BEHAVIORAL ECOLOGY AND POPULATION GENETICS OF TWO POPULATIONS OF BLUE CRAB, *CALLINECTES SAPIDUS* (RATHBUN), IN NEW JERSEY

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A dissertation submitted to

The Graduate School-Newark

Rutgers, the State University of New Jersey

in partial fulfillment of requirements

for the degree of

Doctor of Philosophy

Graduate Program in Biological Sciences

Written under the direction of

Professor Judith S. Weis

and approved by

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Newark, NJ

May 2009

ABSTRACT OF THE THESIS

Behavioral Ecology and Population Genetics of Two Populations of Blue Crab, *Callinectes sapidus* (Rathbun) in New Jersey

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Thesis Director: Dr. Judith S. Weis

Blue crabs are important estuarine organisms, both ecologically and economically. Due to historical differences of human impact between contaminated Hackensack Meadowlands (HM) and cleaner Tuckerton (TK), adult prey capture, juvenile predator avoidance, adult/juvenile aggression, metal accumulation/depuration and population genetics were investigated.

HM adults had reduced prey capture on active prey compared to TK crabs, suggesting HM crabs may have reduced coordination. Stomach analysis revealed HM crabs' stomachs contained ~60% algae/plant and detritus/sediment, and lower crab and fish weights than TK crabs. TK crabs were caged in HM or fed HM food for 8 weeks; their prey capture declined significantly indicating environmental factors were responsible for the behavioral differences.

Crabs were then analyzed for metals in muscle and hepatopancreas. HM crabs were fed clean food or transplanted to TK; TK crabs were fed contaminated food or transplanted to HM. Significant tissue differences were

found for Cu, Hg, Pb and Zn. HM crabs did not show a significant decrease in Hg after switching environment or diet, but showed a significant decrease in Cu, Pb and Zn in hepatopancreas after switching. TK crabs showed a significant increase of Hg in muscle and Cr and Zn in hepatopancreas after switching environment or food.

In the lab, HM juveniles attacked threatening stimuli significantly more and TK juveniles fled or gave a mixed response significantly more. HM juveniles were significantly better at avoiding a crab predator when substrate was present. Follow-up experiments were conducted without substrate to determine if aggression was important. Aggressive juveniles were no more successful than non-aggressive ones at avoiding a predator. Adults were placed in a large tank with a crab pot; significantly fewer HM adults entered the pot. The first HM crab to enter generally prevented others from entering or attacked those that did, suggesting aggression may be causing low pot counts.

Microsatellites were analyzed using four markers. Genotypic differences were not found among the three years which indicates these populations are not genetically distinct. Yearly differences were not found. These results indicate genetics can be ruled out. The ‘switch’ experiments results indicate the environment is causing the behavioral differences.

DEDICATION

To my husband, Chris Reichmuth.

From the beginning, you knew that my education and the degrees I was pursuing were of the utmost importance to me. I thank you for being my support system, my sounding board, my field assistant, and most importantly, for being you: so unselfish and patient. I could not have made this journey without you and am so grateful you have always been one step behind to catch me if I should fall. You will always be my marine scientist-in-training.

ACKNOWLEDGEMENTS

As with every doctoral dissertation, this would not have been possible without the help of many people. First and foremost, I would like to thank my advisor and friend, Dr. Judith Weis, for sharing her vast knowledge, advice, support, patience and continual guidance throughout the time I have been at Rutgers. My committee members, Drs. Claus Holzapfel, Paul Jivoff and Douglas Morrison, provided comments and feedback along the way as I completed research and new questions arose. I would also like to thank Paul for the occasional boat time in Tuckerton when I was fist learning the blue crab ropes, as well as the use of crab traps. Dr. Terry Glover made group field trips to the Meadowlands and Tuckerton possible by driving extra bodies, hauling gear and crabs back and forth as well as shared invaluable insight on statistical analysis and experimental design. This research was funded in part through Rutgers University Marine Field Station Graduate Research Grants and grants from the Meadowlands Environmental Research Institute (MERI).

I spent many hours in other labs throughout my dissertation as well. Thanks to Dr. Peddrick Weis for the use of materials for the metals analysis and lab space at the University of Medicine and Dentistry of New Jersey. The metals analysis would not have been possible without the help and expertise of Ted Proctor, who spent many long hours prepping and running samples and cleaning beakers. Thanks to Dr. Allen Place for the use of materials and lab space at the Center of Marine Biotechnology within the University of Maryland Biotechnology Institute. Jessica Hill and Ernest Williams spent long hours dissecting crabs and

crab legs for tissue, preparing the DNA and running the PCR and sequencing of the samples. Ernest and Xiaojun Feng provided invaluable insight on data analysis.

Brett Bragin, senior naturalist at the New Jersey Meadowlands Commission was an invaluable resource. Thanks for organizing the many hours trawling for crabs on the River and answering many questions on where to fish within the Meadowlands District as well as allowing access to one of the best places to get adult blue crabs within the District without a boat.

I must also acknowledge my colleagues and labmates, who not only provided support and advice along the way, but made the journey so much more enjoyable: Lauren Bergey, Alli Candelmo, James MacDonald, Sona Mason and Tish Robertson. I cannot thank you all enough for the support and guidance you have given along the way. A handful of adventurous high school students (Anthony Catallo, Ryan Weatherford, Karina Loor) and undergraduates (Ross Roudez, Suzan Sharastani, Jonathan Ramirez) spent summers getting wet, muddy and occasionally pinched in the field as well as in the lab, in addition to observing, setting up and taking down experiments and maintaining the crabs in the lab.

Finally, I would like to thank my parents, Bob and Susan Davisson and my sister, Sara, for their unending support as I completed my doctoral program. I have enjoyed explaining the world of blue crabs to you every weekend for the past few years.

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CHAPTER 1

Introduction

IMPORTANCE OF PREDATION IN ESTUARIES

Estuaries and salt marshes are highly productive marine ecosystems. These systems are important because they provide habitat and nursery grounds for many species of fish and shellfish, many of which are both ecologically and economically important. However, the organisms that live in these systems have to be adapted for harsh living conditions, such as major changes in salinity, wave action, exposure and temperature. The organisms and their interactions with the other members of the community form complex food webs. Both biotic and abiotic factors help shape the structure of the plant and animal communities in estuaries and salt marshes (Mann 2000). Phytoplankton, more specifically diatom species, are able to take advantage of the outflow and runoff from the rivers as well as the mixing of these two water bodies. As the productivity of the plankton increase, the organisms that feed upon them can also flourish, making estuaries one of the most productive ecosystems on the planet (Mann 2000).

One of the major biotic factors structuring estuarine communities and food webs is a large guild of predators, in which crustaceans are a major constituent. Their presence represents an abundant and diverse class of opportunistic omnivores instead of one distinct predator. The predators have strong direct effects on the macrobenthos and infaunal community members, such as

bivalves, mussels, polychaetes and other species of crustaceans, which live within the substrate. All the predators vary in space and time creating complex interactions in the organization and patch dynamics of the benthic community (Edwards et al. 1982; Hines et al. 1990). The foraging activity of crabs specifically (i.e., the blue crab, *Callinectes sapidus*) reduces the stability of the sediments, in turn causing the disruption of prey tubes (i.e. polychaetes) and burrows (i.e., bivalves). In addition, several families of shrimp are also important predators: *Crangon* spp. and *Palaemonetes* spp. were found to reduce amphipod populations on a local scale, changing the size structure of the benthic community (Nelson 1981).

One of the most heavily studied estuarine systems is Chesapeake Bay. In this system, predation by both crustaceans and fish has shown to play a significant role in determining infaunal abundances (Virnstein, 1977, 1979). Virnstein and other investigators showed in cage experiments where important predators, such as blue crabs (*C. sapidus*) and spot (*Leiostomus xanthurus*) were excluded, the diversity and density of prey species increased. In the Chesapeake, predation effects are severe and the effects can be seen on adults and on newly settled larvae of many benthic species. In salt marshes within the Chesapeake (and elsewhere), swimming crabs such as the blue crab are important predators, but they have access to the marsh only during high tide.

Most decapod crustaceans are omnivorous predators, preying mainly upon bivalves or gastropods (Virnstein 1977, 1979; Wolcott 1978; Elner 1980; Kneib and Weeks 1980; Whetstone and Eversole, 1981, 1982; Williams, 1982;

Du Preez 1984; Lipcius and Hines 1986; Elner and Campbell 1987; Eggleston 1990; Hall et al. 1990; Hseuh et al.1992; Mayfield et al. 2000; Vannini et al., 2001). It has been noted that diet varies with geographic locations (Laughlin, 1982; Hseuh et al. 1992; Hines et al. 1987; Micheli 1997). Ontogenetic shifts in diet are also common among decapod crustacean predators (Lauglin 1982) and life history may place size classes in exclusive parts of an estuary at times when particular prey items are available.

Predator-prey relationships can be influenced by abiotic factors, such as salinity, however these factors seem to have larger influence over the prey than the predator (Laughlin 1982). Population counts and field observations by Hines et al. (1987) suggested that different size classes of blue crabs utilize different parts of the estuary in a particular season: juveniles and pre-molt crabs are more likely to be found upstream and adults and post-molt individuals are more likely to be found downstream in the Rhode River Estuary. Hence, the predator-prey relationships are determined seasonally by where particular size classes are located (Hines et al. 1987).

How a predator reacts to a stimulus, in this case its prey, can have a major impact on its ecology. When a predator is plastic in its behavior, the animal may be able to take full advantage of new opportunities (i.e. new habitat, food source) (Orians 2000). If a crab shows behavioral plasticity when optimal prey are scarce, it should be able to take advantage of new resource types. *C. sapidus* were found to exhibit flexible behavior when feeding on hard clams (*Mercenaria mercenaria*) of different sizes in aquarium experiments. Shell

strength may be the only cue of prey quality to the crab. This may result in the crab learning which prey can be easily crushed (Cunningham and Hughes 1984; Juanes 1992; Micheli 1995). Blue crabs (or molluscivorous crabs in general) might be able to cue on shell strengths, rather than on the energy content of prey because of the overwhelming importance of the mechanical properties of prey on the life-long fitness of the predator (Juanes 1992; Hughes and Seed 1995). Juanes and Hartwick (1990) showed that claw damage (i.e., chela breakage and claw tooth wear) is a limiting mechanical cost for an organism that has a fixed energy budget. When a crab or lobster chooses a smaller size of prey, less energy is expended, and there is less wear on the claws. Claw damage was shown to have significant effect on feeding, behavior, molting ability, regeneration load, mortality rates, and reproductive success (Smith and Hines 1991; Juanes and Hartwick 1990). The behavioral plasticity exhibited by *C. sapidus* might represent an adaptation to life in unpredictable environments. This modification in behavior may occur because of the following three hypotheses: 1) crabs become more efficient at handling a particular clam size with experience, 2) crabs develop a “search image” for a clam size and become more successful at finding it, and 3) crabs learn to recognize the preferred prey on encounter, thus being more persistent on one size of clams than another. Crabs frequently use different techniques to crush bivalves of different sizes which may allow them to increase their efficiency in handling a particular size class. However, as they mature they may be able to retain enough plasticity to use different techniques when prey becomes large (Micheli 1995).

BLUE CRABS

The life history and ecology of the blue crab, *Callinectes sapidus*, have been widely studied (Van Engel 1958, 1979; Darnell 1959; Fischler 1965; Kennish et al. 1982; Epifano 1995; Pile et al.1996; Moksnes, et al. 1997). The blue crab is a swimming decapod that is estuarine dependent and widely distributed from Nova Scotia to northern Argentina. The species' highest densities occur along the coast of North America between Massachusetts and Texas, which includes the Gulf of Mexico (Williams 1974). They inhabit estuaries and near shore coastal waters to depths of at least 36 m and are a year long resident of New Jersey estuaries (Norse 1977). In New Jersey, blue crabs are active between April and November, but the species reaches its maximum abundance from May through August (Kennish et al. 1982).

Blue crabs are meroplanktonic, meaning that part of their life is spent in the plankton (i.e., in zoea and megalops stages). Gravid female crabs migrate to the mouths of estuaries in early fall to release their larvae later in the season (Jivoff and Hines 1998); some individuals do not spawn until early spring (Turner et al. 2003). The majority of these developing larvae are transported into the ocean by an interaction of seasonal winds and bottom water circulation patterns, and eventually they return upstream to the approximate spawning area by taking advantage of the estuarine flow dynamics (Epifano 1995). After six or seven molts, the zoea changes into a post-larval form known as the megalops (Pile et

al. 1996), which remains in the plankton before metamorphosing into the “first crab” stage and settling out into the benthic community. These juveniles, or “first crabs” gradually move into shallower, less-saline waters in upper estuaries where they grow and mature rapidly; adult size in temperate areas is usually reached in approximately 18 months (Fischler and Walburg 1962; Kennish et al. 1982). It is estimated that blue crabs live anywhere from four years (in Florida) (Laughlin 1982) to seven to eight years (in Chesapeake Bay) (Norse 1977).

Blue crabs are capable of high levels of dispersal in both the adult and larval stages (Cargo 1958). Most movement of adult crabs is restricted to the estuary and surrounding coastal waters, but some females have been known to migrate many kilometers to spawn (upwards of 800 km!) (Kurdos and Burton 1993). The dispersal of the zoea and megalopal stages depends on currents and wind direction (Epifano 1995). On a local scale however, the dispersal of these life stages may be enhanced or restricted by the larval behavior, direction and rate of local currents, and position in the water column (McMillen-Jackson et al. 1994; Diaz et al. 1999)

This species is not only important economically, but it is ecologically important as well. The Chesapeake Bay fisheries harvest approximately 13,000 MT of crabs a year while and in general in the northern Atlantic states, the crab provides a local fishery (Jop et al., 1997). Blue crabs are an important part of a predatory guild within estuaries that structure the dynamics of soft bottom communities. They are important in the estuarine food web because they are not only predators, but they are also scavengers and are prey themselves. The

foraging activity of the blue crab reduces the stability of the sediments, in turn causing the disruption of prey tubes (i.e. polychaetes) and burrows (i.e., bivalves). These behavioral aspects of this species dually impact the benthic community since the blue crab can structure the community through predation as well as physical disturbance (bioturbation) through locomotion, burying, etc. (Virnstein, 1979; Nelson, 1981; Edwards, et al., 1982; Hines et al., 1990). Blue crabs also vary in their foraging rates and predation intensity because of seasonal effects on their size and this can cause significant changes in the mortality of their prey species (mostly bivalves, i.e., *Mercenaria mercenaria, Crassostrea virginica*) (Micheli, 1997). Bivalve population mortality is the highest when blue crab abundance is the highest (Hines et al., 1987).

Blue crabs also affect the adjoining salt marshes. Specific size classes of *C. sapidus* were found to utilize different parts of the marsh when their abundance peaks in mid- to late summer. In these areas, they are important predators, but they have access to the marsh only during high tide. They feed on the marsh's surface on juvenile oysters (*C. virginica*) and ribbed mussels (*Geukensia demissa*), snails (*Littorina* spp.), mummichogs (*Fundulus heteroclitus*), fiddler crabs (*Uca* spp.), and several species of marsh mud crab (Hines et al, 1990; Micheli, 1997). They are also known to be cannibalistic and eat smaller or diseased members of their own species; cannibalized blue crabs can make up as much as 13% of an individual's diet (Moksnes et al., 1997; Clark et al., 1999). Blue crabs can be detrimental to the aquaculture of important

shellfish and can affect the size structure in local populations of fish (Hamilton, 1976; Elner and Lavoie, 1983; Spounagle and Lawton, 1990).

BEHAVIORAL ECOTOXICOLOGY

An organism's behavior reflects the integration of a variety of biochemical and physiological parameters (Peakall 1996). The way an organism responds to its environment can scale up to affects on population and community structure and if such responses are altered by contaminants, this might have effects on the individual, population and community level are the result (Boyd et al. 2002). The mechanisms associated with population and community change following contaminant exposure in the field are quite complex and vary considerably. Direct effects of contaminants on the individual organism may have population effects in terms of reduced abundance through either increased mortality or reduced fecundity. The direct influences of contaminants on predators or grazers can cause altered behavior, which can lead to cascading indirect effects in other trophic levels (Scott and Sloman, 2004). Indirect contaminant effects may lead to increased abundance of a particular trophic level through decreased competition or predation or decreased abundance due to reduced availability of a specific food source (Fleeger et al., 2003). Many studies have investigated the effects of contaminants on the behavior of animals (Boyd et al., 2002; Zala and Penn, 2004).

Impaired behavior can have consequences at the population level through reduced abundance and altered interactions with other members of the same

species, and at the community level through changes in competitive interactions or predation rates. Ultimately, all of this can affect the structure of the ecosystem. Hebel et al. (1997) investigated the effects of copper on the behavior of the European shore crab (*Carcinus maenas*) using a holistic approach to understand how the contaminants affected the crab on the cellular, physiological, and behavioral levels. They found that excess Cu had detrimental effects on enzyme activity, osmoregulatory functions, and locomotive abilities. The authors felt that by investigating how the crab was affected on all levels of organization, the suite of responses to a contaminant could be correlated to changes in population and community interactions. In a mesocosm study, juvenile coho salmon (*Onchorhyncus kisutch*) were fed a zinc-enriched diet (Bowen et al., 2006). The high levels of Zn were found to decrease aggressive behaviors between individuals, but increase overall feeding rates. Even though feeding rates were increased, fish that were fed the Zn-enriched diet had lower growth rates. The authors concluded that these changes in behavior and growth rate could have detrimental effects on competitive interactions and predatory ability in later adult stages.

A large number of studies have also demonstrated the effects of contamination on feeding behavior of aquatic organisms. For example, an investigation conducted by Sandheinrich and Atchison (1989) demonstrated that when bluegills (*Lepomis macrochirus*) are exposed to contaminants, their prey handling time increases, which ultimately causes reduced feeding rates. In laboratory experiments the gastropod, *Nassarius festivus*, was exposed to

varying levels of copper, chromium, cadmium, and zinc (Cheung et al., 2002). Exposure to high levels of Cd and Zn increased the overall time spent feeding. High levels of Zn increased the time required for an individual to reach the food and Cr was found to decrease the success rate of reaching the food, while Cd and Cu had no significant effects.

Several studies have been conducted on how pollutants affect the predatory abilities of the mummichog (*Fundulus heteroclitus*), another important estuarine organism. Weis and Khan (1991) showed in laboratory studies that fish from a polluted environment (Piles Creek in Linden, NJ) caught prey items (guppies) at a reduced rate compared to fish from a cleaner site in Long Island. When another prey species, the grass shrimp (*Palaemonetes pugio*), was used, Piles Creek (PC) fish were again inefficient predators (i.e., reduced prey capture ability) when compared to fish collected from a cleaner, reference site in Tuckerton, NJ (TK). Analysis of the brain tissue of the PC fish found elevated levels of mercury (Hg); this was correlated to their reduced prey capture ability (Smith and Weis 1997). However, this does not mean that Hg is the only possible cause for altered behavior because sediment analysis has shown a suite of contaminants present in the system along with Hg (Weis et al. 2001). These lab data were supported by studies of diets of fish in PC and TK. While TK fish had a considerable amount of active prey species in their guts, PC guts were filled largely by sediment and detritus (Smith and Weis 1997). It has previously been shown that this species does eat detritus, a non-nutritious food source for them. When TK fish were maintained in the laboratory in PC water and

sediments and fed grass shrimp from PC their prey capture ability declined to that of the PC fish over six weeks. When PC fish were maintained in clean water and fed food from TK, their predatory ability improved only very slightly.

Successfully avoiding a predator means to live another day. If juvenile stages of any organism can avoid a predator and live to adulthood, then they have the potential to pass their genes to the next generation (Posey et al. 2005). Juvenile blue crabs are important prey items to various fish species and other carnivorous crab species, including other blue crabs (Fiorenza 1997; Moksnes et al. 1997; Hovel and Lipcius 2001). These early life stages may be affected more by contamination since they must molt more often. When crabs molt, they remove calcium carbonate from the old cuticle through a dissolution process, and can subsequently incorporate it into the new one (Henry and Kormanik, 1985). As a crab's carapace hardens, the calcium used binds with other chemicals, such as harmful metals, thus, exposing the crabs to unnecessary toxicants. These contaminants could be stored within the body and could ultimately affect the individual crab's behavior (Engel and Brouwer 1984; Engel 1987).

The prey responses that are affected by contamination are typically increased susceptibility to predation, decreases are much less common (Fleeger et al. 2003). Setzler-Hamilton et al. (1988) found that larval striped bass experienced increased susceptibility to predation due to increased levels of contamination coupled with habitat loss in the Chesapeake Bay. Weis and Weis (1998) exposed larval mummichogs (*F. heteroclitus*) to lead in laboratory studies, which resulted in reduced predator avoidance when grass shrimp were

predators. In later laboratory studies conducted by Zhou et al. (2001), larval mummichogs exposed to methylmercury were found to have reduced swimming ability, and as a result, experienced increased predation by the grass shrimp, *P. pugio*. In predator avoidance experiments, adult fish from contaminated sites (PC) were "slow" and had reduced activity compared to killifish from cleaner sites (TK), which made them more vulnerable to predation by blue crabs (Smith and Weis 1997). In essence this means that there can be a greater amount of contaminants transferred to higher trophic levels because of reduced predator avoidance by the killifish. The question then arises, whether a similar behavioral difference will be seen in the predatory blue crabs living in a contaminated site compared to cleaner reference sites?

