FIDDLER CRABS (*Uca pugilator*) AS BIOINDICATORS OF ENVIRONMENTAL HEALTH IN COASTAL ESTUARINE COMMUNITIES OF BEAUFORT, SOUTH CAROLINA

A Report of a Senior Study

by

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Fall, 2011

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Editor
ABSTRACT

In the past, coastal environmental health has been analyzed through direct measurements of the sediment and the water. However, there is much evidence that certain species may serve as bioindicators, the ecological and morphological properties of which can predict the level of anthropogenic impact. The purpose of this study is to test the feasibility of utilizing *Uca pugilator* as a bioindicator of anthropogenic impact. In the field, three sites (Reference, Municipal, and Golf Course) were selected in Beaufort, South Carolina as examples of different types and levels of human impact. Crabs were sampled (*n* = 1164) for carapace width, dominant to subordinate claw ratio (males only), and population density; mating behavior was also observed. In the lab, four mesocosms were set up: one to simulate each impacted field site and one control for each. Carapace width and claw size ratio measurements were made before, during, and after treatments were applied. In the field, carapace width was found to be significantly reduced in the Golf Course crabs (*p* < 0.001), and these crabs exhibited increased claw wave behavior. The population densities were significantly greater at both affected sites (*p* < 0.001). The claw size ratios were significantly influenced by site, day, and the interaction of site and day (*p* = 0.005, 0.008, and 0.002 respectively), but there was no clear pattern in these influences. The mesocosm experiment resulted in no significant differences among crab groups. In conclusion the significant differences among crabs in the field are likely attributed to anthropogenic activity. This study supports the notion that *Uca pugilator* could be a valuable bioindicator of estuarine health, and shows that population density, carapace widths, and mating behavior of *Uca pugilator* are influenced by anthropogenic activity. Future studies should use *Uca pugilator* as a bioindicator of estuarine health.
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ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Drew Crain for his constant optimism, encouragement, and wisdom throughout the course of this project. I also would like to extend thanks to Dr. Steve Borgianini and his student Paul Miller at USCB for their assistance in scouting out sites in Beaufort, and for their knowledge of fiddler crabs. Additionally, I would like to thank my friend Miguel Rodriguez for his assistance in the field which made sampling a breeze. I would also like to thank all of my friends for their constant support, and my parents as well as the rest of my family for always being there.

Funding for this study was provided by a U.S. Department of Education Grant (#P116Z100249) and the Maryville College Biology Department.
CHAPTER I

INTRODUCTION

Traditionally, ecological analyses of coastal benthic habitats have been performed through assessing direct measurements of ecological health and pollution content. Because sedimentation is such an important factor in benthic environments, sediment is commonly studied as a means for interpreting the health of the environment. Sediment cores are sampled from the seabed and the contents of the sediment are chemically analyzed with tests such as gamma spectrometry and neutron activation analysis (e.g., Fukuyama et al. 2008). Cores are also analyzed by direct measurements of particle size, water content, nitrogen concentration, and phosphorous content.

However, recently many studies have suggested that there are ecological population trends and animal behaviors that can also accurately determine the health status of benthic communities. For example, there is greater population diversity and density of invertebrate species in environments of favorable total suspended solids (TSS) and conductivity (Quintana et al. 2010). This suggests that certain species can act as bioindicators of environmental stress and environmental health.
Sedimentation

Sedimentation is defined as the process where particles in suspension settle out of the fluid in which they were carried and accumulate at the bottom of the body of water. Sedimentation is facilitated by a number of different mechanisms including varying current velocities, the concentration of suspended sediment, the local bed geomorphology, flooding patterns, and intense weather conditions (Wang 2011). Other sources of the distribution of sediment are nearby bodies of water. For instance, the bulk source of estuarine sediment can be attributed to the river upstream and the ocean downstream. Sedimentation can also be impacted by many anthropogenic root causes, such as pollution, drilling, shore erosion, fish farming, recreational activity, and habitat disturbances to name a few.

Sedimentation occurs at all loci in the ocean, and is responsible for shaping benthic communities. Benthic sedimentation refers to sedimentation occurring at the bottom of any body of water, whereas pelagic sedimentation refers to the accretion of particles in the deep-sea. Deep sea sedimentation is primarily caused by atmospheric, marine, or fluvial sources (Biscaye 1974). In other words, the sediment found on the bottom of the deep sea either comes from airborne constituents, such as gaseous pollutants; marine sources, such as currents from other parts of the ocean; or fluvial sources, such as rivers and streams which empty their sediment into the ocean. Coastal sedimentation is defined as alluviation of sediment at the benthic zone of a shallow coastal area of an ocean. Coastal sedimentation is similar to pelagic sedimentation in that it shares these atmospheric, marine, and fluvial sources. But coastal sedimentation, on the other hand, is also greatly influenced by beach erosion more greatly than deep sea sedimentation. Anthropogenic influences are often primary causes of beach erosion occurring on coasts globally (Crain et al. 1995, Horikawa 1981). In addition to this, coastal ecosystems are also closer to
beaches and human activity, thus rendering them more susceptible to human pollution. Coastal benthos, or life in the benthic zone of coastal regions, is more directly affected by anthropogenic activity than deep-ocean life.

