RESEARCH INFORMATION

Describe your preparation and desire for performing undergraduate research with your chosen mentor

I have worked in the Buston Lab since January 2014. During my time working under Dr. Buston, I have successfully complete a UROP research project during the summer of 2014. I have also worked under my graduate mentor Tina Barbasch on her project, *Integrating the effects of social and environmental context on the plasticity of parental care*, during the present semester.

My responsibilities in the lab include clownfish care and overall maintenance of the tanks in the laboratory. Clownfish care requires me to regularly check on the health and feed them, while lab maintenance requires me to regulate the conditions of the water in the tanks. Both tasks are essential to keeping the fish healthy. Moreover, working under Tina Barbasch requires me to perform various tasks that are important for her current project. These tasks include: collecting eggs and massing them, taking pictures of clutches of eggs, recording the parents while they care for their clutch, and counting the clutch size. These important skills and knowledge that I have gained from caring for the clownfish and collecting data will aid me while conducting my research.

Courses that I have taken that are relevant to this research include statistics MA 115, Marin Biology BI260, and Animal Behavior BI407. I am currently taking Psychology of Learning (PS234), which has allowed me to apply the methods and terminology that I learned about Operant Conditioning to this project.

**Research Project Title**

**Color Discrimination in *Amphiprion percula***

**Describe the research problem**

This section should be about 100-500 words. It should describe background information of the previous work by your mentor and other scientists underlying the scientific question and its significance to the field of biology. There should be a clear sentence near the end that states the hypothesis you plan to test. Be sure to cite any statements of fact that are not common knowledge (see Cited Sources section below).

An organism's ability to predict the consequences of a particular behavior provides an enormous advantage in a variable and dynamic environment. Many organisms are faced with various situations that require them to be able to distinguish whether or not a specific behavior is beneficial or detrimental (Buston, 2004b). For example, it is vital for an organism to be able to discriminate between stimuli that are indicative of either predator or prey (Longley 1917). It is evident then that being able to distinguish stimuli, as well as associate an outcome of a behavior when responding to a stimulus, will be favored by natural selection (Longley 1917).

In order to understand the mechanisms involved with this behavior, many studies of terrestrial organisms have employed operant conditioning experiments in which an organism learns to associate a behavior with a specific outcome. Organisms ranging from passerine bird species, *Motacilla flava* and *Luscinia svecica*, to wasps, *Polybia occidentalis* have shown that they are able distinguish between stimuli and associate a particular reward with a stimulus (Peiponen 1992, Shafir 1996). More specifically, in these studies the organisms demonstrated that they were able to distinguish between color stimuli (Peiponen 1992, Shafir 1996). How applicable these findings are to marine organisms that live in a different light environment remains unclear.

Discrimination of color has been shown in a coral reef fish, *Pomacentrus amboinesis*, in which they were able to discriminate between two colors and receive and reward (Siebeck et al., 2008), though the ecological relevance of this finding was not investigated. The clown anemonefish *Amphiprion percula* are an excellent species for expanding studies on color discrimination because their social and ecological context is well understood. Clownfish have a size-based dominance hierarchy and individual recognition may be important for maintaining that hierarchy (Buston 2004a). Individual recognition, in turn, would be facilitated if clownfish could discriminate and learn colors – the fish exhibit highly variable, conspicuous coloration that may be a signal of individual recognition. Thus, studying color recognition is essential for a deeper understanding of these social interactions (Kelber et al., 2003).

Demonstrating that *A. percula* are able to discriminate between ecologically relevant colored stimuli will allow for a deeper understanding of the role their bright coloration plays in a social and evolutionary context (Longley 1917). Their color patterns include patches of white, black and orange, and there are multiple hypotheses for the function of these patterns, e.g., individual recognition signaling, social status signaling. All of these functional hypotheses rely on the assumption that the fish can learn and discriminate among the colors. I will test this fundamental assumption.

Moreover, demonstrating that *A. percula* can be trained to a specific feeding device will allow the Buston lab to rest various models on their social behavior

(Buston 2004a, *ibid*. 2004b). In particular, since they are monogamous and distribute biparental care, being able to feed each individual parent a different amount of food will allow for future research on parental care negotiation.

In my research I will be testing the following hypothesis: **can *A. percula* discriminate between two distinctly colored stimuli to receive a reward?** To test this hypothesis, I will create a feeding apparatus that allows me to reward an individual for selecting a desired color, collect data based on the number of successful and unsuccessful trails, and analyze whether the data supports or rejects my hypothesis.

**What methods will be used?**

This section should be about 100-500 words. It should begin with a clear "if-then" sentence as follows: "If (restate the hypothesis) is true, then (state what will happen if you perform this experiment)." This is followed by a brief discussion of the methods being employed, needed controls, etc. Be sure to cite any sources for methods or reagents previously developed.