Rationale and Hypotheses

The research described in this dissertation will have three main parts: experiments targeting behavioral ecology (Chapters 2 and 3), description of contamination levels (Chapter 4) and investigations of population genetics (Chapter 5). Blue crabs from the Hackensack Meadowlands (HM), an area with a long history of contamination, and the Great Bay Estuary in Tuckerton (TK), which is a cleaner reference site that has been used for many previous studies are compared.

In section one of this study, behavioral ecology, the following questions were investigated:

1a. Is there a difference in prey capture ability by adult blue crabs of the different populations when juvenile blue crabs are prey? (Chapter 2)

1b. Are adult blue crabs that live in a contaminated site inefficient predators with other prey types, such as fiddler crabs, mummichogs, and/or ribbed mussels?

1c. If the adults from the contaminated site are inefficient predators on the above mentioned prey types, what exactly are they eating under field conditions?

Rationale: Since the Meadowlands have a long history of contamination and the sediments have been demonstrated to have higher levels of metals and other toxicants relative to Tuckerton, blue crabs have been exposed to these contaminants potentially affecting their behavior as major predators within the system. If HM blue crabs have a poor diet due to reduced prey capture ability, this might decrease the overall fitness of individual crabs and result in decreased growth and survival. This could also mean a change in the benthic structure of the estuarine community as well, since blue crabs play an important role as predators (Eggleston et al. 1992; Moksnes et al. 1997; Silliman and Bertness 2002; Jivoff and Able 2001, 2003). Hypotheses for this question are as follows: **(HYP 1)** Adult blue crabs from a contaminated environment will show reduced ability to capture juvenile blue crabs as prey. They should also show reduced ability to prey upon other organisms such as fiddler crabs, mummichogs, and ribbed mussels. Since it is hypothesized that blue crabs from a contaminated

site are inefficient predators, this should be reflected by their natural diets in the field.

2. Is there a difference in predator avoidance ability by juvenile blue crabs of the differently contaminated populations when adult blue crabs are the predator? (Chapter 3).

Rationale: In order to avoid predation, juvenile *C. sapidus* rely on rapidly burying themselves in the substrate and utilizing shelter-providing plants or algae (i.e., *Ulva* spp.) (Moody, 2003). A reduced ability or longer time to bury itself can lead to increased predation risk.

(HYP 2) Juvenile blue crabs that live in a contaminated environment will show a decreased ability to avoid adult blue crab predators.

3. Is there a difference between populations in aggression and response to a threatening stimulus by juvenile blue crabs? (Chapter 3)
    a. If a difference exists, and one population is more reactive to the threatening stimulus, will this allow any advantage with predators (i.e., increased survival with a predator)?

Rationale: If they are exposed to the toxicants that change their behavior to be more or less aggressive, it is possible that this could lead to altered predation risk (Fleeger et al. 2004; Scott and Sloman 2004).

(HYP 3) Blue crabs from a contaminated site should show more aggressive behavior when exposed to a threatening stimulus. Crabs from a contaminated

site should be more agonistic and this in turn should increase its survival with a predator.

4. If altered aggressive behavior continues into the adult life stages, will this altered agonistic behavior affect the probability and rate of other individuals entering a baited crab pot? (Chapter 3)

Rationale: It is possible that since adult blue crabs are exposed longer to the contaminants in the Meadowlands, they would bioaccumulate toxicants that increase their agonistic behaviors (Bryan et al., 1979; Scott and Sloman, 2004). Aggressive behavior could alter the ability of crabs to co-exist with each other in a confined space.

(HYP 4) Any agonistic behavior observed within the juveniles should persist in the adults. These same behaviors should also occur around a baited crab pot. If an aggressive crab enters the pot, it will deter other crabs from entering the same pot.

5. If the environment is the cause of the observed behavioral differences, will "clean" (TK) crabs that are transplanted into the contaminated environment behave similarly to "polluted" (HM) crabs? (Chapter 2)
    a. Is food quality (with contaminants) one of the environmental factors that causes any behavioral differences observed?

Rationale: Previous studies (e.g., Smith and Weis, 1997, Perez and Wallace, 2004) have exposed organisms from reference sites to the conditions of a contaminated site where organisms have aberrant behavior. There are several

environmental conditions that differ between the Meadowlands and Tuckerton area, such as salinity, food quality, tidal creek morphology, and tidal flow. It is possible that one of these parameters, or a combination of them, could be possible for any changes in behavior observed in the reference organisms.

(HYP 5) When TK crabs are transplanted to HM they will show reduced prey capture ability. Contaminants (mainly from food) and the environment are important in causing the observed behavioral differences.

The answers to the above questions will determine if any aberrant behaviors exist among these populations are related to living within a contaminated environment.

The goal of the second part of the thesis is to determine the concentrations of contaminants present in the tissues of blue crabs collected from HM compared to TK (Chapter 4). Previous studies within the Meadowlands have determined that both the biota and sediments have elevated levels of contaminants (Weis et al, 2001). The research in this part will attempt to answer the following questions:

1. Are there differences in the total body burden of metals (copper, chromium, lead, mercury, and zinc) between the two populations?

Rationale: Previous studies (Weis et al. 2001; Weis 2003) have indicated that the sediments and biota within the Meadowlands have higher levels of the aforementioned metals.

(HYP 6) Blue crabs from the Meadowlands will have higher total body burdens of copper, chromium, lead, mercury, and zinc than crabs collected from TK.

(HYP 7) TK blue crabs exposed to HM conditions will show increased levels of metals in their tissues. HM crabs exposed to TK conditions should depurate and decrease total levels of metals within the body.

2. Are there differences among the populations in where within the body the contaminants are stored: muscle (i.e., claw) vs. hepatopancreas?

Rationale: There are two main sites where metals can be stored once they have entered the crustacean body. Either of these sites can serve as a final location for metal storage (Bryan 1971; Ahearn et al, 2004).

(HYP 8) The hepatopancreas serves as the principal organ of absorption, storage, and secretion, therefore, it will have the highest proportion of the total body burden.

The answers to the above questions will provide us with the information of how contaminants are stored and distributed throughout the bodies of crabs living within contaminated areas.

The third portion of the proposal will consider the possibility that some of the behavioral differences may be genetic rather than environmental. Here I investigate the population genetics of the two populations and attempt to answer the following questions (Chapter 5):

1. Is there a difference in the nucleic DNA at six loci between the two populations?

a. If there are differences in nucleic DNA, are they temporal (differences across years/seasons) or geographic differences (differences due to location)?

2. What percentage of the alleles present in the adults is present in the juveniles of the same year?

Rationale: Due to recombination events, it is possible to have a high variety of alleles present at six loci as well as within the mitochondrial DNA. Temporal differences could occur from season to season or year to year; the NJ coastline is only 150 km long and this gives the larvae a short enough distance to travel and mix between sites. Geographic differences could occur if there is not gene flow into a specific location, which would allow the population to become fixed for certain alleles (Graur and Li 2000).

(HYP 9) There will not be a geographical difference in the nucleic DNA. However, a temporal difference would be expected due to recombination events between the two populations leading to which alleles are present within the populations at any given time.

(HYP 10) There should be a high percentage of the alleles found in juveniles also present in the adults of the same year.

3. If differences exist in the nucleic DNA, are the genetic differences correlated to behavioral differences observed?

Rationale: Since very few studies relate population genetics to behavior (Robinson 1999), the answers to the above questions would give us some

information on how the two populations are genetically structured and if observed behavioral differences can be related to genetics of a population.

(HYP 11) Since no genetic difference is expected between the two populations, genetics cannot be one of the causes of the observed behavioral differences.

The answers to the above questions will provide us with the information of how two populations are genetically structured on a small scale and possibly determine which is more important in the observed behavioral difference.

RESEARCH SITES

Hackensack Meadowlands: The Hackensack Meadowlands are an urban wetland in northeast New Jersey, encompassing approximately 83 km^2 in Bergen and Hudson Counties (Appendix 1 for map). This area is one of the largest wetland ecosystems in the Hudson Raritan Estuary and is the largest contiguous open space in the New York metropolitan area. The 34 km^2 of wetland are important to many estuarine bird and fish species. Before the 1970s, the Meadowlands were damaged by industrial pollution, mosquito ditching, suburban and urban development, landfill operations, and dredging. The area still receives run-off from the surrounding highways, towns, and industries. Previous studies (Weis et al. 2001) have shown that the biota has elevated levels of contaminants (Table 1-1 compares concentrations of selected metals).

The main channel of the Hackensack River is less tidally influenced than the control site and has lower salinity (12-17 ppt). Tidal creeks are large and expansive, causing the water movement (i.e., tidal flushing) to be relatively slow.

Tuckerton**:** The Mullica River-Great Bay estuary is comprised of 225 square kilometers of salt marsh and 145 square kilometers of shallow estuarine waters (Appendix I for map). The surrounding area is protected federally by its inclusion in the Edwin B. Forsythe National Wildlife Refuge and locally by the Great Bay Wildlife Management Area. The system is considered one of the cleanest estuaries on the east coast and has been designated as the Jacques Cousteau National Estuarine Research Reserve (NERR). Two collection areas were used: First Bridge (FB), which is on the Little Egg Harbor side of Tuckerton and Graveling Point (GP) located where the Mullica River empties into Great Bay. The TK area has been used as a reference site by the several previous studies (Table 1-1 compares concentrations of selected metals).

The sites within TK are cleaner and have a higher average salinity (28-33 ppt) relative to HM due to the stronger oceanic influence at this location. FB has large, wide tidal creeks similar to the Meadowlands, but GP has narrow creeks and fast water movement relative to the slower water movement of FB.

Table 1-1. Mean concentrations of metals (μg/g ± standard error) found within the sediments. TK data has been in reported in Weis et al. (2001); HM data has been reported in Windham et al. (2004).

Metal	HM	TK
Cu	92 ± 15	43.8 ± 7.6
Cd	n/a	2.1 ± 1.2
Cr	154 ± 3.0	n/a
Hg	2.04 ± 0.53	0.19 ± 0.02
Pb	143 ± 34	73.2 ± 8.0
Zn	178 ± 22	141 ± 13.4

Chapter 2

Prey Capture Behavior

Introduction

An organism's behavior reflects the integration of a variety of biochemical and physiological parameters (Peakall 1996). The way an organism responds to its environment can affect its population and community structure. If the response is altered by contaminants, it might have detrimental effects on the individual, population and community (Boyd et al. 2002). Impaired behavior can have consequences at the population level through altered interactions with other members of the same species and at the community level through changes in competitive or predator/prey interactions. Ultimately, altered behavior can affect ecosystem structure itself since altered predatory behavior will effect populations as well.

The effects of copper on the behavior of the European shore crab (*Carcinus maenas*) were investigated with a holistic approach to understand how the contaminants affected the crab at the cellular, physiological, and behavioral levels (Hebel et al. 1997). Excess Cu had detrimental effects on enzyme activity, osmoregulatory functions, and locomotive abilities. By investigating how the crab was affected on all levels of organization, the suite of responses to a contaminant could be correlated to changes in population and community interactions (Hebel et al. 1997). Mercury inhibits the hatching of blue crab (*Callinectes sapidus*) embryos as well as reduces the survival of the megalopae and juvenile life stages (Engel and Thayer 1998). Additionally, adult fiddler crabs (*Uca pugilator*)

exposed to mercury and cadmium showed slower limb regeneration and molting than controls (Weis, 1976).

Predation is a major form of energy transfer from one trophic level to the next (Bertness and Callaway 1994; Silliman and Bertness, 2002). If a predator's behavior is impaired, the predator may suffer reduced feeding and growth. However, the prey species may benefit from reduced predation if they themselves are less affected than the predator (Santiago Bass et al. 2001). Food web alterations can arise because of changes in behaviors of predator and/or prey (Posey and Hines 1991, Werner et al 1983). Since both predator and prey can occur in the same polluted system, both trophic levels can be affected simultaneously (Boyd et al. 2002; Fleeger et al. 2004). Predator responses to pollution tend to result in reduced predation intensity. Coho salmon (*Oncorhynchus kisutch*) exposed to polyaromatic hydrocarbons (PAHs) in the lab showed reduced feeding rates and were lethargic, i.e., made fewer attempts to capture prey (Purdy 1989).

Impaired prey capture was observed in mummichogs (*Fundulus heteroclitus*) from a contaminated estuarine system in northern New Jersey (Smith and Weis 1997). The ability of the fish to capture grass shrimp (*Palaemonetes pugio*) prey was reduced compared to that of fish from a cleaner reference site. Field-caught fish from the contaminated system also had much lower amounts of live prey, including grass shrimp, in their guts, and high amounts of sediment and detritus. Furthermore, these fish, which had lower

activity levels in general, were more susceptible to predation by blue crabs in the laboratory (Smith and Weis 1997).

Blue crabs vary in their foraging rates and predation intensity because of seasonal effects on their size, and this can cause significant changes in the mortality of their bivalve prey species (i.e., *Mercenaria mercenaria, Crassostrea virginica*) (Micheli 1997). Specific size classes of *C. sapidus* utilized different parts of the marsh in a given season where their abundance peaked in mid- to late summer. Bivalve mortality is the highest when blue crab abundance is the highest (Hines et al. 1987; Micheli 1999). In salt marshes, they are important predators, but they only have access to the marsh surface during high tide. They can be detrimental to the aquaculture of important shellfish and can affect the size structure in local fish populations (Elner and Lavoie 1983; Hamilton 1976; Spounagle and Lawton 1990).

Stomach content analysis and observations of feeding habits revealed they consume at least 99 species. However, a typical diet consists of 20-40% mollusks, 10-26% arthropods, 5-12 % fishes, and 1-7% polychaetes (Hines et al. 1990; Hines 2007). A small percentage of detritus and sediment may also be ingested (Hines 2007).

Since blue crabs are found in the contaminated estuaries (where the mummichogs are easier to catch (Smith and Weis 1997)), a question arises of how and whether blue crabs are also affected in their predator-prey relations. In this investigation we compared adult predatory behavior of blue crabs with several different prey types. We studied crabs collected from the Hackensack

Meadowlands (HM) near Lyndhurst, New Jersey and from the Great Bay-Little Egg Harbor area near Tuckerton, NJ (TK). We hypothesized, based on previous studies on other organisms, that *C. sapidus* from HM, which is contaminated, would be less efficient predators compared to individuals from TK, the reference site. We also hypothesized that HM crabs collected in the field would have increased levels of mercury in their tissues and less full stomachs compared to TK conspecifics. To investigate environmental causes for differences in predatory behavior, we performed two types of studies: (1) we transplanted crabs from one site to the other for a period of time and then tested their prey capture, and (2) in the lab crabs were fed food from the other site. We hypothesized that TK crabs, when transplanted into the contaminated HM or fed contaminated food from HM, would show a decrease in predatory ability and increased mercury (there has been a long presence of mercury in HM), and HM crabs, when transplanted to TK or fed TK food in the lab, would show improved predatory ability and reduced mercury.

Materials and Methods

Experimental Design:

Crabs were collected from HM and TK using a seine net and otter trawl and brought back to the laboratory. Animals were kept in aerated communal tanks with a sand depth of one centimeter and artificial seawater (Instant Ocean®) at their native salinity (HM: 15; TK: 30). A 14/10 light cycle was kept throughout the field season. TK crabs were fed a diet of ribbed mussels (*Geukensia demissa*) and Atlantic menhaden (*Brevoortia tyrannus*) collected from TK, while HM crabs

were fed a diet of menhaden and mummichogs collected from HM; all crabs were fed three times a week, after which the water was changed. Intermolt crabs were acclimated to laboratory conditions for 48 hours prior to beginning of experimentation. Statistical analyses were conducted using Statistix 7.0 and GraphPad Prism 4.0 software.

Prey Capture Experiments:

All prey capture experiments, with the exception of the experiment using fiddler crabs as prey, were conducted in a 76 L aquarium with approximately 3.5 cm deep sand covering the bottom. The aquarium was covered with opaque paper on all sides to prevent distractions by outside movement or the crab's reflection.

Juvenile blue crabs as prey: Four juvenile crabs (mean carapace width, CW: HM = 32 mm; TK = 35 mm), two from each population, were added to the aquarium. Carapace width and sex of the juveniles were recorded. The juveniles acclimated to the tank for one hour. After acclimation, one adult crab, either male or female (mean CW: HM = 101 mm, TK = 94 mm) that had been food-deprived for 48 hours, was placed in the aquarium. In preliminary studies, sex was determined not to be a factor in differences observed. All trials (HM: n=26, TK: n = 29) were recorded using a closed circuit digital camera (Ikegami Tsushinki Co. Ltd) with a 7.5-75 mm lens (Canon) and digital disk recorder (Panasonic, Model WJ-HD 309). The number consumed in 24 hours was recorded and data were analyzed using a two-sample t-test.

Mummichogs as prey: Ten mummichogs (mean total length = 68 mm) from a contaminated site were placed in an aquarium with water depth of 6.5 cm and sand depth of 3 cm. The mummichogs were acclimated to the aquarium for 10 days. One adult food-deprived male crab (mean CW: HM= 105 mm, n= 12; TK= 94 mm, n= 14) was added. A previous experiment (Smith and Weis 1997), as well as pilot studies, suggested two weeks was sufficient time for a blue crab to capture live mummichogs. After two weeks, the number of consumed mummichogs was recorded. The number of mummichogs captured over the two weeks was analyzed using a two-sample t-test.

Fiddler crabs as prey: Twenty adult male fiddler crabs (*Uca pugnax*) were added to a large circular tank (diameter: 40 cm; height: 45 cm) with a mud depth of 15 cm. They were given 24 hours to construct burrows and if they did not, artificial burrows were created using a dowel. Artificial sea water [HM: 15; TK: 30] was then added (depth = 17 cm) to simulate a high tide on the marsh surface and the fiddler crabs were given an additional 30-minute acclimation period before a food deprived adult blue crab, male or female (mean CW: HM= 101 mm, n= 20; TK= 97 mm, n= 23), was added to the tank. After 24 hours the adult blue crab was removed and the number of surviving fiddler crabs was recorded. These experiments were not recorded using the digital recorder because the murkiness of the water upon the addition of the adult blue crab made it impossible to observe interactions between predator and prey. The total number of fiddler crabs consumed was analyzed with a two-sample t-test.

Mussels as prey: Ten ribbed mussels of various lengths (mean length= 5 cm) were used in the following configurations: three single individuals (wet weight, ww = 45 g ± 10 g) were scattered randomly around the tank: one was placed near each end and the third near the middle; a clump of three mussels (ww = 45 g ± 10 g), and a clump of four mussels (ww = 55 g ± 10 g). The configuration was used since mussels are found both by themselves and in clumps, which mimics the mussel beds within estuaries. The thawed mussels were added to the aquarium 30 minutes prior to introduction of the food-deprived predator; since the mussels were previously frozen, each was slightly opened. An air stone was added to the experimental tank so that the 'scent' of the mussels saturated the water. One male or female adult blue crab, (mean CW: HM= 99 mm, n= 17; TK= 92 mm n= 20), was placed in the aquarium. The experiment ended after 24 hours and each configuration was weighed again to calculate the total amount of mussel tissue consumed (ww). The total wet weight consumed was analyzed using a two-sample t-test. A two-way repeated measure ANOVA was used to determine if effects of site, configuration, and site x configuration were present; this was followed by a Tukey test at the 0.05 level to compare means.

Stomach Content Analysis:

Adult crabs, both male and females, from both populations (HM: n = 23; TK: n = 25) were caught by seining and preserved in 10% formalin during May 2006 - October 2007. Crabs remained in formalin for no more than two weeks until the stomach was dissected. Once dissected, the stomach was stored in 70%

ethanol until the contents could be analyzed using a dissecting microscope. Stomach fullness was recorded using a numeric scale ranging from, 1 = empty to 5 = full. Only stomachs that were recorded as 3 and above were dissected further. The contents of the stomach were recorded and weighed (wet weight) in the following categories: crab (recognized by thick pieces of exoskeleton), amphipod and grass shrimp (recognized by thin pieces of exoskeleton), plant and algae, polychaete (recognized by setae), unidentified crustacean, fish (vertebral bones and scales), sediment/detritus, and unidentifiable amorphous material. The overall weight of the stomachs and the amount eaten by category between the two populations were analyzed with a two sample t-test.

Field Transplant Experiments:

Adult blue crabs (only males) from both populations were placed in minnow traps (one per trap) or a divided green crab trap covered in plastic mesh (dimensions: 61 cm x 47 cm x 21 cm) with food (mummichogs and Atlantic menhaden) from the transplanted location and transplanted to the other location for eight weeks HM at TK (n = 8), and TK at HM (n = 11). Traps were checked every 7-10 days and if more food was needed, either 10 adult mummichogs or one large menhaden was added to the trap. After eight weeks in the field, crabs were transported back to the lab for prey capture experiments (using juvenile blue crabs as prey).

Laboratory Trophic Transfer (TT) Experiments:

Adult crabs (only males) were caught in the field and transported back to the lab. Each crab was placed in an individual 20 L aquarium or a divided 76 L aquarium with a water depth of 7 cm, a salinity of 20, and no substrate. Crabs were fed a combination of mummichogs and Atlantic menhaden three times a week. HM crabs were fed TK food (n = 12), and TK crabs were fed HM food (n =14). After eight weeks, these crabs were used in the prey capture experiments (described above) using juvenile blue crabs as prey.