One phenomenon that is strongly coupled with sedimentation and anthropogenic influence is eutrophication. Eutrophication is the accumulation of an overabundance of one or more nutrients in an ecosystem. The results of eutrophication can be just as devastating as the infiltration of toxic contaminants to the environment. This is particularly problematic for estuaries, as they are the sink of fluvial output. In fact, 70% of the sediment input to the sea is from riverine sources (Kaiser 2005, p 475). Coastal eutrophication has been correlated to such anthropogenic sources as fertilizers. Sediment core analyses indicate that nutrient levels increased significantly, coinciding with a threefold increase in use of fertilizers in the agricultural industry in that time period (Kaiser 2005, p 477). Eutrophication has been known to abruptly diminish diversity in benthic communities, while also causing a steep increase in overall density (Wildsmith 2010). This decrease in diversity can cause genetic bottlenecking, and the steep incline in population can cause overpopulation, or an excess of carrying capacity of the habitat.

Sediment Analysis

Sediment analysis can unveil a large quantity of information about a given habitat. Sediment cores can be collected and analyzed to reveal the benthic habitat’s past, much as the layers of soil can be analyzed to give an accurate depiction of terrestrial life history. Studies have utilized this technique to analyze the effects of natural and human processes on the community. In one such study, sediment cores were collected from Ariake Bay Japan, and analyzed using gamma spectrometry and neutron activation analysis (Fukuyama et al. 2008). Cores were also
measured for particle size, water content, nitrogen concentration, and phosphorous content. The cores were also radioactively dated to get accurate ages of the sedimentation. Another study in Long Island Sound extracted a sediment core and analyzed it lengthwise, layer by layer for a large number of elements. Using radioactive half-life analysis, the bioturbation and sedimentation rates were able to be determined at each layer (Benninger et al. 1979). This allows for the development of a detailed history of the seabed, and a picture of how the seabed and its components came to be. Thus, sedimentation can explain both the way the benthos and their habitat has evolved, and also portray the quality of the habitat.

Another study collected sediment cores to analyze them for nitrogen content and carbon content, other organic content, chlorophyll content, as well as nitrogenase activity as measured by acetylene reduction assay. These analyses were performed in order to compare three different marshes: one that was recently restored, one that was restored several years prior, and one that was natural (Pieler et al. 1998). This use of sediment cores allowed for a complete analysis for whether or not the restoration efforts were successful or not. This study shows that direct chemical sediment analysis can be utilized as a means of assessing whether or not anthropogenic alterations intended to restore ecosystems are in fact beneficial.

Sediment can also be analyzed using less direct methodology. Sediment contamination can be analyzed by assessing the toxicological effects of the sediments of interest. Sea urchins, *Paracentrotus lividus*, indicated toxicity through problems in embryogenesis. Therefore, sea urchins showed toxicity in the sediment upon analysis of their embryos. When compared to chemical analysis, the toxicological results coincided flawlessly with the actual chemical results (Fernandez et al. 2008). This shows that benthic fauna can also be used to analyze sediment accurately.
Anthropogenic Effects

It is well known that coastal, benthic sedimentation is a very influential factor on benthic ecosystems, as many organisms make the sediment bed their habitats. There is also evidence to suggest that the rate of sedimentation, that is the rate of sediment deposition, and the content of this sedimentation in a given benthic environment can greatly affect the community inhabiting it. Pollutants in marine sediment are also very important factors. For example, fish farming has been shown to cause twenty times the mortality rate in seagrass *Posidonia oceanica* as normally observed (Diaz-Almela 2008). This is just one example that demonstrates that sediment quality and content as well as rate of sedimentation are key factors that can determine the survival of benthic species. Resulting sedimentation from fish farming also exemplifies another issue: the effects of anthropogenic activity on sedimentation.

Human activity, such as fish farming, mining, and industry have been known to affect benthic communities negatively in numerous ways. The larvae of a certain genus of non-biting midges, *Chironomus spp*, demonstrate the effects of harmful sedimentation on development (Al-Shami et al. 2010). In areas of high metal contamination, the midge larvae show developmental deformities in the form of fluctuating asymmetries (FA). The FA indexes were shown to be most sensitive to the sediments of Mn, Cu, Zn, and Ni, minerals commonly attributed to mining. The asymmetries were measured as non-symmetrical lengths of legs, antennae and other body parts that are normally identical on both sides of the organism. These detrimental deformities show that metal sediment contamination can have detrimental effects on the development of *Chironomus spp* larvae. FA indexes of morphological structures in *Chironomus spp* could be used as tests for sedimentation pollution in bodies of water.
Anthropogenic contamination affects invertebrates most directly by altering the water column. The water column is the conceptual column of layers of sediment in the water, from top to bottom. This stratification of minerals is different from habitat to habitat, but the maintenance of a consistent water column for any one habitat is vital for benthic life. Benthic macroinvertebrates tend to destructure as the result of a changed water column and loss of heterogeneity of the habitat, which can be caused by deposition or sedimentation of new sediment (Marques et al. 2003). For example, mining could cause extreme deposition of zinc and lead which upsets the balance of the water column, causing disappearances or substitutions of taxons. It is quite clear that any alteration in the water column will almost invariably lead to an alteration in the benthic community.

