



## **Fluorescent Protein lures of the hydromedusa *Olindias formosa* Attract Prey.**

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### **ABSTRACT**

Fluorescence is a physical process whereby light is absorbed by an atom/molecule and reemitted at a longer, lower energy, wavelength. Fluorescence in the ocean environment is most common in corals and jellies. The biological of this fluorescence is widely unknown. The unique hydromedusae *Olindias formosa* has fluorescent patches at its bell tentacle tips. This fluorescence has an emission maximum at 528 nm. Attraction experiments were done with two different species of *Sebastes*, under blue, yellow, and white illumination conditions. Both the time the fish spent near the medusa and the number of attacks the fish made toward the lures was measured. The data showed an attraction to the fluorescent lures, most notably with the number of attacks made. These data support the prey attraction hypothesis for marine fluorescence.

### **INTRODUCTION**

Fluorescence is a physical process whereby light is absorbed by an atom/molecule and reemitted at a longer, lower energy, wavelength. Many organisms have evolved fluorescent proteins (FP) that use a fluorophore to harness this process. Green fluorescent protein (GFP) from *Aquoria victoria* (Shimomura et al. 1962) and many GFP-like proteins, once cloned, have become valuable tools to monitor gene expression, examine cellular events, observe protein-protein interactions, and DNA sequences (Chalfie et al. 1994, Chalfie 1995, Tsien 1998, Zhang et al. 2002).

In the light limited ocean, bright fluorescent displays are commonly found in many species of corals and jellies. The biological function of these fluorescent displays is largely unknown, and varies with organism. The most common hypotheses for marine fluorescence include camouflage, communication, protection/predator avoidance, and prey attraction.

Camouflage in marine environments is an important method of hiding from predators or catching prey (Wicksten 1989). The bright colors of coral reef fishes, for

example, are believed to serve as good camouflage at a distance, while being conspicuous at close range (Marshall 2000).

At close distance, these same bright colors maybe used as a form of visual communication (Marshall 2000). This communication hypothesis has also been used to explain behavior in the mantis shrimp (Mazel et al. 2004) and in squid (Mathger 2001). These communication experiments, however, are difficult to quantify in the field or lab. Furthermore, the communication hypothesis, is only valid for species that have eyes in order to detect the fluorescence, and would not explain fluorescence in jellies.

Through fluorescence, prey can also mimic coloration representative of danger or toxins to avoid predation (Wicksten 1989). This again is dependent on the visual system of the predator. By shading photosynthetic zooxanthellae from harmful UV radiation, fluorescent proteins in coral reefs may function as photoprotective molecules (Salih et al. 2000, Dove et al. 2001, Ward 2002) (Gilmore et al. 2003).

Fluorescence may also be used as a means to attract prey. Many animals are positively phototactic and will come near abnormal light patterns and sources. Predators can take advantage of this by having fluorescent lures to attract their prey. One such predator is a deep-sea siphonophore with red bioluminescent tentacle tips (Haddock et al. 2005).

The prey attraction hypothesis is also dependent on the visual system of the prey. (cite mianzan) Based on ocular media transmission, most fish are believed to be able to see a range of wavelength some stretching into the UV. Because it completely dissipates at shallow depths, there is very little long wavelength red light in the ocean and most fish are not thought to be able to see red light.

The *Olindias formosa* (Fig. 1) is a unique hydromedusa and one of the many marine organisms to display fluorescence. *O. formosa* is native to the shallow waters off southern Japan. Typically, hydromedusas have tentacles around the rim of the swimming bell. In addition to these, *O. formosa* has a unique set of tentacles that actually protrude directly from the entire surface of the bell, not just the rim. These bell tentacles, with functional nematocysts, have fluorescent patches adjacent to a pink chromoprotein at the tips (Fig.2). The biological function of this fluorescence is unknown. The typical behavior of *O. formosa* is to remain perfectly still on the sea floor with bell tentacles outstretched moving only slightly with the current. Because of this odd behavior the rare fluorescent tentacle tips are believed to act as lures to attract prey. A similar hypothesis was proposed for *O. tenuis*, (Larson 1986) but no study has ever quantitatively tested this hypothesis. This study examines the proposed attraction of larval stages of two species of *Sebastes* (???) and *Sebastes diploproa* (Fig. 3) to these fluorescent lures.

## **MATERIALS AND METHODS**

### **SPECTRA COLLECTIONS**

The spectra of the blue, yellow, and white LEDs were measured using a USB2G356 spectrometer with an integration time of 10 milliseconds. The emission peak of the FP was measured from whole tissue tentacle tips once removed from the bell of *O. formosa*. The FP was excited using a blue LED with maximum output at 479.1 nm.