The number of juvenile blue crabs consumed was recorded and divided into three groups: HM fed HM food and TK fed TK food; TT (trophic transfer) for HM fed TK food and TK fed HM food; HM transplanted to TK and fed TK food and TK transplanted to HM and fed HM food. These three groups were compared with a one-way ANOVA. This analysis was followed by a Tukey test at the 0.05 level to compare means.

Mercury Analysis:

Crabs (both males and females) freshly caught from the field (HM: n = 12; TK: n = 10) as well as transplants and those from the lab feeding study were frozen. Muscle tissue from the claw was dissected and dried at 58°C for 48 hours. After drying, total sample weights were 0.45 $\pm$ 0.08 g. The samples were acid-digested for 24 hours using trace metals grade nitric acid (Fisher Scientific®) and were digested further using a MARS microwave digestion program (CEM Corporation). The samples were heated to evaporate the nitric acid to a final

volume of ~0.5 ml. All samples were rehydrated to a final volume of 10 ml with 1% nitric acid. For Hg analysis, 1 ml of the rehydrated sample was combined with 3 ml of 5% $KMnO_4$ in an ice bath. Five ml $NH_2OH \cdot HCl$ solution and 1 ml of 10% $SnCl_2$ were added to the sample before analyzing. Hg was analyzed using an Hg Analyzer (cold vapor atomic absorption spectrophotometer; Bacharach Coleman Model 50-D). The following concentrations of Hg standards were used (µg/g Hg): 0, 0.1, 0.3, 0.6, 1.0. Standard reference material used was DORM-2 (dogfish muscle tissue; National Research Council of Canada) and DOLT-2 (dogfish liver tissue; National Research Council of Canada). Mean recovery for DORM-2 and DOLT-2 were, 86.3% and 97.5%, respectively. Statistical analysis of the µg/g mercury within the samples was analyzed with a one-way ANOVA followed by a Tukey test at the 0.05 level to compare the means.

Results

Prey Capture Experiments:

Juvenile Blue Crabs as Prey: Hackensack Meadowlands (HM) adults consumed significantly fewer juvenile crabs in 24 hrs (0.54 $\pm$ 0.26 SE crabs) than Tuckerton (TK) conspecifics (3.25 $\pm$ 0.16 crabs; two-sample t-test: df = 53, t = 12.2, p = 0.0001; Fig. 2). Video observations showed extended periods of inactivity, i.e., resting in one area of the aquarium, by HM crabs adults, which often took days to consume two juvenile blue crabs. On the other hand, TK adults were more active, showing active foraging behavior, i.e., walking around the aquarium and probing the substrate with dactyls and chelae. TK crabs often consumed two juvenile crabs in a few hours to a day.

Mummichogs as prey: HM adult blue crabs captured significantly fewer mummichogs over the two week period (1.10 ± 0.50 SE fish) than TK crabs (3.57 ± 0.57 fish; two sample t-test: df = 24, t = 3.33, p = 0.003; Fig. 2). Most HM crabs did not consume any fish until the second week of the experiment, whereas TK adults were able to successfully catch one or two fish during the first week.

Fiddler Crabs as prey: No significant differences in prey capture ability were found between HM (10.8 ± 1.23 SE crabs) and TK crabs (12.7 ± 1.22 crabs; two sample t-test: df = 41, t = 1.12) when fiddler crabs were prey (Fig. 2). Upon addition to the circular tank, TK and HM adults almost immediately began foraging for fiddler crabs and the water became muddy by crab activity.

Mussels as prey: No significant differences were found in the weight of mussel consumed between HM (0.75 ± 0.54 SE g) and TK crabs (0.87 ± 0.14 g; two sample t – test: df = 37, t = 0.61; Fig. 2-1). Crabs from both populations consumed more single mussels than mussels in clumps, but no differences were found in the amount consumed between populations

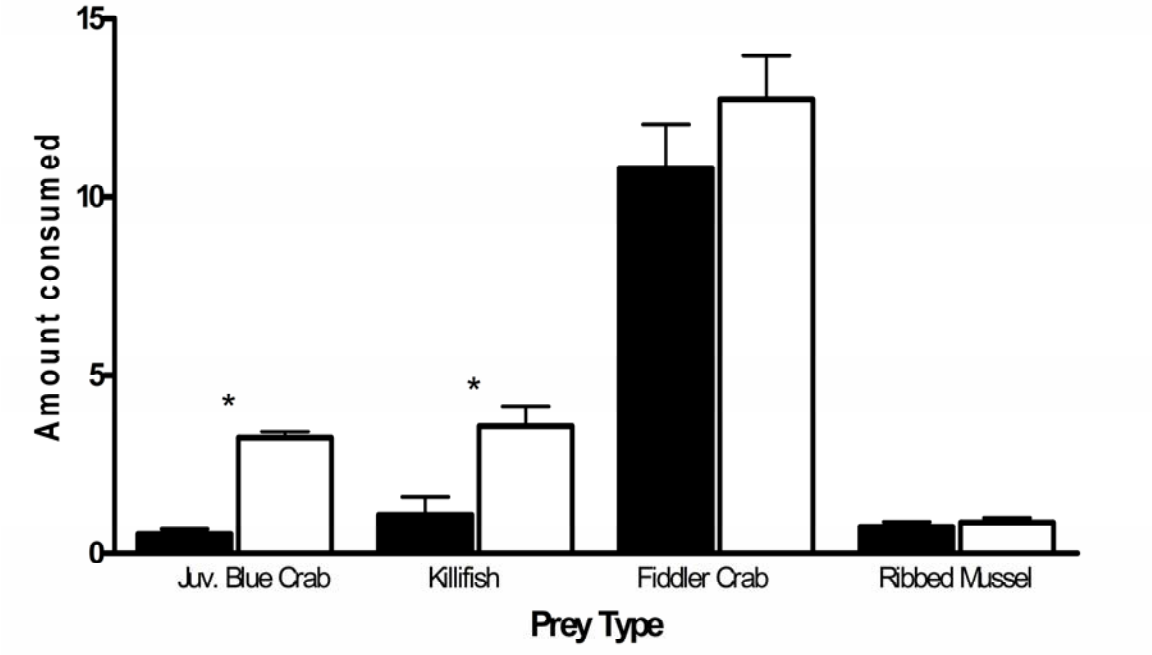


Figure 2-1. Amount of prey types consumed [numbers of juvenile blue crab in 24 hrs, mummichogs in two weeks, fiddler crabs in 24 hrs, and weight of ribbed mussel in 24 hrs] by each population (HM=black; TK= white). Error bar denotes ± one standard error and * designates significant differences

Stomach Content Analysis:

No significant difference occurred in the number of full guts between the two populations. However, TK stomachs had a significantly heavier wet weight overall (HM = 1940 ± 140 SE mg; TK = 2810 ± 250 mg; two sample t-test, df = 46, t = 2.96, p = 0.0048).

HM stomachs contained significantly less crab than TK (HM = 163 ± 55 SE mg; TK= 365 ± 67 mg; two sample t-test, df = 46, t = 1.78, p = 0.03; Fig. 3). HM crabs consumed less fish than TK crabs, but the difference was not significant (HM = 190 ± 43 SE mg; TK= 440 ± 130 mg; two sample t-test, df = 46, t = 1.78, p = 0.08; Fig. 3). HM crab stomachs contained significantly more sediment/detritus than TK stomachs (HM = 610 ± 130 mg; TK = 100 ± 30 mg;

two sample t-test, df =46, t = 3.80, *p* = 0.0004; Fig. 3) and plant/algae (HM = 420 ± 90 mg; TK = 5.0 ± 2.0 mg; two sample t-test, df = 46, t = 5.07, *p* = 0.0001; Fig. 3). These categories together constituted more than 55% of the HM crab diet. Additionally, HM crabs consumed significantly fewer bivalves than TK conspecifics (HM = 30 ± 10 mg; TK = 150 ± 30 mg; two sample t – test, df = 46, t = 3.79, *p* = 0.0004; Fig. 3), but significantly more amphipods and grass shrimp (HM = 94.6 ± 16 mg; TK = 5.40 ± 3.5 mg; two sample t-test, t = 5.68, df = 46, *p* = 0.00001; Fig. 3), and polychaetes relative to TK crabs (HM = 64.0 ± 1.0 mg; TK = 2.0 ± 0.1 mg; two sample t-test, df = 46, t = 6.68, *p* = 0.0001; Fig. 3).

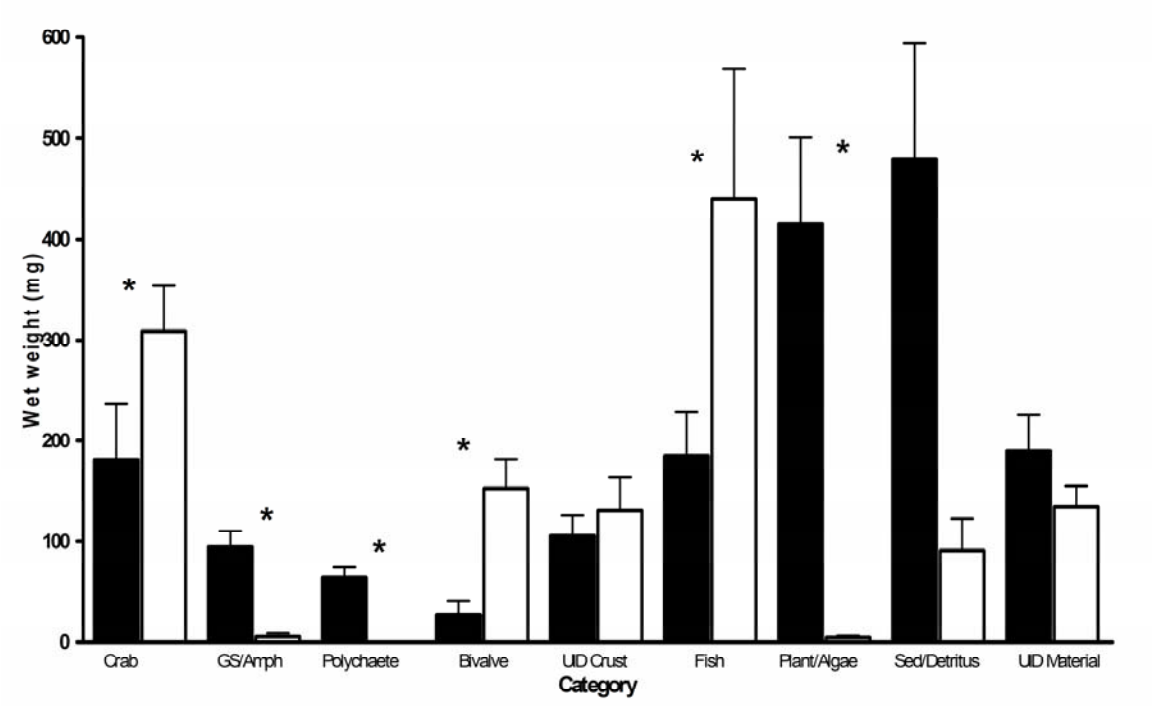


Figure 2-2. Mean wet weight of prey items consumed by adult blue crabs from each population (HM=black; TK=white); error bar represents ± one standard error and * designates significant differences between populations. Grass shrimp = GS, unidentifiable = UID and sediment = Sed.

Field Transplant Experiments:

When compared to the HM baseline data (prey capture of juvenile blue crabs), HM crabs transplanted into the TK environment for eight weeks became significantly better predators (1.78 ± 0.55 SE crabs; Fig. 4). On the other hand, TK crabs transplanted to HM were significantly less efficient predators, decreasing the number of juvenile blue crabs successfully captured (0.55 ± 0.21 crabs). TK transplanted crabs had prey capture efficiency comparable to native HM crabs. There was higher mortality of HM crabs transplanted to the TK environment compared to TK crabs transplanted to HM. At least one third of the HM crabs transplanted to TK died in the three years this experiment was conducted.

Laboratory Trophic Transfer (TT) Experiments:

Similar to the field transplanted crabs, HM blue crabs fed a diet of fish from TK became more efficient predators when preying upon juvenile blue crabs (1.92 ± 0.99 SE crabs); they became significantly better at capturing juvenile blue crabs than native HM crabs. TK adults fed a diet of HM fish became less efficient predators (0.714 ± 0.19 crabs); they exhibited prey capture ability similar to native HM crabs. For comparison, HM fed native food captured 0.54 ± 0.26 crabs and TK fed native food captured 3.25 ± 0.16 crabs (one-way ANOVA: df = 5, F = 34.9, p = 0.0001, Fig. 4).

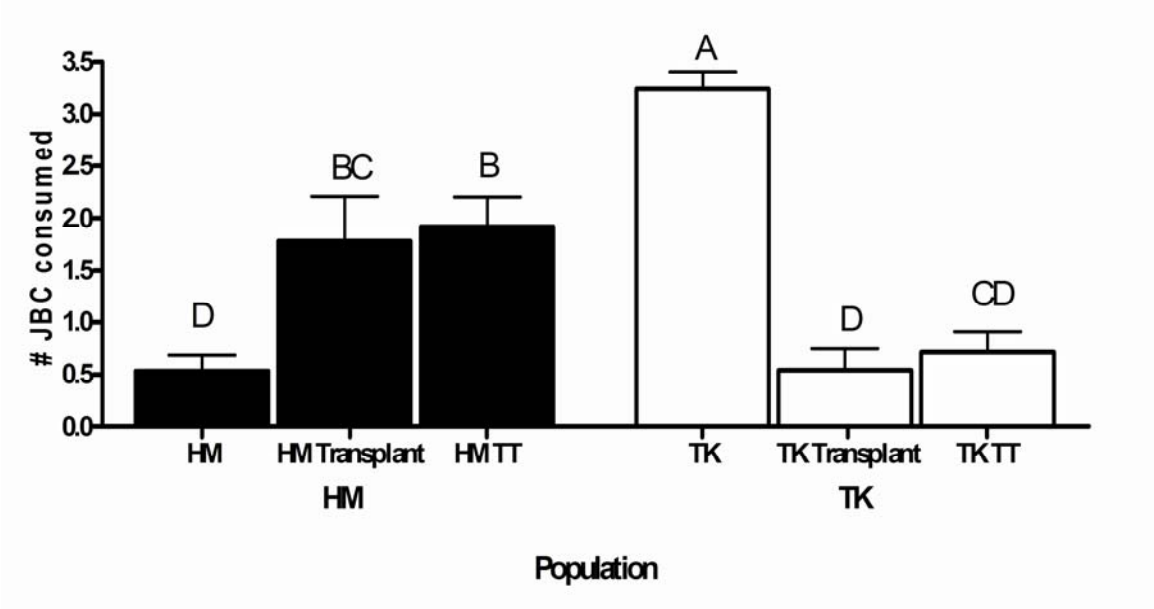


Figure 2-3. Number of juvenile blue crabs (JBC) consumed in 24 hours by treatment and population (HM=black; TK=white); error bar represents ± one standard error and single letters (A, B, etc) denote significant groups as designated by post-hoc Tukey test. TT denotes laboratory trophic transfer.

Mercury Analysis: The baseline level of Hg was not significantly higher in HM than TK crabs. After transplantation to TK or maintenance in the lab on TK food for eight weeks, the Hg levels of HM crabs decreased, but not significantly. However, the TK crabs transplanted to HM or fed HM food in the lab did accumulate significantly higher concentrations of Hg. The transplants showed the largest increase among groups. Moreover, the TK transplants acquired higher concentrations of Hg than HM crabs living in the Meadowlands (HM = 0.445 ± 0.38 SE µg/g; HM Transplants = 0.427 ± 0.35 µg/g; HM TT = 0.316 ± 0.20 µg/g; TK = 0.167 ± 0.05 µg/g; TK Transplants = 1.04 ± 0.93 µg/g; TK TT = 0.605 ± 0.36 µg/g; one-way ANOVA: df = 5, F = 4.33, *p* = 0.002; Figure 5)

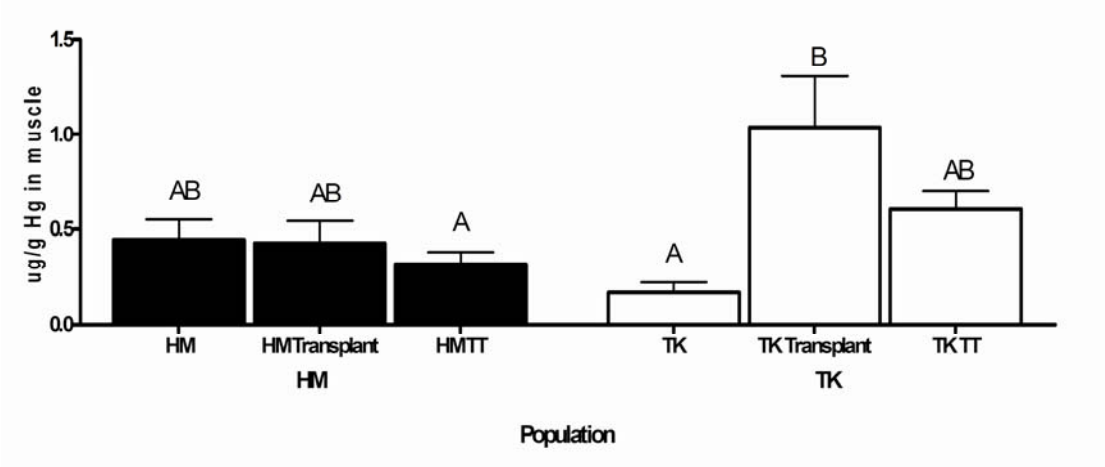


Figure 2-4. Amount of Hg (µg/g) stored in muscle tissue among treatments; error bar represents ± one standard error and single letters (A, B, etc) denote significant groups as designated by post-hoc Tukey test.

Discussion

Blue crabs from contaminated Hackensack Meadowlands (HM) had reduced ability to capture active prey. A significant difference occurred between HM and Tuckerton (TK) capture of both juvenile blue crabs and adult mummichogs, but not of mussels or fiddler crabs. This result is interesting since juvenile blue crabs and mummichogs have complex predator avoidance abilities, e.g., they fight back or dart away, while mussels, and to a lesser extent fiddler crabs, are less active prey. Mussels cannot move at all, but both populations preferred to consume more single mussels clumped ones. Crustaceans in general prefer smaller sized bivalve prey, (Elner and Hughes 1978; Juanes 1992; Micheli 1995) i.e., the single mussels, due to possible wear on their chelae and difficulty in handling larger prey, such as the configurations used in this experiment. Fiddler crabs could not burrow deeper than 15 cm in the

experimental set-up, and were more vulnerable to the blue crab predators, which are capable of putting their claws into the burrows and grabbing the fiddler crab (J. Christy, pers. comm.). The reduced ability to capture only the more active prey suggests that motivation was not affected in the blue crabs, but coordination needed to capture active prey. HM crabs appeared less active than TK crabs in the juvenile blue crab experiments. Additionally, HM crabs were also observed scooping sediment into their mouth when placed into the aquarium for the mussel experiment even though a single mussel or a clump of mussels was in front of the crab.

Estuarine organisms living in contaminated areas have altered prey capture ability. Prey capture of adult mummichogs living in a contaminated New Jersey estuary (Piles Creek) was significantly reduced compared to mummichogs living in a cleaner reference site (TK) (Smith and Weis 1997). The reduction in prey capture was primarily because of reduced attempts to capture the prey (motivation or appetite), as opposed to reduced coordination, which appears to be the case for the blue crabs.

When TK crabs were maintained in HM for two months, their prey capture ability significantly declined, and they became inefficient predators, comparable to the native HM crabs which had lived in the Meadowlands their entire adult lives. This may reflect some degree of tolerance acquired by the HM population living in the contaminated environment (Klerks and Weis 1987). When reference organisms are exposed to contaminants in the lab through food or exposed to field conditions, a change in behavior occurs (e.g., Smith and Weis 1997;

Wallace et al. 2000; Perez and Wallace 2004). Similar results were found when isolating food in lab experiments: a significant decrease occurred in the TK blue crab's ability to catch juvenile blue crab prey. While the decrease was not quite as large as that of the TK crabs that had been transplanted to HM, they were similar to native HM crabs. Contaminants in the fish may have been transferred to the crabs and caused the altered behavior. When the HM crabs were kept in TK or fed TK food for eight weeks, their prey capture improved to a level comparable to that of TK crabs. This differs from results obtained for the contaminated mummichogs (Smith and Weis 1997), which did not show improvement after being kept in clean conditions for 6 weeks. The mummichogs did not depurate Hg from their brains during that time.

The effects of Hg on feeding behavior include an overall decrease in feeding rate (Purdy 1989) and/or a decrease in the number of strikes and search efficiency (Atchison et al. 1987). Blue crabs fed a mercury-rich diet did not significantly depurate Hg after a subsequent four week exposure to cleaner conditions (Evans et al. 2001). If HM crabs had been kept a longer time in a clean environment, they may have depurated more Hg. Interestingly, significant improvements in prey capture occurred prior to any significant reduction in Hg. Mercury levels may have been reduced in the nervous system that controls behavior. There are many other contaminants in the system besides Hg that could have contributed to the observed behavioral differences, and these may have been depurated to a greater degree than the Hg.

TK crabs transplanted to HM in the field or fed HM food in the lab increased the concentration of Hg in their muscle tissue, but only TK transplants showed a significant increase of Hg. A behavioral change occurred in both wherein the crabs became inefficient predators on juvenile blue crabs, similar to HM adults. Since the transplanted TK crabs gained significantly more Hg than the lab-fed crabs, they may have been accumulating contaminants from the water and sediments as well as from their prey. In a similar study, bluefish collected from TK were fed mummichogs from HM; after several months of eating contaminated food, the bluefish had reduced feeding and growth as well as increased levels of Hg and PCBs in their tissues (Candelmo et al. 2007; Candelmo et al. in review). Crabs transplanted from TK to HM significantly increased their Hg body burden, while HM transplants and lab fed crabs decreased their Hg only very slightly, indicating that Hg can be accumulated more rapidly than it can be depurated.