Table 1: Anthropogenic Effects on Benthic Species and Resulting Bioindication Activity

<table>
<thead>
<tr>
<th>Species Common Name</th>
<th>Genus Species</th>
<th>Anthropogenic Cause</th>
<th>Bioindication</th>
<th>Source</th>
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<tbody>
<tr>
<td>Seagrass</td>
<td><em>Posidonia oceania</em></td>
<td>Fish Farming</td>
<td>Indicates pollution by high mortality</td>
<td>Diaz-Almela 2008</td>
</tr>
<tr>
<td>Non-biting midge</td>
<td><em>Chironomus spp</em></td>
<td>Mining</td>
<td>Indicated metallic contamination by fluctuating asymmetry</td>
<td>Al-Shami et al. 2010</td>
</tr>
<tr>
<td>Macroinvertebrates (Not species specific)</td>
<td>Various species</td>
<td>Habitat alteration/Urbanization</td>
<td>Indicate sediment and water quality by population dynamics</td>
<td>Miserendo et al. 2008; Wildsmith et al. 2009</td>
</tr>
<tr>
<td>Crabs</td>
<td>Species</td>
<td>Pollution Type</td>
<td>Indicated Contamination</td>
<td>References</td>
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<tr>
<td>Fiddler Crab</td>
<td><em>Uca annulipes</em> and <em>Uca inversa</em></td>
<td>Sewage Dumping</td>
<td>Indicated sewage contaminants by alterations in feeding behavior</td>
<td>Bartolini et al. 2009</td>
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<tr>
<td></td>
<td><em>Uca pugnax</em></td>
<td>Oil spill</td>
<td>Indicated petroleum contamination by delayed responses and lower population density</td>
<td>Culbertson et al. 2007</td>
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<td></td>
<td></td>
<td>Industrial pollution</td>
<td>Indicated industrialization by larger body size of individuals and lower population density in urban site than the nondeveloped site</td>
<td>Bergey and Weis 2008</td>
</tr>
<tr>
<td></td>
<td><em>Uca pugilator</em></td>
<td>Harmful Pesticide pollution</td>
<td>Indicated contamination with Dimilin pesticide by reduced predator avoidance, burrow construction, and feeding abilities</td>
<td>Cunningham and Myers 1987</td>
</tr>
<tr>
<td>Spider Crabs and Snow Crabs</td>
<td><em>Hyas coarctatus</em> and <em>Chionoecetes opilio</em></td>
<td>Chemical Pollution</td>
<td>Indicated the presence of polycyclic aromatic hydrocarbons (PAHs) found in their muscle and hepatopancreas</td>
<td>Hellou et al. 1994</td>
</tr>
<tr>
<td>Mud Crabs</td>
<td><em>Scylla serrata</em></td>
<td>Chemical Pollution</td>
<td>Indicated presence of pollutants by elevation of biomarkers</td>
<td>Oosterom et al. 2010</td>
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Macroinvertebrates as Bioindicators of an Unimpacted Environment

It has been widely suggested that certain benthic species and communities could act as efficient indicators of overall benthic environmental health. More specifically, many macroinvertebrates have been studied for their denotation of sediment quantity and content. Macroinvertebrates show increased biodiversity and biomass in habitats where sedimentary characteristics are more favorable. Indeed, there is greater diversity and density of invertebrate species in environments of favorable total suspended solids (TSS) and conductivity (highly related to sediment content) (Quintana et al. 2010). Organic matter quality and quantity have been pinpointed as the chief determinants of benthic assemblage. Benthic macrofauna show change in diversity composition, increasing with increased organic matter quality and quantity regardless of physical exposure of the environment (Quintana et al. 2010). These responses of benthic macrofauna show that these communities can be utilized as effective bioindicators of the health of the habitat. The diversity and number of benthic macroinvertebrates can unveil the overall environmental condition, in terms of beneficial sediment and organic content.

Macroinvertebrates as Bioindicators of a Contaminated Environment

As much as benthic invertebrates are great indicators of pristine sedimentation qualities, benthic invertebrates can also signal deteriorating sedimentary environments. Benthic macroinvertebrates are keystone species in the benthos, as they are critical in the function of these communities. This is primarily due to the fact that invertebrates play a key role in nutrient cycling. Therefore, the population dynamics of invertebrates of the benthic zone can denote whether or not the community is stressed. For example, recently a restoration effort was made in
Australia to limit eutrophication by opening a channel between the Peel-Harvey estuary and the Indian Ocean. As a result, the percentage of contribution of particulate organic matter to the sediment decreased, while the mean depth of the transitional zone increased (Wildsmith et al. 2009). Both of these factors indicate that eutrophication has declined. However, the taxonomic spread of the benthic invertebrate species decreased, and the variability in species increased. In addition, crustaceans and mollusks declined in abundance and numbers of species after the channel was built. All of these factors indicate that these species are now more stressed than before. The invertebrates are signifying that the sedimentation and water quality is actually worse now than it was before. Additionally, it has been observed that benthic macroinvertebrates are lower in diversity and number in areas of urban activity with low water quality (Miserendino et al. 2008). Furthermore, benthic invertebrates show preferences for particular sediment compositions. With the exception of gastropods, invertebrates are invariably found by the thousand in the habitats without zinc, whereas almost no invertebrates inhabited the zinc rich environment (Watzin and Roscigno 1997). This illustrates that metal contamination can be predicted by the behaviors and habitation patterns of benthic invertebrates. Therefore, benthic invertebrates, as sensitive as they are to water quality and sedimentation, can be utilized as observable indicators of sediment quality and content. Benthic invertebrates could be used to monitor the ecological rebound of habitats undergoing restoration efforts.

