CHANGE IN RATE OF SWIMMING CONTRACTIONS AND BEHAVIORAL PATTERNS OF *POLYORCHIS PENICILLATUS* IN RESPONSE TO DIFFERENT WAVELENGTHS OF LIGHT

by

SYDNEY WEINANDT

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Approved: *Craig Young, Ph.D.*
Primary Thesis Advisor

Cnidarians have among the most primitive nervous systems, yet they also display diverse behavioral patterns. Previous studies have shown that the hydromedusa *Polyorchis penicillatus* has a light response in which bell contractions increase as light intensity decreases. The role of wavelength had not been studied previously in relation to behavioral photoresponses for *P. penicillatus*. This experiment tested 5 wavelengths of light including blue, red, orange, green, and white light, in relation to rate of swimming contraction and behavioral patterns. Darkness was used as a control. Consistently, exposure to blue, red, and white light stimuli resulted in high proportions of the two non-feeding behaviors. Green and orange light stimuli elicited high proportions of the two feeding behaviors. Results are consistent with the idea that *P. penicillatus* is able to sense different wavelengths through the water column, and therefore engage in feeding behavior in waters with high food concentrations.

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Introduction

The phylum Cnidaria contains organisms such as corals, sea anemones, jellyfish, and their relatives. This phylum includes four classes: Anthozoa, Scyphozoa, Cubozoa, and Hydrozoa. Anthozoans remain polyps throughout their entire life cycle, whereas Scyphozoa, Cubozoa, and Hydrozoa all have a medusa phase. Scyphozoa and Cubozoa have life cycles dominated by medusa stages, whereas most Hydrozoans have a life cycle alternating from the more dominant benthic, colonial polyp phase to a short free swimming medusa phase. Within Hydrozoa, we can further look into the order Anthoathecata and suborder Capitata, to find the family Corynidae, which contains *Polyorchis penicillatus* (Schuchert 2022). In the literature, *P. penicillatus* has generally been classified in the family Polyorchidae which was determined to be synonymous with the family Corynidae in 2010 (Schuchert 2022).

Polyorchis penicillatus, commonly known as Red-eyed Jellyfish, are found in the Pacific Ocean from the Aleutian Islands, Alaska down to the Sea of Cortez, Mexico (Cowles 2006). This species is most often found in the lower meters of the coastal water column, near eelgrass, which supports an environment abundant with its preferred foods. *Polyorchis penicillatus* consumes benthic crustaceans such as tanaids and amphipods, as well as planktonic ones, such as copepods (Arkett 1984). *Polyorchis penicillatus* is a transparent white, gelatinous organism with a bell at least as tall as it is wide. This species can reach approximately 60 millimeters in length, and its shape can vary significantly (Mills 2007). Between 100 and 200 tentacles extend individually from the ring canal. These tentacles are unbranched and can extend and contract when swimming or drifting (Piazzola and Hiebert 2015). Ocelli are purple, red, or brown eyespots, which are also connected to the ring canal. At the center of the bell, the manubrium extends from the peduncle to the mouth, which has 4 oral lips (Piazzola and Hiebert 2015). Cnidocytes, or stinging cells, are

located on both the manubrium and tentacles. Cnidocytes are important in *P. penicillatus* for prey capture.

This species has two distinct feeding mechanisms depending on its location in the water column. While on the bottom, individuals will perch on their tentacles, often touching the sediment with the manubrium (Mills 1981). While floating in the water column, *P. penicillatus* uses sink fishing, where the tentacles are extended, and the position is either maintained or the individual sinks slowly until prey comes into contact with its tentacles. Then, the tentacles are retracted to bring the prey to the manubrium. Two distinct non-feeding behaviors can be observed in *P. penicillatus*. Crumpling is most frequently described as a protective behavior, in which tentacles are retracted and sinking occurs (King & Spencer 1981). *Polyorchis penicillatus* can also be observed executing consistent swimming contractions, resulting in significant displacement from the original location.

While *Polyorchis penicillatus* is most often found near the bottom during the day, they have a vertical diel migration pattern as they migrate up at night, dispersing throughout the water column. This species also demonstrates a light response, similar to other Cnidarians, showing a burst of swimming contractions after rapid changes in light. This is thought to be linked to their diel migration, as the reaction does not correspond with a general predator escape response as seen in other organisms (Arkett 1984). This light response is thought to be facilitated by the photoreceptive organs, called ocelli, and their perception of light intensity (Piazzola and Hiebert 2015). Photoreceptors respond to light intensity changes with graded depolarizations or graded hyperpolarizations, each of which are directly proportional to the change in light level (Martin 2002). Cnidarian ocelli consist of pigment cups, which are complex structures composed of lens,

cornea, and retina, and eye spots (Martin 2002). These structures vary in shape and size among different species of Cnidarians.