Mercury in blue crabs can reach high concentrations in muscle, which poses a high risk to humans, since blue crabs constitute a major fishery. In Superfund sites where Hg levels are extremely high this metal may not have an effect on the survival of the blue crabs themselves (Brouwer and Lee 2007; Engel and Thayer 1998).

Similar effects of contaminants on feeding behavior have been reported (Smith and Weis 1997, Wallace et al. 2000, Perez and Wallace 2004). We found the contaminants within the HM estuary system cause adult blue crabs to have impaired coordination and become inefficient predators on more active prey. We

suggest that contaminants such as Hg are the cause of the impaired predatory behavior observed in the HM crabs, as well as in TK crabs transplanted into the HM environment or fed contaminated food.

The investigation into the diets of the adults from these two estuaries supported the laboratory studies. Blue crabs are well known for their predatory abilities within estuaries (Hines 2007; Virnstein 1977; 1979). Stomach content analysis revealed that HM crabs are primarily scavengers, as suggested by large amounts of algae/plant material, detritus and sediment found in their stomachs. This category comprised almost 60% of the HM blue crab diet, compared to 8% in the TK crab diet, which suggests more than incidental ingestion. The stomach contents of field caught crabs may also reflect the availability of different prey types at the different habitats, i.e., HM crabs consumed significantly fewer bivalves. Bivalves are not very common in the Meadowlands (personal observation), but are abundant in the Tuckerton area. However, mummichogs and juvenile blue crabs are abundant within the Meadowlands, but comprise only a small percentage of the diet. Blue crab diets in Florida were often representative of what was available to them at a particular time and season (Laughlin 1982). A larger amount of floating algae and reduced numbers of bivalve beds occurs within HM (personal observations), probably due to habitat degradation over the years. Even though remains of fish were found within the HM stomachs, whether an individual crab actually captured a live fish or found one that was already dead or dying is unknown. The larger numbers of

sediment-dwelling amphipods and polychaetes in the HM stomachs may have been ingested with the sediments crabs were consuming.

If given a choice between algal and animal diets, Asian shore crabs (*Hemigrapsus sanguinaeus*) that were food-deprived consumed more algae combined with animal food, suggesting a wider diet breadth, i.e. ingestion of algae, for an organism that is normally a predator (Brousseau and Baglivo 2005). If HM blue crab adults are poorly coordinated for capturing active prey, but can ingest large amounts of algae, detritus, and other low protein food types, they can increase their diet breadth and ensure their survival within the estuary. Even predators such as blue crabs can consume plants/algae and extract some nutrition (Wolcott and O'Connor 1992).

A side benefit of HM blue crabs consuming more algae, plant material, and sediments, could be a reduction of contaminant uptake. Plants and algae generally have lower levels of metals and organic contaminants (particularly those that biomagnify, including Hg) than animal foods. Bluefish (*Pomatomus saltatrix*) fed a diet of menhaden and mummichogs from the Meadowlands greatly increased their body burden of mercury and PCBs (Candelmo et al. 2007). With regard to the consumption of the contaminated sediment, how much of the adsorbed contaminants are available once mineralized within the sediments remains questionable (Chapman and Wang 2001). Although blue crabs may be ingesting contaminated sediment, much of the contaminants may not be available. As a result of their diet, the Hg body burden of HM crabs that

have lived their entire life in that location tended to be lower than that of TK crabs which were fed a diet of fish from HM for only two months.

The HM population might be comprised of smaller crabs with reduced fitness if they have a diet of presumably less or lower quality food. When three species of amphipods were fed a diet of low quality food, two of the three species experienced reduced survivorship, growth, and fecundity (Cruz-Rivera and Hay 2000). Reduced growth and lifespan were observed in a mummichog population because the fish were poor predators and consumed a considerable amount of detritus (Toppin et al. 1987). This may not be the case with HM crabs, however. In more than five years of collecting blue crabs within the Meadowlands we have found significantly larger individuals relative to TK (Appendix II), but their fitness is unknown. High levels of contaminants within the Meadowlands have resulted in fishing bans on many species, including blue crabs. The absence of fishing may be the reason for the larger crabs at HM. It is possible that top-down effects are occurring within HM and is similar to that seen in grass shrimp at contaminated sites where mummichogs were poor predators, small in size and few in numbers (Santiago Bass et al. 2001).

Even with a diet of low quality food, the blue crab population appears to be increasing. The Meadowlands Environmental Research Institute (MERI) conducted two surveys, one during 1987-1988 and again from 2001-2003 (Bragin et al. 2005); the total abundance of blue crabs collected with the same amount of effort increased three-fold. This is probably a result of a combination

of the ban mentioned above as well as improving environmental conditions in a degraded habitat.

Cannibalism is a major feature of blue crab predator-prey interactions. Adult blue crabs could be responsible for 75-97% of the mortality in small blue crabs (Hines and Ruiz 1995). Reduced predation on juvenile blue crabs, if it occurs in nature in HM, suggests that there might be less cannibalism occurring in HM than at other sites. Cannibalism is considered an important way of population regulation (Hopper et al. 1996). If there is less cannibalism by adults occurring at HM, it could ultimately affect the size, density, and the distribution of juvenile blue crabs within the Meadowlands (Mosknes 2004) and could theoretically increase the population.

HM blue crabs have a reduced feeding ability on more active prey such as juvenile blue crabs and mummichogs. Since predation is a major form of energy transfer and since this process also structures the estuarine community, food web changes within the Meadowlands may be a result of the altered feeding and diet of the blue crabs. However, HM blue crabs, even though they are feeding on a low quality diet, do not appear to have a reduced fitness, and probably because of the fishing bans, appear to be doing well in an estuary that has a long history of contamination.

Chapter 3

Aggression and Predator Avoidance

Introduction

Recent animal behavior research has focused on behavioral types of individuals and classifying the 'behavioral syndrome' of a population (Sih et al. 2004). The most common of these behavioral types are aggression and the shy-bold regime, which have been investigated mostly in vertebrate species, such as fish (e.g., Coleman and Wilson 1998; Ward et al. 2004). Boldness has been classified as an individual's tendency to take risks and be exploratory in novel situations, where shyness is at the opposite end of the regime. Literature suggests that the range of behaviors of this regime can have ecological impacts ranging from effects on diet, predator risk, and parasite load (Wilson et al. 1993). The behavior of brachyuran crabs has also been widely studied (e.g., Crane 1975; Hazlett 1971, 1972; Jachowski 1973), but not necessarily in this context. Just as in other animal populations, the plasticity of aggressive and defensive responses can vary across a population as well as between populations. Recently aggression and bold behavior have been correlated with the success of the invasive crayfish, *Pacifcastacus leniusculus* (Pintor et al. 2008). In another study, risk-taking behavior predicted aggression and mating success in a species of fiddler crab, *Uca mjoebergi*, (Reaney and Backwell 2007).

An organism's propensity for aggression is likewise important as it may affect interactions between individuals. An individual's tendency for or against aggression may also help determine its predation or foraging success (e.g.,

subduing resisting prey and pressing attacks) or defending a prey item from other individuals (Kaiser et al. 1990). Both adult and juvenile blue crabs (*Callinectes sapidus*) are known to be aggressive, especially towards conspecifics (Clark et al. 1999a; Moksnes et al. 1997). Such encounters can leave a crab injured or missing appendages. Hence, an individual can have presumed competitive and/or energetic disadvantages as well as increased predation risk (Juanes and Smith, 1995). The blue crab's most formidable defensive (or offensive) weapons are its sharp, strong chelae. How an individual uses these weapons may affect its prospects for survival in an encounter with a predator or conspecific competitor. A field study found an increase of antagonistic behavior in crabs with certain shapes of commercial pots (Vasquez-Archdale et al. 2003). Since crab pots are sometimes used in population estimates, it is possible that increased agonistic behaviors may lead to underestimates in population counts.

Predator avoidance is another behavior that can vary within and between populations. If juvenile stages of any organism can avoid a predator and live to adulthood, then they have the potential to pass their genes to the next generation (Posey et al. 2005).

A number of studies have investigated effects of contaminants on aggressive behavior and predator avoidance. Henry and Atchison (1979a; 1979b; and 1986) investigated the effects of metals (i.e., Cu and the combination of Cd and Zn) on the hierarchy structure in bluegills; the dominant fish in schools became more dominant and exhibited higher levels of agonistic behavior when exposed to Cu and the combination of Cd and Zn. Subordinate fish in the

schools exhibited less agonistic behaviors when exposed to the contamination relative to the control subordinate fish. Higher rates of aggression have been observed in cats (Li et al. 2003) and juvenile rainbow trout exposed to lead. Increased levels of aggression as a result of chemical exposure (DDT) have been observed previously in blue crabs (Lowe 1965). Since juvenile blue crabs often bury themselves in soft sediments to escape predators and to molt (which they do more often than adults), and may eat detritus and other materials found in the substrate (Moody 2003) this species might be vulnerable to contaminants in the sediment. In general, early life stages may be affected more by contamination than adults.

There is also a body of literature indicating that toxic contaminants can impair predator avoidance responses such that more contaminated prey are generally more easily captured by predators (Fleeger et al. 2003). Larval striped bass (*Morone saxatilis*) experienced increased susceptibility to predation due to increased levels of contamination coupled with habitat loss in the Chesapeake Bay (Setzler-Hamilton et al. 1988). Three-spined sticklebacks (*Gasterosteus aculeatus*) exposed to bis(tributyltin)oxide (TBTO) chose more exposed areas of the water column increasing the time visual to the predator and ceased predator avoidance behaviors sooner (Wibe et al. 2001). *Daphnia* exposed to a suite of contaminants increased their drift time in the water column, thus increasing their exposure time and susceptibility to fish predators (Taylor et al. 1994).

Weis and Weis (1998) exposed larval mummichogs (*Fundulus heteroclitus*) to lead in laboratory studies, which resulted in reduced predator

avoidance when adult grass shrimp (*Palaemonetes pugio*) were predators. In later laboratory studies conducted by Zhou et al. (2001), larval mummichogs (*F. heteroclitus*) exposed to methylmercury were found to have reduced swimming ability, and as a result, experienced increased predation by the grass shrimp, *P. pugio*. Impaired responses have also been seen in populations living in contaminated environments. Adult mummichogs from a contaminated site had reduced activity levels compared to those from a cleaner reference site, and were also more vulnerable to predation by blue crabs in laboratory studies (Smith and Weis 1997).

In this investigation of aggression, we compared the response to a threatening stimulus and predator avoidance of juvenile blue crabs from the Hackensack Meadowlands (HM), an estuarine system with a long history of contamination, and a cleaner reference site in southeastern NJ, Tuckerton (TK). It was hypothesized that *C. sapidus* from HM would show signs of aberrant behavior and inferior predator avoidance abilities compared to individuals from TK. We also examined behavior in adult blue crabs from the same two systems when confined to crab pots. We expected aggressive individuals, once inside a pot, would keep other crabs out of the baited pot, which could have implications for using crab traps in population estimates.

Materials and Methods

Experimental Design:

Crabs were collected from HM and TK using a seine net and otter trawl and brought back to the laboratory. They were kept in aerated tanks with a sand

depth of one centimeter and artificial seawater (Instant Ocean®) at their native salinity (HM: 15; TK: 30). A 14/10 light cycle was kept throughout the field season. TK crabs were fed a diet of ribbed mussels (*Geukensia demissa*) and Atlantic menhaden (*Brevoortia tyrannus*) collected from TK while HM crabs were fed a diet of menhaden and mummichogs collected from HM; all crabs were fed three times a week after which the water was changed. Intermolt crabs were acclimated to laboratory conditions for 48 hours prior to beginning of experimentation.

Response to a Threatening Stimulus:

Crabs (mean carapace width, CW, = 52 mm) were restrained inside a small, inverted opaque container positioned at one end inside a 38 L aquarium with a depth of 5 cm artificial sea water (no sand on the bottom) and allowed to acclimate for 10 minutes. The aquarium was covered on three sides with opaque, non-reflective paper to restrict the crab's peripheral vision and prevent it from reacting to its own reflection or movement outside the aquarium. The top two thirds of the remaining side were covered as well for the same reason; the bottom third was left clear for observation.

While the crabs were isolated in the opaque containers, a stimulus consisting of black rubber stopper 44 mm diameter (crabs smaller than 35 mm were tested with a stopper 19 mm diameter) attached to a dowel 54 cm in length, was slowly lowered into the other end of the aquarium. After 10 minutes in isolation, the opaque container was removed. The stopper was slowly pushed toward the crab by an experimentor who stood beyond the opaque side of the

aquarium. No part of this experimentor ever appeared directly over the aquarium. A second observer, stationed back several feet from the aquarium to avoid provoking a reaction from the crab, recorded the nature of the crab's reaction.

Three response types were recorded. A "flee" reaction was defined as the crab quickly moving away from the stopper, and "attack" was a lunge toward it. A "mixed" response was lunging toward the stopper and then quickly retreating to the opposite end of the tank. If the crab was facing away from the stimulus, especially active, or moved immediately across the aquarium before the stimulus was activated, then the trial was discontinued. The observer recording the reaction was blind in regard to the identity or population of crab being tested. Statistical analyses were conducted using Statistix 7.0 and GraphPad Prism 4.0 software. Differences in the responses to the stimulus were analyzed using Chi-Square tests.

Survival with an adult blue crab predator with substrate:

The experiment was conducted in a 76 L aquarium with approximately 35 mm sand covering the bottom. The aquarium was covered with opaque paper on all four sides so that the crabs did not get distracted by outside movement or reflections. Four juvenile crabs (CW: HM = 30 mm; TK = 35 mm) two from each population, were added to the aquarium and allowed to acclimate for one hour. The sizes and sexes of the juveniles were noted and recorded. After acclimation, one food-deprived adult crab, either male or female (mean CW: HM = 101 mm,

TK = 94 mm) was added. Adults were usually 90-100 mm larger than the juveniles. In 45 trials, we used 20 HM adults and 25 TK adults. Preliminary studies determined sex was not a factor in differences observed. All trials were recorded using a closed circuit digital camera (Ikegami Tsushinki Co. Ltd) with a 7.5-75 mm lens (Canon) and digital disk recorder (Panasonic, Model WJ-HD 309). The number and identity (which population) of juveniles consumed in 24 hours was recorded and data were analyzed using chi-square tests.

Survival with a predator without substrate:

Baseline Threatening Stimulus Test: One juvenile crab at a time (mean CW = 31 mm) was placed in an opaque rectangular Nalgene® container (dimensions: L = 23 cm, W = 13.5 cm, H = 13.5 cm) with no substrate and water depth of 4 cm and allowed to acclimate for 30 minutes before testing. Each crab was tested three times using a threatening horizontal stimulus: a #2 stopper (diameter: 2 cm) attached to a wooden dowel (length: 52.5 cm) with a 10 minute rest period between tests. Each response to the stimulus was classified as an attack, flee, or mixed (a combination of attack and flee response). The crab was classified as either 'aggressive,' 'non-aggressive' or 'mixed' as a result of the most dominant response from the baseline test. Only TK juveniles were used for this experiment due to the inability to collect an adequate number of juveniles from HM that year. After classification, they were used in the predator avoidance experiment.

Predator Avoidance: Similar to the predator experiment described earlier, this experiment used a 76 L aquarium, but without sand as substrate to allow better

visual observations; in addition, white paper was placed underneath the aquarium. Only two juveniles were used in each experiment, and in the following combinations: one aggressive + one non-aggressive or, one aggressive + one mixed, or one non-aggressive + one mixed. All trials were recorded using a closed circuit digital camera (Ikegami Tsushinki Co. Ltd) with a 7.5-75 mm lens (Canon) and digital disk recorder (Panasonic, Model WJ-HD 309). The juveniles were marked with a Sharpie® in order to distinguish them on the video, and allowed to acclimate to the tank for one hour. After the acclimation period, an adult male blue crab that had been food-deprived for 48 hours was added to the tank. The experiment ended after 48 hours. The first juvenile to be consumed and any other interactions with the predator were recorded and analyzed using chi-square tests.

Adult crab pot experiment:

Crab pot experiments were conducted outdoors in a large circular tank (diameter: 1.7 m; height: 1.5 m) with a sand depth of 1 cm and water depth of 0.5 m. Five food deprived adult crabs from one population (HM: n = 11 trials; TK: n = 9 trials) were placed in the tank and allowed to acclimate for 30 minutes. After acclimation, one commercial crab pot with bait (Atlantic menhaden, *Brevoortia tyrannus*) was placed in the bottom of the tank. Crabs were checked after 24 and 48 hours. After 48 hours, the experiment ended and crab positions within the tank and pot were noted. The number of crabs that had entered the pot at 24 hrs and 48 hrs were analyzed using student's t-tests.

Results

Response to a threatening stimulus:

In 99 trials, HM juveniles attacked a threatening stimulus significantly more often than TK juveniles; TK juveniles either fled or gave a mixed response significantly more often than HM juveniles.(χ^2 = 8.45, df = 2, $p \leq 0.015$; Figure 3-1). Figure 6 represents the percentage of each response.

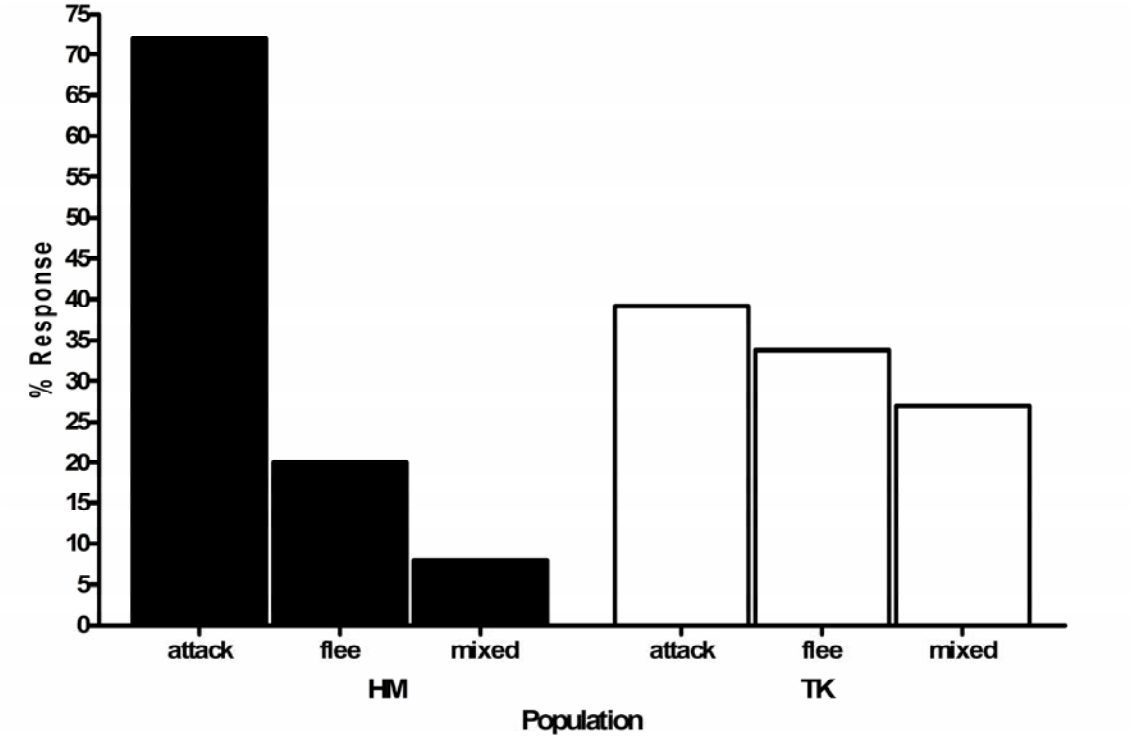


Figure 3-1. Percentage of responses to a threatening stimulus by HM and TK juvenile blue crabs.

Predator avoidance with substrate:

In 45 trials, HM juveniles were significantly more successful at avoiding being eaten by an adult than TK juveniles.(χ^2= 10.06, df = 1, $p \leq 0.002$; Figure 3-2). Even though these experiments were videotaped, it was often hard to see the

actual details of the interactions between the juveniles and adult predator due to the sandy substrate. Some flees and other interactions between juveniles and the adult were observed, but even though the juveniles were marked it was hard to definitely determine which population it was from. Figure 3-2 represents the percentage of juveniles of each population that survived.

Survival with a predator without substrate:

In 11 trials, aggressive juveniles were not significantly better at avoiding an adult predator than non-aggressive ones (although all the juveniles in this study were from TK). Figure 3-2 represents the percent survival. Only two trials were conducted with crabs classified as mixed.

Observations of the videotapes indicated that when aggressive crabs went into a threat posture with their claws extended, they were likely to be captured by the adult, which was much larger and not daunted by the threat display. Only in one trial did this allow survival: the adult stopped pursuit of the aggressive juvenile and pursued the non-aggressive one until cornered. Viewing the tapes indicated that, in general, fleeing to the opposite end of the tank was the best strategy for survival with an adult blue crab; this was observed for all three behavioral types.