Crabs as Bioindicators

As many studies suggest, macroinvertebrates could potentially be utilized as bioindicators of aquatic habitat quality. In particular, crabs specifically are effective bioindicators of a myriad of different biological factors. For instance, stenohaline and euryhaline oyster reef crabs effectively confirm the fluctuations of salinity in the Fakahatchee Bay estuary
(Shirley et al. 2004). Because stenohaline crabs can only live in a strict range of salinity, the changing ratio of stenohaline crabs to euryhaline crabs shows the change in salinity, which increased in dry seasons and decreased and wet seasons. These results demonstrate that crabs can be a valuable bioindicator of alterations in freshwater flow in estuaries.

The morphology and population dynamics of crabs can also answer questions of environmental sustainability. One example of crab morphology affirming the presence of pollution is crab size. Males and females of fiddler crab species *Uca pugnax* are significantly larger in polluted areas of high industrialization than *Uca pugnax* in a cleaner, less polluted environment. The crabs from the more polluted habitats had significantly lower population density, lowered recruitment, reduced reproductive season, and lower survivorship of offspring (Bergey and Weis 2008). This shows that crabs’ morphology as well as population dynamics could both be key indicators of pollution and/or anthropogenic disturbances in the benthic community.

The content of surface sediment can also be predicted through a chemical analysis of crab muscle and hepatopancreas. Spider crabs, *Hyas coarctatus*, and snow crabs, *Chionoecetes opilio*, contained polycyclic aromatic hydrocarbons (PAHs) in their muscle and hepatopancreas tissue that strongly correlated with findings in the surface sediment of their habitat (Hellou et al. 1994). This illustrates another way that crabs are bioindicators. Crabs can show the content of their environment by a simple chemical analysis (ultraviolet/fluorescence gravimetry and gas chromatography-mass spectrometry) (Hellou et al. 1994). Mud crabs, *Scylla serrata*, also showed pollutants through their chemistry. The biomarkers GST, ChE, NPH and BaP were analyzed in crabs of a pollution gradient. Significant elevation of GST and BaP activity and
inhibition of ChE were found, as indications of pollution. These results suggest the prospect that
mud crabs have great potential as indicators of water quality (Oosterom et al. 2010) It is
therefore apparent that crabs are bioindicators by chemistry as well as biology.

Crustaceans have been observed as indicators of many ecological features. Crustaceans
living in sediment were responsive to metal contamination and water quality. Additionally,
crustaceans showed change in abundance and richness depending on changes in the fluvial
habitat and sediment (Tall et al. 2008). This illustrates that communities of crabs, among other
crustaceans, are responsive to sedimentary change, and therefore can indicate sedimentary
conditions in benthic habitats.

Crabs can also indicate the predominant sediment type of a benthic ecosystem. Rock
crabs show a surprisingly strong preference for fine, sandy sediment (Wong et al. 2006). This
preference can be utilized to analyze the sediment type predominant in an area, just by
examining rock crab abundance. Other crab community tendencies and preferences could also be
utilized as indicators of benthic habitats.

Crabs not only show evidence of environmental features such as salinity and sediment
types and content, but they also respond to anthropogenic factors, making them key bioindicators
of human impact on benthic communities. For example, the behaviors of fiddler crab species
*Uca annulipes* and *Uca inversa* are “strongly affected by domestic sewage dumping, and have
the potential to be used as bioindicators” (Bartolini et al. 2009). These crabs are very sensitive
bioindicators of pollution, as they react immediately even to very low sewage loadings. The
crabs showed alterations in feeding behaviors in response to sewage dumping (Bartolini et al.
2009). *Uca pugnax* also indicate other environmental problems through their behavior. Fiddler
crabs exposed to petroleum avoided burrowing into layers of sediment with oil (Culbertson et al. 2007). These crabs also experienced delayed evasive responses, lowered feeding rates and developed lower population densities. Another species of fiddler crab, *Uca pugilator*, are potential bioindicators of a pesticide called Dimilin- indicating its presence through hindrances in several of their behaviors. These behaviors were observed in juvenile fiddler crabs which experienced reduced ability to avoid predators, construct burrows, and feed adequately for survival (Cunningham and Myers 1987). These findings, coupled with the fact that fiddler crabs are easy to study due to their high density populations and the short time needed to observe them (Bartolini et al. 2009), advocate the utilization of fiddler crab morphology, population dynamics, and behavior for monitoring areas of suspected pollution and other anthropogenic perturbations.

