1971). For exotherms, RNA activity has been demonstrated to increase with environmental temperature and is commonly featured in protein metabolism models (Foster 1992, Juinio 1992, Fraser et al. 2002, Peck 2003, Mercaldo-Allen 2006, 2008). Despite its importance in governing biochemical reactions, temperature was a non-significant model term in this study. This result however is not entirely uncommon in developing nucleic acid-based growth models for larval fish Buckley (1984) notes that when calibration temperature ranges are limited, other factors such as feeding regime had significant effect on tissue nucleic acid ratios. The experimental temperatures in this study ranged by $8°C$ and may not have produced a detectable significant effect in the laboratory experiment.

In refining the nucleic acid-based model, I explored the type of relationship between nucleic acid concentrations and specific growth. A plot of *R:D* to observed *G* suggested the potential for a linear or curvilinear shape relationship between *R:D* and *G*. This prompted a Box-Cox power transformation analysis and indicated equal weighting for both non-transformed and square root transformed explanatory variable. Subsequent AIC_c comparisons indicated a significant improvement in model fit using the transformed model over the non-transformed model. The transformation depicts an asymptotic relationship between observed *R:D* ratios and

32

growth rate. This same asymptotic relationship has been demonstrated in other marine species including adult mussels and sardine larvae (Chicharo and Chicharo 2008). The curvilinear response may be the result from other bioenergetics constraints that were not measured inhibiting high growth rates despite high tissue *R:D* ratios.

Growth estimations derived from field animals and the laboratory calibration data, indicated that growth of laboratory animals was less than observed in the field (Chicharo and Chicharo 2008). Indeed, if *R:D* serves as an index of growth, the fastest growing individuals in the laboratory (ad-libitum conditions) exhibited a maximum *R:D* of 6.62. Thirty-eight percent of field collected crabs had *R:D* greater than the laboratory maximum, with the highest *R:D* observed in the field of 10.34. The higher *R:D* values observed in the field may indicate laboratory conditions supported reduced growth even under optimal feeding conditions, a possible result of food quality or stress of captivity. Food quality has been linked to differences in juvenile blue crab growth and condition. Studies focused on rearing juvenile blue crab have found crabs fed fresh brine shrimp tend to grow more rapidly than those fed on artificial pelleted diets (Millikin et al. 1980). The pelleted food used in the calibration experiment may have contributed to the lower observed growth for the laboratory crabs. Growth may have also been reduced in the laboratory due stress from captivity. Although housed in separate compartments within aquaria, crabs were able to visually and chemically detect conspecifics which may have led to elevated stress levels. Future work to improve the *R:D* growth model to emulate field-observed *R:D* may require adjusting food quality and laboratory conditions.

Although the nucleic acid index developed here provides a useful tool for estimating growth in the field, estimates derived from any statistical model should be interpolated within the laboratory index values and not extrapolated beyond the calibration range. Nucleic acid indices

are particularly sensitive to specific life stages and body size. For example, comparisons between the megalopae and the juvenile stages of the western rock lobster (*Panulirus cygnus*) indicate significant *R:D* differences between life stages (Lemmens 1995). Even within an ontogenetic stage, body size for some species can influence *R:D* ratios (Buckley 1984). Therefore it is advisable to interpolate the nucleic acid-based growth model developed here for juveniles within the calibration size range for reliable *G* estimations. Unfortunately this size restriction greatly reduced the number of field animals that could be used in this study.

Juvenile blue crab growth in the Patuxent River

Overall, estimates of *G* for individuals crabs collected in the Patuxent River were independent of site. Site-specific growth means were not significantly different across the sampling period or pooled over time. Predicted *G* did not significantly vary by site type, site location, or any measured environmental conditions.

Interestingly, despite observed variability in dissolved oxygen and salinity there was no strong pattern of environmental conditions influencing our indicator of growth, *R:D*. Numerous studies have pointed to the bioenergetics constraints from various levels of environmental conditions and rate of change of these conditions leading to changes in growth and *R:D* in crabs (e.g. Holland et al. 1971, Findley et al. 1978) and fish (e.g. Stierhoff et al. 2009)The small sample sizes used for the analysis may have lacked power to detect these environmental influences.

Previous studies using caged individuals also experience have mixed results with habitat effects growth within the Chesapeake Bay. For example, within the southern portion of the Chesapeake Bay, the York River VA, juveniles had distinct differences in habitat-associated

34

growth (Seitz et al. 2005), meanwhile studies in the northern portion of the Bay in the South River MD, found a lack of habitat effect on growth (Long et al. 2011). This variability between tributaries within the Chesapeake Bay may indicate a longitudinal pattern of discrete nursery habitats in the lower portion of the estuary with less discrete juvenile habitats in the upper portion of the estuary with our study aligning with the mid to northern portion of the Bay.   This may be the result of juveniles dispersing from areas of greater densities (the southern portion of the Chesapeake Bay) to areas of lower conspecific densities leading to less competition for northern habitats.

Juvenile blue crab nursery habitat and mode of habitat selection

The site-related growth investigated in this study was driven by a goal to understand habitat selection and potentially identify important nursery habitat. Although we sampled diverse habitats over 78km of the Patuxent River, MD, we found little evidence for site effects on growth with a lack of either outstanding high or low quality habitats. Although growth is not the only potential criteria in identifying nursery habitat (Beck et al. 2001), it does play an important role in juvenile recruitment into the adult stock. This work may indicate a lack of distinct nursery habitat for juvenile blue crab within this tributary. Two alternative hypotheses may explain the lack of site-specific differences in growth.

First, we could hypothesize that the Patuxent River during 2013 was recruitment limited and only high quality habitats were occupied and therefore all estimated growth was optimal to high. Evidence from the Chesapeake Bay blue crab winter dredge survey suggests that 111 million juvenile blue crab in the Chesapeake Bay during the winter of 2012-2013[2]. This is the

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[2] Maryland Department of Natural Resources http://dnr2.maryland.gov/fisheries/Pages/blue-crab/dredge.aspx

third lowest abundance of juvenile blue crab observed in the winter dredge survey since 1990. Although the abundance of juveniles does not, by itself, indicate recruitment limitation, it does suggest that the density of juvenile crabs in the summer of 2013 would have likely been much lower than in other years. Lower juvenile density reduces competitive pressure for high quality habitats. As a consequence, there would be a lower likelihood of low quality habitats being occupied in 2013. Interestingly, the comparison of G in the field vs the laboratory indicates the in situ growth was much higher than optimal laboratory conditions which may suggest also habitats sampled in the field were of higher quality.

Alternatively, if the blue crab stock in the upper portion of the Chesapeake Bay was not recruitment limited during the sampling period in summer 2013, the homogenous habitat growth pattern may be the result of habitat selection guided by the "ideal free distribution" (referred to as IFD, Fretwell and Lucas 1970). IFD predicts equal per capita growth rates across all habitats, where differences in habitat growth potential are mediated by individual distribution. The density of settler in the habitat balances the differences in habitat quality as individuals settle in the most optimal habitat. Each crab that settles in a habitat exploits resources and competes with conspecifics, reducing realized growth of all crabs in the habitat. Individuals will continue to settle into the high quality habitat until their density and resource usage reduces the realized growth to that of a lower quality habitat. At this level of abundance, a newly settling crab could do equally well in the high and lower quality habitats. At the landscape scale, IFD processes produce equivalent growth rates in all habitats, but inherently the high quality habitats will have higher densities of settling crabs (Jaap van der Meer and Ens 2007).

These two mentioned alternatives on habitat selection both could potentially yield equivalent G across habitats observations but they make very different predictions regarding

juvenile abundance. In the recruitment limited case, abundance would be low across all occupied habitats. In contrast, under IFD, crab abundance within a habitat should be proportional to the habitat's potential quality (Fretwell and Lucas 1970). To tease apart these hypotheses on juvenile blue crab habitat usage and value, the density of juveniles within habitats must be quantified. Regrettably, seine samples proved challenging in areas of complex habitat structure and were not a reliable index of relative abundance. Thus I am unable to evaluate these two alternative hypotheses with the data currently available. However, to further explore this question there are approaches to obtaining more reliable indices of relative crab abundances in structured habitats (e.g., suction dredging; Orth and van Montfrans 1987). Studies incorporating techniques to better estimate juvenile density should be a high priority in the future for elucidating juvenile blue crab habitat usage and potential juvenile nursery habitat.

Although we were unable to estimate density for a clearer insight into juvenile blue crab habitat selection and potential nursery habitat identification, our estimations of growth indicate a tributary wide pattern with greater growth achieved earlier in the summer (e.g., July) than for individuals of the same size later in the summer (e.g., August). This study further emphasizes the need to further investigate habitat quality and juvenile densities to understand juvenile blue crab habitat associations.

As an economically and ecologically important species in the Chesapeake Bay, the long term management of the stock requires clear understanding of blue crab ecology including the value of juvenile blue crab habitat. If there are distinct habitats that encourage juvenile growth, the identification of these habitats can aid in restoring and protecting the stock. This is especially important given that juvenile blue crab utilize shallow water habitats, which are often the same habitats are at elevated risk of human-caused disturbances. My study demonstrates equivalent

growth across various habitats and was unable to identify critical nursery habitats, therefore, one

potential indication from this work may be that juvenile blue crab rely on a mosaic of juvenile

habitats for development. If this is the case, biologists and managers may need to map

connectivity among habitat types to protect networks of habitats in the estuary to protect critical

juvenile habitat.

Table 2.1 Table of results of calibration experiment by ration and temperature treatment including initial carapace width, CW_0 (mm), change in carapace width, ΔCW (mm), initial weight, W_0 (g), change in weight, ΔW (g), absolute growth rate, AGR (mg.d^{-1}), and the ratio of RNA to DNA, $R{:}D$. Values reported in the table are the means ($\pm$ standard deviation) of 6 individuals, except for starvation 28°C and ad libitum 28°C which include only 5 individuals.

