FINAL PROJECT REPORT TO THE UNIVERSITY OF HAWAI'I AT HILO

MARINE OPTIONS PROGRAM

Shell color polymorphism in the marine gastropod *Mitrella fusiformis*

Mikayla Jones

Marine Science Department

University of Hawai'i at Hilo

MOP ADVISOR

Dr. Marta deMaintenon, Professor

Division of Natural Sciences

University of Hawai'i at Hilo

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ABSTRACT

Gastropod mollusks are extremely important in marine ecosystems as predators and prey, especially in coral reefs. It is critical to understand where and how these benthic organism interact with their communities and with the physical aspects of their ecosystems. Our project is to study polymorphism among shallow columbellids, a common and diverse family of marine neogastropod snails. Columbellids are small (most less than 10 mm in length) and can be common epibenthic carnivores in shallow marine ecosystems. A number of nearshore species in this group are polymorphic for shell color and pattern, a factor that may correlate to their individual survival by making them more or less visible to predators and prey. Our objective was to investigate correlations between polymorphism, environment and sex in common local epibenthic marine gastropods. This study will focus on Mitrella fusiformis (Pease, 1868), one of the most common Hawai'i columbellid species. To study these organisms, we made collections in several locations around the Island of Hawai'i, where they are typically in 1 m depth of water or less. Individual, adult animals were sexed, measured and their shell color and pattern documented photographically using a dissecting microscope with camera. Most animals were returned alive to their original habitat after data was collected. Mitrella fusiformis were collected from August of 2014 to March of 2016 (n=300). The resulting data set allowed us to investigate correlations between shell color and pattern, substrate habitat and location. There was a significant relationship between location and shell color and pattern ($p<0.001$). Although, there was no significant correlation between sex, substrate habitat and shell color and pattern some trends where elucidated. For example, locations around the Island of Hawai'i had different ratios of male to female to juvenile snails. Those locations with a similar number of individuals across the maturity levels had higher abundance throughout the year. In addition, *Mitrella fusiformis* do not completely dictate what substrate habitat they live in based on their shell color and pattern. However, there is a trend of cream shell snails being more prevalent in light sand colored substrates and dark shell snails on basalt.

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INTRODUCTION

Many species of marine invertebrates display color polymorphism. The frequency of these color morphs varies based on a number of different factors including age, diet, crypsis, predation intensity, predator identity or pathogens (Harley et al.2006). Conspicuous morphological distinctiveness between species of the same family or in the same community is also characteristic of tropical marine mollusk fauna (Clarke 1978). "Sexual selection, referred to the component of natural selection occurring during mating, has been considered one of the most important selective components capable of maintaining natural polymorphism by different mechanisms" (Rolan-Alvarez et al. 2012). A common hypothesis states that color variation differs among geographically isolated populations and thus polymorphism experiences genetic drift.

Different geological and chemical environmental conditions produce varying polymorphisms. Genetic sequences that code for these different polymorphisms are found through mitochondrial DNA, which is passed down to the offspring from the mother. For example, Harley et al. (2006) investigated color polymorphism in the sea star *Pisaster ochraceus* by characterizing geographic patterns of genetic structure from southern Alaska to southern California using the sequence variation in the mitochondrial cytochrome oxidase I gene. He concluded that geographic differences in the frequencies of *Pisaster* color morphs (brown, orange, or dull purple) most likely reflect phenotypic plasticity driven by diet or other environmental factors. Another marine environmental factor that can influence why a particular polymorphism exists versus another is salinity. Along the same lines as Harley et al. (2006) and Sokolova & Berger (1999) investigated the effect of moderate to extreme salinity change on shell color polymorphism as a driving force of the phenotypic structure of *Littorina saxatilis* along a salinity gradient in the White Sea estuaries. Sokolova & Berger (1999) concluded that the brown tessellated un-banded morph had an advantage over the purple tessellated morph in fluctuating ocean salinity and temperature because the brown tessellated morph was found predominately in estuaries, whereas the purple tessellated morph was found farther up river.

Gastropod shell color has three primary functions: communication, crypsis and thermoregulation (Endler 1978). Substantial evidence is accumulating that many predators, and especially those in the tropics, feed selectively; they hunt by sight and are able to distinguish colors and/or color patterns (Clarke 1978). Thus any rare morph is eliminated from predatory selection because it has not been selected as a viable food source. If a polymorph is more abundant than it is preyed upon first, making diversity a much needed refuge from predation. In a study of the supertidal gastropod *Littoraria filosa* and parasitoid flesh fly *Sarcophaga megafilosia,* frequency dependent selection can be detected by exposing samples of snails containing different relative frequencies of two or more morphs to the flies and then comparing the proportion removed to the proportion that survived (Mckillup 2007).