Ocelli are composed of two types of cells: supportive and sensory. Supportive cells are much larger than the sensory cells and are pigmented by smaller granules (Eakin & Westfall 1962). Sensory cells are spindle shaped, narrow structures, that are slightly swollen at the base from which a nerve fiber extends (Eakin & Westfall 1962). Ocelli are evenly spaced along the ring canal of *P. penicillatus*, coinciding with the base of a tentacle. The paired ocellar nerves cross over the base of the tentacles, and connect to the outer nerve ring (Lin, Gallin, & Spencer 2001). The eyecups, which are underneath the visible pigmented lens, are composed of cylindrically shaped receptors and melanocytes, which are arranged radially (Eakin & Westfall 1962). Melanocytes are much larger and more abundant in the epithelium, or the outermost layer of the jellyfish bell. These supportive pigmented cells are found on either side of the receptor cells. The end of the receptor cell supports many connected microvilli, which intermingle with other structures. Microvilli are microscopic membrane protrusions, similar to cilia but immotile. Within the microvilli exists a structural fibrillar system with 9 peripheral filaments and 2 central filaments (Eakin, & Westfall 1962). Many organelles also intermingle with this structure.

The ocellar nerve fibers descend into the outer nerve ring. This is where the oscillating system (O system) and the buster system (B system) are centralized. With photic stimulation, simultaneous neuronal spikes can be seen in the B system, which suggests electron coupling (Spencer & Arkett 1984). With the decrease of light intensity, spontaneous oscillations of the membrane potential in the O system stop simultaneously, also suggesting electron coupling (Spencer, & Arkett 1984). Additionally, this shows that light stimulation perceived by one

individual ocellus is communicated to the entire individual through the nervous system, as only one ocellus was exposed to the stimulus in this experiment.

While many studies of light responses in hydromedusa exist, few studies have documented the response of *Polyorchis penicillatus* to different wavelengths of light (Arkett 1985). This prior study observed spectral sensitivity paired with a shadow response and did not observe extended exposure response or behavioral responses (Arkett 1985). The observation of this system has the potential to contribute to knowledge about how these systems have evolved and what the organism's behavior may imply from an ecological standpoint. To address this gap in knowledge, it is important to observe the change in swimming rate with each wavelength, but also to determine if the behavior is similar after the light exposure, and how behavior changes with each wavelength of light. I hypothesize that the swimming rate will increase with blue light compared to the other light stimuli, as this is the wavelength that protrudes deepest into the water column. I expect that feeding behavior will increase with orange and green light as compared to red and blue light due to the closer similarity to a yellow wavelength. I also expect that the high levels of variation will be seen across individual specimen tests within a single light stimulus.

Methods

This experiment consisted of 6 trials of different wavelengths of light, all of which were repeated with different specimens, resulting in 3 full runs. Specimens of approximately the same size were collected from the Outer Boat Basin of the Charleston Marina (Figure 1). Sixteen individuals were collected for run 1, 11 for run 2, and 12 for run 3. During non-testing periods, specimens were kept in a 10-gallon tank in a seawater table with flowing water and natural light cycles. Medusae were fed with brine shrimp only after testing was completed and were not tested again until 10 hours after feeding. Light testing occurred in a completely dark room that was temperature controlled between 10° C and 13° C. In the darkened room was a 10-gallon tank with still water that was replaced every 3 hours. Specimens underwent trials in varying wavelengths of light: none (dark), white, red, orange, green, and blue.

