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Assessing the effect of beach substrate color, size, and sex on the carapace color of common shore crabs (Hemigrapsus oregonensis) in the Puget Sound

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Assessing the effect of beach substrate color, size, and sex on the carapace color of common shore crabs (*Hemigrapsus oregonensis)* in the Puget Sound

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Abstract

The shore crab *Hemigrapsus oregonensis* is highly variable in the coloration of its carapace, with morphologies ranging from dark brown to yellow-green, reddish, and white. This polymorphism may serve as an asset against predation if there exists selective pressure on the crabs to select substrates based on their phenotype. We sampled crabs from multiple sites along the Puget Sound, using image analysis to quantify the "whiteness" of the carapace and the substrate. The size and sex of the crabs was also recorded. Though there was not a significant relationship between carapace whiteness and substrate whiteness (possibly due to lack of significant difference between sites), female crabs were found to be significantly smaller and whiter than male crabs; furthermore, the total number of white crabs of either sex significantly declined as body size increased. These results suggest that white crabs, and especially male white crabs, experience differential mortality as their size increases. The sex difference in whiteness could be attributed to behavioral differences between males and females that lead to higher male mortality in white crabs.

Introduction

Because the physical appearance of an organism plays an instrumental role in avoiding detection by its predators, selection favors those individuals most able to emulate their respective environments (and thus reduce predator attacks) through their color, texture, or pattern. Mobile species face unique challenges in avoiding predation because their heterogenous background is constantly subject to change; therefore, polymorphic species possess an advantage due to the phenotypical variation in populations. Polymorphism—or phenotypical variation—of color within a species can arise from diet or be caused by genetics. However, most polymorphism is caused by a combination of both genetic and environmental factors; for example, the variable color of the common sea star *P. ochraceus* seems to be exaggerated by diet with an underlying genetic basis (Harley et al. 2006).

Regardless of the underlying cause of variable color in a species, polymorphism is an asset to populations occupying heterogenous terrain. Variation in color among individuals greatly decreases a predator's ability to form a search image (Pietrewicz and Kamil 1979, Cooper and Allen 1994). Therefore, natural selection (and sometimes sexual selection) reinforces and maintains polymorphism in many prey species; though natural selection alone will not account for microhabitat variation (Chunco et al. 2007). Individual behavior can enhance camouflage even further should individuals choose microhabitats that conceal them best. A recent study involving island lizards demonstrated that wild lizards chose to rest against backgrounds that most closely matched their dorsal patterning (Marshall et al. 2016).

In many cases, predation acts as a factor to balance or otherwise mediate the prevalence and proportion of polymorphism in a population; for example, male fiddler crabs are preyed upon more often than their female conspecifics because their large claws are conspicuous to avian predators (Koga et al. 2001). Additionally, differences in behavior and habitat selection between sexes may further contribute to differential mortality in populations (Jormalainen and Tuomi 1989). In general, conspicuous behavior or large size (especially when paired with a brighter morphotype) can leave individuals more susceptible to predators (Bond and Kamil 1998, Jensen and Egnotovich 2015).

A common polymorphic species native to the Puget Sound region is the yellow shore crab *Hemigrapsus oregonensis,* a small (with a maximum carapace width of 34.1 mm for males) crustacean which inhabits intertidal regions on rocky beaches (Oliver and Schmelter 1998). As they are a common prey item of gulls as well as predatory fish, the crabs face pressure to blend in with the substrate they inhabit. Several color morphologies are present in *H. oregonensis*, with carapaces ranging from white, green, brown, yellow, reddish, or a mottled combination (Oliver and Schmelter 1998). Juvenile *H. oregonensis* tend to be more variable in color than their adult counterparts, likely because smaller crabs run a greater risk of predator attack (Palma and Steneck 2001). Additionally, phenotype-environment matching is most prevalent at the micro-scale in shore crabs, reflecting local adaptation, while larger-scale matching may be a result of phenotypic plasticity or "predator mediated differential mortality" (Todd et al. 2012).

