Papers & Publications: Interdisciplinary Journal of Undergraduate Research

Volume 2 Article 13

2013

Fiddler Crabs (Uca pugilator) as Bioindicators of Environmental Health in Coastal Estuarine Communities of Beaufort, South Carolina

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Introduction

Traditionally, environmental health analyses of coastal benthic habitats have been performed through assessing direct measurements of water, sediment, and tissue samples for contaminant content (Fukuyama et al. 2008, Pulkrabová et al. 2007) or physiological responses of benthic animals (Bamber and Depledge 1997, Medesani et al. 2011). Many recent studies have suggested there are population trends and animal behaviors that can be used to accurately determine the health status of benthic communities (Table 1). For example, environments with favorable total suspended solids and conductivity exhibit increased diversity and density of invertebrate species (Quintana et al. 2010), whereas anthropogenically induced loss of habitat heterogeneity causes changes in community structure of benthic macroinvertebrates (Marques et al. 2003). Collectively**,** these data suggest that certain invertebrate species can act as bioindicators of environmental health, and the population dynamics of these species can be used to determine whether the community is stressed (Miserendino et al. 2008, Wildsmith et al. 2009).

Of the macroinvertebrates, crabs hold promise as effective bioindicators of a myriad of different natural environmental factors. For instance, stenohaline and euryhaline oyster reef crabs have been used to monitor fluctuations of salinity in the estuaries (Shirley et al. 2004). Similarly, the sand fiddler crab, *Uca pugilator,* is an excellent bioindicator of temperature changes, as elevated temperatures increase mortality and shell blanching (Wilkens and Fingerman 1965).

Crab morphometrics, population dynamics, and behaviors have been used to address environmental health. Male and female mud fiddler crabs, *Uca pugnax,* are significantly larger in industrial polluted areas than crabs in a less polluted environment (Bergey and Weis 2008). Crabs from these same polluted sites have significantly lower population density, lower recruitment, a reduced reproductive season, and lower survivorship of offspring. Whereas population dynamics and morphometrics are obvious indicators of environmental health, crab behaviors have been used as a subtler indicator. Mangrove fiddler crabs (*Uca annulipes* and *Uca inversa*) exhibit alterations in feeding behaviors in response to increased sewage dumping (Bartolini et al. 2009), *Uca pugnax* exposed to petroleum avoid burrowing into layers of sediment with oil (Culbertson et al. 2007), and *Uca pugilator* exposed to the pesticide Dimilin show reduced ability to avoid predators, construct burrows, and forage (Cunninghan and Myers 1987). These findings, coupled with the fact that fiddler crabs are relatively easy to study due to their high density populations and the short time needed to observe them (Bartolini et al. 2009), suggest the use of fiddler crab, *Uca spp*., morphology, population dynamics, and behavior as a way to monitor areas of suspected anthropogenic perturbations.

This study examined the feasibility of using *Uca pugilator* as a bioindicator of anthropogenic impact on coastal South Carolina. The goal of this study was to examine the influence of various human-altered habitats on *Uca pugilator.* To accomplish this, *Uca pugilator* from three sites of varying human impacts (one reference site, one golf course site, and one municipal site) were assessed for population densities, morphological measurements, and behaviors. It was hypothesized that different anthropogenic activities would influence *Uca pugilator* population density, morphology, and behaviors.

Table 1. Benthic species used as bioindicators

Species	Common Name	Anthropogenic activity	Bioindication	Reference
Chironomus spp.	Non-biting midge	Mining	Indicated metallic contamination by fluctuating asymmetry	Al-Shami et al. 2010
Various macro-invertebrates		Habitat alteration/ Urbanization	Indicated sedimentation and changes in water quality by differences in population dynamics	Miserendino et al. 2008; Wildsmith et al. 2009
Uca annulipes and *Uca inversa*	Mangrove fiddler crabs	Sewage dumping	Indicated sewage contaminants by alterations in feeding behavior	Bartolini et al. 2009
Uca pugnax	Fiddler crabs	Oil spill	Indicated petroleum contamination by delayed responses and lower population density	Culbertson et al. 2007
		Industrial pollution	Indicated industrialization by larger body size of individuals and lower population density in urban site than the nondeveloped site	Bergey and Weis 2008
Uca pugilator	Sand fiddler crabs	Harmful pesticide pollution	Indicated contamination with Dimilin pesticide by reduced predator avoidance, burrow construction, and feeding abilities	Cunninghan and Myers 1987
Scylla serrata	Mud crabs	Chemical pollution	Indicated presence of pollutants by elevation of enzyme and urinary metabolite biomarkers	van Oosterom et al. 2010