Ration	*Temperature*	*CW_0*	*ΔCW*	*W_0*	*ΔW*	*AGR*	*R:D*
	20	40 (3)	3 (5)	4.45 (0.68)	0.48 (1.38)	0.8 (0.64)	1.95 (0.35)
Starvation	24	41 (3)	1 (2)	4.90 (0.91)	0.51 (1.43)	0.19 (0.62)	1.80 (0.29)
	28	41 (3)	3 (4)	4.93 (0.60)	0.83 (1.65)	0.41 (0.81)	2.06 (0.31)
	20	42 (3)	9 (3)	5.39 (1.18)	3.57 (0.96)	1.43 (0.40)	4.23 (0.48)
Mid ration	24	41 (4)	6 (4)	5.05 (1.13)	3.36 (1.49)	1.40 (0.39)	3.78 (0.65)
	28	42 (4)	5 (2)	4.88 (0.88)	2.67 (1.42)	1.17 (0.48)	3.33 (0.55)
	20	41 (4)	5 (5)	4.71 (2.22)	2.56 (2.35)	1.21 (0.86)	3.79 (1.09)
ad libitum	24	41 (5)	9 (4)	4.79 (1.36)	4.46 (2.27)	1.80 (0.77)	3.92 (0.80)
	28	44 (3)	13 (6)	5.43 (1.28)	8.20 (4.82)	2.39 (1.08)	4.45 (1.40)

Table 2.2 Comparison of linear models to predict specific growth rate (G) of juvenile *C. sapidus* (n = 52). The performance of seven different models (A-G) are compared based on their structure, K - the number of parameters estimated including the intercept and error term, AICc – a sample size bias corrected Akaike's Information Criterion, Δmodel – differences in model AIC and the most parsimonious model AICc, adjusted r^2 – the coefficient of determination and w_i' – the Akiake weight of each model. Model structures show combinations of muscle RNA (R, $\mu g.mL^{-1}$) and DNA (D, $\mu g.mL^{-1}$) concentrations, the ratio of RNA:DNA ($R:D$), body weight (W, g), temperature (T, fixed factor with three levels), sex of the crab (S), and a normally distributed error term. The best model according to Akaike's Information Criterion with small sample bias adjustment (AICc) is highlighted in bold.

Model	*Model structure*	*K*	*AIC$_c$*	*Δ$_{model}$*	*adjusted r^2*	*w_i'*
A	*R:D, R, D, T, W, S*	12	64.58	15.25	0.6647	0
B	*R:D, R, D, T, W*	7	54.30	4.98	0.6698	0.05
C	*R:D, R, D, W*	6	52.63	3.30	0.6705	0.12
D	*R:D, R, W*	5	51.63	2.30	0.6676	0.20
E	***R:D, W***	**4**	**49.33**	**0.00**	**0.6734**	**0.63**
F	*R:D*	3	66.70	16.83	0.5372	0
G	*W*	3	64.70	15.38	0.5499	0

Table 2.3 Comparison of linear models to predict specific growth rate (G) of juvenile *C. sapidus* (n = 52). Performance of seven different models (E-H) is based on their structure, K - the number of parameters estimated including the intercept and error term, AICc – a bias corrected Akaike's Information Criterion, Δmodel – differences in model AIC and the most parsimonious model AICc, adjusted r^2 – the coefficient of determination and $w_i{}'$ – the Akiake weight of each model. Candidate models include the non-transformed model (E) and second degree polynomial model (H). Models include the ratio of RNA:DNA ($R{:}D$), body weight (W, g), and a normally distributed error term. The best model according to Akaike's Information Criterion with small sample bias adjustment (AICc) is highlighted in bold.

Model	Model structure	K	AIC_c	Δ_{model}	adjusted r^2	$w_i{}'$
E	$R{:}D$, W	4	49.33	4.91	0.6734	0.08
H	$R{:}D^{1/2}$, $(W)^{1/2}$	**4**	**44.42**	**0**	**0.7028**	**0.92**

Table 2.4 Variables coefficients and standard errors for the nucleic acid-based index model (H) estimating specific growth (*G*) of juvenile *C. sapidus*. The model relies on the ratios of RNA:DNA (*R:D*) and wet weight (*W*, g).

Variable name	Coefficient	Coefficient value	Standard errors
Intercept	β_0	-77.810	9.982
R:D	$\beta_{R:D}$	29.522	6.803
W	β_W	20.043	3.893

Table 2.5 Site specific data from each sampling event in 2013 with number of crabs collected (n), and mean (± standard deviation) of carapace width (CW), weight (W), RNA:DNA ratios (*R:D*), and estimated specific growth (*G*)