Though *H. oregonensis* is commonly found throughout the Puget Sound region, little local research has been conducted on the tendency of these crabs to select microhabitats on the basis of their phenotype, and the influence of size and sex on these patterns. Using image analysis technology as well as a color index scale, we will compare shore crab carapace color with substrate color at both large (beach) and small (quadrat) scales. Because selection favors crabs that choose to inhabit microhabitats matching their carapace, we should see a strong correlation between the color of the substrate and the color of the even at a small scale. Additionally, we should expect to find greater color variation in small crabs. Though we cannot determine through this study if carapace-substrate matching in *H. oregonensis* is a result of local adaption or phenotypic plasticity, the results of this study will further reveal patterns in shore crab behavior in relation to phenotype, with larger implications in predator-avoidance techniques and the reinforcement of polymorphism.

Methods:

Data collection:

Sampling was conducted at two beach sites at low tide along Ruston Way in Tacoma, WA. At each site the water line (where *H. oregonensis* is most abundant) served as a transect, and 0.25-m^2 PVC pipe quadrats were randomly placed along it. Five quadrats were sampled per site. Before sampling a given quadrat, all algae, litter, and other non-substrate material were removed, and the substrate within the quadrat was photographed with an iPhone camera under standard lighting conditions, with a grey card to standardize exposure. After photographing substrate, all *H. oregonensis* present in the quadrat were captured and placed in a clear Tupperware container; with a maximum number of 15 crabs per quadrat. When lifting rocks, the methods used by Jensen and Egnotovich were emulated: a homemade tube-shaped sandbag was placed around rocks to be turned to prevent escapees (Jensen and Egnotovich 2015). Each crab captured was sexed, and the length and width of its carapace was measured with digital calipers to the nearest 0.01 mm. The carapace of each crab was photographed with an iPhone camera under standard lighting conditions, including a color card to maximize color integrity. The color of each crab was also manually indexed, following the methodology of Jensen and Egnotovich. The four categories of color included white, off-white, mottled, and normal (Jensen and Egnotovich 2015). After all data were collected to satisfaction, crabs were released to their respective quadrats and all rocks and algae were returned to their prior positions.

Image analysis

All images (of substrate and of crabs) were analyzed using ImageJ. To determine the whiteness of a carapace or section of substrate, the desired section was manually selected and copied onto a new image. To standardize scale, the length of one square on the color card (1.2 cm) was also copied onto the new image, and the scale of the image was set in centimeters. Following this step, the area of each carapace or substrate section was measured in cm^2. The color threshold tool was used to select all white areas of the carapace or substrate, with the goal being that all white in the original image was highlighted. The area of only those white sections was measured in cm^2. The ratio of white area to total carapace or substrate area was calculated to produce the percentage of whiteness.

Data analysis

All analytical and descriptive statistics were performed using RStudio. We performed a linear regression test to determine if whiter crabs occupied whiter substrates significantly more than non-white crabs. Because data for crab size and whiteness did not display equal variance between the sexes, non-parametric tests were conducted. Two separate Spearman correlation tests along with two Wilcox tests were performed to assess the relationship between crab whiteness and body size for male and female crabs.

Results

Sampling and morphological observations

A total of 13 quadrats (the substrates ranging from 1-5.5% white) were sampled along the two Puget Sound beach sites. 156 crabs were photographed and measured, with a 71:85 male to female ratio. Carapace size ranged from 3-20 mm in length. While the rarer red and purple morphologies were not encountered during sampling, a vast range in brown, green, and white carapaces were observed. While some “white” crabs were ivory white, many others were yellowish or had symmetrical brown and white patterning. While 28% of crabs’ carapaces were over 25% white, the majority of crabs sampled resembled the normal coloration of greenish-brown.

Phenotype-substrate matching

Across all quadrats sampled, barnacles, crushed oyster shell, and plastic comprised the whitest substrate material. The average whiteness of crabs sampled at a site was not significantly impacted by the whiteness of the sampled substrate (linear regression, F=0.24, df=2,11, p=0.634, R^2=0.02, Figure 1).

Effects of sex and size on whiteness

The relative whiteness of the carapace was significantly affected by both the size and sex of the crabs. Carapace size was significantly negatively correlated to its percent whiteness for both female (correlation, rho=-0.36, p <0.001 Figure 2a) and male (correlation, rho=-0.34, p=0.004 Figure 2b) crabs. Furthermore, while 10% of females over 10 cm in area were white, only 2.8% of large (over 1 cm^2) males possessed white coloration, indicating that male white crabs do not persist at larger sizes as strongly as female white crabs. These results are exaggerated by the fact that median (±IQR) body size was significantly higher in male crabs than in female crabs (Wilcox test, w=1991, $p < 0.001$, Figure 3a). While 29% of females were over 1 cm^2 in size, 55% of male crabs were as large.