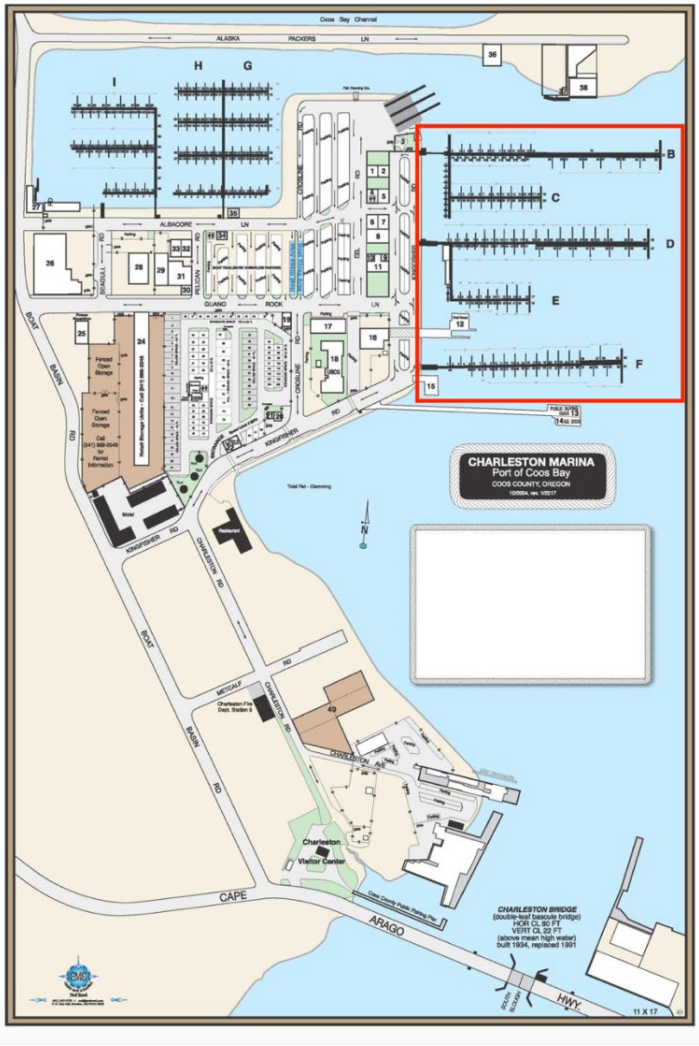

Figure 1. Diagrammatic map of the Charleston Marina. A red box encloses the area defined as the Outer Boat Basin, where experimental animals were collected. Image credit: 2020 Oregon International Port of Coos Bay.

Wavelengths of the light filters were determined before testing along with the wavelengths of the water collected from the harbor (Figure 2) using a spectrophotometer. Light was projected onto the tank using a Kodak 4600 slide projector. Light intensity was controlled for by adjusting the distance between the projector and tank, and by using grey window mesh as a neutral density filter to reduce light intensity. Light intensity was approximated during the

experiment by using the Photone (Version 3.2.6) light meter application on a cell phone, which measured lux. Afterwards, powers of each wavelength of light in Watts were measured both with and without the filters using a Powermax USB PS10Q sensor from a close distance (0.036m). Using these measurements, light intensities were found by dividing power by the surface area of the projector lamp. Then, the measurement was scaled using the Inverse Square Law, $\frac{I_1}{I_2} = \frac{d_2^2}{d_1^2}$. Finally, a ratio was created of the mesh covered light filters to the uncovered light filters (Table 1). Different wavelengths have different intensities due to the number of rays within each wavelength per second. Because of this, a ratio is necessary for comparison of the different wavelength filters.

Color/ wavelength of filter	**Distance from tank (cm)**	**Power of filter with mesh (W)**	**Power of filter without mesh (W)**	**Ratio of intensities**
Red (670 nm)	200	0.0079	0.0366	0.22
Orange (660 nm)	200	0.0118	0.0708	0.17
Blue (490 nm)	190.6	0.0051	0.0160	0.31
Green (530 nm)	200	0.0033	0.0143	0.23
White	190.6	0.0105	0.1290	0.08

Table 1. This table shows the distances from which the trials were completed, the measurement of power for each light filter with and without the mesh, and the ratio of the calculated light intensities. Power was measured from 0.036 m away.