In some of the trials juvenile-juvenile interactions were observed. In two trials, the aggressive crab 'bullied' the other crab into the area of the tank where the adult was, resulting in capture. In one case, the aggressive individual held its ground in an area of the tank and the other crab was forced to another area of

the tank where the adult was, and was captured. In another case, the scuffle between juveniles alerted the adult and the aggressive individual was captured.

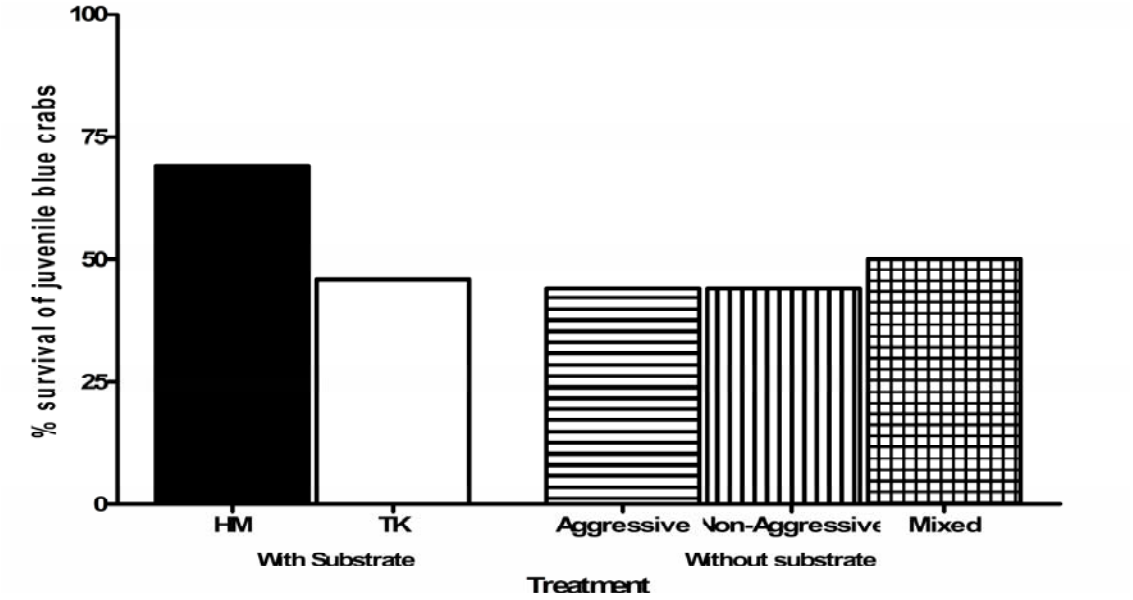


Figure 3-2. Percentage of HM and TK juvenile blue crabs that successfully avoided an adult blue crab predator with substrate and percentage of aggressive, non-aggressive and mixed individuals that successfully avoided an adult blue crab predator.

Adult Crab Pot Experiment:

After 24 hours, significantly more TK crabs entered the crab pot than HM crabs. The same result was true after 48 hours (Figure 3-3). In many HM trials, aggressive crabs in the pot killed and/or cannibalized other crabs that entered the pot. If this occurred, no other crabs entered the pot during the experiment. In all TK trials, individuals did not prevent other crabs from entering the pot and cannibalism did not occur.

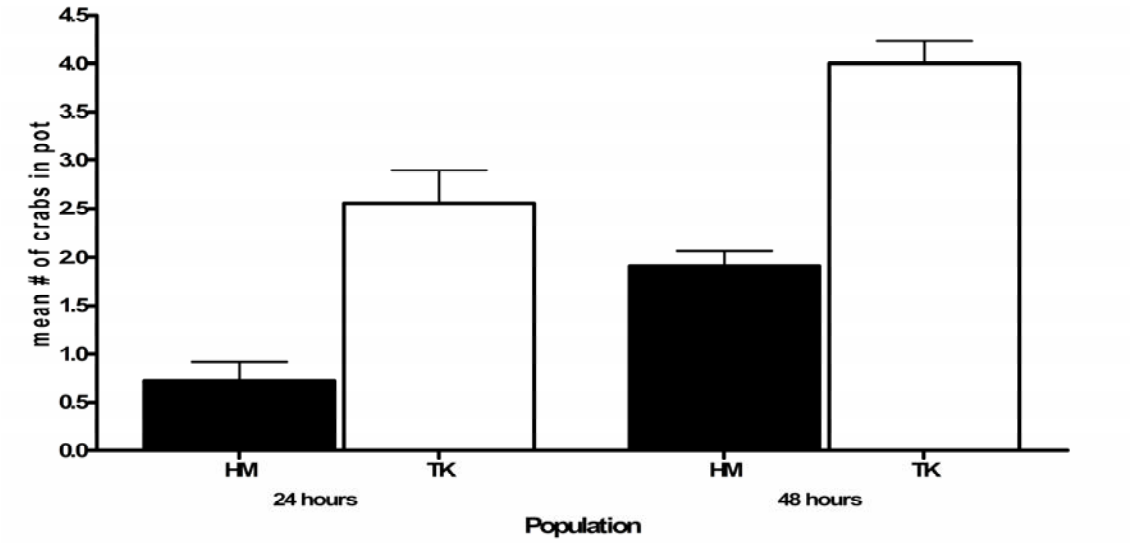


Figure 3-3. Mean number of crabs entering a baited crab pot after 24 hours and 48 hours respectively; error bar represents one ± standard error.

Discussion

HM juveniles were found to attack a threatening stimulus significantly more often than their TK counterparts, while TK juveniles fled the stimulus or showed a mixed response significantly more often. HM juveniles were also significantly better at avoiding an adult blue crab predator, which was unexpected. It appears that HM adults continue to show aggressive behavior in the adult life stages, which causes significantly fewer individuals to enter a baited crab pot.

HM juveniles showed a significantly increased percentage of attacks towards a threatening stimulus compared to TK conspecifics. Blue crabs are known to show a range of agonistic behavior ranging from displays, fending and striking (Clark et al 1999a; Hines 2007). It is possible that the greater aggression seen in the HM population is a neuro-behavioral effect of contaminants in the

area, which is known to have elevated levels of many contaminants including mercury and PCBs (Ludwig and Ianuzzi 2005; Weis et al. 1986). Sublethal concentrations of contaminants have been found to affect behavior such as agonistic/aggressive responses in a wide variety of organisms including crustaceans (Boyd et al. 2002; Weis & Perlmutter 1987). It is possible that altered neurotransmitters as a result of contaminant effects may be the cause of increased aggression observed in the juvenile HM blue crabs. Serotonin has been linked to aggression in other species of crustaceans, such as the crayfish *Astacus astacus* and the lobster *Homarus americanus* (Kravitz 2000). Subordinate individuals injected with the amine serotonin continued to engage their opponents and continued fighting in situations that would ordinarily result in a withdrawal (Huber et al. 1997).

In a pilot study, TK juveniles were transplanted to HM and vice versa; crabs were left at each location for two months and once returned to the lab were subjected to both the aggression tests described earlier. A behavioral switch was observed whereby TK crabs attacked the stimulus more often suggesting that the environment e.g., the contaminants, may be the cause of this behavior change. However, due to high mortality in the field, further study is needed (MacDonald, unpublished data).

The finding that HM juveniles survived longer in the presence of a predator was unexpected. It is unlikely that the HM crabs survived longer because they were unpalatable. If that had been the case, they would have been killed and not consumed. The parameters of the predator avoidance experiment

with substrate gives the juveniles many options to escape the predator through fleeing and then rapidly burying themselves, fighting back while staying buried or standing ground to offer resistance. With the presence of the sand in the aquarium, it was impossible to observe the actual interactions between the adult and juveniles, but it seems that this suite of behaviors in conjunction with the substrate allowed HM juveniles to successfully avoid predators. However, there is limited amount of area in which to flee in the aquarium and this predisposition to flee may be more useful in the open estuary.

To test whether it is indeed the aggressive tendencies of the HM juveniles that confer an advantage over their TK counterparts in dealing with a predator, we conducted the experiment without substrate allowing us clear observations of the interactions between the juveniles and adult predator. The results indicate that it is not aggressive behavior per se that protects the crabs since aggressive and non-aggressive individuals were equally likely to be captured. The crabs showed a wide range of interactions and even though a juvenile may have been classified as aggressive, it often fled when the adult approached. The juvenile blue crabs may be able to distinguish the difference between a 'real' threat, the adult blue crab vs. a stopper, which may not be as threatening. These results suggest that the aggression observed in the HM juveniles could be context-dependent (Coleman and Wilson 1998). However, the most common scenario preceding capture was displaying and standing ground, a type of aggressive behavior that is not apparently useful when the predator is much larger. These experiments were conducted with juveniles from the TK population (since HM

juveniles were scarce that summer) and only provide insight into whether or not aggression per se increases the survival of these individuals.

Interactions of the aggressive juveniles with the other juvenile in the tank were also relevant. In several trials, the aggressive juvenile bullied the other juvenile to the area of the tank where the adult was present. However, the aggressive individual was not necessarily the survivor in the trials in which this type of interaction occurred. This suggests that aggressive individuals may be better at holding ground or defending their space/refuge against other juveniles, but does not indicate that they are less likely to be captured. Additional studies will be needed to ascertain exactly what aspects of behavior are most closely associated with the survival of HM juveniles with a predator, since it appears that the increased propensity for aggression per se is not the critical factor.

Adult blue crabs are responsible for 75-97% of mortality in juvenile blue crabs within Chesapeake Bay (Hines and Ruiz 1995). In the Gulf of Mexico this value ranges from 85-91% (Heck and Coen 1995); in New Jersey, mortality ranges from 10-45% (Wilson et al. 1990a, 1990b). However, adult blue crabs from HM were found to be poor predators on juvenile blue crabs under laboratory conditions. In addition, few crab parts were found in stomach contents of field-collected individuals, suggesting that HM adult blue crabs are scavengers, rather than voracious predators as in ‘normal’ estuarine systems (Reichmuth et al. 2007; Reichmuth et al. 2009). This suggests that less cannibalism may be occurring within the Meadowlands system allowing larger numbers of juveniles to survive (notwithstanding their scarcity in 2008). If this is the case, juvenile blue

crabs may be overcrowded in a degraded, patchy habitat. Studies have shown that increased agonistic encounters occur in populations of blue crabs under over-crowded conditions (Clark et al. 1999a; 1999b). This may be why HM blue crabs were found to have increased propensity for aggression. Funnel-web spiders (*Agelenopsis aperta*) were also found to be highly aggressive under low food availability conditions (Maupin and Riechert 2001). In these situations, resources are limited and competition is increased, which favors aggressive individuals (Sih et al. 2004).

The juvenile crabs that were determined to be aggressive before the predator avoidance experiment did not always show an aggressive posture (i.e., bold behavior) toward the predator suggesting that this behavior may be context dependent. Similar results were found in sunfish, where individuals were classified as bold when approaching a meter stick, but the same bold individual did not inspect a novel food source (Coleman and Wilson 1998). The behavioral plasticity observed in the predator avoidance experiments is adaptive to the circumstances.

The results of the crab pot experiment suggest that HM crabs remain aggressive into the adult life stages. Aggressive behavior can have serious implications on the ecology of an organism if the individuals are aggressive in novel or inappropriate situations (Sih et al. 2004). (In our initial collections of juveniles from HM we put them in containers together, and by the time we returned to the laboratory in less than an hour many had been killed by others,

unlike TK crabs. This experience prompted the current behavioral studies on aggression.).

A few of the crab pot trials with HM crabs also resulted in a crab being killed and/or eaten by another crab in the pot. The other crabs may not have entered the trap due to the scent of the injured conspecifics. Field experiments using crab pots baited with an injured blue crab caught fewer crabs than traps baited with menhaden (Ferner et al. 2005). Another study using odor plumes containing metabolites of injured crabs found crabs reduced their foraging behavior and the amount of movement (Moir and Weissburg 2008). It is possible that metabolites released from the injured crab in our mesocosm acted as a deterrent.

Our results suggest caution when using crab pots in population estimates and are supported by field observations. When we used crab pots within the Meadowlands, few crabs were caught and on occasion, an inhabitant would be dead or severely damaged. Using the same effort in TK, many more crabs were caught (personal observation) without damage to the inhabitants. The poor field catch using the baited crab traps is not an accurate representation of the blue crab abundance within the Meadowlands since other fishing techniques (seines and trawls) were far more successful.

Despite the increased aggression observed in both HM juveniles and adults, the HM blue crab population seems to be doing well. Individuals from this population are significantly larger than those in TK (Appendix II) and a survey conducted in the Meadowlands in 1987-88, and again in 2001-03 showed a

three-fold increase in abundance of blue crabs within this system (Bragin et al. 2005). Juveniles may experience reduced predation because of two behaviors: (1) their enhanced ability to avoid predation by adults, and (2) the poorer predation ability of blue crab adults (Reichmuth et al. 2009). There is also a fishing ban within the HM area due to elevated contaminants in blue crabs. Reduced fishing is effectively the removal of a top-down control on the population. All of these factors together appear to contribute to population growth of this species in the contaminated environment.

Chapter 4

Bioaccumulation and Depuration of Metals

Introduction

Estuarine sediments are major sinks for many materials (i.e., metals, PAHs, PCBs, pesticides, etc) in run-off from the land. Urban estuarine sediments usually contain elevated levels of metals including copper, cadmium, chromium, lead, mercury, and zinc (Benoit et al. 1999; Ludwig and Iannuzzi 2005; Sanger et al. 1999). Most of the copper in storm water that runs-off from urban sources is contained in automobile brake pads, building supplies (i.e., siding, gutters), and pesticides that contain copper (Hoenicke et al. 2003). Cadmium is used in smelting and electrolytic refining practices; it is discharged into the environment during the manufacture of cadmium-containing materials and when these products are discarded (i.e., batteries) (Hutchinson and Meema 1987; Kostochka et al. 1998). Most of the time chromium is mobilized by the weathering of serpentine sediments and is usually in the form of a usable nutrient (Abu-Sab, 1998). The lead found in estuarine sediments is deposited from atmospheric sources such as industry, but it can also have local sources (i.e., street runoff) (Legra et al. 1998). Mercury occurs in several forms (methylmercury is most toxic) and is released from many industrial processes such as paper processing, mining processes, coal burning plants, and oil and gas combustion (Jones et al. 1996).

Crustaceans can accumulate metals in their systems by absorption from the surrounding water/sediment through the gills or through ingestion of food (Bryan 1971; Bryan 1979). Recent studies suggest that diet, however, may be the major source of metals for many estuarine invertebrates (Wang 2002). High concentrations of metals have been shown to be toxic to crustaceans. They affect growth, reproduction, molt cycle, limb regeneration, biochemistry, and physiology (Bryan 1979). A study on female shore crabs (*Carcinus maenas*) found that excess Cr and Cu affected reproductive hormone activity, which could affect reproduction (Elumalai et al. 2004). Morgan et al. (2006) correlated high concentrations of metals with developmental abnormalities in the embryos of the lined shore crab (*Pachygrapsus crassipes*). Weis et al. (1992) reviewed studies that demonstrated that metals retarded limb regeneration and molting in fiddler crabs. As shown in an earlier study, exposure to mercury and cadmium inhibited regeneration and the molt cycle (Weis 1976). Metals can also affect osmoregulation; Thurberg et al. (1973) found that excess copper caused loss of osmoregulatory function in the green crab and excess cadmium caused reduced rate of oxygen consumption in both *C. maenas* and the Atlantic rock crab, *Cancer irroratus*.

For decapod crustaceans, copper and zinc are essential for hemocyanin and enzymatic activity, but elevated concentrations can be toxic if not stored or excreted (Ahearn et al., 2004; Rainbow, 2007; Vernberg and Vernberg, 1974). These metals are regulated to specific concentrations, but once these thresholds are reached, the regulatory process breaks down and accumulation begins

(Engel and Brouwer 1987; Rainbow 1985, 2002; Wang and Rainbow, 2007). Non-essential metals, such as Cr, Hg, and Pb are not regulated and accumulation can occur at all concentrations (Brouwer and Lee 2007; Rainbow 1985).

There are three organ systems in which crustaceans are most vulnerable to the toxic effects of contaminants: the gills, hepatopancreas, and the antennal glands (Bryan 1979). The gills are one of the entry points for harmful substances since they are large adsorptive organ systems. Further damage to the gills can occur when substances are excreted in the metabolized form (Bryan 1971). The hepatopancreas serves as the principal organ of absorption, storage, and secretion and could be damaged since it is a storage site for toxicants. The antennal gland is also a site of particular vulnerability since this is where excretion takes place (Malins et al. 1980).

There are several possible fates of a metal once inside the crustacean: binding to metallothionein, transport to the mitochondria, accumulation by lysosomes, transfer to the endoplasmic reticulum, or discharge back to the blood (Ahearn and Zhuang 1996; Ahearn et al. 2004). Most recent research supports the idea that probably all of these processes are important and the organism probably uses a combination of these (Ahearn et al. 2004). In blue crabs, several metallothioneins have been identified that bind copper, cadmium, mercury and zinc (Brouwer and Lee 2007).

Because of the historical differences in these two locations, the purpose of the current investigation was to quantify the concentrations of metals (Cr, Cu, Hg,

Pb, and Zn) in the muscle and hepatopancreas of crabs within these two sites. In addition, we wanted to determine the pathway of uptake, i.e., if the environment or diet, has an affect on the accumulation of metals within these two tissue types. This has been investigated by quantifying the concentrations of metals in individuals transplanted to the "other" site or fed food from the other site in the lab for eight weeks. We expected that the hepatopancreas would have elevated concentrations of these metals since this organ is one of the main sites of storage and adsorption of toxicants. We also expected that crabs from contaminated HM would have higher levels of metals than crabs collected from TK, and that TK crabs that were fed contaminated food in the lab or transplanted to HM for eight weeks would have elevated levels of contaminants (similar to baseline crabs from HM). Conversely, HM crabs fed clean food or transplanted to TK were expected to reduce their body burden of metals.

Materials and Methods

Experimental Design

Crabs were collected from the Hackensack Meadowlands (HM) and Tuckerton (TK) using a seine net and otter trawl and brought back to the laboratory in buckets filled with water from the site; crabs were separated by burlap to prevent cannibalism en route to the lab. Some were frozen and prepared for metal analysis. Others were kept in aerated communal tanks (76 L) with a sand depth of one centimeter and artificial seawater (Instant Ocean®) at their native salinity

(HM: 15; TK: 30) for a maximum of 48 hours with a 14/10 light cycle before being transplanted or fed switched diets in the trophic transfer experiment.

Laboratory Trophic Transfer (TT) Experiments

Adult males were placed in individual 20 L aquaria or a divided 76 L aquarium with a water depth of 7 cm, a salinity of 22, and no substrate. Crabs were fed mummichogs *(Fundulus heteroclitus)* and Atlantic menhaden (*Brevoortia tyrannus)* three times a week. HM crabs were fed TK food (n = 10), and TK crabs were fed HM food (n =14). After eight weeks, these crabs were used in a behavioral experiment for one week, during which time they were not fed (Reichmuth et al., in press), and then frozen and prepared for metal analysis.

Field Transplant Experiments

Adult male blue crabs from both populations were placed in minnow traps (one per trap) or a divided crab trap covered in plastic mesh (dimensions: 61 cm x 47 cm x 21 cm) with food (mummichogs and Atlantic menhaden from the transplanted location) and transplanted to the other location for eight weeks - HM at TK (n = 9), and TK at HM (n = 13). (The n was initially higher, but there was mortality in the transplants; approximately one-third of HM crabs transplanted to TK died over the three summers this field experiment was conducted). Traps were checked every 7-10 days. The transplants with the minnow traps were 'self-feeding,' but the crabs in the traps were provided extra food to ensure an adequate amount of food was consumed (collected by minnow traps set in the same area). After eight weeks, these crabs were used in a behavioral

experiment for one week, during which time they were not fed (Reichmuth et al., in press), and then frozen and prepared for metal analysis.

Metals Analysis:

Crabs collected from the field (TK: n = 10; HM: n = 13), as well as transplants (TK transplants: n = 11; HM transplants: n = 9) and those from the lab trophic transfer study (TK: n = 13; HM: n = 10) were frozen. Claw muscle and the hepatopancreas were dissected and dried at 58°C for 48 hours. After drying, individual sample weights were 0.45 ± 0.08 g for muscle and 0.29 ± 0.06 g for hepatopancreas. The samples were acid-digested for 24 hours using trace metal grade nitric acid (Fisher Scientific®) and were digested further using a MARS-5 microwave digester (CEM Corporation). The samples were heated to 150ºC to evaporate excess nitric acid to final volumes of ~0.5 ml, and then restored using 1% nitric acid to a final volume of 10 ml. For Cr, Cu, Pb, and Zn analysis, 10-20% dilutions were analyzed in an AA Flame Spectrophotometer (Perkin-Elmer AAnalyst 400). Standard reference materials (SRMs) used were DORM-2 (dogfish muscle tissue) and DOLT-2 (dogfish liver tissue) from the National Research Council of Canada. Mean recovery for DORM-2 and DOLT-2 were, 91.3 ± 9.8% and 85.8 ± 7.3%, respectively for Cr; 96.5 ± 1.8% and 106 ± 5.5%, respectively for Cu; 88.3 ± 2.3% and 63.5 ± 9.2%, respectively for Pb; and 98.6 ± 2.9% and 102 ± 3.9%, respectively for Zn. (The Pb in both reference materials is near the threshold level of analysis in our method.) SRMs and method blanks were included with each 12 samples.