Materials and Methods

Study Areas

The study was conducted on three ~ 30 m^2 sites in Beaufort County, South Carolina from June 16 to July 10, 2011. (A video of the sites is available at www.youtube.com/mcbiology.) One site was relatively undisturbed and served as the reference site, a second was a municipal site directly receiving sewage effluent, and a third site was downstream from a golf course (thus receiving fertilizer and pesticides). Each site was divided into three replicated plots of 10 m^2, which were selected based on areas of the highest concentration of observed *Uca pugilator*. Due to the confined geographic location of this study (Beaufort County, SC), multiple affected golf course and municipal sites were not available for examination, and thus the present study suffers from pseudoreplication (Hurlbert, 1984). Nonetheless, the three distinct sites were selected to ascertain the potential use of *Uca spp*. as a bioindicator.

The reference site, Lemon Island Preserve, was virtually undisturbed by human activity. The preserve is a large island (approximately 400 acres) of protected estuarine salt marsh on the southern side of Broad River in Beaufort, SC (32.371382° N, -80.812937° W). The reference site included large salt pans and tidal pools surrounded by *Spartina spp.* and *Juncus spp*. Bordering this salt marsh were maritime forests. *Uca pugilator* frequented the tidal pools and salt pans but burrowed on the edges of the salt pans adjacent to the *Spartina spp.* and *Juncus spp*. *Uca minax* and *Uca pugnax* were also seen in this site, although *Uca pugilator* was the most abundant species.

The municipal site (32.357410° N, -80.696090° W) was located on Parris Island, a portion of Port Royal. This island houses the U. S. Marine Corps Recruit Depot Parris Island training facility. The estuary receives effluent from the Parris Island Wastewater Treatment Plant (958 m southeast of the site) and the unlined Causeway Landfill (1.66 km southwest of the site). The study 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 (Table 2). The habitat consisted of a narrow strip of salt pans and tidal pools, which quickly transitioned into a moist and muddy field of *Spartina spp*. *Uca pugilator*'s burrows were along this transition. *Uca pugnax* were observed, but no *Uca minax* were seen at this site.

The third site, adjacent to an active golf course, was located near Harbour Town, in Sea Pines on Hilton Head Island (32.139670° N, -80.808602° W). The study site was along the banks of the Heddy Gutter Creek near Deer Island Road Bridge. This creek is the sink of the Harbor Town Golf Course, contributing insecticides and fertilizers. Flat banks consisting of mud and sand characterized the site. Surrounding these embankments were patches of *Spartina spp*. 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.

Endpoints Measured

Morphometrics, population density measurements, and behavioral observations were taken at every site during low tide. The sites were visited one site per day in rotation for three weeks between June 16, 2011 and July 10, 2011, such that each site was visited every three days. Environmental conditions were also monitored. A Vernier LabQuest was used to assess the water temperature, salinity, and turbidity twice each week at each site.

Similar numbers of *Uca pugilator* were captured at the reference, municipal, and golf course sites (n = 388, 406, and 370 respectively). Crabs were captured from the surface or coaxed from burrows by lightly prodding the back of the burrow with a spade. The crabs were collected individually by hand and placed into a 19-L bucket.

Morphological measurements were made using a plastic Vernier Caliper DY-VC01 (+/- 0.05 mm). Carapace width was measured at the widest point for all crabs. Crab gender was determined by abdomen shape. Male fiddler crabs possess the secondary sexual characteristic of typically having one large, dominant claw for attracting mates and one subordinate feeding claw. Both their dominant and subordinate claws were measured from the tip of the immovable finger to the base of the propodus. (The ratio of dominant claw to subordinate claw was used in our analysis as an indicator of claw regeneration after predation.) All crabs were then marked on the left side of the carapace with a permanent marker so that recaptures could be recorded and released near the vicinity of their collection.

The three sites were also surveyed for population density following the methods of Bergey and Weis (2008). A 1 m plastic ring was thrown randomly at 30 points per site (10 per plot). The number of burrows within the ring were counted and divided by the area to determine 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 provides for a much more accurate estimation than only counting individuals outside the burrow (Bergey and Weis 2008).