Finally, white crabs were much more likely to be female than male, as the median (±IQR) whiteness of female crabs was significantly higher than that of male crabs (Wilcox test, w=4355, $p << 0.001$, Figure 3b). While 38% of female crabs were white, only 15% of male crabs displayed white coloration.

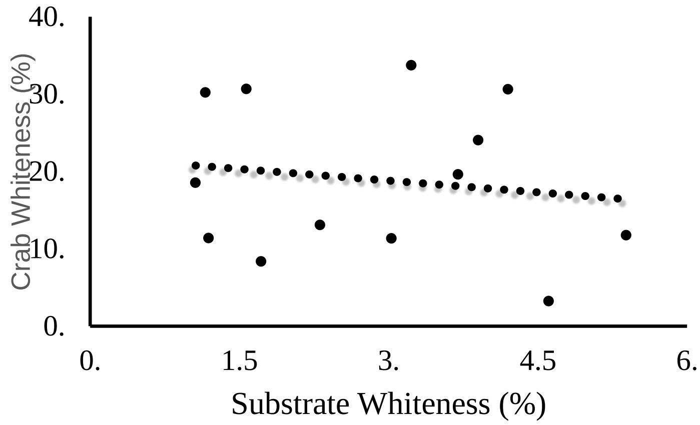

Figure 1. Average crab whiteness (%) as a function of substrate whiteness across each sampling location (n=13). The crabs' average whiteness was not significantly impacted by the substrate in which they were found (p=0.634, R^2=0.02).

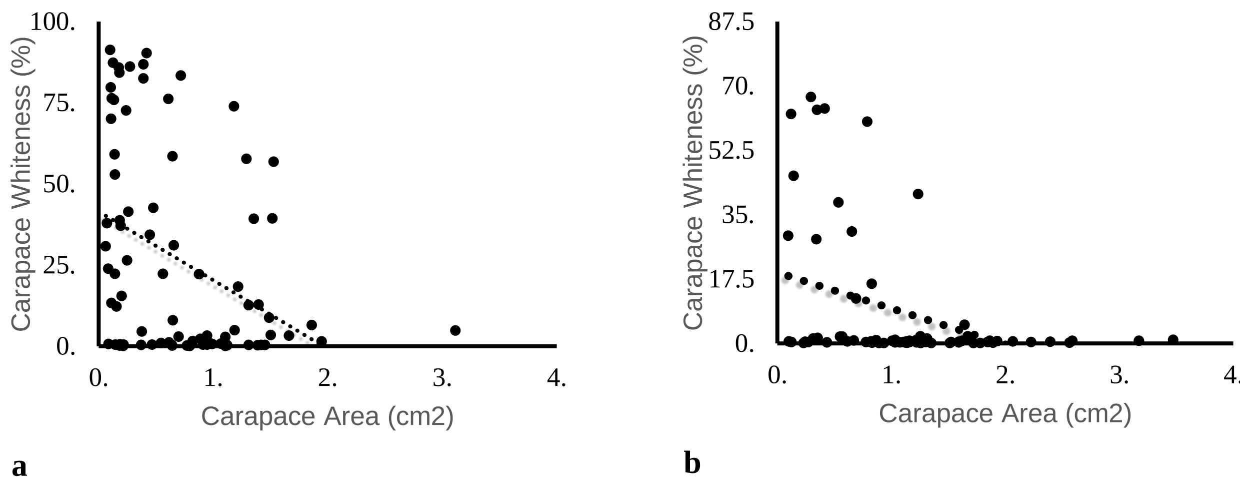

Figure 2. Carapace area (cm^2) and percent carapace whiteness in female (a, n=85) and male (b, n=71) *Hemigrapsus* collected from all sites. Whiteness significantly declined as body size increased for both females (rho=-0.36, p <0.001) and males (rho=-0.34, p=0.004).