Site ID	Site Name	Latitude	Longitude	Position in river	Habitat	Date	Temp (ºC)	Salinity (ppt)	Dissolved Oxygen (mg/L)	n	CW (mm)	W (g)	R:D	G (mg·g^{-1}·d^{-1})
LM	Lower Marlboro	38.661283	-76.682319	Upper	Gravel	10-July	29.2	0.33	3.86	2	38 (1)	3.78 (0.98)	6.82 (0.07)	10.6 (1.5)
						25-July	28.8	1.27	3.65	5	55 (7)	11.62 (4.38)	9.24 (2.06)	21.9 (5.2)
						9-Aug	26.2	1.79	4.92	3	49 (4)	7.91 (2.30)	4.53 (0.05)	11.4 (2.2)
						29-Aug	26.4	1.88	5.47	4	51 (11)	9.96 (6.49)	5.36 (1.02)	14.3 (0.55)
KL	Kings Landing	38.620362	-76.672446	Upper	Mud	19-Jun	24.6	0.29	3.55	4	35 (4)	2.92 (0.95)	6.53 (1.62)	08.6 (3.0)
						10-July	28.0	0.85	3.70	7	47 (9)	7.18 (3.59)	7.92 (3.23)	15.6 (7.4)
						25-July	27.2	2.67	3.62	8	58 (11)	14.24 (6.73)	7.33 (1.32)	20.9 (6.0)
						9-Aug	24.7	3.12	4.05	12	55 (17)	13.23 (11.45)	5.05 (2.69)	15.0 (8.5)
						29-Aug	26.4	1.88	5.47	2	52 (16)	8.37 (6.16)	4.91 (0.53)	12.1 (0.71)
EH	Eagle Harbor	38.573321	-76.684403	Upper	Sand	10-July	29.9	3.49	5.31	5	56 (21)	14.75 (13.22)	5.48 (0.97)	16.9 (8.7)
						25-July	28.4	5.08	3.97	5	63 (24)	19.23 (15.91)	8.20 (5.26)	23.4 (15.6)
						9-Aug	25.7	6.44	5.20	3	62 (12)	18.02 (8.39)	4.01 (0.74)	18.0 (4.8)
GG	Gods Grace	38.538695	-76.668553	Upper	Sand	10-July	29.3	4.86	6.60	8	71 (23)	27.61 (18.01)	7.57 (3.43)	27.7 (13.3)
						25-July	27.8	7.57	4.40	3	75 (27)	34.33 (24.80)	8.10 (2.22)	31.9 (14.6)
TP	Teague Point	38.530423	-76.678620	Lower	Sand	10-July	30.1	5.12	7.62	2	57 (30)	20.98 (25.65)	5.74 (0.88)	20.1 (16.5)
						25-July	28.0	6.03	6.72	3	62 (18)	17.01 (9.81)	10.02 (0.61)	26.5 (07.9)
SP	Sheridan Point	38.467747	-76.649422	Lower	Sand	10-July	29.2	8.11	8.19	1	35 (-)	2.48 (-)	4.74 (-)	05.0 (-)
						25-July	28.8	9.30	6.11	2	26 (2)	1.16 (0.29)	6.71 (5.11)	04.7 (09.2)
						9- Aug	26.0	9.48	5.38	1	51 (-)	7.94 (-)	5.49 (-)	13.3 (-)
CR	Coatigan Run	38.425819	-76.645544	Lower	Sand	10-July	30.3	8.88	8.79	1	58 (-)	17.97 (-)	3.24 (-)	16.8 (-)
						25-July	27.0	8.77	7.33	2	85 (14)	41.94 (15.57)	12.2 (10.56)	41.2 (6.4)
						9- Aug	25.8	9.57	4.90	1	94 (-)	56.37 (-)	7.15 (-)	42.1 (-)
BI	Broome's Island	38.411311	-76.546461	Lower	Sand	19-Jun	25.8	9.46	8.00	1	57 (-)	12.62 (6.06)	7.27 (-)	20.3 (-)
						9-July	28.7	9.37	7.43	5	42 (13)	6.75 (14.77)	5.54 (2.05)	10.7 (10.1)
						26-July	27.2	-	6.17	4	72 (19)	25.53 (16.98)	7.87 (2.21)	28.4 (8.1)
						7-July	28.0	10.81	6.58	4	56 (27)	15.99 (6.06)	5.14 (1.31)	16.5 (10.8)
JP	Jefferson Patterson Park	38.400877	-76.513372	Lower	Marsh	9-July	28.6	8.96	7.02	5	46 (13)	8.42 (0.95)	7.17 (2.14)	15.5 (7.6)
						26-July	27.0	10.16	5.34	5	48 (18)	12.68 (3.59)	5.20 (3.02)	13.7 (15.2)
						7-Aug	25.3	10.54	6.30	6	65 (16)	23.13 (6.73)	5.24 (1.45)	21.7 (12.9)
						27-Aug	25.8	10.86	3.59	1	38 (-)	4.29 (11.45)	2.80 (-)	3.6 (-)
SB	Solomons Beach	38.325152	-76.462381	Lower	Sand	9-July	27.9	10.55	7.55	11	53 (13)	11.89 (6.94)	7.14 (2.46)	18.3 (6.3)
						26-July	28.0	10.81	6.58	13	55 (11)	12.86 (6.84)	6.50 (2.12)	18.4 (7.4)
						7-Aug	26.7	11.34	6.29	7	54 (18)	12.35 (11.89)	5.02 (1.16)	14.8 (8.7)
						27-Aug	26.7	11.7	3.64	5	55 (11)	13.19 (6.83)	6.17 (0.89)	18.4 (5.1)
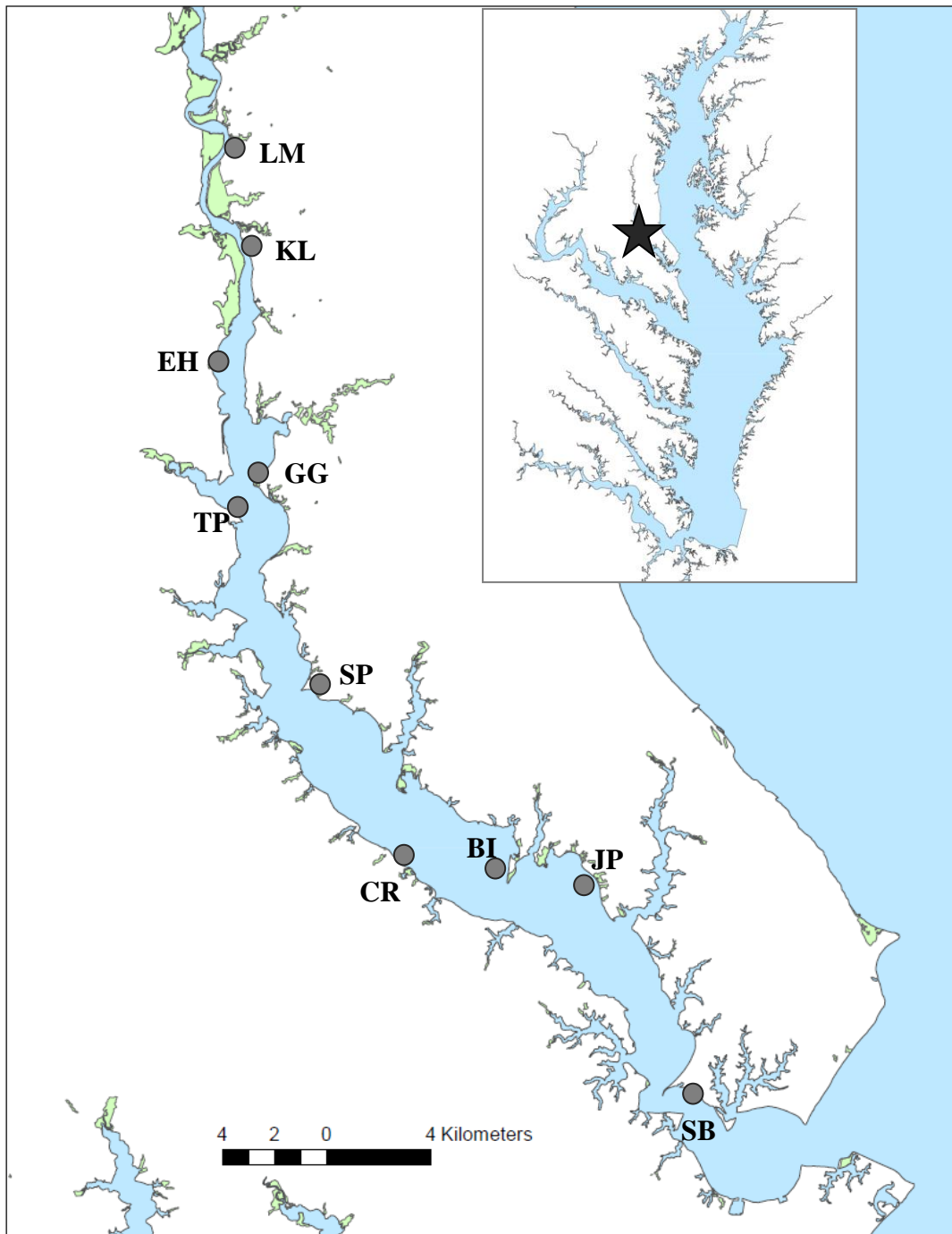
Figure 2.1 A map of the Patuxent River, MD (denoted by star) with the sampling locations (dark circles). Sample site abbreviations are moving upstream from the river mouth: SB – Solomons Beach, JP – Jefferson Patterson Park, BI – Broome's Island, CR – Coatigan Run, SP– Sheridan Point, TP – Teague Point, GG – Gods Grace, EH – Eagle Harbor, KL – Kings Landing, and LL – Lower Marlboro . The green shading indicates area where the shoreline is dominated by marshland.
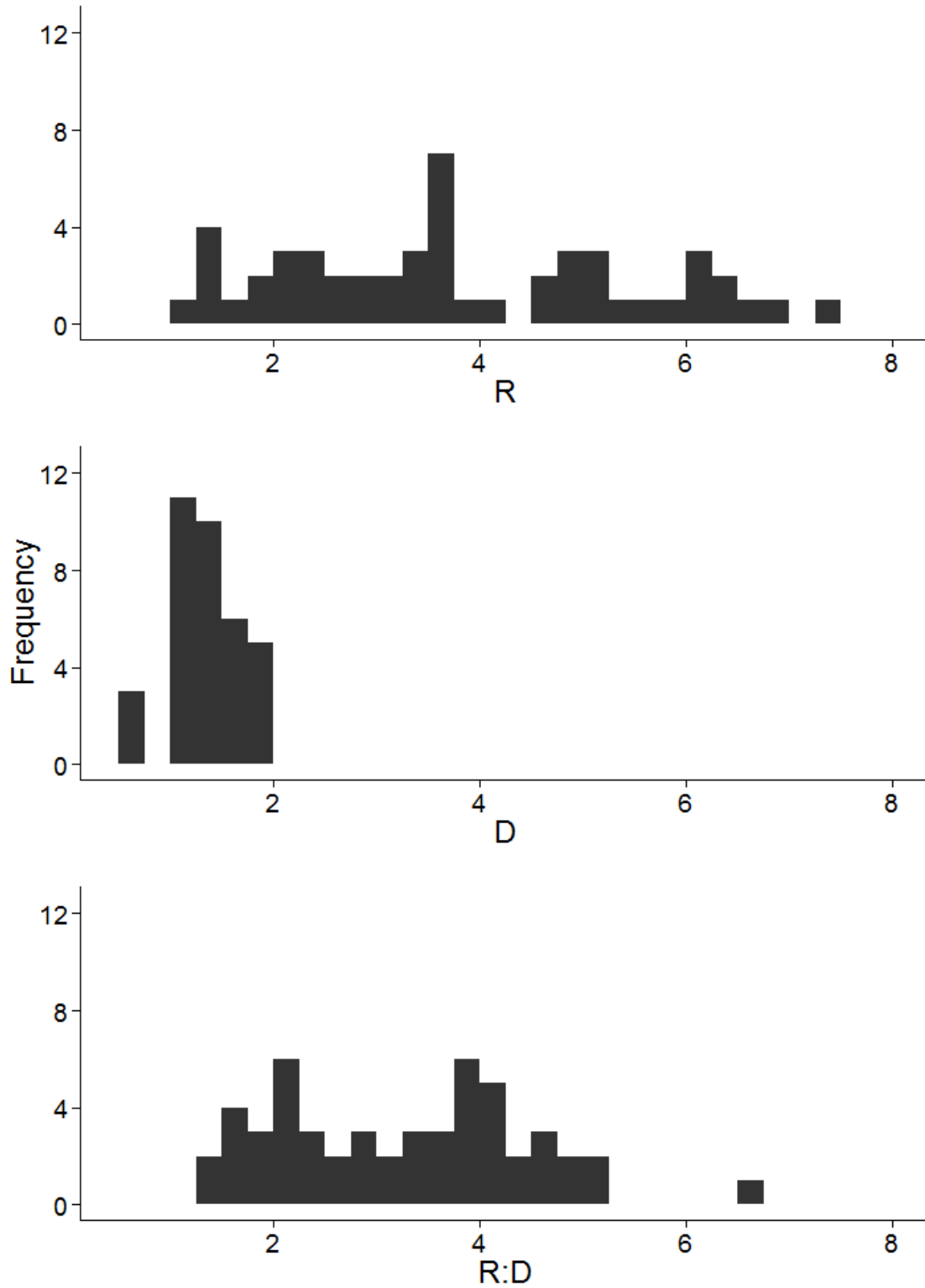
Figure 2.2 Histograms of RNA concentration (R, μg.mL^{-1}), DNA concentration (D, μg.mL^{-1}) and the ratio of RNA:DNA (*R:D*) from the juvenile crab growth-calibration experiment.