If ***A. percula* can discriminate between two distinctly colored stimuli to receive a reward, then there will be a difference between the groups that favors the trained population.**

In this experiment I will be using pair bonded clownfish from the Buston Laboratory. These clownfish have been housed in the lab for over 2 years. There will be 20 pairs used, each consisting of 1 male and 1 female for a total of 40 clownfish.

The clownfish in this experiment will be fed via a feeding apparatus similar to the one described in Siebeck et al. (2008). The first major component will be the laminated circle shaped sheets that will be attached to the feeding apparatus. These sheets will act as the discriminative stimuli; both sheets will be orange with either a white or black square in the middle. These colors are ecologically relevant since they represent the body color patterns of clownfish, which are orange, black, and white. The food distributing apparatus will consist of a thin tube that is attached to a syringe. Since I will be testing two colors, each color will have its own food distributing apparatus. In order to remove any olfactory cues during the trails, I will fill both tubes with food, but only dispense the food reward for the correct color choice.

Familiarization Phase

The first step will be to train the clownfish to feed from the apparatus; I will do this as in Siebeck et al. (2008). Currently, the clownfish tap a pipette in order to obtain food. This means that the time it will take to train them to feed from the apparatus is predicted to be short. I will use the apparatus to feed them for approximately 1 week. This will ensure that they are conditioned.

Stimulus Phase

Once the clownfish are familiarized, I will place on of the laminated circles in front of the apparatus This stimulus will designate where the clownfish will tap to receive food. The clownfish will have to tap the stimulus at least 10 times in order to receive food. They will then perform this task in 10 trials to show that they have formed an association between the food and the stimulus. This association took 2-3 days to form in the Siebeck et al. (2008) experiment; however the learning phase is expected to be shorter since clownfish are already acclimated to captivity.

Conditioning Phase

Once the association has been established, I will present a new laminated circle and the original laminated circle simultaneously. The fish will then be scored on the number of correct responses, where an incorrect response would be to tap the new circle and the correct response would be to tap the original. Incorrect responses will result in no food given.

During the trials, I will insert a partition that will divide the two clownfish to separate parts of the tank. The training side will contain one fish and one anemone. Once both individuals have been trained to different color stimuli, I will put them back together to determine how robust their learning is to extinction in a social context.

**How will the data be collected and analyzed?**

This section should be about 100-500 words. It will require you to describe the format the data will be collected in (e.g., measurements taken through an electron microscope). This could include a description of the expected result if your hypothesis is supported. Be sure to describe how your data will be analyzed so as to confirm or reject any of your hypotheses. This section should end with a description of the next steps for each outcome. Be sure to cite sources for any proposed analytical methods previously developed by other scientists in the field.

The first week will be devoted towards creating the feeding apparatus and attachable colored targets. I will spend the next week acclimating the first to feed from the apparatus, without presenting a color cue. They normally feed from a pipette, so the estimated time for the fish to feed from the apparatus should be relatively short. The next month will be performing the learning trails and collecting data. Since there 20 breeding pairs, I will perform approximately 10 training trials for every breeding pair every day. This would mean that I would be training both of the individuals from each breeding pairs to feed from the feeding apparatus without the color stimulus. Eventually, I will add one color stimulus to the apparatus. Once they are trained to feeding to a color, I will begin scoring them by presenting another color stimulus attached to another feeding apparatus, with one correct color that provides a reward and one incorrect that does not provide a reward. I will perform these discrimination trials 10 times per day for each fish.

These data will be analyzed using appropriate statistical analyses.

**Cited Sources**

The three previous sections (Problem, Methods, Data Analysis) should be well cited for all statements of fact that are not common knowledge and any previously developed methods and reagents. There is no limit to this section. This section should NOT be a simple bibliography list, but rather the citations should be connected to the aforementioned statements in the sections above. The style can be either footnotes, citations numbers, or author-date. You may view citation examples here.


Buston, PM (2004b). Territory inheritance in the clown anemonefish. Proc. R. Soc. Lond. B. (Suppl.) 271: S252-S254.

Buston, PM (2004a). Does the presence of non-breeders enhance the fitness of breeders? An experimental analysis in the clown anemonefish *Amphiprion percula*. Behav. Ecol. And Sociobiol. 57: 23-31.

Kelber, A., Vorobyev, M. and Osorio, D. (2003). Animal colour vision – behavioral tests and physiological concepts. Biol. Rev. 78: 81-118.

Longley, W.H. (1917). Studies upon the biological significance of animal coloration. I. The colors and color changes of West Indian reef-fishes. J. Exp. Zool. 23: 533-601.

Peiponen, V.A. (1992): Colour Discrimination of Two Passerine Bird Species in the Munsell System. Ornis Scandinavia 23: 143-151.

Shafir, Sharoni. (1996): Color Discrimination Conditioning of a Wasp, Polybia Occidentalis (Hymenoptera Vespidae). Biotropica 28.2: 243-251.

Siebeck, U.E., Litherland, L., and Wallis G.M. (2009). Shape learning and discrimination in reef fish. Journal of Experimental Biology 212: 2113-2119.

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