## SEBASTES ATTRACTION EXPERIMENTS

Custom-built tanks (33.75 cm x 18.75 cm x 22.5 cm) were used. Three sides were black, and they had black lids with a total of 8 holes for different LEDs. LEDs were evenly distributed in order to uniformly illuminate the tank. This best mimicked the natural setting (both fish and jelly illuminated), and also ensured the observer could see the fish at all times. These tests were carried out with larval eastern Pacific *Sebastes* and *Sebastes diploproa*, both obtained from Shane Anderson at UCSB. The natural prey of *O. formosa* is unknown, but because of similar habitat characteristics and their visual feeding habits, these larval *Sebastes* are a reasonable substitute. Because of ethical concerns and scarcity of experimental organisms, actual feeding on the fish by *O. formosa* was not allowed. Instead, a clear barrier was put in place so the fish could see the medusa, but not come into actual contact. The barrier was put into place so that the tank was divided into two volumes with a ratio of 3:1. The larger volume side was the fish side, and the smaller was the jellyfish side. A trial consisted of placing a fish into its side of the tank, turning on the LEDs and watching the behavior of the fish. Each trial was 15 minutes long, and was performed under dark room conditions. The time the fish spent in the region of interest (ROI) (Fig. 4) was measured, along with the number of times the fish attacked the clear barrier. An attack was defined to be a fast burst speed or swimming energy directed at the clear barrier cutting the fish off from the medusa side of the tank; in most cases the mouth was open. These tests were done under blue (479.1 nm), yellow (566.2 nm), and white lighting illumination. Blue light excited the fluorescence and best mimics the color of the natural ocean environment. Yellow light did not excite the fluorescent, but did illuminate the tank and jelly. White light excited the fluorescence and also fully illuminated the tank and entire body of the medusa. Tests under each different lighting scheme were conducted both with and without a medusa present. This was to record the fish's typical behavior with out the distraction of the medusa. For tests with a jelly present, the jelly was placed into its side of the tank and allowed to settle into its typical "fishing" behavior (not swimming, tentacles out stretched, dome shaped on bottom) before the test began, typically 30 minutes. Commonly the fish would sit still in one area of the tank; to account for this, experimental time was not started until the fish crossed the halfway mark of the tank to signal active swimming. Time measurements and counts of attacks were then averaged over the multiple trials and error bars reported are the standard error of the mean.

## RESULTS

### SPECTRA COLLECTION

The blue LED shows a maximum emission at 479.1 nm and the yellow LED at 566.22 nm. The white LED showed two peaks, a sharp peak at 463.5 nm and a broader less intense peak at 548.7 nm (Fig. 5). The spectrum of the fluorescence from whole tissue tentacles shows a peak fluorescent emission at 528.7 nm (Fig. 6).

## PREY ATTRACTION EXPERIMENTS

Time Spent Data: After averaging over all the different trials, the fraction of time spent in the ROI ( $t_{roi}$ ) under control conditions (no jelly present) for the different lighting schemes are as follows. For blue light (479.1 nm) with  $n=13$ ,  $t_{roi} = 30 \pm 2\%$ . For yellow light (566.22 nm) with  $n=9$ ,  $t_{roi} = 38 \pm 8$ . And for white light with  $n=9$ ,  $t_{roi} = 42 \pm 9\%$ . Once the jelly was added,  $t_{roi}$  for the different lighting schemes changed as follows. For blue light with  $n=15$   $t_{roi} = 50 \pm 5\%$ . For yellow light with  $n=9$ ,  $t_{roi} = 34 \pm 9\%$ . For white light with  $n=9$   $t_{roi} = 32 \pm 7\%$ . The increase in time spent in the region of interest under the blue light conditions is 20%. Due to large error bars, however, this is not a significant difference. These data are summarized in figure 7.

Attack Data: The number of times the fish attacked the clear barrier ( $N_a$ ) was also measured. Under control conditions, the  $N_a$  for the different colors is as follows. For blue light with  $n=13$ ,  $N_a = 2.7 \pm .4$ . For yellow with  $n=9$ ,  $N_a = 3 \pm 1$ . And for white with  $n=9$ ,  $N_a = .7 \pm .5$ . When the jelly was added these data changed to the following. For blue light, with  $n=15$   $N_a = 8 \pm 2$ . For yellow with  $n=9$ ,  $N_a = 2 \pm 1$ . And for white with  $n=9$ ,  $N_a = .4 \pm .2$ . This indicates over a three-fold increase in  $N_a$  when fluorescence is present (blue light). These data are summarized in figure 8.

## DISCUSSION

These data show an attraction to the fluorescent lures, the  $N_a$  data more so than the  $t_{roi}$ . The  $t_{roi}$  data does show a mild attraction to the lures, although not significantly so. One possible explanation for this lack of time spent in the ROI under fluorescent conditions can be explained by the fish's attack behavior. The fish would see the lures from outside the ROI, make a quick attack entering the ROI, the barrier would block the attack, and the fish would retreat to a safe distance, outside the ROI, and repeat this cycle. Under this attack cycle, the fish would not spend significantly more time in the ROI from control conditions.

The  $N_a$  for blue light conditions, the best approximation to the ocean, does show a significant increase in the number of attacks the fish made at the barrier. Under blue light conditions, the visibility of the body of the medusa is minimized, and appears mostly transparent. The fluorescent patch would appear to be a free-floating particle that the fish could mistake for food, thus the increase in the number of attacks under blue light.

The reason why the fish did not attack the fluorescent lures under white illumination, even when fluorescence was present, may be that the entire body of the medusa was illuminated, along with the stalk that holds the lure. The pink chromoprotein was also visible under white light; however, the fish do not have full color vision, especially in the red/pink wavelength range, and this tip most likely would appear as black or dark to the fish. So under white light, both the tentacle stalk and some form of chromoprotein tip are visible, the fish would not see a bright free-floating speck that appeared to be food, and not attack.

A lure is most effective when it resembles something appealing, in this case, a free-floating particle of food. Thus, as these data support, not only is fluorescence important, but more so, the appearance that the fluorescent patch is isolated from the body of the jelly and tentacle stalk.

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