For total Hg analysis, 1 ml of the rehydrated sample was oxidized with 3 ml of 5% $KMnO_4$ in an ice bath. Five ml $NH_2OH \cdot HCl$ solution and 1 ml of 10% $SnCl_2$ were added to the sample immediately before analyzing. Hg was analyzed using a cold vapor atomic absorption spectrophotometer (Bacharach Coleman Model 50-D). Mean recovery for DORM-2 and DOLT-2 were, 86.3 ± 6.9% and 97.5 ± 3.9%, respectively. Minimum detection levels for both analytical methods were three times the standard deviation of method blanks. SRMs and method blanks were included with each 12 samples.

Statistical analysis:

Each metal was analyzed with a three-way mixed model ANOVA in order to test for differences among tissue type (muscle vs. hepatopancreas), sampling site (HM vs. TK), and treatment (native vs. trophic transfer vs. field transplant). The ANOVAs were followed by post hoc Tukey tests to compare the means at the 0.05 level. SPSS v. 15.00 for Windows was used to perform statistical analyses, GraphPad Prism 4.0 was used to generate the graphs, and Microsoft Excel 2003 was used to generate the table.

Results

Cr:

The concentration of this metal was significantly higher in Hackensack Meadowland (HM) crabs relative to Tuckerton (TK) (Figure 4-1A). A significant difference was also found in each tissue due to treatment: Cr was higher more

often in the hepatopancreas relative to the muscle (Figure 4-1, B). Significant differences were found in the tissue due to treatment when compared to the baseline as well: an increased concentration in the hepatopancreas and muscle was observed in TK crabs fed contaminated food (Figure 4-1B) or transplanted in the field for eight weeks (Figure 4-1C). HM concentrations were very variable across the treatments (Figure 4-1B, C; see Table 4-1 for results of three-way repeated measures ANOVA).

Cu:

Native TK and HM crabs both had significantly higher concentrations of Cu in their hepatopancreas vs. muscle; however, a population difference was not found (Figure 4-1A). A significant difference was found between tissues due to the trophic transfer and transplant experiments: HM crabs transplanted to TK for eight weeks (Figure 4-1C) showed a significant decrease in the muscle and hepatopancreas Cu. In both trophic transfer and field transplant treatments, TK muscle had significantly higher Cu than hepatopancreas (Figure 4-1B, C; see Table 4-1 for results of three-way mixed model ANOVA).

Hg:

Native HM crabs had significantly higher Hg concentrations than TK crabs (Figure 4-2A) and muscle was found to have significantly more Hg than hepatopancreas (Figure 4-2A). TK crabs fed contaminated food or transplanted to HM for eight weeks showed significantly elevated Hg (Figure 4-2B, C). HM

crabs fed clean food had a slight decrease in hepatopancreas Hg, but this was not significant and HM crabs transplanted to TK were more variable (Figure4-2B, C; see Table 4-1 for results of three-way mixed model ANOVA).

Pb:

The hepatopancreas was found to have significantly more Pb than the muscle, overall (Figure 4-2A). HM crabs fed clean food did not show a significant decrease in tissue Pb (Figure 4-2B), but HM crabs transplanted to TK showed a significant decrease in Pb in the hepatopancreas (Figure 4-2C). TK crabs fed clean food or transplanted to HM did not show any decrease in Pb (Figure 4-2B, C). However, population differences were not seen (see Table 4-1 for results of three-way mixed model ANOVA).

Zn:

Native HM crabs had elevated concentrations of Zn compared to TK crabs, but this difference was not significant (Figure 4-1A). The hepatopancreas was found to have significantly higher concentrations of Zn (Figure 4-1A). TK crabs fed contaminated food or transplanted to HM for eight weeks showed significantly increased levels of Zn, especially in the hepatopancreas (Figure 4-1B, C). HM crabs fed clean food or transplanted to TK showed decreased Zn in the hepatopancreas (Figure 4-1B, C; see Table 4-1 for results of three-way mixed model ANOVA).

Table 4-1. Three-way mixed model ANOVA results.

Metal	Tissue [A (df = 1)]	Site [B (df = 1)]	Treatment [C (df = 2)]	A x B (df = 1)	A x C (df = 2)	B x C (df = 2)	A x B x C (df = 2)
Cr	F = 0.963	F = 2.74	F = 2.29	F = 1.923	F = 4.621[a]	F = 5.254[a]	F = 9.466[a]
	p = 0.330	*p* = 0.103	*p* = 0.109	*p* = 0.171	*p* = 0.014	*p* = 0.008	*p* = 0.0001
Cu	F = 0.953[a]	F = 2.705	F = 0.988[a]	F = 0.362	F = 3.428[a]	F = 2.250	F = 0.305
	p = 0.003	*p* = 0.105	*p* = 0.012	*p* = 0.550	*p* = 0.039	*p* = 0.089	*p* = 0.738
Hg	F = 14.21[a]	F = 4.772[a]	F = 4.183[a]	F = 0.553	F = 2.349	F = 4.587[a]	F = 2.597
	p = 0.0001	*p* = 0.033	*p* = 0.020	*p* = 0.460	*p* = 0.104	*p* = 0.014	*p* = 0.083
Pb	F = 47.50[a]	F = 1.099	F = 1.221	F = 0.909	F = 4.081[a]	F = 0.678	F = 1.828
	p = 0.0001	*p* = 0.299	*p* = 0.302	*p* = 0.344	*p* = 0.022	*p* = 0.391	*p* = 0.170
Zn	F = 10.91[a]	F = 3.053	F = 1.649	F = 3.63	F = 1.951	F = 5.529[a]	F = 5.270[a]
	p = 0.002	*p* = 0.086	*p* = 0.201	*p* = 0.061	*p* = 0.151	*p* = 0.006	*p* = 0.008

[a] designates significance

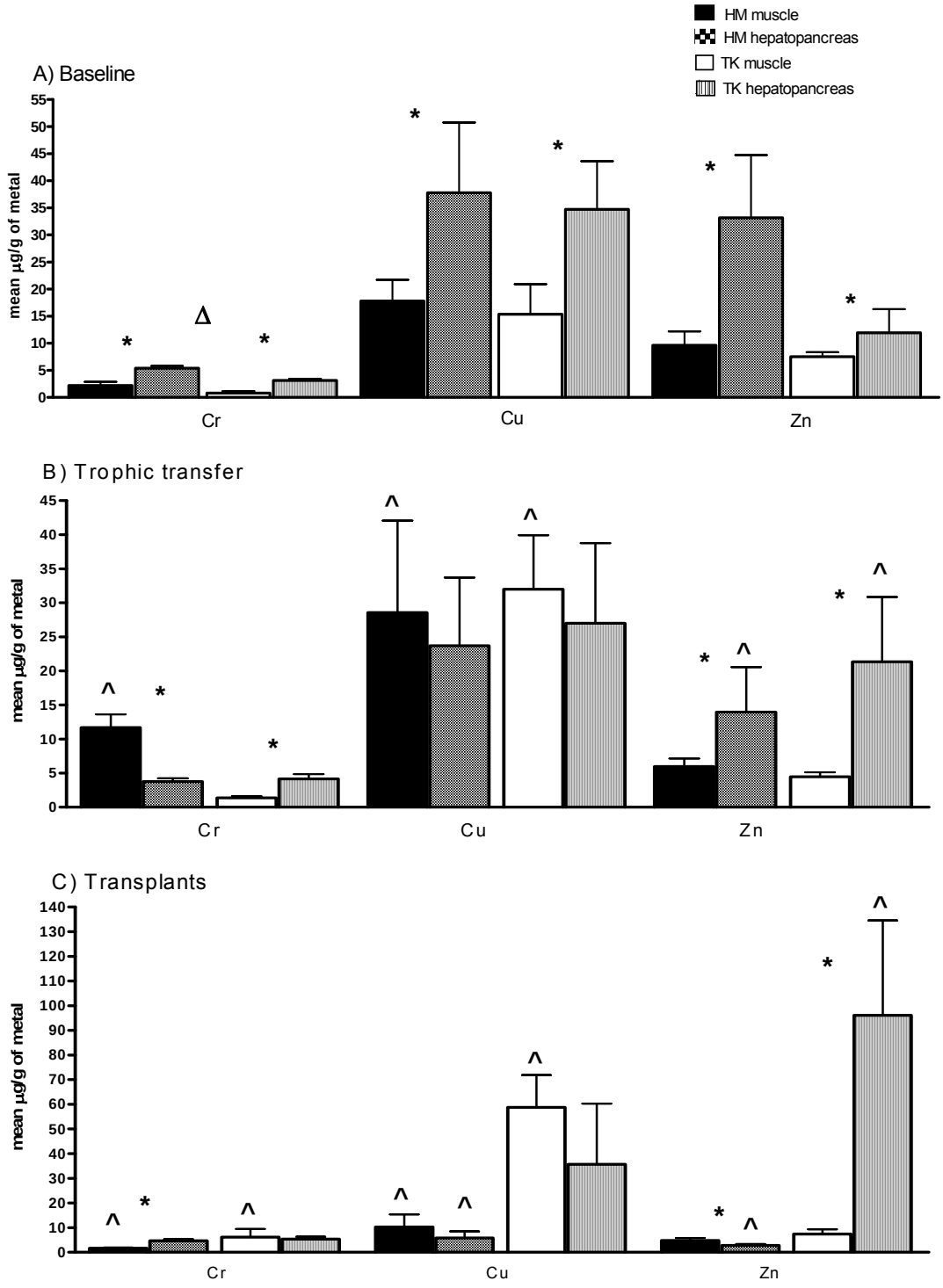


Figure 4-1. A) Mean concentrations ± one standard error of chromium (Cr), copper (Cu), and zinc (Zn) in native Hackensack Meadowlands (HM) and Tuckerton (TK) blue crabs (baseline); ***** denotes significant difference in tissue types and ∆ designates significant site differences in the baseline. B) Mean concentrations ± one standard error of Cr, Cu and Zn in HM crabs fed clean food from TK and TK crabs feed contaminated food from HM. C) Mean concentrations ± one standard error of Cr, Cu and Zn in HM crabs transplanted to TK and TK crabs transplanted to HM; ***** denotes significant difference in tissue types and **^** designates significant difference from baseline concentrations in this tissue.

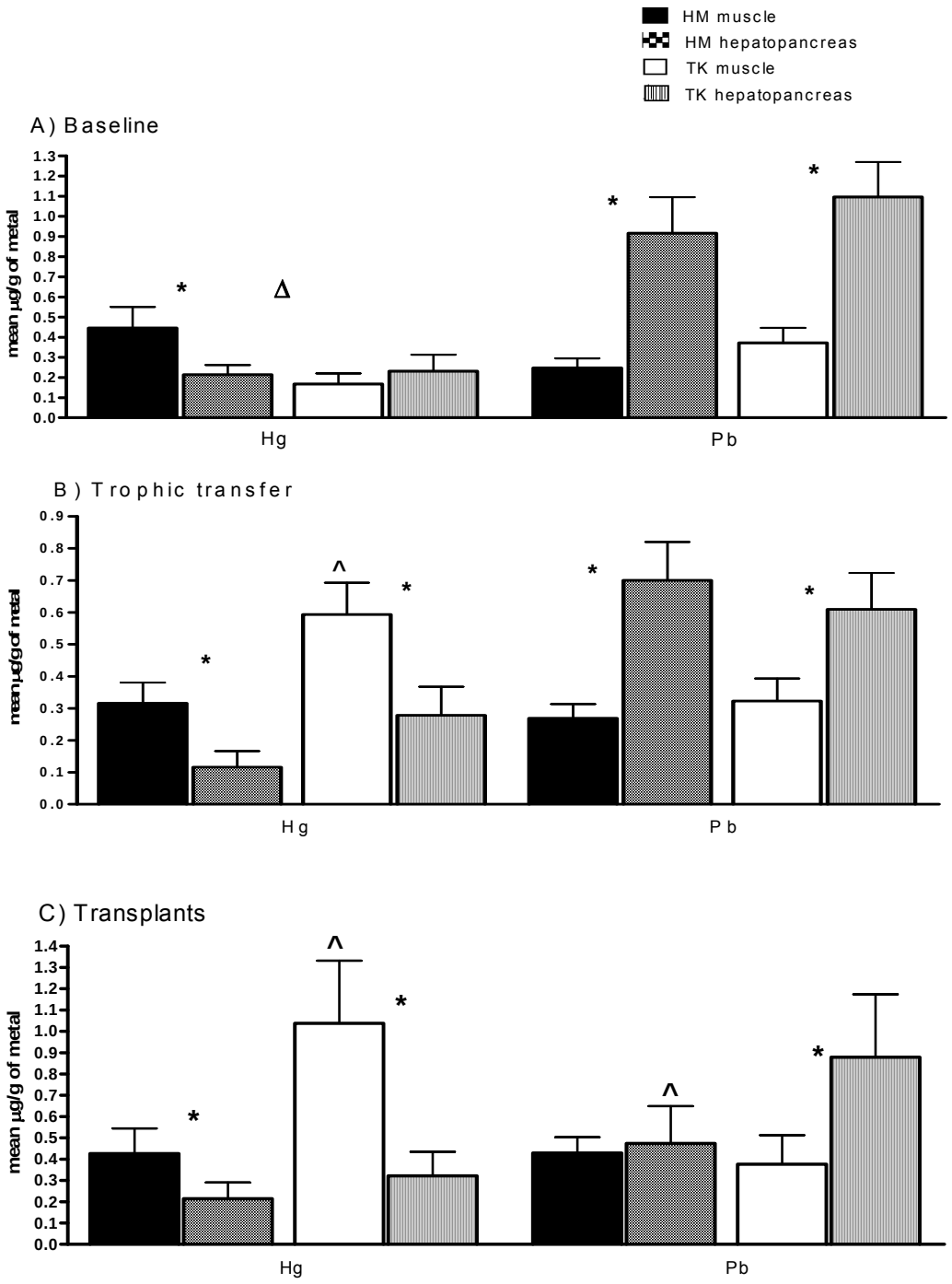


Figure 4-2. A) Mean concentrations ± one standard error of mercury (Hg) and lead (Pb) in native Hackensack Meadowlands (HM) and Tuckerton (TK) blue crabs (baseline); * designates significant tissue differences and Δ designates significant population differences. B) Mean concentrations ± one standard error of Hg and Pb in HM crabs fed clean food from TK and TK crabs feed contaminated food from HM. C) Mean concentrations ± one standard error of Hg and Pb in HM crabs transplanted to TK and TK crabs transplanted to HM; * denotes significant difference in tissue types and ^ designates significant differences from baseline concentrations in this tissue.

Discussion

Overall, a number of our hypotheses were not supported: metal concentrations in the hepatopancreas were not always higher than in muscle; Hackensack Meadowlands (HM) crabs were not always higher in metals than Tuckerton (TK) crabs; TK crabs did not always increase their metal levels when fed contaminated food or transplanted to HM; HM crabs did not always decrease their metal levels when fed clean food or transplanted to TK.

The results of this study suggest that the accumulation of essential and non-essential metals within the muscle and hepatopancreas of blue crabs is highly variable between HM and TK. Significant differences in baseline levels were only found for Hg, which is unexpected due to well-known contamination in the Hackensack Meadowlands (Ashley and Horwitz 2002; Iannuzzi et al. 2004) and previous studies suggesting the biota and sediments have elevated metals (Weis et al. 2001; Windham et al. 2004). However, subtle differences were found for the other metals investigated.

A significant population difference was found for Cr concentrations when combined with other factors and this is not surprising because of the long history of contamination within the Hackensack Meadowlands (Iannuzzi et al., 2004; Ludwig and Iannuzzi 2005). However a degree of variability was observed within some of the experimental treatments. Similar variable results among the tissues studied (e.g., exoskeleton, gonads, claw muscle, hepatopancreas) were found in a similar investigation exposing crayfish to Cr (Bollinger et al. 1997). At the highest exposure, the hepatopancreas was found to have the greatest

concentration (Bollinger et al. 1997). TK crabs across all treatments had higher concentrations of Cr in the hepatopancreas relative to muscle with the exception of the TK transplants to HM. The same was true for HM crabs with the exception of the trophic transfer crabs, in which the Cr was higher in muscle. Crayfish exposed to Cr were allowed a 1-3 week period to depurate any accumulated metal and Bollinger et al. (1997) found that Cr has a slow clearance rate, especially from the hepatopancreas. This may explain why we observed only a small decrease in Cr in hepatopancreas of HM crabs fed clean food or transplanted to TK for 8 weeks.

The significantly higher concentration of Hg found in the muscle relative the hepatopancreas was unexpected since the hepatopancreas is one of the main storage sites for toxicants (Brouwer and Lee 2007). Several recent studies also found elevated concentrations of Hg in muscle tissue of blue crabs (Karouna-Reiner et al. 2007; Sastre et al. 1999) and green crabs, *Carcinus maenas* (Coelho et al. 2007). High Hg could pose severe health risks if consumed by species on higher trophic levels including humans (Karouna-Renier et al. 2007). HM blue crabs had significantly elevated Hg due to contamination within the Meadowlands. Berry's Creek, a tributary of the Hackensack River, has been designated as a Superfund site due to elevated levels of mercury. It is possible that Hg from Berry's Creek has been transported throughout the Meadowlands system from sediments due to currents, etc. (Cardona-Marek et al. 2007).

TK crabs fed contaminated food in the lab for 8 weeks showed an increase in Hg in muscle, as did those transplanted to HM. These results indicate that Hg was transferred through diet, but the greater increase seen in the transplanted crabs suggests that exposure to the sediments and water in addition to food caused a further increase in muscle concentrations. Several studies (Morrel et al. 1998; Lawson and Mason 1998) using phytoplankton, zooplankton, and fish, found organic Hg (methylmercury) was the form most assimilated and thus transferred up the food chain. However, HM crabs fed clean food or transplanted into the clean environment did not show a significant decrease in Hg, which indicates that Hg may be harder or slower to depurate than to accumulate. Similar findings were seen in mummichogs (*Fundulus heteroclitus*) by Smith and Weis (1997). These data have major implications for risk assessment of contaminated sites and should be taken into consideration when blue crabs support local fisheries.

Higher concentrations of Pb were found in hepatopancreas relative to the muscle. Literature suggests that Pb is not transferred through the food chain, and concentrations may decrease as trophic level increases (Chen et al. 2000). Overall significant site differences were not found and concentrations of Pb were similar in the tissue and hepatopancreases of native TK and native HM crabs. TK sediments have higher concentrations of Pb than expected (Windham et al. 2004). Tuckerton has a long history of waterfowl hunting and it is possible that the lead shot has accumulated in the sediment.

Blue crabs may be accumulating high levels of metals in the exoskeleton (not measured) and depurating them through molting, as was found with fiddler crabs (Bergey and Weis 2007). This mechanism of depuration could also explain the significant change in the concentrations of Pb in HM crabs transplanted to TK relative to the native HM crabs. When the transplanted crabs were periodically checked, molts were often noticed in the traps. Bergey and Weis (2007) also found that fiddler crabs from a contaminated site were able to depurate metals, such as Pb, more efficiently via molting than crabs from a reference site. Freshwater crabs, *Potamonautes perlatus,* collected from a riverine system in South Africa, were found to accumulate the majority of the body burden in the exoskeleton (Reinecke et al. 2003); another species from the same area also accumulated Pb into its exoskeleton (Du Preez et al. 1993). The fact that some of the blue crabs in this experiment molted during the eight weeks and some did not may partially explain the high variability in tissue levels of metals.

The higher concentration of Cu in the hepatopancreas vs. the muscle was expected since the hepatopancreas has higher levels of metallothioneins, which help sequester this metal. Other studies have found most of the Cu is stored in this organ (Brouwer and Lee 2007; Engel and Brouwer 1984; Rainbow 1985, 2007; Sastre et al. 1999). While Cu is essential for oxygen transport in hemocyanin, if uptake is in excess, accumulation can occur. This seems to be the case for TK crabs fed contaminated food or transplanted to HM for eight weeks. A significant increase in Cu in the muscle suggests TK crabs are accumulating the extra Cu in this tissue rather than the hepatopancreas.

Whether or not this Cu is being stored in a detoxified form after being bound to a metallothionein is unknown. On the other hand, the hepatopancreas in HM crabs transplanted to TK showed a significant decrease in Cu. It is possible that excess Cu was bound to metallothioneins and excreted while the HM crabs exposed to the cleaner environment. HM crabs fed clean food in the lab for eight weeks did not lose a significant amount of Cu and this may be due to the dispersion of metals within a confined water column within the aquarium (approximately 25 L of water), relative the to the open water column in the field. It is possible that crabs confined in laboratory conditions could have taken up metals through the gills even though the water was changed every couple of days.

Zn is also an essential metal that can be toxic if accumulated above necessary levels. The higher concentration of Zn in the hepatopancreas vs. muscle is expected since the hepatopancreas is a storage site of this metal (Engel 1987; Rainbow 2007). TK crabs fed contaminated food or transplanted to HM showed a significant increase in Zn in the hepatopancreas, while there was not a change in muscle. In laboratory feeding experiments conducted with two predatory gastropods (*Babylonia formasae habei* and *Nassarius teretiusculus*), Wang and Ke (2002) found that Zn could be trophically transferred and then biomagnified with an increase in trophic level. Their results suggest that diet was the main cause of the bioaccumulation, which could also explain the increase in Zn we observed. HM crabs fed clean food in the lab or transplanted to TK had a significant decrease in Zn in the hepatopancreas. In a recent study,

oysters from a contaminated coastal lagoon were transplanted to a cleaner reference site for three months, after which Zn concentrations were three-fold lower than initial levels (Reboncasdo Amaral et al. 2005). These results indicate that the oysters were able to depurate in the cleaner environment and may also help explain the results observed with the HM transplants. As with Cu, dispersion of the Zn in the open water column may also play a role in the depuration observed in the HM crabs transplanted to the TK environment. Molting in the field may also explain some of the depuration as well.