45
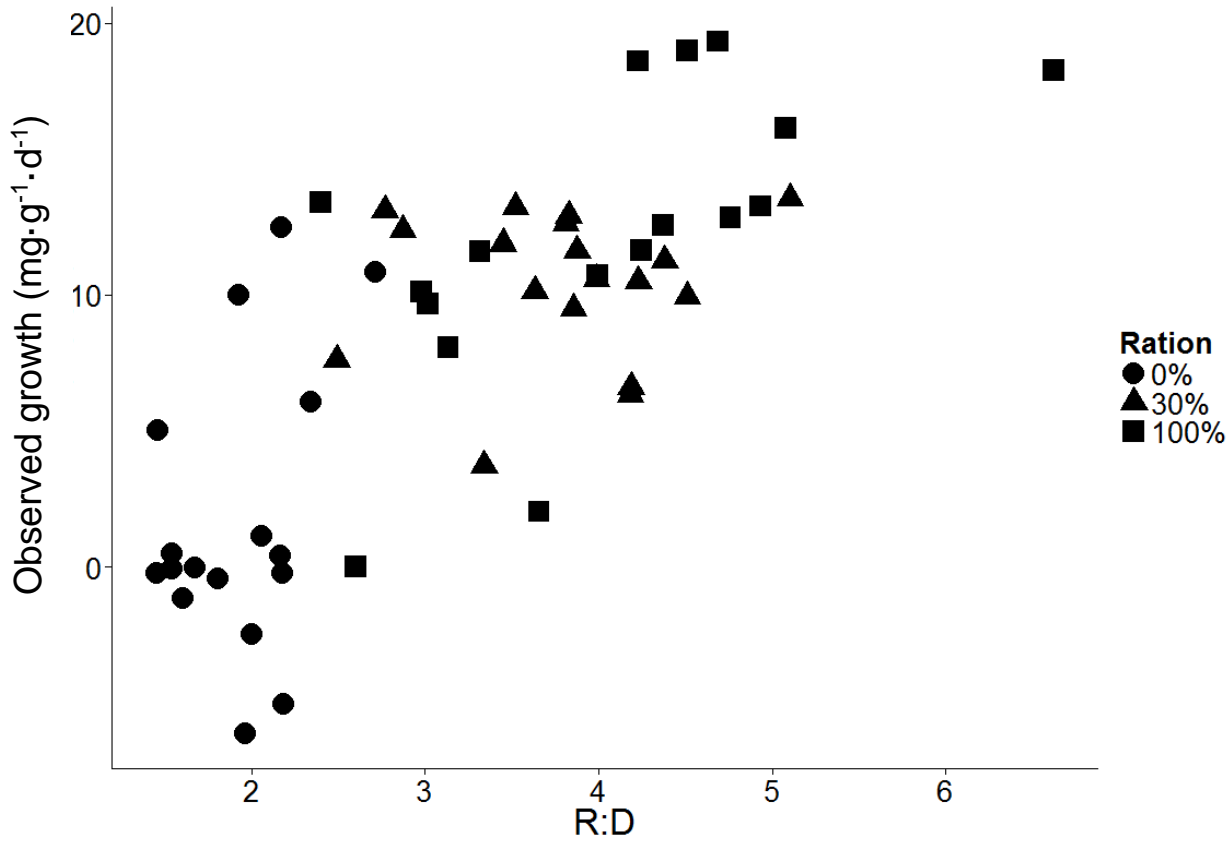
Figure 2.3 Relationship between RNA:DNA ratios (*R:D*) and observed growth (mg·g^{-1}·d^{-1}) in juvenile *C. sapidus*. Ration treatment for each individual is identified by circle for 0%-starvation, triangle for 30% of max ration, and square for *ad libitum* or 100% ration.
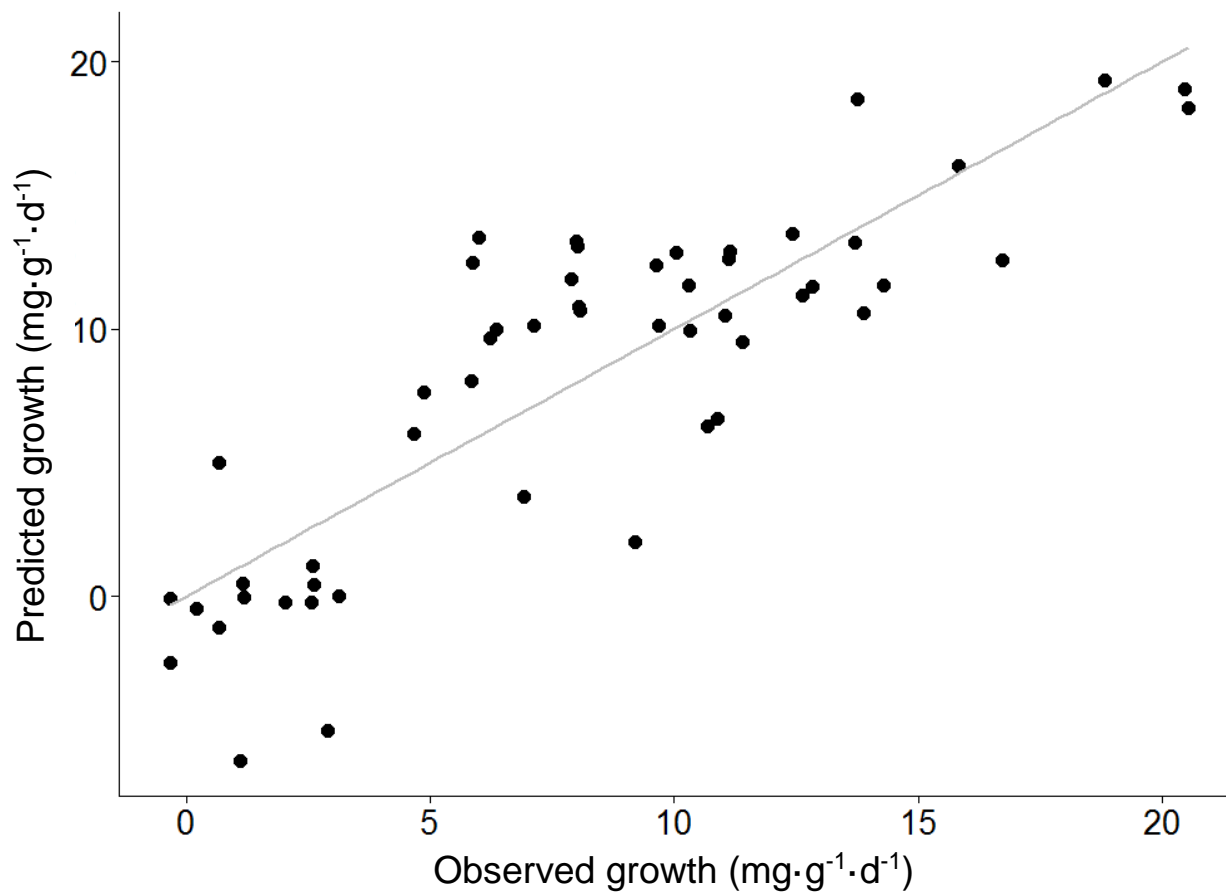
Figure 2.4 Relationship between observed growth (mg·g^{-1}·d^{-1}) and predicted growth for *C. sapidus* using the individual observations and the derived growth model from the calibration experiment compared to a 1:1 line.
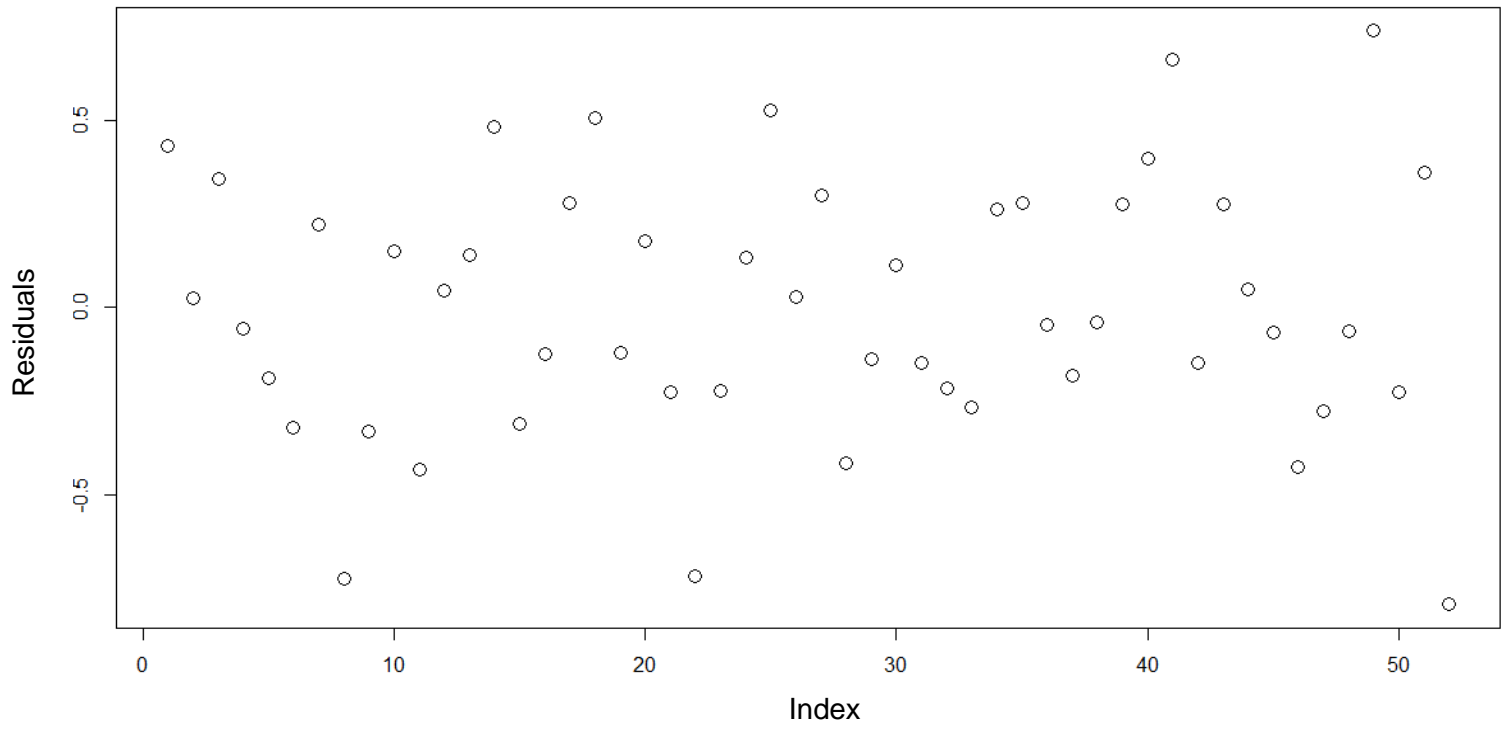
Figure 2.5 Residual plot of observed growth (*G*) and predicted growth for *C. sapidus* using the individual observations and the derived growth model from the calibration experiment.
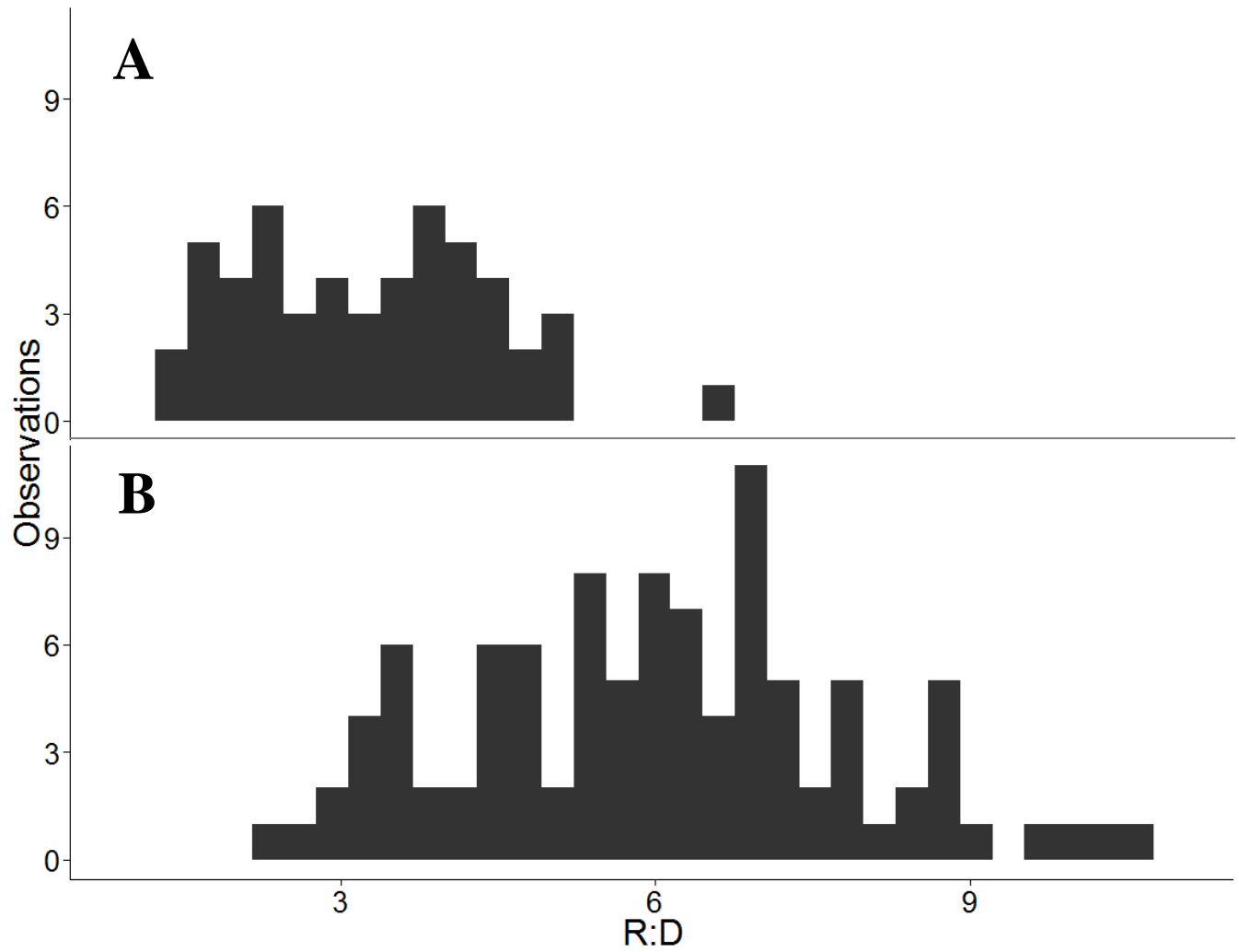
Figure 2.6 Frequency diagram of A) *R:D* values observed in the laboratory calibration experiment and B) RNA:DNA ratios (*R:D*) of field collected individuals.
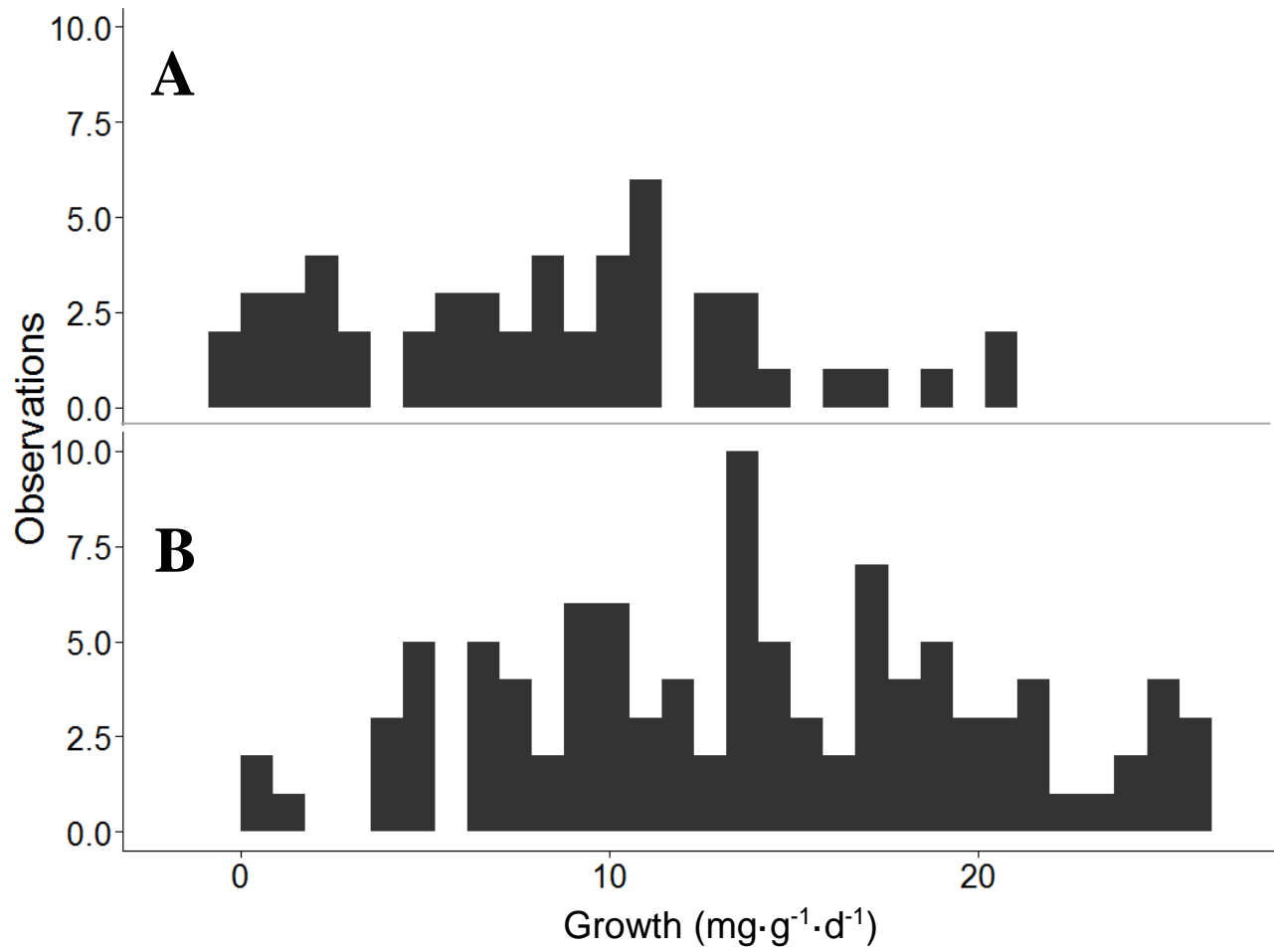