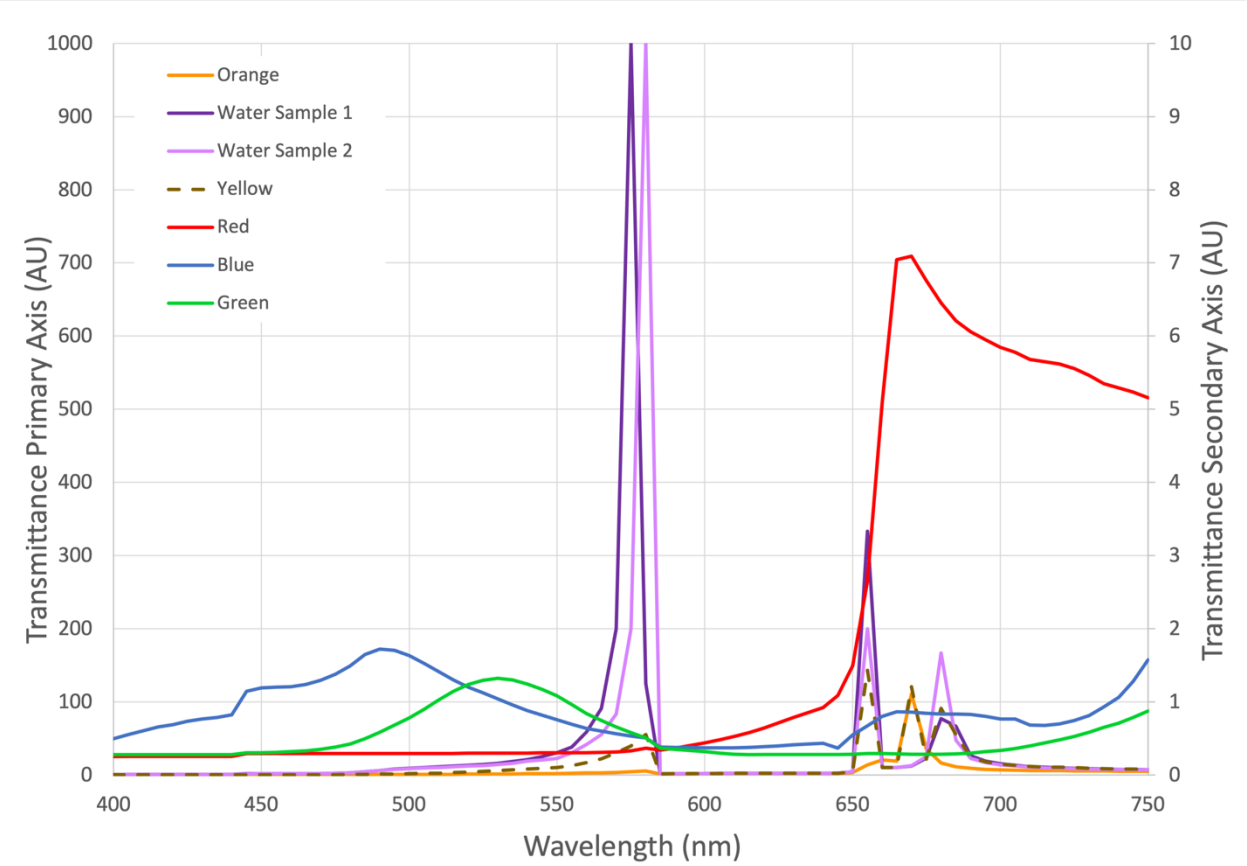

Figure 2. Transmittance of light measured by a spectrophotometer on each light filter and two samples of marina water. Values for the green, blue, and red light filters are placed on the secondary y-axis (right side), while values for orange, water sample 1, water sample 2, and yellow are on the primary y-axis (left side). Yellow light was not used in the experimental procedures but is presented for comparison.

Each individual medusa was tested individually for each treatment. No specimen was exposed to two types of light in one day. Each individual was given a 15-minute acclimation period in the darkened room prior to light testing. Light stimulus was given for 5 minutes immediately following the acclimation period. Four types of behavior were observed and documented: perching, sink-fishing, swimming, and crumpling. Initial response to light exposure (in the first minute) was recorded. Immediately after the light stimulus was turned off, swimming

contractions were counted for 2 minutes, and behavior was recorded. Specimens were kept separately following each test to ensure no individual was repeated for the same stimulus.

The data sheet contains the information from these trials- specimen count, date, trial, number of swimming contractions per 2 minutes, number of swimming contractions per minute, observed behavior in first minute of exposure, observed behavior after exposure, and behavior classification (feeding or nonfeeding). The data were originally collected in separate data sheets for each light stimulus and combined into one data sheet for analysis. Data and statistics were analyzed using R. Helmert contrast coding and chi squared analyses were run. Data are available at

https://www.dropbox.com/s/t479ah38k0mlu98/Final%20Project%20Thesis%20Data.xlsx?dl=0

Results

Light intensity ratios showed some variation across each wavelength of light. The ratios ranged from 0.08 with white light to 0.31 with blue. While the difference between these two is only 0.23, this is a relatively significant number. Red and orange wavelengths showed a 0.05 unit difference between each other (Table 1). Blue and green wavelengths showed a 0.08 unit difference between each other (Table 1). White light was the most dissimilar from the other stimuli's light intensity ratios.