On each sampling day before collecting began, 15 min were spent watching male crabs for mating behavior. Care was taken not to disturb crabs, and, as such, the crabs were watched from a distance. During mating season, in order to attract female mates and ward off conspecifics, 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 was recorded.

Statistical Analyses

Data were analyzed using Minitab 16 Statistical Software. A two-way ANOVA was performed on carapace width, claw size ratio, and population density comparing the three sites. The software was also used to perform Fisher's LSD post hoc test on all significant data sets. Environmental conditions—temperature, salinity, and turbidity—were compared among the three sites by a one-way ANOVA.

Results

The environmental data collected from each site demonstrated some variability among sites (Table 2). Salinity was not significantly different among sites ($F = 0.39$, $DF = 41$, $p = 0.681$). However, temperature was significantly lower at the golf course site ($F = 9.68$, $DF = 41$, $p < 0.001$), and turbidity was significantly higher at the municipal site ($F = 20.01$ $DF = 41$, $p < 0.001$). Recapture rates were extremely low at all sites (reference = 0.0062 ± 0.003, municipal = 0.0083 ± 0.008, and golf course = 0.0038 ± 0.004).

Table 2. Environmental data from each site

	Temperature (°C)		Salinity (ppt)		Turbidity (NTU)	
Site	Mean	SE	Mean	SE	Mean	SE
Reference	34.26	0.408	28.62	3.150	125.9	24.10
Municipal	34.81	1.260	30.43	2.760	226.1	32.50
Golf Course	30.28	0.158	31.57	0.356	23.37	2.700

Figure 1 shows the crab carapace width over time at each of the three sites. Site, day, and the interaction of site and day all had significant influence on crab carapace width ($p < 0.001$, < 0.001, and 0.005 respectively). Crabs from the golf course site were smaller throughout the time period. 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 (Figure 2). Claw size ratio (the ratio between the dominant and subordinate claw sizes) was 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 (Figure 3).

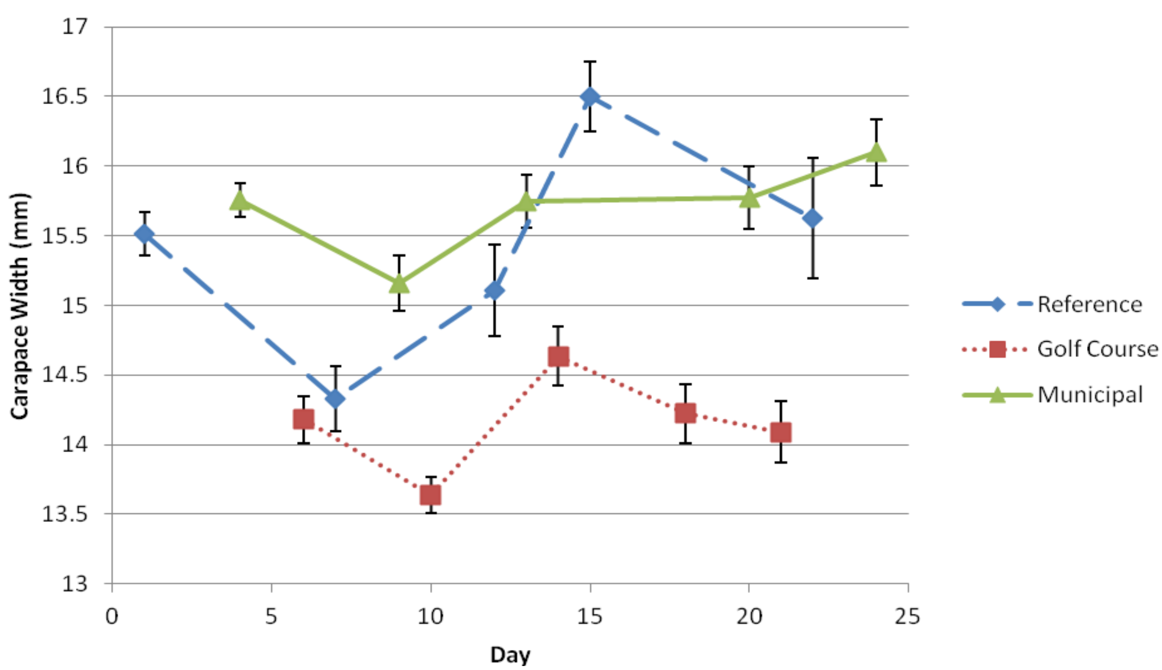

Figure 1. Mean crab carapace widths (+/- 1 SE) over the duration of the study. Crabs from the golf course were smaller throughout the study ($p < 0.005$).