Figure 2.7 Frequency diagram of nucleic acid-based growth estimates of growth (*G*) of A) laboratory estimates derived in the calibration experiment and B) field collected individuals.
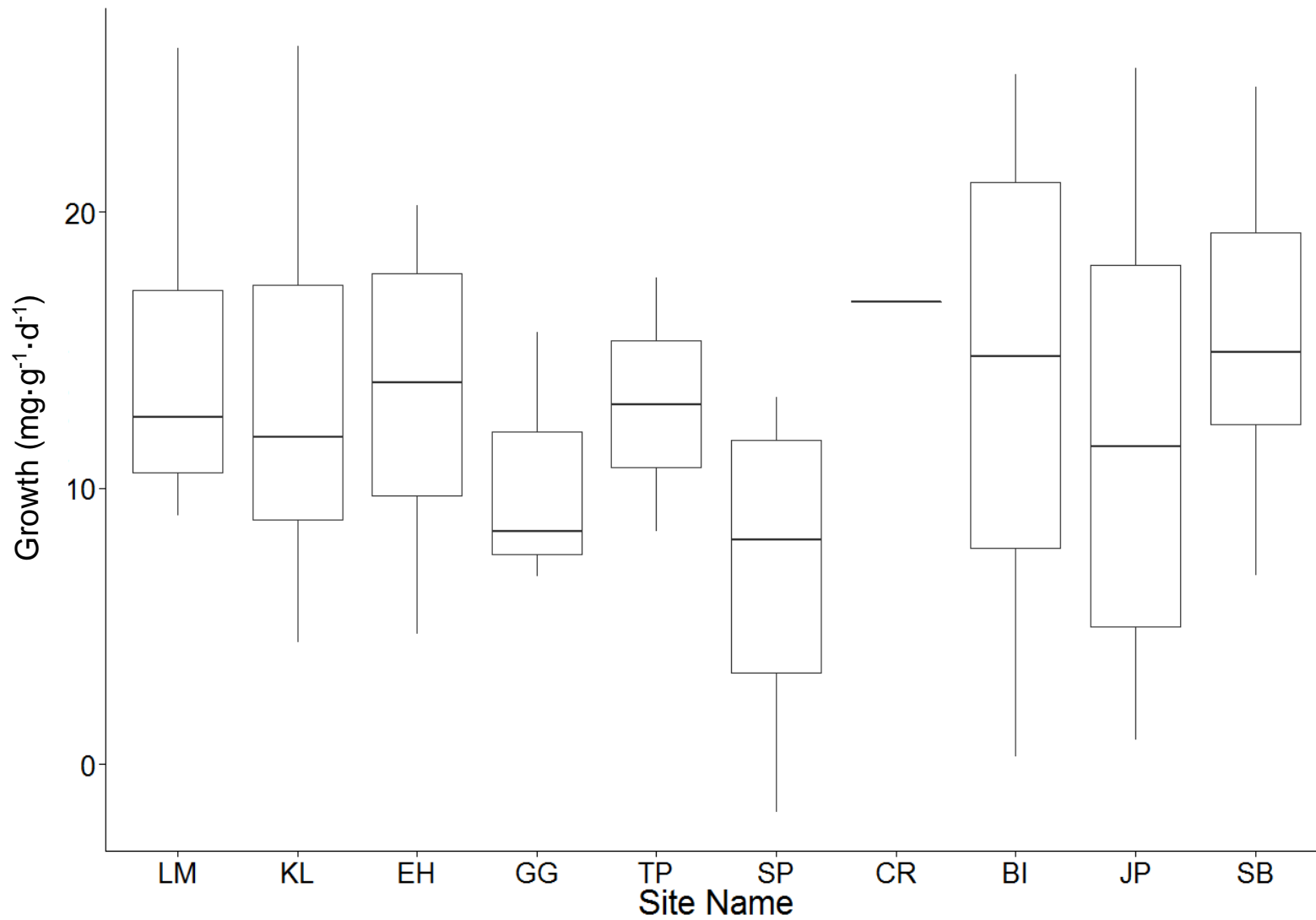
Figure 2.8 Box plots of estimated specific growth (G) of field collected juvenile *C. sapidus* by collection site. Boxes indicate a median value (dark line) and the 25th and 75th percentiles. Whiskers indicate 1.5* interquartile range. Single median lines represent sites with insufficient data to generate boxplots. Sites are arranged by decreasing latitude.
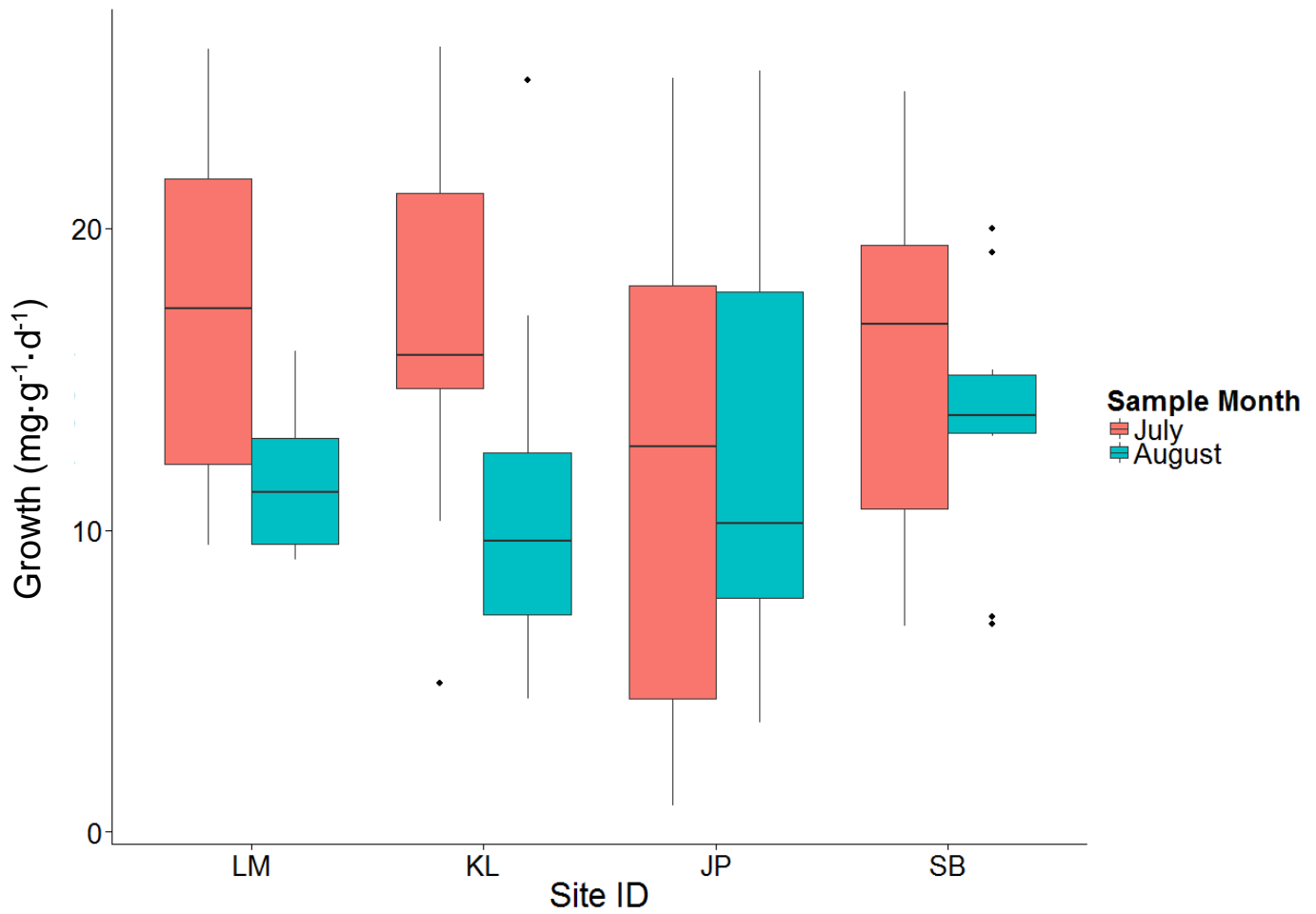
Figure 2.9 Box plots of estimated specific growth (*G*) of field collected juvenile *C. sapidus* by collection site. Boxes indicate a median value (dark line) and the 25th and 75th percentiles. Whiskers indicate 1.5* interquartile range with points representing outliers. Light blue shaded boxes represent pooled samples for July 2013 and darker orange shading represent pooled samples for August 2013. Sites are arranged by decreasing latitude.