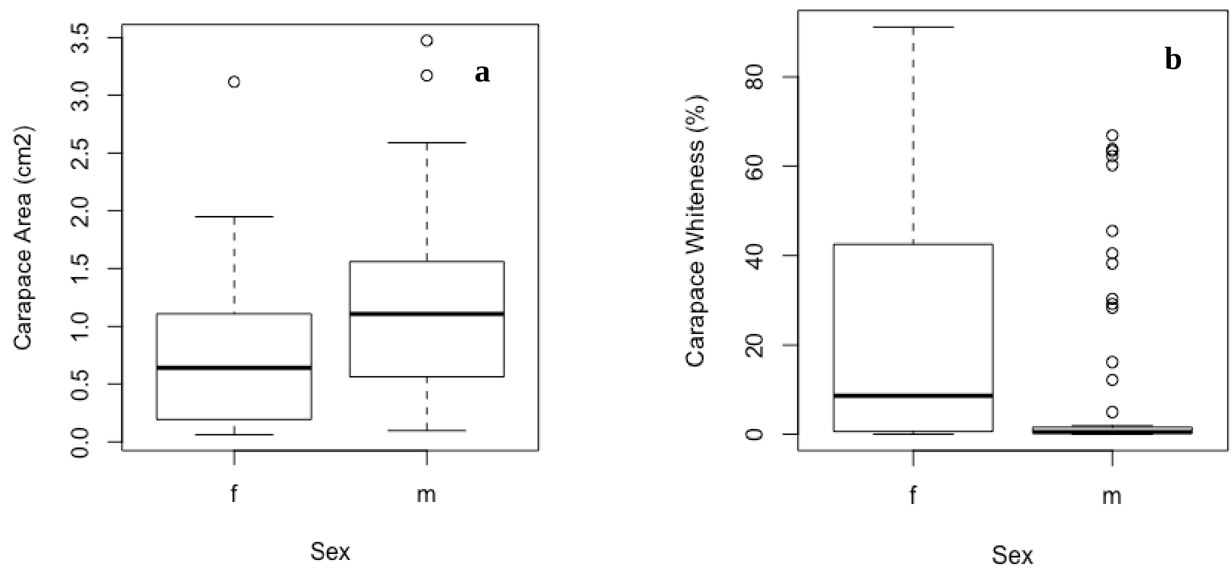

Figure 3. Median (±IQR) carapace area (a) and carapace whiteness (b) for male and female *Hemigrapsus.* While male crabs were significantly larger than females ($p<0.001$), females were significantly whiter than males ($p << 0.001$).

Discussion

Phenotype-substrate matching

Our results indicated no significant relationship between whiteness of substrate and carapace whiteness; contrary to our initial predictions and the findings of a previous local study (Jensen and Egnotovich 2015). Due to municipial restrictions, our sampling was limited to a relatively short stretch of beach with little variance in substrate: while the Jensen study sampled beaches ranging from 1-25% white, our quadrats ranged from 1-5% white. This may not have been enough variance to produce a phenotype-substrate matching behavior in shore crabs. Repetition of the experiment using sampling locations that contrast more heavily in their whiteness would produce more variance substrate data; additionally, it would be interesting to determine the percent difference in whiteness necessary to observe a phenotype-substrate matching response in shore crabs. A laboratory experiment determining if crabs will choose to occupy a lighter or darker substrate based on their carapace color is also justified.

Effects of size and sex on whiteness

The whiteness of an individual was significantly influenced by both its carapace area and sex. White crabs, especially white males, were virtually absent from the population when carapace area exceeded 1 cm^2. A significant decline in whiteness as carapace size increases suggests that while whiteness at a smaller size can serve as an asset against predation, large white crabs experience differential mortality or a change in color with age. The ability of off-white and mottled *Hemigrapsus* to darken over time when exposed to darker substrate has been confirmed in a laboratory setting; however, as pure white crabs lack the melanophores necessary to change color, color change cannot be the sole mediating factor in the absence of large white crabs (Jensen and Egnotovich 2015). Although smaller crabs are at a greater risk of predator attack (Palma and Steneck 2001), a large white crab's movements may be more easily detected by a visual predator than that of a smaller crab due to the small, fragmented nature of white substrate on Puget Sound beaches.

Our results, along with those of Jensen and Egnotovich, indicated that white crabs were twice as likely to be female; furthermore; while white females were more persistent in the population at larger sizes, large white male crabs were rarely encountered in the duration of our study. The dramatic difference in the proportion of white male to female crabs could be explained by behavioral differences between sexes that are exacerbated by an increase in size. An empirical study of shore crab behavior observed that male *Hemigrapsus* tend to feed on the exposed sections on the beach and rocks, while females forage along the lower sections and crevices of the rocks. Additionally, male crabs usually froze when startled, while startled females immediately scurried away from any potential threats (Knudsen 1964). The tendency of male crabs to forage in the open and freeze when threatened puts them at a much greater risk of mortality by visual predators; additionally, male sexual and aggressive behaviors (fighting, claw waving, and lunging) that correspond with larger size may make them more conspicuous to a predator (Lindberg 1980).