The Present Study

The present study brings into question the feasibility of utilizing *Uca pugilator* as a bioindicator of anthropogenic impact. The goal of this study is to examine the influence of various human-altered habitats on the morphological measurements, population densities, and behaviors of *Uca pugilator*. 
CHAPTER II

MATERIALS/METHODS

Methods for this study included both field and laboratory procedures. *Uca pugilator* was studied in the field from 19 June to 10 July 2011. Four mesocosms were then set up on 12 July 2011, and treatments were applied from 18 July to 7 September 2011.

Field Sites

The study was conducted on three sites in the Beaufort, South Carolina area (see Figure 1). Each site surveyed was representative of different types of anthropogenic impact. Every site was divided into three subsites; replicated plots within each site. The subsites were selected based on areas of the highest concentration of observed *Uca pugilator* on the surface as well as occupied burrows.

The first site exemplified a habitat that was virtually undisturbed by human activity. The Lemon Island Preserve is a large plot of protected estuarine salt marsh on the southern side of the Broad River. Because of its protection as an undeveloped sea island of about 400 acres of salt marsh and maritime forest (protected by a partnership between the Open Land Trust and
Beaufort County) as well as its isolated proximity from human activity, this unimpacted site was considered the “Reference” site. Characteristics of the Reference site included large salt pans and pools of tidal water. These pans were surrounded by *Spartina* grass as well as *Juncus*. Bordering the boundaries of the salt marsh were maritime forests. *Uca pugilator* frequented the tidal pools and the salt pans, and lived in burrows on the edges of the salt pans where the *Spartina* and *Juncus* began. *Uca minax* and *Uca pugnax* were also seen in this site, although *Uca pugilator* was the most abundant species.

The second site was located on Parris Island, a portion of Port Royal. This island houses the U. S. Marine Corps Recruit Depot Parris Island training facility. This site was an example of industrial impact, as it received effluent from the Parris Island Wastewater Treatment Plant, as well as the unlined Causeway Landfill (hereafter, this site is referred to as the “Municipal” site). The site was located in the salt marsh north of Malecon Drive, and is within fluvial impact of both the water treatment plant and the landfill. The site was notably more turbid than the Reference site (see Table 2 in Results). The site consisted of a narrow strip of salt pans and tidal pools, which quickly transitioned into a moist and muddy field of *Spartina* grass. *Uca pugilator*’s burrows were found along this transition. Further out into the *Spartina*, the sediment got muddier and the populations of fiddler crabs were mostly comprised of the mud fiddler crab *Uca pugnax*. *Uca minax* were not observed at this site.

The third site was a model of agricultural impact. This site was located near Harbour Town, in Sea Pines within Hilton Head Island. The site was located along the banks of the Heddy Gutter Creek near the Deer Island Road bridge. This creek is the sink of the Harbor Town Golf Course which is the major source of impact for this site, consisting of agricultural pollutants such as insecticides and fertilizers. As a result, this site was named the “Golf Course” site. This
site was characterized by flat banks consisting of mud and sand. Surrounding these embankments were patches of *Spartina*. Burrows of both *Uca pugilator* and *Uca pugnax* were found along the borders of these patches. As observed in the other two sites, *Uca pugilator* and *Uca pugnax* tended to live in separate but nearby colonies. Also observed at this site was the blue crab *Callinectes sapidus*. No *Uca minax* were observed at this site.

**Figure 1:** Map of the three field sites studied.

1. Lemon Island Preserve: “Reference” site, little to no anthropogenic impact (32.371382, -80.812937).

2. Parris Island: “Municipal” site, impacted by a nearby landfill and water treatment plant (32.35741, -80.69609).

Field Measurements

*Uca pugilator* were sampled from the Reference site, Municipal site, and Golf Course site at low tide (n = 388, 406, and 370 respectively). While the tide was low the crabs were collected from the surface. Burrowed crabs were coaxed from burrows by lightly prodding the back of the burrow with a spade. The crabs were individually collected by hand and placed into a 5 gallon bucket. Morphological measurements were conducted using a plastic Vernier Caliper DY-VC01 (+/- 0.05mm). First, every crab's carapace width was measured at the widest point. The crabs were sexed by the shape of their abdomen (see Figure 2). The males had both their dominant and their subordinate claws measured. The claws were measured from the tip of the immovable finger to the base of the propodus. The dominant claw size was divided by the subordinate claw size to get a claw size ratio for each male crab. The crabs were then marked on the left side of their carapace with permanent marker so that recaptures could be recorded. The crabs were then released in the vicinity of their collection.

Each of the sites was also surveyed for population dynamics. A plastic ring with a diameter of approximately 1.02 meters was thrown in thirty random areas per site (ten per subsite) populated by *Uca pugilator* burrows. The number of burrows within the ring was then counted, and this number was divided by the area to find the population density. This was repeated once a week for every site. This method has been demonstrated to not damage the community (e.g. in comparison to excavation), and it is a much more accurate estimation than only counting individuals outside the burrow (Bergey and Weis 2008).
Each of these data acquisition techniques were repeated at every site. The sites were visited one site per day in rotation for three weeks between June 16, 2011 and July 10, 2011. Additionally, environmental conditions were also monitored during this period at each site. A Vernier LabQuest was utilized to assess the water temperature, salinity and turbidity twice each week at each site.

Figure 2: A female (left) is compared to a male (right) sand fiddler crab. The female's abdomen is one large shield shape, while the male has a column that runs down the middle of its abdomen.