Chapter 3

Past Reflections and Future Projection

In this work, we developed a growth model that was effective for estimating short term juvenile blue crab growth. This study provides a two-fold advancement in growth estimation for blue crab- through short term and intermolt growth measurement and single observation growth estimation. Although growth is commonly measured over short timescales in freshwater organisms, the marine-estuarine environment poses special challenges that typically preclude short term estimates. For example, the large spatial scale of marine systems makes mark and recapture studies intractable for many organisms. Moreover, the discrete nature of crustacean growth creates additional technical challenges for measuring growth. This study addresses both of these issues by facilitating an instantaneous measurement of growth based on a single encounter with an individual. This method allows for *in situ* estimates that otherwise would only be feasible through rigorous tagging efforts or using individuals that are caged or tethered as well as the ability to measure growth during the intermolt stage.

Contrary to our hypotheses, we did not see growth vary across space or among habitats within the Patuxent River in 2013. Consequently, we were unable to identify the prime mode of habitat selection or definitively identify nursery habitat based on the metrics provided by Beck et al. (2001). Our result suggest homogenous growth across the tidal portion of the Patuxent River which may indicate juvenile growth may vary across a larger spatial scale than sampled. If this is the case, effective

juvenile habitat management may require more concerted efforts to explore inter-tributary growth. Our results also suggest a lack of environmental effects on juvenile blue crab growth, it should be noted that the environmental conditions were taken during crab collection and future studies would be improved to include continuous environmental monitoring for a better picture of the conditions that are integrated during a particular intermolt period.

Despite rigorous efforts, field sample sizes were relatively low which in turn reduced our power to detect significant differences within the observed range of variability. Although we sampled multiple habitats that had high densities of juvenile blue crabs in 2012, our catch rates in 2013 were low for juveniles in the size range covered by my growth model. Our struggle to collect juvenile crabs during this study is likely related to unusually low juvenile recruitment as noted by the winter dredge survey. This low number of juvenile recruits may also indicate that the habitats sampled were note near the carrying capacity which would influence the growth pattern observed in our field work. It was impractical to estimate juvenile density at our sites using seine surveys (due to complex habitats). Without reliable density measures, we were unable to infer juvenile blue crab production and habitat selection method.

In the context of this project, we also attempted to examine the latency of RNA:DNA to better understand the time window that nucleic acid ratio condition represented. However, in an effort to boost our samples sizes during this experiment, large numbers of crabs were housed together in tanks. Although the tanks were heavily furnished with structure to provide refuge, extensive cannibalism occurred.

This unanticipated feeding activity (and loss of sample size) precluded useful data from being generated. However, I believe that understanding the timeframe represented by nucleic acid ratios is a critical step in understanding the context of measured growth in the field. Future researcher may want to separate crabs to eliminate cannibalism which can impact the results of a latency experiment.

There are also other opportunities to expand the scope and fully develop this project. First it would beneficial to attain growth rates in the laboratory comparable to the apparent rapid growth observed in the field. This will likely require alternate food and less stressful housing conditions. In addition, the calibration experiment may be strengthened by starting individual crabs immediately after they have molted so the growth observed would be less affected by preexisting conditions. Expanding the thermal range and size of crabs used in the calibration experiment would also further expand the utility of this approach. Although there was some unexplained variability in the growth model, this appears to be due to the physiology of the crabs rather than measurement error. Potentially expanding the calibration to include molt cycle or other aspects of blue crab physiology may improve model performance. Lastly, exploring the effects of the molt cycle on RNA:DNA ratios would assist in when crabs should be sampled for nucleic acid tissue concentrations.

Overall, blue crab growth is still difficult to quantify in the field and this study represents an advance towards an effective in-situ and intermolt growth. Although some work remains to be done, this approach seems to be feasible for juvenile blue crab and warrants exploration in other related taxa. I encourage future researchers to

build on this thesis research and develop a fully vetted nucleic acid-based approach

for measuring blue crab growth.

References

Anderson, D. R., and K. P. Burnham. 2002. Avoiding pitfalls when using information-theoretic methods. The Journal of Wildlife Management 912-918.

Anger, K. and H. J. Hirche. 1990. Nucleic acids and growth of larval and early juvenile spider crab, *Hyas araneus*. Marine Biology 105(3): 403-411.

Baird, D. and R. E. Ulanowicz. 1989. The seasonal dynamics of the Chesapeake Bay ecosystem. Ecological Monographs 59(4): 329-364.

Beck, M. W., K.L. Heck Jr, K.W. Able, D.L. Childers, D. B. Eggleston, B.M. Gillanders, … and R. J. Orth. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates: a better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. Bioscience 51(8): 633-641.

Bergeron, J. P. 1997. Nucleic acids in ichthyoplankton ecology: a review, with emphasis on recent advances for new perspectives. Journal of Fish Biology 51(sA): 284-302.

Boesch, D. F. and E. R. Turner. 1984. Dependence of fishery species on salt marshes: the role of food and refuge. Estuaries 7(4): 460-468.

Box, G. E. and D. R. Cox. 1964. An analysis of transformations. Journal of the Royal Statistical Society Series B (Methodological): 211-252.

Brylawski, B. J. and T. J. Miller. 2003. Bioenergetic modeling of the blue crab (*Callinectes sapidus*) using the fish bioenergetics (3.0) computer program. Bulletin of marine science 72(2): 491-504.

Brylawski, B. J. and T. J. Miller. 2006. Temperature-dependent growth of the blue crab (*Callinectes sapidus*): a molt process approach. Canadian Journal of Fisheries and Aquatic Sciences 63(6): 1298-1308.

Buckley, L. J. 1984. RNA-DNA ratio: an index of larval fish growth in the sea. Marine Biology 80(3): 291-298.

Buckley, L., E. Caldarone, and T. L. Ong. 1999. RNA—DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. Molecular Ecology of Aquatic Communities 265-277.

Burnham, K. P. and D. R. Anderson. 1998. Introduction. In Model Selection and Inference. Springer New York. 1-31

Butler, A. J. and P. Jernakoff. 1999. Seagrass in Australia: Strategic Review and Development of an R&D Plan. Collingwood (Australia): CSIRO Publishing.

Caldarone, E. M., M. Wagner, J. St Onge-Burns, L. J. and Buckley. 2001. Protocol and guide for estimating nucleic acids in larval fish using a fluorescence microplate reader. Northeast Fisheries Science Center Reference Document 1(11): 1-22.

Caldarone, E. M., J. M. S. Onge-Burns, and L. J. Buckley. 2003. Relationship of RNA/DNA ratio and temperature to growth in larvae of Atlantic cod *Gadus morhua*. Marine Ecology Progress Series 262: 229-240.

CBSAC (Chesapeake Bay Stock Assessment Committee). 2014. 2014 Blue crab advisory report. CBSAC, Annapolis, Maryland.