Correlation between color polymorphism and individual physiology is a result of the pleiotropic effects of genes responsible for shell color or a linkage between them and genes determining certain physiological features (Raffaelli 1979, 1982). Saura (2011) investigated the dietary effects on shell growth and shape in an intertidal marine snail *Littorina saxatilis* and determined that snails with faster growth rates also have larger shell apertures. McKillup (1983) proposed that a mark-release-recapture experiment done using juvenile snails could provide evidence of relative growth and mortality of *Nassarius pauperatus* and could be reimagined to provide a track of color polymorphic traits over many generations in different habitats.

Gastropod mollusks are extremely important in marine ecosystems as predators and prey, especially in coral reefs. It is important to understand where and how these benthic organisms interact with their communities and with the physical aspects of their ecosystems. Our project is to study polymorphism among shallow water columbellids, a common and diverse family of marine neogastropod snail. Columbellids are small (most are less than 10 mm in length) and can be very common epibenthic carnivores in shallow marine systems. A number of near shore species in this group are polymorphic for shell color and pattern, a factor that may correlate to their individual survival by making them more or less visible to predators and prey.

Our objective is to investigate correlations between polymorphism, environment and sex in common local epibenthic marine gastropods. This study will focus on *Mitrella fusiformis* (Pease, 1868), one of the most common local columbellid species. Last year, we determined that there were trends for different morphs on the leeward and windward sides of Hawaii Island but our sample size was not large enough to show statistical significance. Thus, this year our project focuses on increasing sample size to better elucidate trends. In addition, further emphasis will be placed on collecting environmental data with the hopes of correlating environmental factors with certain polymorphisms. The resulting data set will allow us to investigate correlations between shell color and pattern, and factors including sex, substrate habitat, salinity, and location. The hope is to gain more insight into how these organisms interact with their habitats in our Hawaiian waters.

<u>METHODS AND MATERIALS</u>

Field Methods:

Common environmental factors to be recorded are salinity, temperature, dissolved oxygen, food source and habitat. Salinity, temperature and dissolved oxygen will be measured via a YSI borrowed from the University of Hawaii at Hilo Marine Science Department. Food source and habitat will be described in the field and jotted down in short hand in the field. Pictures will also be used to recall memory about the particular environment in which the snails were collected. We will make collections at several known locations including Onekahakaha, Richardsons and Chocks on the windward side of the Island and Waikoloa on the leeward side of the Island of Hawai`i. However an additional goal of this project is to expand the locations at which *Mitrella fusiformis* are found around the Island of Hawai'i. *Mitrella fusiformis* is typically found in 1m depth of water or less.

Lab Methods:

Individual adult animals were sexed, measured, and their shell color and pattern documented photographically using a dissecting stereomicroscope with camera. *Mitrella fusiformis* are collected in plastic containers and the seawater is kept oxygenated with an air pump and air stones until animals are analyzed. Most animals were returned alive to their original habitat after data sets are collected. The animals were not kept longer than 2 to 3 days after field collection.

<u>RESULTS</u>

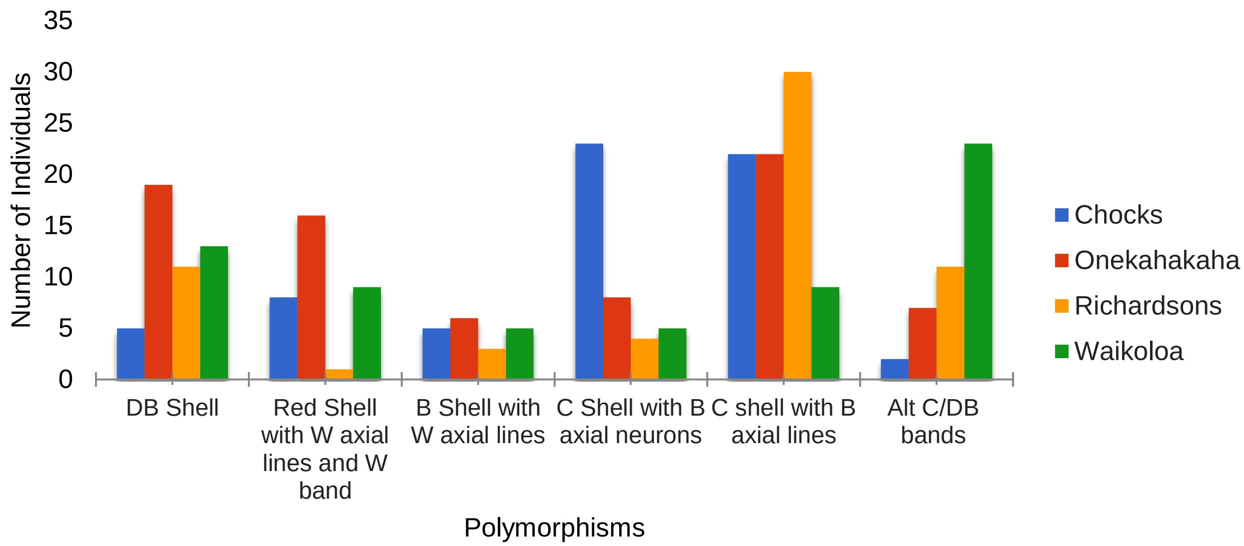

Figure 1. Dispersal of Polymorphisms across the four study sites.