Within each light stimulus, clear behavior patterns were observed. Green and orange light stimuli each elicited high counts of sink fishing and perching behavior (Figure 3). Blue and red light stimuli elicited higher counts of swimming and crumpling compared to green and orange light stimuli (Figure 3). The white light stimulus exhibited the highest counts of swimming behavior (Figure 3). While results showed a lower rate of swimming contraction with exposure to red light, the proportion of individuals exhibiting swimming behavior was higher than both green and orange light stimuli (Figure 3 and Figure 4). The red light stimulus also elicited the highest count of crumpling behavior compared to all other stimuli (Figure 3). Pearson's Chi-squared test showed that there is a statistical difference between the light stimuli results in regards to feeding behavior versus non-feeding behavior ($p < 0.0001$). Within this test, it was evident that there was significantly more non-feeding behavior with blue light stimulus, with 20/38 individuals demonstrating non-feeding behavior, than green light stimulus with 7/37 individuals. Similarly, it was evident that there was significantly more non-feeding behavior with red light stimulus as compared to orange light stimulus, showing 20/38 and 9/35 non-feeding individuals respectively. Discrepancy in the total number of individuals is due to deaths of

specimens during trials. White light stimulus elicited mostly non-feeding behavior responses, with 73.5% of individuals not engaging in feeding behavior.

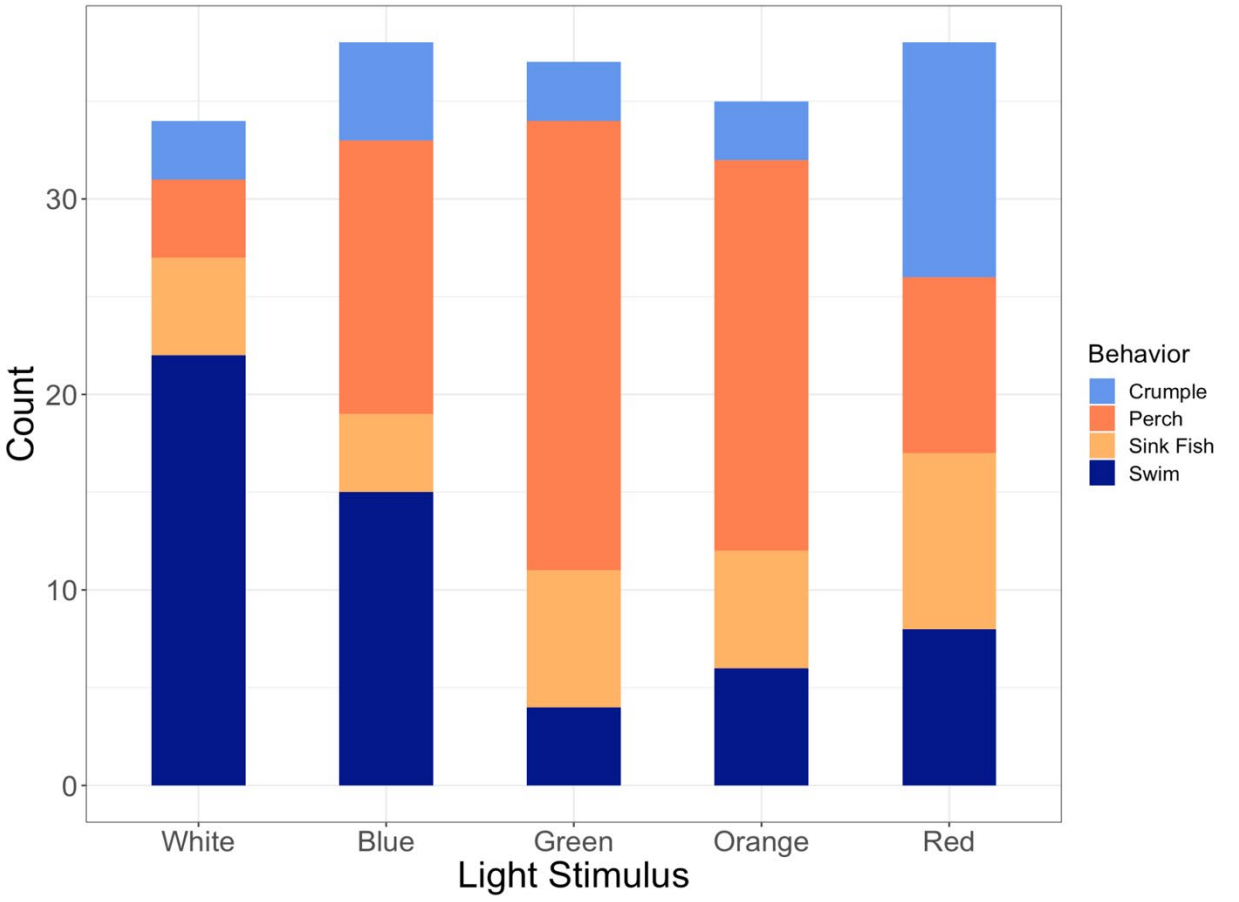

Figure 3. Number of times each behavior was observed at each wavelength across all individual tests.