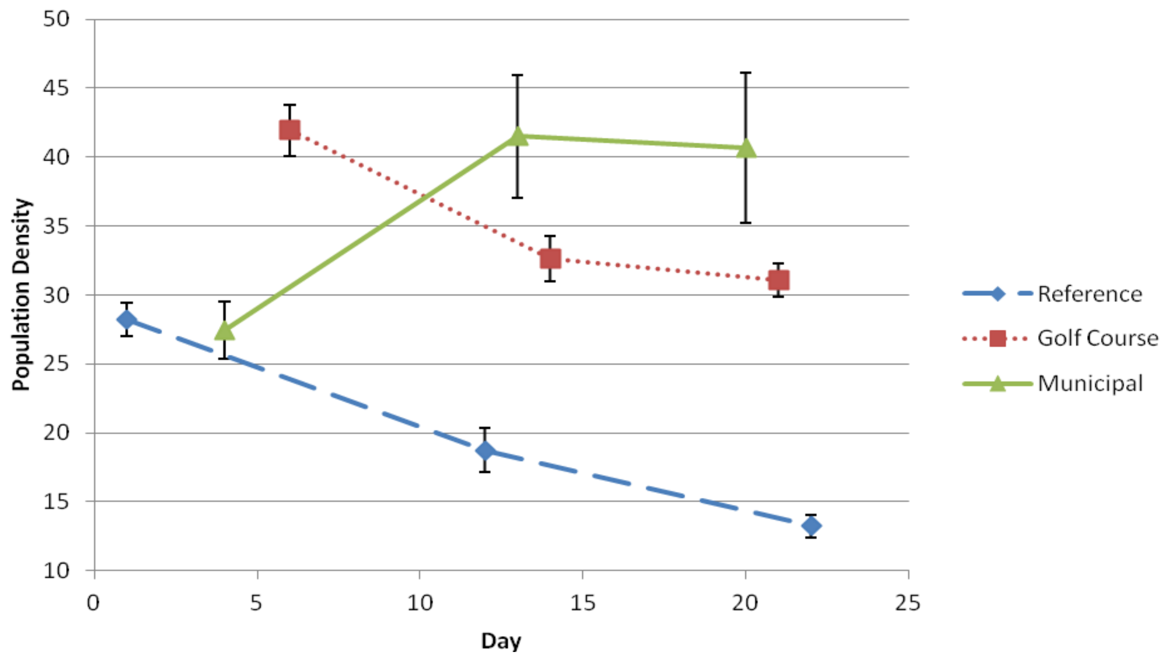

Figure 2. Mean population densities (+/- 1 SE) of crabs over the duration of the study. Throughout the study, densities were higher at both affected sites compared to the reference site ($p < 0.001$).