Chang, E. S. 1995. Physiological and biochemical changes during the molt cycle in decapod crustaceans: an overview. Journal of Experimental Marine Biology and Ecology 193(1): 1-14.

Chenery, M. A. 2002. Population Dynamics of Blue Crab (*Callinectes sapidus*) in The Hudson River, New York. Doctoral dissertation, University of Maryland.

Chícharo, M. A. and Chícharo, L. 2008. RNA: DNA ratio and other nucleic acid derived indices in marine ecology. International Journal of Molecular Sciences 9(8): 1453-1471.

Chung, S. J. and N. Zmora. 2008. Functional studies of crustacean hyperglycemic hormones (CHHs) of the blue crab, *Callinectes sapidus*–the expression and release of CHH in eyestalk and pericardial organ in response to environmental stress. FEBS Journal 275(4): 693-704.

Ciotti, B. J., T. E. Targett, R. D. M. Nash, R.S. Batty, T. M. Burrows, and A. J. Geffen. 2010. Development, validation and field application of an RNA-based growth index in juvenile plaice *Pleuronectes platessa*. Journal of Fish Biology 77(10): 2181-2209.

Colton, A. R. 2011. An evaluation of the synchronization in the dynamics of blue crab (*Callinectes sapidus*) populations in the western Atlantic. Master's thesis, University of Maryland.

Dahlgren, C., G. Kellison, A. Adams, B. Gillanders, M. Kendall, C. Layman, J. Ley, I. Nagelkerken, and J. Serafy. 2006. Marine nurseries and effective juvenile habitats: concepts and applications. Marine Ecology- Progressive Series 312:291-295.

Edsman, L., T. Järvi, and B. Niejahr. 1994. The RNA concentration as an index of current growth rate in juvenile signal crayfish, *Pacifastacus leniusculus*. Nordic Journal of Freshwater Research.

Epifanio, C. E. 2007. Biology of Larvae. The Blue Crab, *Callinectes sapidus*. Maryland Sea Grant, College Park, MD, 513-533.

Etherington, L. L., and D. B. Eggleston. 2000. Large-scale blue crab recruitment: linking postlarval transport, post-settlement planktonic dispersal, and multiple nursery habitats. Marine Ecology Progress Series 204:179-198.

Etherington, L. L. and D. B. Eggleston. 2003. Spatial dynamics of large-scale, multistage crab (*Callinectes sapidus*) dispersal: determinants and consequences for recruitment. Canadian Journal of Fisheries and Aquatic Sciences 60(7): 873-887.

Everett, R. A., and G. M. Ruiz. 1993. Coarse woody debris as a refuge from predation in aquatic communities. Oecologia 93(4): 475-486.

Figueira, W. F. and L.B. Crowder. 2006. Defining patch contribution in source-sink metapopulations: the importance of including dispersal and its relevance to marine systems. Population Ecology 48(3):215-224.

Findley, A. M., B. W. Belisle, and W. B. Stickle. 1978. Effects of salinity fluctuations
    on the respiration rate of the southern oyster drill *Thais haemastoma* and the
    blue crab *Callinectes sapidus*. Marine biology 49(1): 59-67.

Foster A. R., D. F. Houlihan, S. J. Hall, L. I. Burren. 1992. The effects of temperature
    acclimation on protein synthesis rates and nucleic acid content of juvenile
    cod (*Gadus morhua*). Canadian Journal of Zoology 70:2095–2102.

Fraser, K. P., A. Clarke, and L. S. Peck. 2002. Low-temperature protein metabolism:
    seasonal changes in protein synthesis and RNA dynamics in the Antarctic
    limpet *Nacella concinna* Strebel 1908. Journal of Experimental Biology
    205(19): 3077-3086.

Fretwell, S. D. and H. L. Lucas Jr. 1970. On territorial behavior and other factors
    influencing habitat distribution in birds I. Theoretical development. Acta
    Biotheor 19:16–36.

Fry, F. E. J. 1971. The effect of environmental factors on the physiology of fish. Fish
    Physiology 6:1-98.

Gilliam, J. F. and D. F. Fraser. 1987. Habitat selection under predation hazard: test of
    a model with foraging minnows. Ecology 68(6): 1856-1862.

Gorokhova, E. and M. Kyle, M. 2002. Analysis of nucleic acids in Daphnia:
    development of methods and ontogenetic variations in RNA-DNA content.
    Journal of Plankton Research 24(5): 511-522.

Grimm, C., K. Lehmann, C. Clemmesen, and H. Brendelberger. 2015. RNA/DNA
    ratio is an early responding, accurate performance parameter in growth

experiments of noble crayfish *Astacus astacus*. Aquaculture Research 46(8): 1937-1945.

Hare, J. A., and R. K. Cowen. 1997. Size, growth, development, and survival of the planktonic larvae of *Pomatomus saltatrix* (Pisces: Pomatomidae). Ecology 78(8): 2415-2431.

Hayes, D. B., C.P. Ferreri, and W. W. Taylor. 1996. Linking fish habitat to their population dynamics. Canadian Journal of Fisheries and aquatic sciences 53(S1): 383-390.

Heyer, C. J., T. J. Miller, F. P. Binkowski, E. M. Caldarone, and J. A. Rice. 2001. Maternal effects as a recruitment mechanism in Lake Michigan yellow perch (*Perca flavescens*). Canadian Journal of Fisheries and Aquatic Sciences 58(7): 1477-1487.

Hines, A.H., R. N. Lipcius, and A. M. Haddon. 1987. Population dynamics and habitat partitioning by size, sex, and molt stage of blue crabs *Callinectes sapidus* in a subestuary of central Chesapeake Bay. Marine Ecology Progress Series, 36(1): 55-64.

Hines, A.H. and T. G. Wolcott. 1990. Blue crab movement and feeding measured by ultrasonic telemetry. Bulletin of Marine Science 46: 246.

Hines, A.H., T. G. Wolcott, E. González-Gurriarán, J.L. González-Escalante, and J. Freire. 1995. Movement patterns and migrations in crabs: telemetry of juvenile and adult behaviour in *Callinectes sapidus* and *Maja squinado*. Journal of the Marine Biological Association of the United Kingdom, 75(1): 27-42.

Hines, A.H. 2007. Ecology of juvenile and adult blue crabs. The Blue Crab,
Callinectes sapidus. University of Maryland Sea Grant Press, College Park,
MD 565-654.

Holland, J. S., D. V. Aldrich, K. Strawn. 1971. Effects of temperature and salinity on
growth, food conversion, survival and temperature resistance of juvenile blue
crabs, *Callinectes sapidus* Rathbun ([Sea grant publication], TAMU-SG-71-
222; Sea grant publication (College Station), no. TAMU-SG-71-222).
College Station: Texas A & M University, Sea Grant Program.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. American
Fisheries Society Symposium Series 2:17-29.

Houlihan, D. F., C. P. Waring, E. Mathers, and C. Gray. 1990. Protein synthesis and
oxygen consumption of the shore crab *Carcinus maenas* after a meal.
Physiological Zoology 735-756.

Houlihan, D. F. 1991. Protein turnover in ectotherms and its relationship to
energetics. Advances in comparative and environmental physiology 7:1–43.

Hovel, K. A., and M. S. Fonseca. 2005. Influence of seagrass landscape structure on
the juvenile blue crab habitat-survival function. Marine Ecology Progress
Series 300: 179-191.

Jaap Van Der Meer and B.J. Ens. 1997. Models of interference and their
consequences for the spatial distribution of ideal and free predators. Journal
of Animal Ecology 66(6): 846-858.

Jensen, O. P. and T. J. Miller. 2005. Geostatistical analysis of the abundance and
winter distribution patterns of the blue crab *Callinectes sapidus* in

Chesapeake Bay. Transactions of the American Fisheries Society, 134(6): 1582-1598.

Juinio M.A.R., J. S. Cobb, D. Bengston, and M. Johnson. 1992. Changes in nucleic acids over the moult cycle in relation to food availability and temperature in *Homarus americanus* postlarvae. Marine Biology 114:1–10.

Juinio M.A.R. and J. S. Cobb. 1994. Estimation of recent growth of field-caught postlarval American lobsters, *Homarus americanus*, from RNA:DNA ratios. Canadian Journal of Fisheries Aquaculture Science 51:286–294.

Kerkhof, L. and B. B. Ward. 1993. Comparison of nucleic acid hybridization and fluorometry for measurement of the relationship between RNA/DNA ratio and growth rate in a marine bacterium. Applied and Environmental Microbiology 59(5): 1303-1309.

King, R. S., A. H. Hines, F.D. Craige, and S. Grap. 2005. Regional, watershed and local correlates of blue crab and bivalve abundances in subestuaries of Chesapeake Bay, USA. Journal of Experimental Marine Biology and Ecology 319(1): 101-116.

Lemmens, J. W. T. J. 1995. Nucleic acid levels, cellular activity and growth during the puerulus stage of the Western Rock Lobster (*Panulirus cygnus* (George); Decapoda: Palinuridae). Journal of Experimental Marine Biology and Ecology 194(2): 143-156.

Levin, P. S. and G.W. Stunz. 2005. Habitat triage for exploited fishes: Can we identify essential "Essential Fish Habitat?". Estuarine, Coastal and Shelf Science 64(1): 70-78.

Lipcius, R. N., W. T. Stockhausen, D. B. Eggleston, L. S. Marshall Jr, and B. Hickey.
1997. Hydrodynamic decoupling of recruitment, habitat quality and adult
abundance in the Caribbean spiny lobster: source–sink dynamics?. Marine
and Freshwater Research 48(8): 807-816.

Lipcius, R. N., D. B. Eggleston, K. L. Heck Jr, R. D. Seitz, and van Montfrans. 2007.
Post-settlement abundance, survival, and growth of postlarvae and young
juvenile blue crabs in nursery habitats. The Blue Crab *Callinectes sapidus*.
Maryland Sea Grant College, College Park, Maryland 535-564.

Long, W.C., J. N. Grow, J. E. Majoris, and A. H. Hines. 2011. Effects of
anthropogenic shoreline hardening and invasion by *Phragmites australis* on
habitat quality for juvenile blue crabs (*Callinectes sapidus*). Journal of
Experimental Marine Biology and Ecology 409(1):.215-222.

Lovette, I.J. and W. M. Hochachka.. 2006. Simultaneous effects of phylogenetic
niche conservatism and competition on avian community structure. Ecology
87:S14–S28.