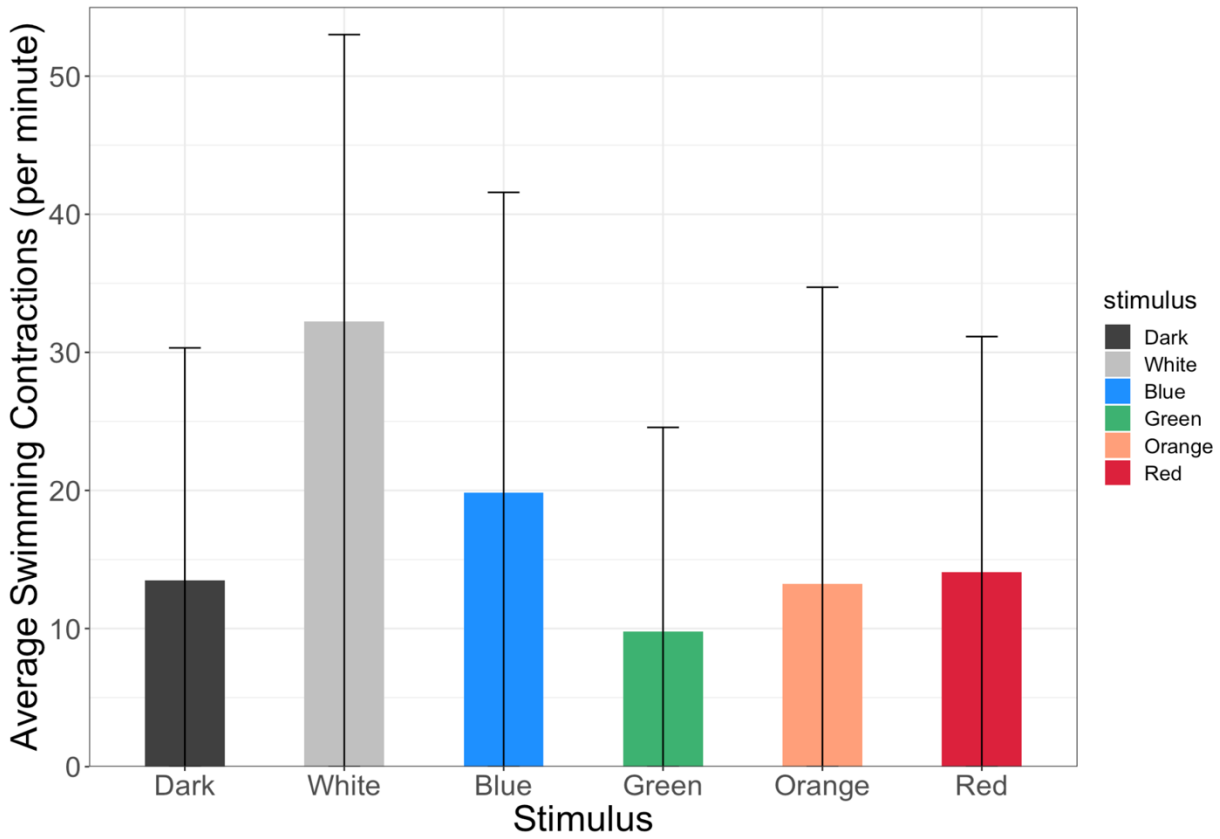

Figure 4. Average swimming contractions per minute within each color stimulus. Standard errors across the individual tests are represented at the top of each bar.