Male-biased predation due to sexual behavior is an ecological pattern reflected in many populations; for example, polymorphic isopods experience male differential mortality due to the high diurnal male activity during the breeding season (Jormalainen and Tuomi 1989a). The idea that white large males experience differential mortality due to their showy behavior would require experimental testing; currently, there is little evidence underlying the idea of white male-biased mortality in shore crabs. Obtaining a sex ratio of individuals consumed by the crabs' primary predators could determine if large white males really are being preyed upon more often; additionally, an updated study of differential male and female behavior could help provide more reliable circumstantial evidence for this theory.

While brighter or rarer morphs are advantageous in some polymorphic species (Bond and Kamil 1998), this theory does not seem to hold true with *Hemigrapsus* due to the relative lack of large white crabs. Because white male crabs are relatively uncommon even at smaller sizes, it appears that most whiteness persists in the population due to the success of white female crabs. The genetic heritability of carapace whiteness would be an interesting follow-up experiment in analyzing the maintenance of polymorphism in these crabs. The stark differences in the variability in these shore crabs due to size and sex have broad implications regarding the true complexity of selective forces on morphology and predator prey interactions. In general, polymorphic species serve as an excellent vessel for studying the selective pressures posed by predation, and the mechanisms by which polymorphism is maintained and reinforced.

References

Bond, A. B., and A. C. Kamil. 1998. Apostatic selection by blue jays produces balanced polymorphism in virtual prey. Nature 395:594.

Chunco, A. J., J. S. McKinnon, and M. R. Servedio. 2007. Microhabitat variation and sexual selection can maintain male color polymorphisms. Evolution: International Journal of Organic Evolution 61:2504–2515.

Cooper, J. M., and J. A. Allen. 1994. Selection by wild birds on artificial dimorphic prey on varied backgrounds. Biological Journal of the Linnean Society 51:433–446.

Harley, C. D. G., M. S. Pankey, J. P. Wares, R. K. Grosberg, and M. J. Wonham. 2006. Color polymorphism and genetic structure in the sea star *Pisaster ochraceus*. The Biological Bulletin 211:248–262.

Jensen, G. C., and M. S. Egnotovich. 2015. A whiter shade of male: color background matching as a function of size and sex in the yellow shore crab *Hemigrapsus oregonensis* (Dana, 1851). Current Zoology 61:729–738.

Jormalainen, V., and J. Tuomi. 1989a. Sexual differences in habitat selection and activity of the colour polymorphic isopod *Idotea baltica*. Animal Behaviour 38:576–585.

Knudsen, J. W. 1964. Observations of the reproductive cycles and ecology of the common *Brachyura* and crablike *Anomura* of Puget Sound, Washington.

Koga, T., P. R. Backwell, J. H. Christy, M. Murai, and E. Kasuya. 2001. Male-biased predation of a fiddler crab. Animal Behaviour 62:201–207.

Lindberg, W. J. 1980. Behavior of the Oregon mud crab, *Hemigrapsus oregonensis*. Crustaceana: 263–281.

Marshall, K. L., K. E. Philpot, and M. Stevens. 2016. Microhabitat choice in island lizards enhances camouflage against avian predators. Scientific Reports 6:19815.

Oliver, J., and A. Schmelter. 1998. Life history of the native shore crabs *Hemigrapsus oregonensis* and *Hemigrapsus nudu*s and their distribution, relative abundance, and size frequency distribution at four sites in Yaquina Bay, Oregon. [Online] Oregon State University.

Palma, A. T., and R. S. Steneck. 2001. Does variable coloration in juvenile marine crabs reduce risk of visual predation? Ecology 82:2961–2967.

Pietrewicz, A. T., and A. C. Kamil. 1979. Search image formation in the blue jay (*Cyanocitta cristata*). Science 204:1332–1333.

Todd, P. A., J. Oh, L. H. Loke, and R. J. Ladle. 2012. Multi-scale phenotype-substrate matching: Evidence from shore crabs (*Carcinus maenas L*.). Ecological Complexity 12:58–62.