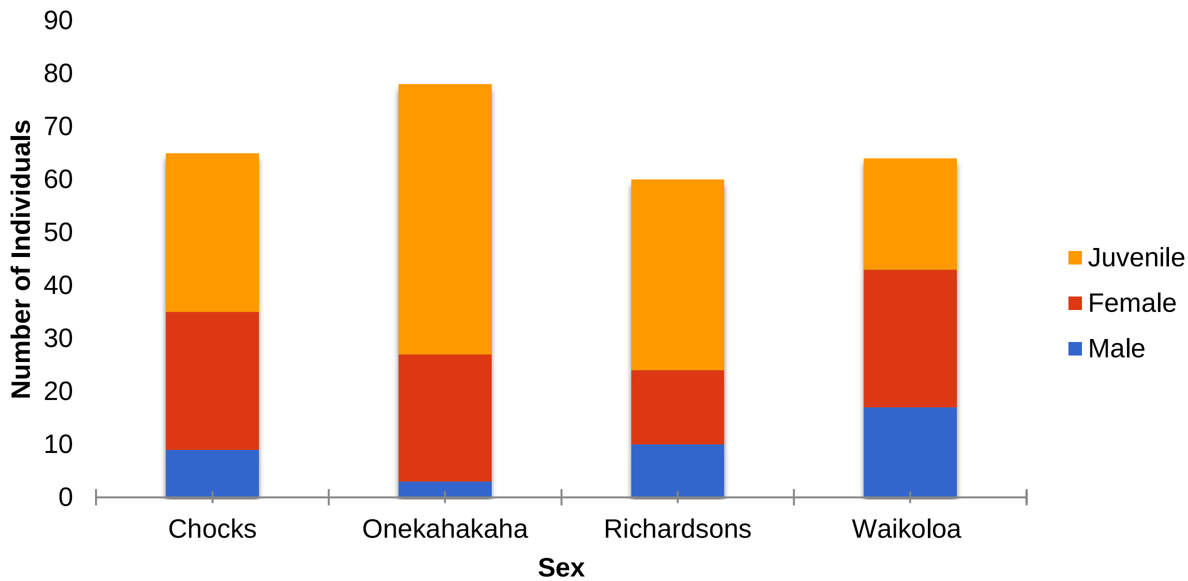

Figure 2. Distribution of maturity in *Mitrella fusiformis* across the four study sites.

<u>DISCUSSION</u>

There is a preference for a cream shell with brown axial lines at all study sites except Waikoloa (Figure 1). Chocks, Onekahakaha and Richardsons' substrate is light and silty covering basalt rock. However, Waikoloa is dominated by basalt cobbles on sandy substrate which may explain why dark brown shell and alternating cream/dark brown band polymorphisms have such prevalence. Chocks also has a competing preference for cream shell with brown axial neuron lines. Investingating why red shell polymorphism is present at all four locations but only extremely low at Richardsons would be interesting as it could be related to diet avaliability. Chocks and Onekahakaha are the closest study sites to one another but even they have distinct differences in the dispersal of polymorphsims at their locations. Figure 2 shows the distribution of maturity in *Mitrella fusiformis* across all four study sites. Waikoloa has the most stable ratio of sexually mature to immature individuals. In addition, Onekehakaha has the most juveniles which could suggest that the population is growing.

We found a significant association between shell pattern and location (Chi-Square = 78.4, df =15, p-value = 0.000). Camoflauge seemes to be the major factor driving the diversity of these Shell color and pattern polymorphisms in *Mitrella fusiformis*. There is a preference for a cream shell with brown axial lines at all four study sites (Figure 2). The habitat of all the sites is dominated by cobbles or boulders of basalt surrounding a mix of light and dark sandy substrate with light to moderate wave action. *Mitrella fusiformis* are found underneath or in basalt rocks on the sandy substrate. Thus if the geological morphology of the study sites are not what define the difference then perhaps physical factors such as salinity, temperature or oxygen levels explain why there are different polymorphisms. Another hypothesis could be that diet is an influence on why there are different dominating polymorphisms. Further work on this project include investigating the factors listed above to determine what factor(s) influence the dispersal of polymorphisms at these study sites.

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