There were high levels of variation across individual tests of swimming contraction rate with each light stimulus, including the control variable of darkness. Despite variation, a clear density of swimming contraction rates can be observed with each stimulus (Figure 5). The average rate of swimming contraction was highest with white light stimulus at 32 swimming contractions per minute and lowest with green light stimulus at 10 swimming contractions per minute (Figure 4). The dark control stimulus was the second lowest average at 13 swimming contractions per minute (Figure 4). Helmert contrast coding showed that there was a mean difference of 18 swimming contractions between dark and white stimuli ($F = 18.05$, $p < 0.0001$). The swimming rate was higher with blue light stimulus, however, red light stimulus resulted in a

similar swimming rate to the dark control (Figure 4). There was a statistical difference in rate of swimming contractions of the higher rate white, red, and blue stimuli compared to lower rate orange, green, and dark stimuli ($F = 14.44$, $p < 0.001$).

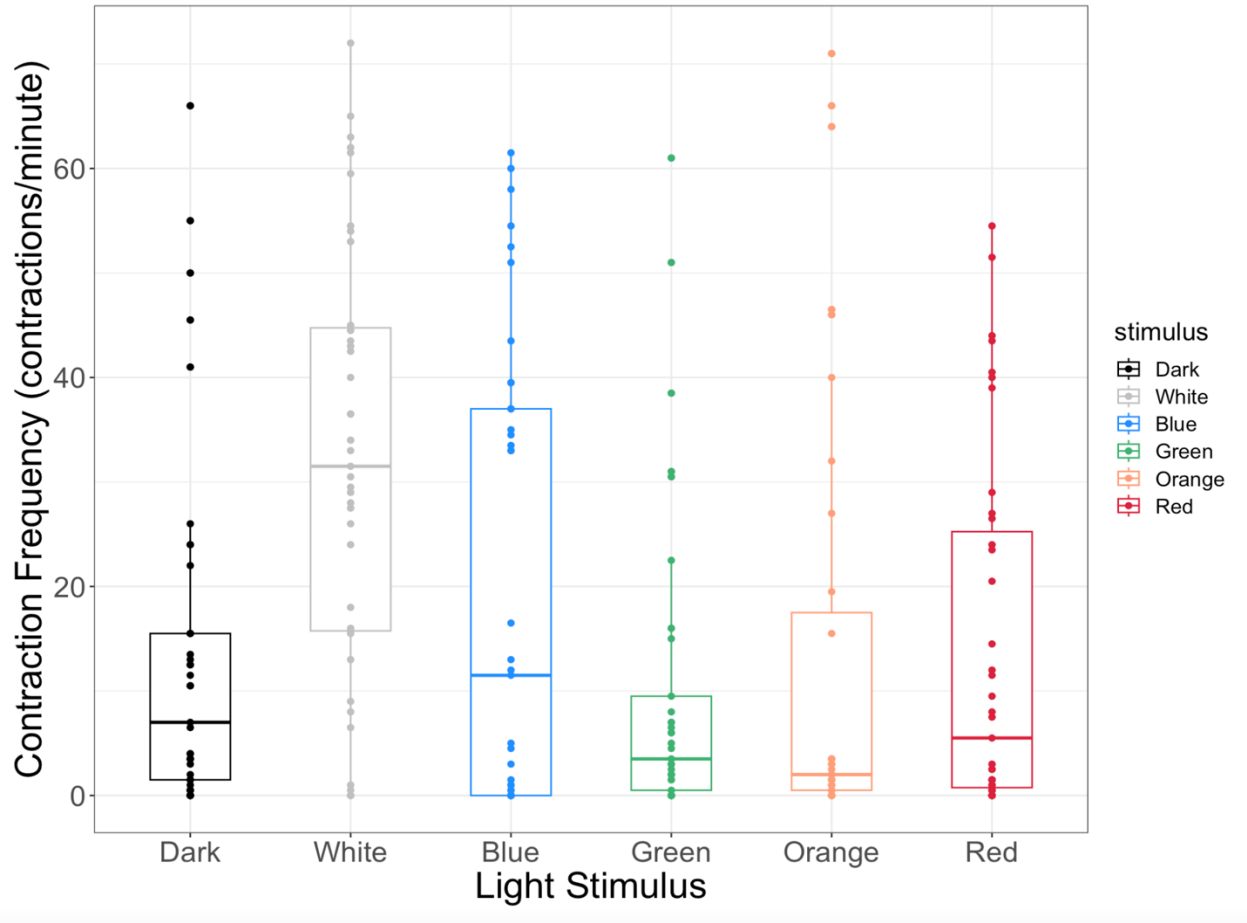

Figure 5. Distribution of swimming contractions per minute observed across tests of individual *P. penicillatus*, separated by light stimulus. Each box shows the interquartile range of the individual swimming contractions frequencies, with the line that splits each box shows the median of the data. Each individual point represents an observation of swimming contraction frequency. Vertical lines show the upper adjacent value, with the remaining points representing outliers.