It is generally accepted that metals are more bioavailable when the salinity is lower (Bryan and Langston 1992; Lee et al. 1998), as it is in HM. If salinity is playing a role in the uptake of metals, one would expect TK crabs fed HM food in the lab (but at an intermediate TK salinity) would not accumulate as much metal as those transplanted to HM. This was the case for Cr in both the muscle and hepatopancreas, Cu and Hg in the muscle only and Zn in the hepatopancreas. This was not true for Pb in either tissue types.

Several studies have indicated that decapod crustaceans such as blue crabs are good bioindicators for polluted systems. However, the data presented in this investigation show that while they generally reflect environmental concentrations for some metals, there is a high degree of variability within a population. Blue crabs have a wide diet, in which they accumulate metals in different forms and concentrations. In a previous study (Reichmuth et al. 2009), HM blue crabs were found to consume more detritus, sediment, algae, and plant material and less crab and fish than TK crabs. The HM crabs appear to be

feeding lower on the food chain than typical blue crabs due to impaired predatory behavior, and this diet may result in reduced bioaccumulation of toxicants through the food chain. The concentrations of metals accumulated in individuals of this population may be more representative of what is present in the sediments rather than what is present in other organisms a blue crab would “normally” consume. This may explain the observation that TK crabs in trophic transfer experiments or transplanted to HM for only 8 weeks sometimes acquired higher metal levels than HM crabs that had lived there for their whole life.

Recent literature (Wang 2002; Wang and Rainbow 2008) has suggested that closely related species of crustaceans can vary greatly in the levels of various contaminants that they bioaccumulate. The data presented here echo this finding, but focus on two populations of the same species, blue crabs, in New Jersey estuaries. For some metals, there was either a clear increase or decrease in concentration across treatments, but for others there were only subtle differences that did not yield a clear pattern. This reflects the suite of environmental conditions to which each population is exposed. Since HM blue crabs have been exposed to contaminants their whole life, they may have become tolerant to them, or better able to regulate their uptake, whereas TK crabs exposed to the HM environment appear to be less able to regulate the uptake, and therefore in some cases accumulated the higher levels of metal contaminants. Our data also suggest and reinforce previously known information that it is easier to accumulate than it is to depurate some metals.

Chapter 5

Population Genetics

Introduction

An organism's traits (which culminate in the phenotype, i.e., suite of behaviors, physical traits) may or may not be strongly influenced by the environment. Either way, the end result is a wide variety of phenotypes represented by one genotype, or one phenotype represented by a few genotypes (Graur and Li 2000). There can be many different alleles present at different frequencies in a single population. Population geneticists are interested in these genetic polymorphisms for several reasons:

- As a means of DNA fingerprinting. This reveals the identity of all the individuals within the population for purposes of determining genetic relatedness (Hartl 2000).
- To monitor levels of genetic diversity. This is especially important for key indicator species present in communities in environments that are exposed to chemical, biological, and/or physical stress (Place et al. 2005).
- To understand population history. By looking at the genetic polymorphisms within subpopulations of a species, we can understand how migration and other factors affect the genetic structure of the population (Steven et al. 2005).

Microsatellites, a sequence of variable number of tandem repeats and are used because they are highly polymorphic and can evolve rapidly (Hartl 2000). As individuals in a population breed over time, their microsatellites will get

recombined; because of this, the population will maintain a variety of microsatellites that is characteristic for that population and distinct from other populations (Balloux and Lugon-Moulin 2002).

Populations of organisms almost always exhibit some sort of geographical structure. In turn, the geographical structure affects the genetic organization of the species. Members are rarely distributed homogenously in space; therefore there is always some sort of clumping or aggregation (Hartl 2000). The subdivision of the population is often caused by environmental patchiness, good habitat mixed with unsuitable habitat; or the observed patchiness can be caused by some type of social behavior (Johnson and Black 1982). The reason the population is so important is because it is within these groups that the systematic changes in allele frequency can occur (Graur and Li 2000).

Allele frequencies in most populations are affected by mutation, migration, and natural selection. These processes can cause directional changes in allele frequency over time (Schlöterrer 2000). Random fluctuations in allele frequency (i.e., genetic drift) can also occur strictly through chance since populations are not infinitely large and their sizes are rarely constant. Mutations, e.g., substitutions, insertions and deletions, transposable elements, create new variation, and are, thus, the ultimate source of genetic variation (Graur and Li 2000). Migration brings new individuals into the population, but usually has a homogenizing effect because the exchange of migrants between subpopulations does not allow one to diverge from another (Burton and Feldman 1982). Selection allows the genetic variation created by mutation to be organized,

maintained, deleted, or dispersed among the populations through the balance of migration and the random changes in allele frequency. In essence, the role of selection is to increase the frequency of alleles that allow increased survival and reproduction (Hartl 2000).

Most population genetic studies relate the allele frequencies and other information to the genetic structure of the population. There are few studies relating the genetics of an organism to its behavior. Only recently have geneticists have been asking, “why” and ecologists have been asking, “how?” (Robinson 1999; Fitzpatrick et al. 2005).

The understanding and characterization of the population genetics of the blue crab is critical to the future success of the population and the fishery (Place et al. 2005; Steven et al. 2005). If the genetic structure of a population is distinguished from other populations, one could predict if immigrants will successfully repopulate a locally depleted population. Dispersal is the main component of gene flow from one population to the next in marine environments. However, high potential for dispersal does not necessarily mean high potential for gene flow since the individuals that make it to the new location may have a very similar genetic structure as the current inhabitants. (McMillen-Jackson et al. 1994; Kurdos and Burton 1993).

In a previous study, behavioral differences were found between two populations of blue crabs: one with a long history of contamination, the Hackensack Meadowlands, and the other, a cleaner, reference site, Tuckerton (Reichmuth et al. 2009). The purpose of the current investigation is to determine

if genetics could be a cause of the observed behavioral differences. We hypothesize there will be a geographical difference in the nucleic DNA (microsatellites). Geographic differences can occur if gene flow is not occurring in a specific location, which would allow the population to become fixed for certain alleles (Graur and Li 2000). Additionally, a temporal difference is expected due to recombination events between the two populations leading to which alleles are present within the populations at any given time. Due to recombination events, it is possible to have a high variety of alleles present at four loci as well. Temporal differences can occur from season to season or year to year; the NJ coastline is only 150 km long and this gives the larvae a small enough distance to travel and mix between sites.

Material and Methods

Collection of samples

Crabs were collected from HM and TK using a seine net and otter trawl and brought back to the laboratory. They were kept in aerated tanks with a sand depth of one centimeter and artificial seawater (Instant Ocean®) at their native salinity (HM: 15; TK: 30). A 14/10 light cycle was kept throughout the field season. TK crabs were fed a diet of ribbed mussels (*Geukensia demissa*) and Atlantic menhaden (*Brevoortia tyrannus*) collected from TK while HM crabs were fed a diet of menhaden and mummichogs collected from HM; all crabs were fed three times a week after which the water was changed.

Microsatellite Analysis

One walking leg (see Table 5-1 for sample information) was removed from crabs in the field or in the lab once all behavioral experiments were concluded and stored in 95% ethanol until tissue extraction. DNA was extracted using a Qiagen DNEasy 96 Tissue Kit (animal tissue protocol). Primer sequences, annealing temperatures, and PCR conditions used were the same as described in Stevens et al., 2005. PCR products were diluted to 1:10 in distilled water and 1μL of this solution was added to 8.5 μL of Hi-Di Formamide and 0.5 μL of 500 GeneScan 500 LIZ size standard. This mixture was denatured at 95°C for 5 minutes and cooled on ice for 5 minutes then transferred to an ABI 3100 Genetic Analyzer for 30 min at 60°C and 15 kV. The resulting data was analyzed and scored with GeneMapper v 3.7 software (Applied Biosystems). GeneMapper data were analyzed using an Excel Microsatellite Toolkit.

Table 5-1. Blue crab sample information. Adults and juveniles are designated with "A" and "J," respectively.
HM designates Hackensack Meadowlands and TK designates Tuckerton

Sampling Period	Sample Code	Sample Size
Summer 2005	HMA	15
	TKA	74
Summer 2006	HMA	83
	HMJ	25
	TKA	88
	TKJ	89
Summer 2007	HMA	41
	TKA	71
	TKJ	24

Data and Statistical Analysis

The number of alleles, allelic richness, gene diversity and Weir and Cockerham estimates of F_{is} (*f*) and F_{st} (θ) were calculated using Fstat (Goudet, 2002). Hardy Weinberg equilibrium, genotypic differentiation and linkage disequilibrium were calculated using the web-based program GENEPOP ver. 3.4 (Raymond and Rousset, 1995). Statistics were calculated for overall population differences (HM and TK), differences among sampling years (2005, 2005, 2007), and differences between adults and juveniles of the same sampling year (2006 HM adults and 2006 HM juveniles, and so on). Exact tests and *p*-values were also calculated using GENEPOP; statistical significance was a *p*-value of 0.00001 (after sequential Bonferroni correction).

Results

Genetic variation and Hardy-Weinberg equilibrium:

A total of 510 individuals (adults and juveniles combined) were sampled over the three year period (Table 5-1) and a total of 268 alleles were observed (Table 5-2). The four loci were polymorphic and were similar in genetic diversity (0.857 – 0.975; Table 5-2; see Appendices III and IV for selected markers). Additionally, all the samples had similar allelic richness. However, the number of alleles observed was variable (42-86; Table 5-2).

No significant linkage disequilibrium was detected among the loci between the two populations, across years or comparing adults and juveniles of the same year. All loci have deviations from Hardy-Weinberg equilibrium in the direction of

heterozygote deficiency. Only one locus was significantly heterozygote deficient: CSC-094 (Table 5-2).

Genetic differentiation

Over all samples, exact tests of genotypic differentiation did not detect population structure ($p > 0.05$; Table 5-2). However, significant yearly population structure was detected at all loci from HM adult and TK adults ($p \leq 0.0000$; Table 5-2). The global estimate of F_{st} was low ($\theta = 0.065$; Table 5-2). and was not significantly different from zero.

Adult and juvenile blue crabs collected the same year from the same population did show significant genotypic differences at some loci: CSC-094 and MIH for 2006 HM adults and juveniles; CSC-094 and MIH for 2006 TK adults and juveniles. Genotypic differences also existed CSA-073, CSC-094 and MIH for 2007 TK adults and juveniles, but these results were not significant after the sequential Bonferroni correction (Table 5-2).

Table 5-2. Per locus and global allelic richness, gene diversity, Weir and Cockerham estimates of Fis (f) and Fst (θ) and exact tests of genotypic differentiation (bold denotes significance).

	CSA-035	**CSA-073**	**CSC-094**	**MIH**	**ALL**
Number of alleles	86	71	42	69	268
Allelic richness	32.7	29.5	20.7	34.1	29.3
Gene diversity	0.975	0.963	0.857	0.972	0.942
Fis	0.051	0.218	**0.438**	0.091	0.200
Fst	0.001	0.003	0.257	-0.002	0.065
Exact test	0.317	0.019	0.0009	0.580	0.229
Fst (all HM)	0.049	0.204	0.313	0.076	0.161
Exact test	0.004	0.037	0.00	0.009	0.012
Fst (all TK)	0.044	0.141	0.214	0.076	0.119
Exact test	0.0605	0.0173	0.00	0.102	0.045
Fst (HMA 2005, 2006, 2007)	0.019	0.026	0.132	0.025	0.051
Exact test	**0.00**	**0.00**	**0.00**	**0.00**	**0.000**
Fst (TKA 2005, 2006, 2007)	0.024	0.021	0.086	0.016	0.037
Exact test	**0.00**	**0.00**	**0.00**	**0.00**	**0.000**
Fst (HMJ 2006)	0.100	0.173	0.439	0.022	0.184
Exact test	0.570	0.013	0.029	0.237	0.212
Fst (TKJ 2006 and 2007)	0.016	0.050	0.123	0.167	0.089
Exact test	0.0014	0.00	0.0005	0.0001	0.001
Fst (2006 HMA and HMJ)	-0.0015	0.0085	0.1075	0.0568	0.043
Exact test	0.744	0.015	0.000	0.002	0.190
Fst (2006 TKA and TKJ)	0.004	0.0046	0.0559	0.0732	0.034
Exact test	0.0282	0.0066	0.00	0.0001	0.009
Fst (2007 TKA and TKJ)	0.019	0.0278	0.0334	0.0199	0.025
Exact test	0.001	0.0001	0.001	0.001	0.001

Discussion

In this study, the authors investigated the genetic structure of two blue crab populations in New Jersey. No spatial differences were found between the populations, but a significant temporal difference was observed over the three sampling years. Significant differences between juveniles and adults of the same sampling years were not detected.

Genetic variation and Hardy Weinberg deficiency

The allele richness of our samples is comparable to other studies investigating blue crab microsatellites (McMillan-Jackson and Bert, 2004) and the allele frequency distribution is similar among the four loci (Appendices III-VII).

Several assumptions are made under the Hardy-Weinberg model: large (infinite) population size, no mutation, no natural selection, and random mating. Our results indicate only one locus was significantly heterozygote deficient, CSC-094, as determined by the F_{is} indicator *f*. Departures from HWE can be explained by deviations from the assumptions above in addition to the Wahlund effect. It could be that the populations are small, but this does not seem to be the case. In a survey conducted by the Meadowlands Environmental Research Institute (Bragin et al. 2005), total numbers of blue crabs have increased from approximately 400 crabs in 1986-87 to over 1000 in 2000-01; this density does not account for an infinite population size, however. A small sample size collected in 2005 for HM adults (n = 15) may have biased the result. The Wahlund effect is a reduction in heterozygosity resulting from sampling across

subdivided populations and it cannot be a major contributor here because the two populations lack any spatial/geographic structure. Natural selection can be ruled out since it must be acting on all four loci instead of just one (CSC-094), unless selection is occurring for adaptive conditions. The last possible explanation could be null alleles.

Genetic Differentiation

Populations of organisms almost always exhibit some sort of geographical structure. In turn, the geographical structure affects the genetic organization of the species. However, it appears this is not the case for these two populations of blue crabs. The exact test of genotypic differentiation supports the null hypothesis of no difference of genotypes between the populations. Similar findings were reported for blue crabs within the Chesapeake Bay (Cole and Morgan 1978). The large possibility for dispersal of this species may create a homogenizing affect between the two populations studied here (Johnson and Black 1982). The low Fst (*f*) also indicates little genotypic structure, suggesting a panmictic population. A low fixation index value suggests that the probability of mating pairs being related increases. This can occur in organisms with overlapping generations, such as blue crabs (Hines et al. 2007).

It is interesting to note that populations of a related species, *Callinectes danae,* were not found to be panmictic due to estuarine conditions possibly creating a barrier preventing larval mixing (Weber and Levy 2006). We previously expected a similar result with the populations investigated in this study

due to the urbanization and filling that has taken place within the Hackensack Meadowlands, possibly creating barriers for crabs migrating into and within the system.

A few other possibilities of why a spatial difference was not observed include: a) the length of the New Jersey coastline, approximately 150 km; this provides a short enough distance for larvae from both populations to mix before settling in their final location in northern NJ or southern NJ (and possibly places in between) (Epifano 1995); b) adult males and females could possibly be migrating from one of these places to the other and mating with individuals in those particular locations-this would lead to a high variation in alleles (gene flow), but no spatial difference between the sites of collection (Kordos and Burton 1993). Female blue crabs have been observed migrating over 800 km around the panhandle of Florida, just to release their larvae into the southern Atlantic Ocean (McMillen-Jackson et al, 1994).

A significant temporal difference exits only in HM adults and TK adults among the sampling years. Random fluctuations in allele frequency (i.e., genetic drift) can also occur strictly through chance since populations are not infinitely large and their sizes are rarely constant. A small sample was collected in 2005 for HM adults (n = 15) and this could cause bias in the results observed here. In addition, a large gene pool may in fact exist for this species and the individuals that were collected over the three year period were a small representation of the larger gene pool (McMillen-Jackson and Bert 2004). Finally, random genetic drift or mutations, which create new variation may also explain the temporal

differences observed. Allele frequencies in most populations are affected by mutation, migration, and natural selection. These processes, the size of the population, and random chance, can cause directional changes in allele frequency over time (Schlöterrer 2000).

Genetics related to Behavior

The results of this investigation suggest that genetics are not the cause of the previous behavioral differences observed between the two populations of blue crabs (Reichmuth et al. 2009). That study provided evidence of environmental causes of behavioral differences. Even though these loci do not code for the behavior observed per se, these data indicate that genetic structure does not exist between the two populations. This suggests that environmental conditions, e.g., the contaminants within the Hackensack Meadowlands, are the probable cause.

The New Jersey population is panmictic and knowing the genetic structure of the population has important management implications. Even though the blue crab supports only a local fishery in northeastern states (Jop et al., 1997) should overfishing occur, new genotypes will not be repopulating the northern populations. Since the blue crab has both an important ecological and economic role, it is important to note all biological data including ecological, behavioral, and genetics.

Chapter 6

Discussion & Conclusions

Many ecotoxicology studies incorporate the physiology or biochemical aspects of the organism, but only recently has the organism's behavior become of equal importance. My aim was to determine if differences existed in adult prey capture behavior, juvenile predator avoidance, metal accumulation and depuration between a population living in an estuary with a long history of contamination, and the other living in a cleaner reference estuary. If such differences exist, I further inquired whether these can have a genetic base by searching for molecular differences between the two populations.

BEHAVIOR

Predator-prey interactions are often studied in polluted environments because both the predator and prey are simultaneously affected by the contaminants (Fleeger et al. 2003). Blue crabs are an excellent organism to study: they are both prey for estuarine fish and adult crabs as juveniles, and as they mature into adults, they become predators that exert an immense amount of pressure on the benthic community. There were several hypotheses concerning behavioral differences between the two populations. First, adult blue crabs from the Hackensack Meadowlands (HM) were expected to have poor prey capture ability on various prey types. Blue crabs exposed to oil contamination were found to have a reduced feeding rate (Wang and Stickle 1987). However, the

data in Chapter 2 supported the hypothesis for active prey only, such as mummichogs and juvenile blue crabs, not for mussels, which suggests a lack of coordination, rather than a loss of appetite. The concentration of Hg in the muscle of HM crabs was significantly more than what was detected in TK conspecifics, could have caused the lack of coordination. Hg, especially methyl-mercury has been linked to such behavior in other estuarine species such as mummichogs (Zhou et al. 2001). Organic contaminants such as pesticides (although not studied here), could also have caused lack of coordination (Ren et al. 2007).

It was expected that HM blue crabs would also be consuming less nutritious food in the field. The data also support this hypothesis: HM blue crab stomachs contained more detritus/sediment and plant/algae, these categories comprising approximately 60% of their diet, relative to conspecifics from TK. Polychaete spines and amphipods, both sediment dwelling invertebrates were also present in larger quantities. This may be a reflection of what is available, but could also reflect lack of coordination to capture active prey, resorting to scavenging behavior. Even though it appears that predatory crabs consume some plant and algae material and can extract nutrition from these sources. (Woolcott and O'Connor 1992), the scavenger diet of HM crabs does not reflect Optimal Foraging Theory (OFT) per se. Predators such as blue crabs would be classified as a searching predator according to OFT (Hughes 1979), with bivalves being the optimal prey. As the first and second types of prey become limited, diet should expand to the third, fourth (and so on) ranked prey items

(Hughes, 1979) and this may be what HM crabs are doing in the absence of bivalves, which would in fact satisfy OFT requirements. The environment also plays an important role in OFT. The Meadowlands are a degraded habitat and distances between high quality prey patches may be immense and affect the crab's perception of where the patch may be (Hines et al. 2003), in that feeding upon algae, plant, and smaller invertebrates becomes optimal. A switch of diet due to patchy prey has been observed in blue crabs within the Chesapeake Bay (Hines et al. 2003), amphipods (Cruz-Rivera et al. 2003) and invasive species, such as the Asian shore crab, *Hemigrapsus sanguinaeus* (Brousseau and Baglivo 2005).

The present study attempted to determine if contaminants from food or the overall environment were the cause of the observed behavioral differences. I expected TK crabs fed contaminated food in the lab or transplanted to the HM environment for eight weeks would decrease the number of juvenile blue crabs consumed and vice versa. As seen in Chapter 2, the data support this hypothesis. Contaminants in the fish may have been transferred to the crabs and caused the altered behavior. The field transplant experiments also supported the hypothesis: TK crabs maintained in HM for eight weeks showed a significant decrease in their prey capture ability, and they became inefficient predators, comparable to the native HM crabs which had lived in the Meadowlands their entire adult lives. A change in behavior has also been observed in several other studies that have transplanted reference organism to polluted environments or exposed reference organisms to contaminated food

(e.g., Smith and Weis 1997; Perez and Wallace 2004; Temara et al. 1999). However, mummichogs from a contaminated environment exposed to cleaner conditions for six weeks did not improve their ability to capture prey (Smith and Weis, 1997).