On each sampling day before collecting began, 15 minutes was spent watching the male crabs for mating behavior. During mating season, in order to attract female mates and ward off male competition, male fiddler crabs wave their enlarged dominant claw up and down while just outside their burrow. The number of males observed engaging in this courtship behavior on each collection day at each site was recorded.
Mesocosm Experiment

Figure 3 presents a flowchart of the mesocosm experiment. Two-hundred and three sand fiddler crabs were transported from the reference site Lemon Island preserve in Beaufort, SC to Maryville, TN in styrofoam containers with sediment, water, and some *Spartina* and *Juncus*. The crabs were distributed randomly into 4 mesocosms, about 50 crabs in each (50 crabs in Mesocosm 1, 52 crabs in Mesocosm 2, 51 crabs in Mesocosm 3, and 50 crabs in Mesocosm 4). The mesocosms were set up identically, with sediment, water, and plants collected from the reference site. Instant Ocean seawater was mixed with dechlorinated water (tap water treated with Top Fin Tap Water Dechlorinator) for additional water for each of the mesocosms. For the duration of the mesocosm experiment, salinity was kept between 25-30 ppt and the temperature was kept at 24 degrees Celsius. Each tub containing each mesocosm was propped up at an angle to make a pool and a sediment embankment. Each mesocosm was fitted with an aerator to keep oxygen dissolved in the water. Turtle food was tossed in each mesocosm daily as a food supplement. Heat lamps were positioned over the mesocosms and were on timers set to simulate day and night. Before the start of the experiment, on 14 July 2011, every crab was measured for carapace width, and every male was measured for claw size ratio.

Then, every mesocosm was converted to simulate the sites studied in the field. Mesocosm 1 was the control, analogous to the reference site in South Carolina. Mesocosm 1 was labeled the “Control.” No treatments were added to the water of this mesocosm. The water consisted solely of dechlorinated water and Instant Ocean seawater.

Mesocosm 2 simulated the Parris Island site, impacted by a landfill and a waste water treatment plant. Therefore, Mesocosm 2 was named the “Municipal” mesocosm. It was determined that the most likely contaminants of this site were Bisphenol A (BPA) and synthetic
estrogen ethinylestradiol (EE2). Saltwater was mixed with EE2 in ethanol to make a solution of 4.5 pg/L EE2, and with enough BPA in ethanol to make a solution of 1µg/L BPA. This saltwater treated with EE2 and BPA replaced the clean water in Mesocosm 2 on 18 July 2011.

Mesocosm 3 simulated the Seapines Hilton Head site, impacted by the golf course. It was hypothesized that the major pollutants in this site were insecticide, herbicide, and fertilizer. This mesocosm was named the “Golf Course” mesocosm. Sevin is a common insecticide commonly used on golf courses. Sevin was mixed with saltwater at a concentration of 0.3µl/L. A high nitrogen fertilizer was added to the saltwater at 10 mg/L. Roundup is a very commonly used herbicide used on golf courses. Roundup was added to this water 0.75 mg/L. This water treated with Sevin, fertilizer, and Roundup replaced the water of Mesocosm 3 on 18 July 2011. All of the crabs from the “Golf Course” mesocosm died on 17 August, so no third measurement was made for this mesocosm on 7 September.

Mesocosm 4 was the control site for Mesocosm 2. Because Mesocosm 2 has ethanol introduced to it due to the need to dissolve the contaminants in it, Mesocosm 4 served as a control where nothing except the same amount of ethanol was added. This mesocosm was labeled “Control w/Ethanol.” The water in Mesocosm 4 was replaced with ethanol treated water on 18 July 2011.

A half water change was performed on each mesocosm every 4 days in order to replenish any contaminants that were broken down over time. This was done every 4 days because the contaminant with the shortest half-life was EE2 with a half-life of 7 days in water (Seahill 2002).
During the period of treatments, morphological measurements were conducted on every crab on 17 August 2011. At the end of the experiment, measurements were repeated on 7 September 2011.

Figure 3: Flowchart demonstrating the layout of the mesocosm experiment.
Data Analysis

Data were analyzed using Minitab 16 Statistical Software. A two-way ANOVA test (95% Confidence interval) was performed on carapace width, claw size ratio, and population density comparing field data for all three sites. The software was also used to perform Fisher’s LSD Post Hoc test on all data sets.

The mesocosm data were treated the same way for carapace width and claw size ratio. A two-way ANOVA and a Fisher’s Post Hoc test were run for each data set: before the treatment, during the treatment, and after the treatment; for all four mesocosms.

The environmental conditions- temperature, salinity and turbidity – were also analyzed for variance between the three sites by means of a one-way ANOVA.
CHAPTER III

RESULTS

Field

The environmental data collected from the nearest source of water at each site is displayed in Table 2 along with the recapture rates of each site. The salinity and recapture rates were found to be not significantly different between any of the sites (p = 0.681 and p = 0.856 respectively). However temperature was significantly lower at the Golf Course site (p < 0.001), and turbidity was significantly higher at the Municipal site (p < 0.001).