Discussion

Overall, these results show a difference among wavelengths in average rate of swimming contraction per minute, but more importantly they show qualitative differences in behavior among light stimuli. Within each light stimulus, high levels of variation can be seen in measurements of swimming contraction rate. Some variation could be attributed to the relative sizes of the test animals, as smaller *P. penicillatus* have been observed to maintain higher rates of swimming (Arkett 1984). The average rate of swimming contractions correlated with the proportion of swimming behavior, as it was observed that there were higher average rates in the stimuli with the higher counts of swimming individuals.

Moving down through the water column, red light is the wavelength that penetrates the least, with the blue light wavelength penetrating the furthest. Behaviorally it would make sense for *P. penicillatus* to have a higher swimming contraction rate after blue light exposure, as in the water column this would indicate more space to swim upwards. Additionally, during sunset periods, a shift towards shorter wavelengths occurs in the water column, however the large amount of detrital matter in the water would likely shift the transmission towards green (Arkett 1985). If *P. penicillatus* were to migrate upwards solely based on the sunset cue as previously hypothesized, results should show a higher proportion of swimming behavior upon green light exposure as well as blue light exposure. However, this study shows significantly higher proportions of perching and sink-fishing upon green light exposure. Zooplankton, the prey of *P. penicillatus* can be mixed in with detrital matter and make up a yellow color, similarly shifting the absorption of light to more of a green wavelength. With this context, it seems that *P. penicillatus* swim when exposed to a wavelength that indicates low food concentration and prioritize feeding when exposed to a wavelength that indicates higher food concentration.

The crumpling behavior was seen frequently in response to red light stimulus. Crumpling has been cited with many different potential causations, but overall, the behavior consists of a cease in swimming contractions while the tentacles are pulled into the bell (Hyman 1940). Crumpling behavior results in sinking for the individual. As red light is transmitted only into the shallowest depths of the water column, it could indicate that the swimming space is below the individual. The orange light stimulus, the wavelength falling between red and yellow on the visible light spectrum, shows low proportions of crumpling and swimming behavior and high proportions of each feeding behavior. This result coordinates with the resulting difference between green and blue stimuli, indicating that it is possible that *P. penicillatus* exhibit feeding behavior when exposed to a wavelength that indicates higher food concentration, and exhibit non-feeding behavior when exposed to a wavelength that indicates lower food concentration.

More testing with a higher sample size is necessary to ensure that these trends are consistent across *P. penicillatus*. During the experiments, some injuries and illnesses occurred within individuals during each trial. Injured specimens were not tested again until they returned to health. With a higher sample size, instances of injury will have a smaller effect on the data in addition to reinforcing any trend that occurs. It is also possible that the difference in light intensity between trials influenced behavior. Previous studies have only shown a difference in swimming rate with logarithmic differences between light intensities (Arkett 1985), so it is unclear what effect these smaller differences could have in this experiment. Additionally, a taller tank with a treadmill as used in prior studies would be beneficial to ensure that behavioral reactions are not occurring due to individuals touching the tank (Arkett 1985). If repeated, this study could also be expanded to break up the data by different sized individuals to reaffirm that smaller *P. penicillatus* have a higher rate of swimming contraction.

These results demonstrate a clear difference in behavior in response to different wavelengths. The dark and white light control data aligns with previous studies (Arkett 1985) showing consistent maintenance and stimulated swimming contraction rates. The average swimming contraction rate does appear with large error bars which is likely due to the large amounts of variation in individual testing. Consistently, exposure to blue, red, and white light stimuli resulted in high proportions of the two non-feeding behaviors. Green and orange light stimuli showed high proportions of the two feeding behaviors. This result is consistent with the idea that *P. penicillatus* is able to sense each of the different wavelengths through the water column, and therefore engages in feeding behavior around wavelengths where food concentrations might be higher. *Polyorchis penicillatus* are constantly hunting and feeding, and therefore with this logic would be searching for high food concentrations by moving up and down through the water column, as the results show them engaging in this behavior with red and blue light stimuli. Demersal zooplankton are known to migrate up and down through the water column predictably at night, fluctuating with lunar cycles (Alldredge & King 1980). It is commonly accepted that *P. penicillatus* also undergo diel vertical migrations to follow their prey (Arkett 1984; Larson 1986; Piazzola & Hierbert 2015). The results of the present study justify further investigation to determine the cause of the vertical diel migration in *P. penicillatus*.

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