HM juvenile blue crabs were also expected to show aberrant behavior, specifically in their response to a threatening stimulus and predator avoidance. . In Chapter 3, the data support this hypothesis for HM juvenile blue crabs; in approximately 70% of the trials, HM juveniles attacked a threatening stimulus, while TK conspecifics fled significantly more often. Higher rates of aggression have been observed in cats (Li et al. 2003) and juvenile rainbow trout exposed to lead (Atchinson et al. 1987). More specifically, increased levels of aggression as a result of chemical exposure (DDT) have been observed previously in blue crabs (Lowe 1965). The increased agonistic behavior observed with this population could be due to altered neurotransmitters, such as serotonin (Kravitz, 2000), contaminants, or an over-crowded, patchy habitat where competition for resources is high (Clark et al. 1999a; 1999b).

It was expected that HM juvenile blue crabs would also show impaired predator avoidance behavior due to the long history of contamination within the Meadowlands. Weis and Weis (1998) exposed larval mummichogs (*Fundulus heteroclitus*) to lead in laboratory studies, which resulted in reduced predator avoidance when adult grass shrimp (*Palaemonetes pugio*) were predators. Larval mummichogs (*F. heteroclitus*) exposed to methylmercury were also found to have reduced swimming ability, and as a result, experienced increased

predation by the grass shrimp, *P. pugio* (Zhou et al. 2001). However, HM juveniles successfully avoided predation by an adult blue crab significantly more than TK juveniles, and this was unexpected. The results of the additional videotaped predator avoidance experiment without substrate suggest that aggression per se, is not the reason for increased survival since an equal number of both aggressive and non-aggressive juveniles survived and aggressive crabs were not necessarily aggressive toward the predator. In sunfish, individuals classified as bold when approaching a meter stick did not necessarily inspect a novel food source (Coleman and Wilson 1998). The behavioral plasticity observed in the predator avoidance experiments indicates behavior adaptive to the circumstances.

In the literature, enhanced predator avoidance due to contamination has been given little attention in the terms of behavior (Fleeger et al. 2003). *Daphnia* exposed to lindane experienced a decrease in swimming behavior, which decreased their susceptibility to a *Hydra* predator (Taylor et al. 1995). Similar results were found with winter flounder exposed to a toxic dinoflagellate (Samson et al. 2007). Morphological changes (e.g., spines) which resulted in increased predator avoidance have also been observed in *Daphnia* exposed to pesticides (Hanazato 2001). A decrease in activity and/or a morphological change could result in increased protection from predators. However, metal analysis was not conducted on juveniles in this investigation so it is hard to if say contaminants are related to the increased predator avoidance.

The observed aggression in the HM juveniles as well as the poor collection of HM crabs using crab traps suggested that increased aggression might cause fewer crabs to enter pots. A previous study found an increase of antagonistic behavior in the Japanese rock crab, *Charybdis japonica,* with certain shapes of commercial pots (Vasquez-Archdale et al. 2003). I expected the aggression to continue into adult life stages and keep individuals out of a trap, which was the case. Some of the HM trials resulted in an injured or killed pot inhabitant. Field catches also resulted in an injured crab in the pot. Recent literature suggests that injured conspecifics release metabolites that can deter a conspecific's movement and/or foraging behavior (Moir and Weissberg 2008), deterring the crab from entering a trap (Ferner et al. 2005). It may be that aggression per se is not the reason for adults not entering a trap, but the scent of injured conspecifics that deters their movement around the trap. These results have implications for using crab pots in population counts.

Even though behavioral differences were found between the two populations, HM blue crabs do not appear to have any maladaptive behaviors that are affecting their fitness. The many years of pollution within the Meadowlands may have in fact helped blue crabs by removing a top predator, humans, through fishing bans.

METAL ACCUMULATION

Because other studies (Weis et al., 2001; Iannuzzi et al., 2005) have indicated elevated levels of contaminants within the sediments and biota in the

Hackensack Meadowlands, it was expected that a population difference would be found for body burdens of five metals. In Chapter 4, a population difference was detected for most metals, although some were not significant. Similar results have been found for species of mangrove crabs from polluted and reference sites (Harris and Santos 2000). We also observed that for some metals, there was either a clear increase or decrease in concentration across treatments, but for others there were only subtle differences that did not yield a clear pattern.

Most of the literature describing field transplant and/or laboratory trophic transfer experiments and metal accumulation involves bivalve species (i.e., Regoli and Orlando 1994; Roesijadi et al. 1984; Wepner et al. 2008) or small crustaceans such as amphipods (i.e., Lenihan et al. 1995; Morris and Keogh 2002). These organisms are easy to study in these situations and are warranted for this type of research because they are good bioindicators. This study is the one of the firsts using a larger decapod crustacean and has implications for management of the species. A high degree of variability was detected among individual crabs, but overall, reflected environmental conditions. Land use change (e.g., the increase of impervious surfaces) threatens most estuarine habitats where blue crabs spend most of their life. Contaminants enter estuarine waters from both point and non-point source run-off and this can increase with land use change (Bhaduri et al. 2000). If we understand how reference organisms, especially those that are of great commercial and ecological importance, accumulate and depurate metals as well as how their behavior

changes in relation to contaminants, we may be better prepared to make laws or policy changes that will better suit the survival of this species.

POPULATION GENETICS

The main hypothesis concerning population genetics was the null hypothesis (i.e., overall genetic differentiation between the two populations would not be detected). The data in Chapter 5 support my hypothesis and supports most current literature (McMillan 1994; McMillan and Bert 2004). Genetic differentiation was not found in blue crab subpopulations within the Chesapeake Bay (Cole and Morgan 1978). Panmictic populations are often the case with species that have overlapping generations, such as blue crabs (Hines 2007) and when there are short distances (150 km) between sites. Larger scaled studies of blue crab microsatellites (e.g., McMillan-Jackson and Bert 2004) have shown a decrease in haplotype diversity along a latitude gradient, suggesting the northern latitudes (e.g., New York) are the least diverse. Overall genetic diversity of NJ blue crabs was similar to what was found in this particular study and were highly variable. McMillan-Jackson and Bert (2004) suggest that short-term gene flow is on a regional basis and long-term gene flow is long distance. By characterizing populations on a small scale, such as in this investigation, an understanding of the genetic structure of at least two populations is achieved, such understanding has potential having an impact the sustainability of blue crabs in this region.

CONCLUSIONS

There is a long history of habitat degradation and contamination within the Meadowlands. It was not until the construction of a water treatment plant in the 1960s and the enactment of the Clean Water Act in the 1970s that untreated wastewater and dumping ceased (Crawford et al. 1994). As a result, many toxicants contaminate the sediments, water column, and biota of the Meadowlands (Durrell and Lizotte 1998; Iannuzzi et al. 2005). In the early 1980s, a fishing ban on most species including blue crabs was put into effect.

The contaminants within the Meadowlands have affected the behavior of both the adults and juvenile age classes. The adults are poor predators on active prey, including juvenile blue crabs, and have fulfilled the role as a scavenger, rather than the active predator that exerts an immense amount of pressure structuring the benthic community. As a result, less cannibalism, the blue crab's way of the population regulation, is occurring (juvenile crabs can make up ~15% of an adult's diet; Pile et al., 1996; Moksnes et al., 1997). Thus, more juveniles (that are good at defending themselves), as well as other prey items, are surviving. In turn, this can result in more prey being available for other predatory estuarine species, such as striped bass and bluefish. This may have detrimental affects as well. As blue crabs continue to consume detritus and other small mud-dwelling organisms, the ability for the system to recycle nutrients may be decreased.

HM juveniles are not cannibalized by adults as often, which appears to result in a HM juveniles being more aggressive relative to TK counterparts. More

individuals are possibly overcrowding in suitable habitat patches, which increases the competition for resources (Clark et al. 1999a; 1999b). In a degraded habitat, such as the Meadowlands, quality patches are at a premium and may make the need to defend these patches even more important. It may not be aggression per se that allows the juveniles to survive longer with the adult, but the ability to defend a suitable patch from other juveniles that may be the key.

It is ironic that pollution may have helped blue crabs living within the system because it removed a top predator, humans, from overfishing. The indirect effect of predator removal has lead to a population of larger individuals relative to individuals living within the cleaner reference site (see Appendix II). In addition, the population also appears to be increasing in number as the results of survey conducted in the Meadowlands in 1987-88, and again in 2001-03 which showed a three-fold increase in abundance of blue crabs within this system (Bragin et al. 2005). It is unexpected that a diet consisting mainly of plant, algae and sediment can support a predator such as the blue crab, but in HM, it appears to be working as a substitute until the crab can find other suitable prey.

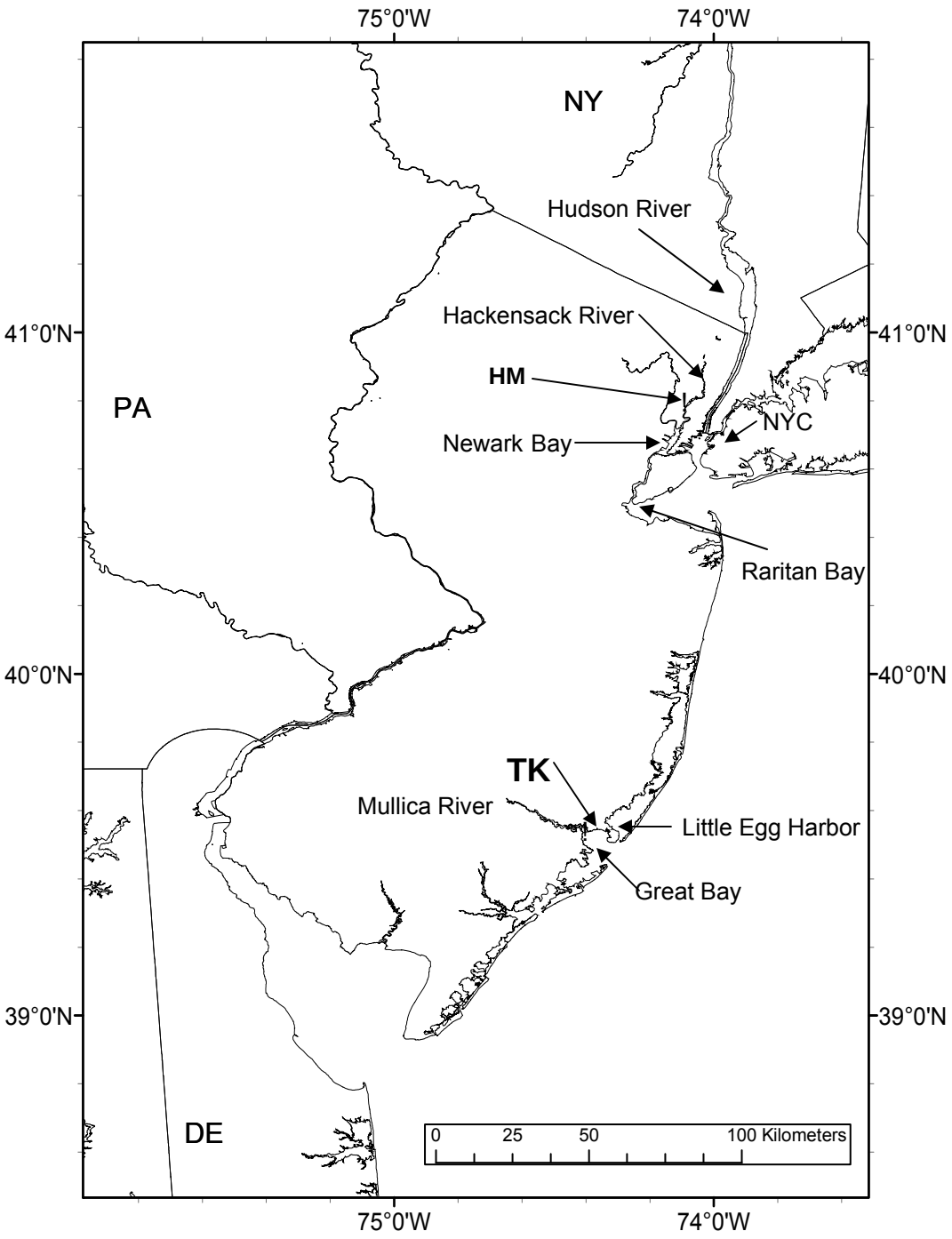
There is also recent evidence that the Meadowlands system is becoming cleaner (B. Bragin, personal communication). This trend could continue for the entire watershed and its inhabitants, including the blue crab, and possibly one day (not in my lifetime) support a fishery. The results of this study suggest that through the removal of a predator, such as humans, the abundance of blue crabs can increase. This result in itself has major management implications and could possibly change fishing strategies within locations where blue crabs are heavily

fished (e.g., Chesapeake Bay). The opposite could also happen with the cleaner reference site.

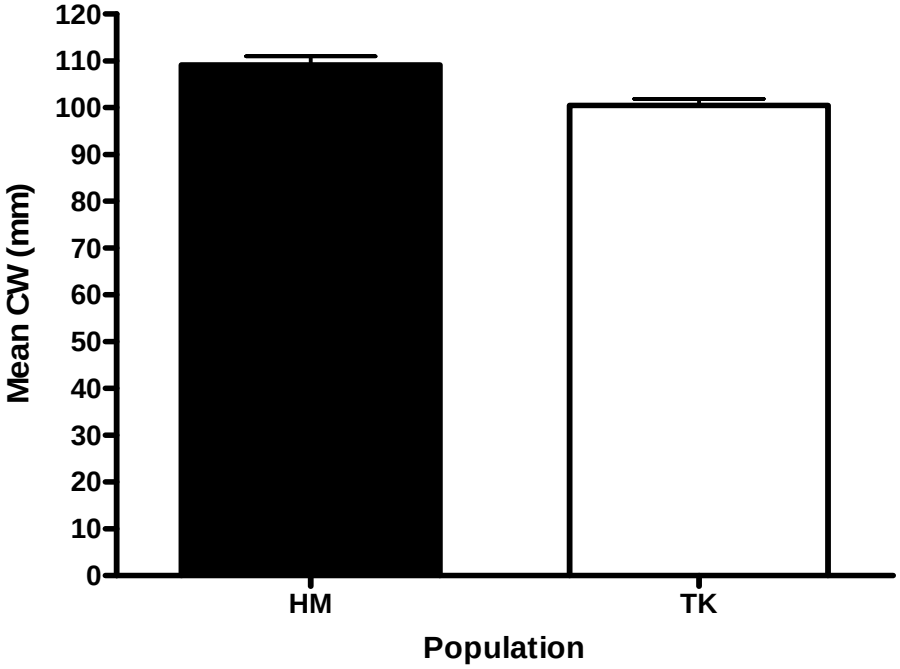
The results of the genetic data supports a panmictic population along the NJ coast, and compared to other studies, shares similarities with alleles found within the western Atlantic. These results in itself wholly support the hypothesis and help solidify the idea that the environment, more specifically the contaminants, is the cause of the behavioral differences observed in the native HM and TK blue crabs from either population as well as the crabs exposed to the "switch" experiments. The switch experiments also showed that for some metals accumulation was possible and the corresponding behavioral switch occurred, further supporting the hypothesis that the environment is the cause.

This study aimed at using as many parameters as possible to assess the impact of contaminants on blue crabs, an important ecological and economic species. There are significant behavior differences between HM adults and juveniles compared to TK conspecifics of the same age class, but it appears that these differences are not detrimental or maladaptive to the population as long as there isn't fishing pressure.

Appendix I: Site Locations



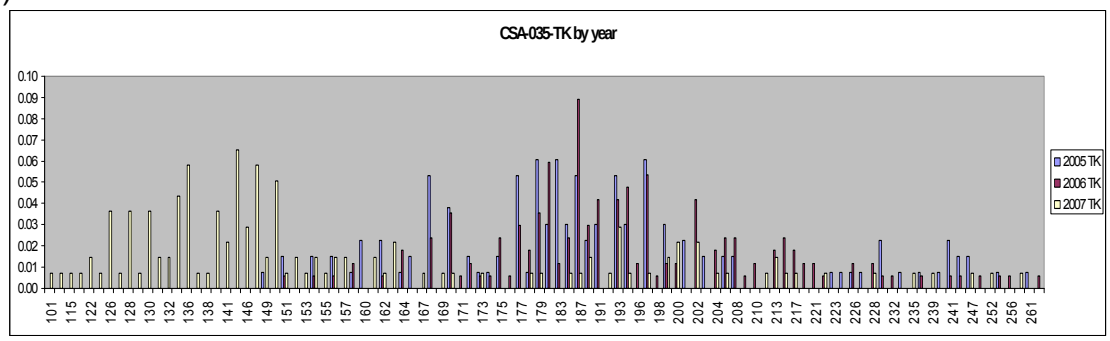
Appendix II: Growth Data



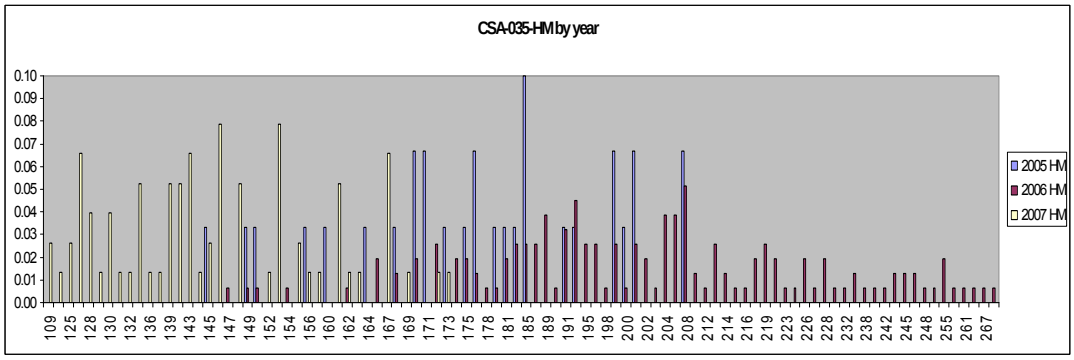
Mean carapace width (CW) of blue crabs collected from the Hackensack Meadowlands (HM) and Tuckerton (TK). No significance difference was observed.

Appendix III: Allele Frequencies-CSA-035

A)



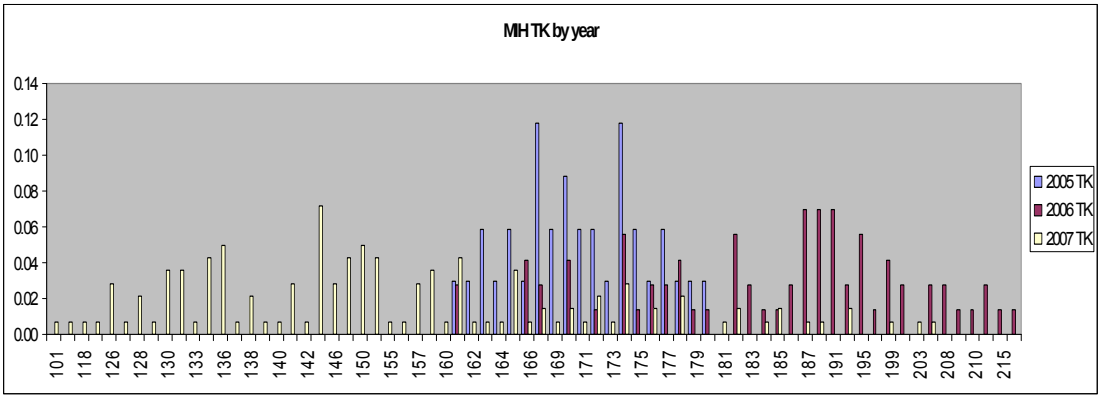
B)



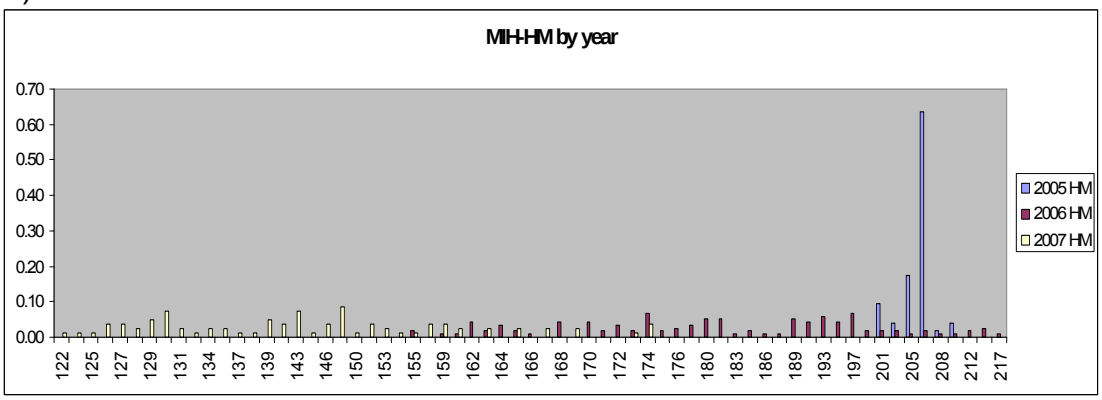
A) Allele frequencies for Tuckerton (TK) blue crabs (all age classes) among the three sampling years for marker CSA-035. B) Allele frequencies for Hackensack Meadowlands blue crabs (all age classes) among the three sampling years for marker CSA-035.

Appendix IV: Allele Frequencies-MIH

A)



B)



A) Allele frequencies for Tuckerton (TK) blue crabs (all age classes) among the three sampling years for MIH (molting inhibitor hormone). B) Allele frequencies for Hackensack Meadowlands blue crabs (all age classes) among the three sampling years for MIH.

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