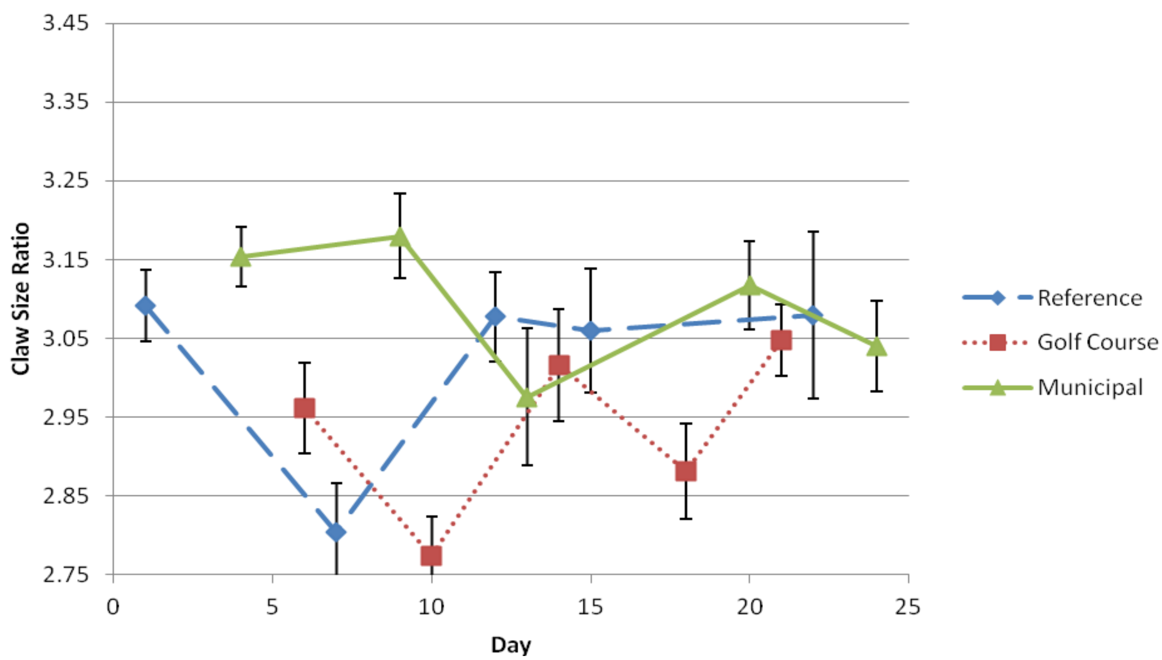

Figure 3. Mean claw size ratios (+/- 1 SE) of male crabs over the duration of the study. Claw size ratio was significantly influenced by site, date, and the interaction of site and date ($p < 0.01$ in all cases), but there was no clear pattern to these influences.

Behavioral data are shown in Table 3. These data show the proportion of male crabs observed engaging in the mating behavior of claw waving. The majority of crabs at the golf course site exhibited this mating behavior, but very few of the crabs at the other sites were observed in this activity.

Table 3. Ratio of male crabs observed claw waving to the total number of crabs observed

Observation Duration	Day of Observation June 19 – June 24	June 25 – June 28	June 30 – July 2	July 3 – July 6	July 7 – July 10
Reference	0/200	3/200	1/100	0/200	1/200
Municipal	0/200	1/200	1/50	0/200	0/200
Golf Course	95/100	95/100	95/100	95/100	95/100

Discussion

These data suggest that fiddler crab morphometrics, population densities, and behaviors can be used as indicators of environmental health in coastal South Carolina. Whereas previous studies have shown that *Uca spp*. are influenced negatively by natural disturbances (Brandt et al. 2010), the present study shows that fiddler crabs may also be influenced by anthropogenic disturbances. The hypothesis that anthropogenic activities would influence *Uca pugilator* was supported by data from population density, morphology, and behaviors.

Crabs at the golf course site had a much higher population density and were smaller. It is not unreasonable to consider that size of the crabs and population density could be related, as Bergey and Weis (2008) found that sites with a smaller population density could reduce the amount of competition, allowing for greater growth. There were significant differences in the temperatures and turbidities of the sites, so it is possible that these factors influenced the results. It is also possible that the cause of these crabs being smaller and in higher population densities is human impact. For instance, the golf course runoff—pesticides and fertilizers—could have inhibited growth in the crabs. Smaller crabs require less living space and thus have a higher population density. An alternative explanation is that some of the contaminants, such as high nitrogen fertilizer, caused eutrophication and an overabundance of one of *Uca pugilator*'s food sources, algae. This could have caused the population of *Uca pugilator* to thrive, and thus over-populate. The overpopulation could have resulted in increased competition, resulting in a scarcity of resources. Future studies should examine whether physical (e.g., temperature or turbidity) or anthropogenic factors (e.g., pesticides or fertilizers) influence crab morphology and population density.

The claw size ratio showed no clear patterns. This is not surprising, as *U. pugilator* often shed 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 stage in this growth pattern, and because any of the crabs could have dropped a claw at any date prior, it is not surprising that the ratio between dominant and subordinate claw would be random among all sites.

Mating behavior was different among the sites. At the reference and municipal sites, very few crabs were observed exhibiting claw waving behavior. However, every day at the golf course site, virtually all male crabs were engaged in this behavior. One possible explanation is 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 increased 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 does not support this possibility. Future studies should examine the influence of particular pollutants on crab mating behaviors.

The purpose of this study was to test the feasibility of utilizing *Uca pugilator* as a bioindicator of anthropogenic impact in coastal South Carolina. At the three distinctly different sites, fiddler crabs had different body size, population density, and behaviors. This study illustrates relatively inexpensive, timesaving, low-impact methods to assess estuarine health.

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