The behavioral data from each site on each collection date is shown in Table 3. These data show the number of male crabs observed engaging in the mating ritual of the claw wave.
Table 2: Environmental Field Data and Recapture Rates

<table>
<thead>
<tr>
<th>Site</th>
<th>Temperature (°C)</th>
<th>Salinity (ppt)</th>
<th>Turbidity (NTU)</th>
<th>Recapture Rate (recaptures/n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Reference</td>
<td>34.26</td>
<td>0.408</td>
<td>28.62</td>
<td>3.150</td>
</tr>
<tr>
<td>Municipal</td>
<td>34.81</td>
<td>1.260</td>
<td>30.43</td>
<td>2.760</td>
</tr>
<tr>
<td>Golf Course</td>
<td>30.28</td>
<td>0.158</td>
<td>31.57</td>
<td>0.356</td>
</tr>
</tbody>
</table>

Table 3: Number of Male Crabs Observed Initiating the Claw Wave Mating Behavior

<table>
<thead>
<tr>
<th>Site</th>
<th>Day of Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Municipal</td>
<td>0 1 4 0 0</td>
</tr>
<tr>
<td>Golf Course</td>
<td>ALL* ALL* ALL* ALL* ALL*</td>
</tr>
</tbody>
</table>

*ALL*: practically every male crab observed was engaged in the claw wave mating ritual

Figure 4 shows the widths of the crabs’ carapaces over time at each of the three sites in the field. Site, day, and the interaction of site and day all had significant influence on the width of the crabs (p < 0.001, < 0.001, and 0.005 respectively). The crabs from the golf course site were smaller throughout the time period, as shown in the graph. Population density was not influenced by day of sampling (p = 0.162); however, site (p < 0.001) and the interaction of site...
and day (p < 0.001) did influence density, with densities being higher at both of the affected sites (see Figure 5). The claw size ratio (ratio between the dominant and subordinate claw sizes) were significantly influenced by site, day, and the interaction of site and day (p = 0.005, 0.008, and 0.002 respectively), but there was no clear pattern in these influences, as shown in Figure 6.

**Mesocosm**

For the Municipal treatment, the width of the carapace at the end of the experiment was not significantly influenced by treatment, date or the interaction of treatment and date (p = 0.320, 0.829, and 0.910 respectively; see Figure 7). Likewise, for the Golf Course treatment, carapace width was not influenced by treatment, date, or the interaction of treatment and date (p = 0.437, 0.323, and 0.248 respectively; see Figure 8). For the Municipal treatment, the claw size ratio at the end of the treatments was not significantly influenced by treatment, date, or the interaction of treatment and date (p = 0.894, 0.923, 0.830 respectively; see Figure 9). Similarly, the Golf Course treatment saw no significant influence on claw size ratio by date or the interaction of treatment and date (p = 0.443 and 0.251 respectively); however the treatment was shown to have significant influence (p = 0.046; see Figure 10).
Figure 4: The mean widths (+/- 1 SE) of the carapaces of the crabs in the field over time.
Figure 5: The mean population densities (+/- 1 SE) of the crabs in the field over time.
Figure 6: The mean claw size ratios (+/- 1 SE) of the male crabs in the field over time.
Figure 7: The mean widths (+/− 1 SE) of the carapaces of the crabs in the Municipal treatment and its control over time.
Figure 8: The mean widths (+/- 1 SE) of the carapaces of the crabs in the Golf Course treatment and its control over time.
Figure 9: The mean claw size ratios (+/- 1 SE) of the male crabs in the Municipal treatment and its control over time.
Figure 10: The mean claw size ratios (+/- 1 SE) of the male crabs in the Golf Course treatment and its control over time.
Crabs were significantly different in size at each of the sites. The site and the interaction of site and day did influence the population densities of these crabs as well, confirming that the crabs live in significantly varying densities at each of the three sites. Claw size ratio was also confirmed as significantly different, however no clear pattern was shown. The crabs at the golf course were the most different in size from those in other populations, and were clearly significantly smaller than the crabs of the other two sites. The crabs at the golf course also had a much higher population density. It is not unreasonable to consider that population density and size of the crabs could be connected. Bergey and Weis (2008) found that their site with a smaller population density could reduce the amount of competition, allowing for greater growth. This could explain why the Golf Course site has smaller crabs, but higher densities of them: competition is higher in a dense population leading to less ability for growth.
Because the environmental conditions of the three sites were relatively consistent, with the only significantly different condition in the Golf Course site being temperature, it is likely that the cause of these crabs being smaller and in higher population densities is human impact. One possibility is that the Golf Course pollution – pesticides, herbicides, and fertilizers – stunted growth in the crabs. It is possible that the chemicals interfered with the development of these crabs and resulted in a smaller average adult crab. Because the crabs were smaller, they require less living space and thus lived in a higher population density. The alternative is that the contaminants, such as high nitrogen fertilizer, caused eutrophication and thus an overabundance of one of *pugilator*’s food sources: algae. This could have caused the population of *pugilator* to overly thrive, and thus over-populate. The overpopulation could have resulted in increased competition, which in turn caused a scarcity in resources, leaving the crabs smaller.