MacCall, A. D. 1990. Dynamic geography of marine fish populations. Seattle,
Washington: Washington Sea Grant Program 153.

MSFCMRA (Magnuson–Stevens Fishery Conservation and Management
Reauthorization Act). 2007. Public Law No. 109-479, 120 Stat. 3575.

Mathers, E. M., D. F. Houlihan, and M. J. Cunningham. 1992. Nucleic acid
concentrations and enzyme activities as correlates of growth rate of the *saithe*
Pollachius virens: growth-rate estimates of open-sea fish. Marine Biology
112(3): 363-369.

Mayrand, E., H. Guderley, and D. J. Dutil. 2000. Biochemical indicators of muscle growth in the snow crab *Chionoecetes opilio* (O. Fabricius). Journal of Experimental Marine Biology and Ecology 255(1): 37-49.

McMillan, D. N. and D. F. Houlihan. 1988. The effect of refeeding on tissue protein synthesis in rainbow trout. Physiological Zoology 429-441.

McNamara, P. T., E. M. Caldarone, and L. J. Buckley. 1999. RNA/DNA ratio and expression of 18S ribosomal RNA, actin and myosin heavy chain messenger RNAs in starved and fed larval Atlantic cod (*Gadus morhua*). Marine Biology 135(1): 123-132.

Mente, E., P. Coutteau, D. Houlihan, I. Davidson, and P. Sorgeloos. 2002. Protein turnover, amino acid profile and amino acid flux in juvenile shrimp *Litopenaeus vannamei*: effects of dietary protein source. Journal of Experimental Biology 205(20): 3107-3122.

Mercaldo-Allen, R., C. Kuropat, and E. M. Caldarone. 2006. A model to estimate growth in young-of-the-year tautog, *Tautoga onitis*, based on RNA/DNA ratio and seawater temperature. Journal of Experimental Marine Biology and Ecology 329(2): 187-195.

Mercaldo-Allen, R., C. Kuropat, and E. M. Caldarone. 2008. An RNA: DNA-based growth model for young-of-the-year winter flounder *Pseudopleuronectes americanus* (Walbaum). Journal of Fish Biology 72(6): 1321-1331.

Miller, T. J., S.J. Martell, D.B. Bunnell, G. Davis, L. Fegley, A, Sharov, ., ... and R. N. Lipcius. 2005. Stock assessment of blue crab in Chesapeake Bay. Final

report to NOAA Chesapeake Bay Office, Annapolis, MD. Chesapeake Bay Stock Assessment Committee 2014.

Millikin, M. R., G.N. Biddle, T.C. Siewicki, A.R. Fortner, and P.H. Fair. 1980. Effects of various levels of dietary protein on survival, molting frequency and growth of juvenile blue crabs (*Callinectes sapidus*). Aquaculture 19(2): 149-161.

Millward, D.J., P.J. Garlick, W. P. T. James, D. O. Nnanyelugo, and J. S. Ryatt. 1973. Relationship between Protein Synthesis and RNA Content in Skeletal Muscle. Nature 241:204-205.

Minello, T. J., K.W. Able, M.P. Weinstein, and C.G. Hays. 2003. Salt marshes as nurseries for nekton: testing hypotheses on density, growth and survival through meta-analysis. Marine Ecology Progress Series 246:39-59.

Moksnes, P. O. and K. L. Heck Jr. 2006. Relative importance of habitat selection and predation for the distribution of blue crab megalopae and young juveniles. Marine Ecology Progress Series 308: 165-181.

Morris, D. W. 2003. How can we apply theories of habitat selection to wildlife conservation and management?. Wildlife Research 30(4): 303-319.

Moss, S. M. 1994. Growth rates, nucleic acid concentrations, and RNA/DNA ratios of juvenile white shrimp, *Penaeus vannamei* Boone, fed different algal diets. Journal of Experimental Marine Biology and Ecology 182(2): 193-204.

Moss, S. M. 1999. Use of nucleic acids as indicators of growth in juvenile white shrimp, *Penaeus vannamei*. Marine Biology 120(3): 359-367.

Moya-Laraño, J., R. Macías-Ordóñez., W. U. Blanckenhorn, and C. Fernández-
 Montraveta. 2008. Analyzing body condition: mass, volume or density.
 Journal of Animal Ecology 77(6): 1099-1108.

Neufeld, D. and J. Cameron. 1994. Mechanism of the net uptake of water in moulting
 blue crabs (*Callinectes sapidus*) acclimated to high and low salinities.
 Journal of Experimental Biology 188(1): 11-23.

NMFS. 2007. NMFS strategic plan for fisheries research. U.S. Department of
 Commerce, NOAA Technical Memorandum NMFS-F/SPO-80: 170

Orth, R. J. and Van Montfrans, J. 1987. Utilization of a seagrass meadow and tidal
 marsh creek by blue crabs *Callinectes sapidus*. Marine ecology progress
 series 41(3): 283-294.

Pardieck, R. A., Orth, R. J., Diaz, R. J., and Lipcius, R. N. 1999. Ontogenetic changes
 in habitat use by postlarvae and young juveniles of the blue crab. Marine
 Ecology Progress Series 186: 227-238.

Parslow-Williams, P. J., R. J. A. Atkinson, and A. C. Taylor. 2001. Nucleic acids as
 indicators of nutritional condition in the Norway lobster *Nephrops
 norvegicus*. Marine Ecology Progress Series 211: 235-243.

Peck, M. A., L. J. Buckley, E. M. Caldarone, and D. A. Bengtson. 2003. Effects of
 food consumption and temperature on growth rate and biochemical-based
 indicators of growth in early juvenile Atlantic cod *Gadus morhua* and
 haddock *Melanogrammus aeglefinus*. Marine Ecology Progress Series 251:
 233-243.

Perkins-Visser, E., T. G. Wolcott, and D. L. Wolcott. 1996. Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (*Callinectes sapidus* Rathbun). Journal of Experimental Marine Biology and Ecology 198(2): 155-173.

Pile, A. J., R. N. Lipcius, J. van Montfrans, and R. J. Orth. 1996. Density-Dependent Settler-Recruit-Juvenile Relationships in Blue Crabs. Ecological Monographs 66(3): 277-300.

Post, J. R. and E. A. Parkinson. 2001. Energy allocation strategy in young fish: allometry and survival. Ecology 82(4): 1040-1051.

Pulliam, H. R. 1988. Sources, sinks, and population regulation. American Naturalist 652-661.

Reyns, N. B., and D. B. Eggleston. 2004. Environmentally-controlled, density-dependent secondary dispersal in a local estuarine crab population. Oecologia 140(2): 280-288.

Rice, J. A. 1990. Bioenergetics modeling approaches to evaluation of stress in fishes. In American Fisheries Society Symposium 8:80-92.

Rosenzweig, M. L. 1981. A theory of habitat selection. Ecology 62(2): 327-335.

Searcy, S. P., D. B. Eggleston, and J. A. Hare. 2007. Is growth a reliable indicator of habitat quality and essential fish habitat for a juvenile estuarine fish? Canadian Journal of Fisheries and Aquatic Sciences 64(4): 681-691.

Seitz, R. D., L. S. Marshall Jr, A. H. Hines, and K. L. Clark. 2003. Effects of hypoxia on predator-prey dynamics of the blue crab *Callinectes sapidus* and the Baltic clam *Macoma balthica* in Chesapeake Bay. Marine Ecology Progress Series 257:179-188.

Seitz, R. D., R. N. Lipcius, and M. S. Seebo. 2005. Food availability and growth of the blue crab in seagrass and unvegetated nurseries of Chesapeake Bay. Journal of Experimental Marine Biology and Ecology 319(1): 57-68.

Shirley, M. A., A. H. Hines, and T. G. Wolcott. 1990. Adaptive significance of habitat selection by molting adult blue crabs *Callinectes sapidus* (Rathbun) within a subestuary of central Chesapeake Bay. Journal of Experimental Marine Biology and Ecology 140(1-2): 107-119.

Smith, S. G. and E. S. Chang. 2007. Molting and growth. The blue crab *Callinectes sapidus*. University of Maryland Sea Grant College, College Park 197-254.

Sogard, S. M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of marine science 60(3): 1129-1157.

Stierhoff, K. L., T. E. Targett, and J. H. Power. 2009. Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics using RNA: DNA. Canadian Journal of Fisheries and Aquatic Sciences 66(7): 1033-1047.

Sutcliffe Jr, W. H. 1970. Relationship between growth rate and ribonucleic acid concentration in some invertebrates. Journal of the Fisheries Board of Canada 27(3): 606-609.

Traganos, F., Z. Darzynkiewicz, Z., and M. R. Melamed. 1982. The ratio of RNA to total nucleic acid content as a quantitative measure of unbalanced cell growth. Cytometry 2(4): 212-218.

Van Horne, B. 1983. Density as a misleading indicator of habitat quality. The Journal of Wildlife Management 893-901.

Vrede, T., J. Persson, and G. Aronsen. 2002. The influence of food quality (P: C ratio) on RNA: DNA ratio and somatic growth rate of Daphnia. Limnology and Oceanography 47(2): 487-494.

Wagner, M. M., R. G. Campbell., C. A. Boudreau, and E. G. Durbin. 2001. Nucleic acids and growth of Calanus finmarchicus in the laboratory under different food and temperature conditions. Marine Ecology Progress Series 221: 185-197.

Wang, S. Y., and W. B. Stickle. 1987. Bioenergetics, growth and molting of the blue crab, *Callinectes sapidus,* exposed to the water-soluble fraction of South Louisiana crude oil. Pollution Physiology of Estuarine Organism 17: 107.

Wang, S. Y., and W. B. Stickle. 1988. Biochemical composition of the blue crab *Callinectes sapidus* exposed to the water-soluble fraction of crude oil. Marine Biology 98(1): 23-30.

Williams, A. B. 1974. The swimming crabs of the genus Callinectes (Decapoda: Portunidae). Fishery Bulletin 72(3): 685-798.

Williams, A. H., L. D. Coen, and M. S. Stoelting. 1990. Seasonal abundance, distribution, and habitat selection of juvenile *Callinectes sapidus* (Rathbun) in the northern Gulf of Mexico. Journal of Experimental Marine Biology and Ecology 137(3): 165-183.

Wolcott, T.G. and A. H. Hines. 1989. Ultrasonic biotelemetry of muscle activity from free-ranging marine animals: a new method for studying foraging by blue crabs (*Callinectes sapidus*). The Biological Bulletin 176(1): 50-56.