The claw size ratio showed no clear patterns and no strong significance. This is expected as *pugilator* can drop their dominant claw when threatened, and regenerate a new dominant claw in its place (Ahmed 1978). Because sampling of the male crabs could have shown a ratio for any of the stages in this growth pattern, and because any of the crabs could have dropped a claw at any date prior, it was expected that these data would be fairly random.

The mating behavior was very different between the sites. At the Reference and Municipal sites, few of the crabs observed were performing the ritualized claw wave. However, every day at the Golf Course site, every male crab spotted was engaged in the ritual. Because of the consistency of the environmental conditions among the sites and the independence of sampling date, it is assumed that the reason for the increased mating behaviors of the crabs at the Golf Course site was the result of golf course pollution. It is proposed that the crabs’ increased mating behavior is due to individual pollutants or a mixture of fertilizers, herbicides, or
insecticides. Another possibility is that the difference is due to the population density. Pratt et al. (2005) found that the boldness of courting behavior (measured by number of reemergences to court and time elapsed until reemergence when crabs were purposely startled into their burrows) was significantly higher in populations of higher population densities. However, the similarity of population densities between the Golf Course and Municipal sites precludes this possibility. Future studies should examine the influence of particular pollutants on crab mating behaviors.

Mesocosm

None of the crab groups from the mesocosms showed any significant differences, and none of the graphs indicated patterns. It can therefore be asserted that the crabs did not vary in growth rates from one mesocosm to another. This could be attributed to a number of reasons.

One potential reason the mesocosm experiment failed to show significant differences in the morphological measurements of the crabs under different treatments is that the time span was not long enough. The span of the mesocosm experiment was approximately two months, which is not a very long time to allow for growth to occur.

Another reason could be attributed to the fact that all of the crabs used in the mesocosm experiment were adults (width \( \geq 10 \) mm). Adult crabs grow at a much lower rate than juvenile crabs. Thus, if juvenile crabs were used in the experiment, growth patterns would be more apparent within a shorter period of time. Thus, a more successful mesocosm experiment may use juvenile crabs over a longer period of time. The treatments would show changes in these juvenile crabs’ growth rates in their more influential developing stages. Extending the experiment over a longer period of time would allow for a more long-term observation of growth patterns, and how they are impacted by treatments.
These rationales also explain the lack of significant claw size ratio data as well. But additionally, claw size ratio may not be the best measure for analyzing treatment impacts in a mesocosm experiment. Claw size ratio can be an important feature to measure in the field, as claw size ratio is often an indicator of predation rates. However, in a closed environment such as a mesocosm, predation is not a factor. Therefore, treatments are not likely to impact claw size ratios.

Other potential problems in the design of the mesocosm experiment are the treatments themselves. One factor to consider is the concentration of each treatment. The concentrations of the treatments were based on normal data found in impacted environments. The actual concentrations found in the environment of the actual field in Beaufort, SC are unknown. So these treatments may not accurately reflect the concentrations in the field. Additionally, the actual fertilizers, pesticides, insecticides and other pollutants are unknown - they were just chosen because they are the most likely and most popular.

The mesocosms themselves may have been a problem on growth rates. Living in a mesocosm could potentially cause stress on any animal that has lived in the wild all of its life. Therefore, just by living in a mesocosm, potential data from treatments could have been skewed by stress, or by other factors missing that a mesocosm cannot emulate from the habitat.

Finally, another reason the mesocosm experiment does not show significant data is because of the sample size. Each mesocosm contained only 50 crabs. The actual field sites contained hundreds of thousands, or even millions of crabs. Statistically, this is a fairly small and perhaps biased sample size for the mesocosm experiment.
One important occurrence was the fact that all of the crabs in the Golf Course treatment died on 17 August, 2011. None of the crabs in the other three mesocosms were affected. There were no visible changes to the environment of the Golf Course mesocosm, and the mesocosms were isolated and locked from outside disturbances. Additionally, salinity and temperature were monitored and maintained constant and consistent between all of the mesocosms. All four mesocosms were identical besides treatment. The most reasonable assumption, therefore, is that the crabs in the Golf Course mesocosm experienced the fatality due to the treatment. This assumption suggests the lethality of *Uca pugilator* from prolonged subjection to high nitrogen fertilizer, insecticides, herbicides, or the combination of all three contaminants.

There could, however, be alternative explanations for the deaths of all crabs in the Golf Course mesocosm. In fact, an alternative explanation is almost warranted, because they all died in the matter of 24 hours without showing preceding signs of weakening or dying off slowly. The most logical alternative is that one or more of the crabs in this mesocosm carried a rare but fatal disease that was passed onto the other crabs over time. Another possibility is the transmission of a parasite that eventually kills its host, as crabs are often the hosts to parasites.

**Summary and Future Studies**

The purpose of this study was to test the feasibility of utilizing *Uca pugilator* as a biindicator of anthropogenic impact. In conclusion, the significant differences in the field appear to be attributed to anthropogenic activity. This study supports the notion that *Uca pugilator* is a valuable biindicator of estuarine health and also indicates that carapace widths, population densities, and mating behavior can be important attributes of *pugilator* in relationship to anthropogenic activity.
Future studies are encouraged to use *Uca pugilator* as a bioindicator of estuarine health.

It is suggested that a longer time frame is utilized, and that environmental concentrations of particular contaminants are measured in future studies.


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