

# TOUCH, SHADOW, AND LIGHT: A COMPARATIVE ANALYSIS OF SENSORY SYSTEMS AND STRESS RESPONSES IN *SPIROBRANCHUS GIGANTEUS* ON MO'OREA (FRENCH POLYNESIA)

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**Abstract.** The sessile polychaete worm *Spirobranchus giganteus* is a filter feeder that retracts into its calcareous tube under stress from threats and predators. When retracted, it cannot use its branchial crown for breathing or feeding purposes, creating a cost-benefit balance between obtaining resources and protecting itself from harm. Stimulating different sensory organs via three different tests produced different reemergence times depending on the threat level induced by each test and the flow rate environment the test was performed in. This study focused on stimulating either the tactile sensory system or visual sensory system. Both shadows and light were used to stimulate the visual sensory system. The tactile tests induced the largest proportion of hiding and the longest reemergence times, with the visual tests using shadows inducing a much smaller proportion of hiding and shorter reemergence times. The visual light tests were inconclusive. High flow rate environments also produced longer reemergence times, whereas low flow rate environments produced shorter reemergence times.

**Key words:** Christmas tree worm; *Spirobranchus giganteus*; reemergence time; flow rate; Mo'orea, French Polynesia; stress

## INTRODUCTION

The investigation of sensory systems different from our own reveals valuable insight into evolutionary patterns that have created variations in species' interactions and responses to their environment. Newly developed traits are only evolutionarily conserved if they increase an individual's fitness for their particular environmental circumstances, thus an organism's morphology is a product of their environment (Krebs and Davies 1993). In order to accurately respond to the surrounding environment, an organism must be able to sense and interpret it to a functional degree. The sensory systems of an organism must benefit and contribute to increased survival and fitness. If a sensory system is too energetically costly for the benefit it provides, it will be selected against until the

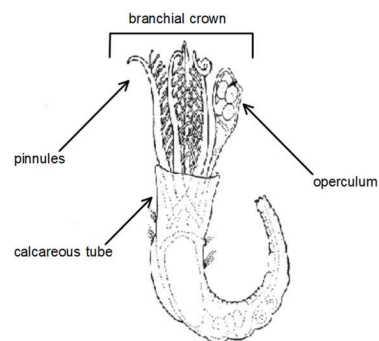


FIG. 1. *Spirobranchus giganteus* anatomy.

benefit and cost are balanced (Niven and Laughlin 2008).

The serpulid polychaete worm *Spirobranchus giganteus* (Pallas 1776) is an invertebrate obligate associate of corals that can interpret both tactile and visual stimuli, maximizing its ability to avoid predation (Hunte *et al.* 1990, Pezner *et al.* 2017, Bok *et al.*

2017). Christmas tree worms have two feather-like whorled stalks composing a branchial crown that extends outside of its burrow for foraging and breathing purposes (Strathmann *et al.* 1984). The branchial crown is the only visible part of the worm, but an abdomen comprising more than half of the worm's total body weight is hidden within the calcareous tube inside the coral (Hunte *et al.* 1990).

Strathmann *et al.* (1984) found that the branchial crown consists of tentacles made up of ciliated pinnules that draw water and nutrients up from the base of the crown to the tip of the worm, contrary to how most suspension feeders obtain nutrients (Fig. 1). This method of feeding directs waterflow upwards from the coral head, potentially enhancing water circulation to make more nutrients available for the coral polyps around the worm, forming a mutualistic relationship between Christmas tree worms and their host coral (Hunte *et al.* 1990). Additionally, Christmas tree worms have been found to defend the coral from predation by Crown-of-Thorns Starfish (*Acanthaster planci*) as they agitate the underside of the star and prevent it from being able to feed on the coral (DeVantier *et al.* 1986). The worms also create a hard calcium carbonate operculum spine (Fig. 2.) that extends up from their tube and further debilitates the starfish, protecting the coral the worms inhabit (Rippy and Kelleher 2004). The operculum (Fig. 2.), a modified radiole, covers the entrance to the worm's tube when it is withdrawn, acting as a protective plug (Vin



FIG. 2. a. branchial crown, b. operculum, c. operculum spine, d. calcareous tube.

and Tove 2011). When threatened or disturbed, the worm withdraws into its tube, sealing its branchial crown off with its operculum.

Studies have been conducted to evaluate Christmas tree worms' ability to receive and interpret both tactile and visual signals. To test the magnitude of the worms' fear response due to a tactile stressor, a UCLA study on Moorea in 2017 used a 2m rod to prod a subject worm, inducing hiding (Pezner *et al.* 2017). The data collected from these experiments showed that Christmas tree worms have prolonged reemergence times when grouped together in large aggregates with other worms since they are preyed upon more heavily in large groups (Pezner *et al.* 2017).

Despite the increased chance of predation, Christmas tree worms have been found to settle in large aggregates, potentially to facilitate reproduction by increasing their proximity to individuals of the same species because they are broadcast spawners (Hunte *et al.* 1990). When the larvae settle, they do not burrow into the coral, but rather build up and extend their calcium carbonate tube as the coral grows up to encase it, causing it to appear as if the tube was burrowed into the coral (Hunte *et al.* 1990).

Many studies have examined the visual system of Christmas tree worms to find that they possess both simple and compound ocelli eyes on their branchial crowns (Bok *et al.* 2017). Their eyes cannot recognize shapes clearly, but are very susceptible to shadows, aiding in the detection of passing predatory fish (Bok *et al.* 2017). Reef fish that graze on corals have been observed preying on Christmas tree worms. Tangs, surgeonfish, and wrasses have been observed consuming Christmas tree worms, and stomach contents of butterfly fish have been found to contain up to 13% sedentary polychaete worms (Harmelin-Vivien and Bouchon-Navaro 1983, Vine and Bailey-Brock 1984).

J. R. Marsden *et al.* (1984) conducted a study on Christmas tree worm larvae's visual ability in Barbados and Martins Bay. It was found that the larvae responded positively to visible light of wavelengths shorter than 590nm

(Marsden 1984). Additionally, shading played a part in how accurately the larvae responded to light. The visual system of adult Christmas tree worms has been studied extensively by Michael Bok, whose research has shown that the pigment of Christmas tree worm eyes absorbs the maximum amount of light at 464 nm, allowing the radiolar photoreceptors on the feeding tentacles and operculum to sense shadows to trigger a mechanical defensive withdrawal (Bok *et al.* 2017).

Although there have been multiple studies on the sensory systems of Christmas tree worms, few have studied the effect of shadows on their behavior, and even less have cross examined the worms' reactions to tactile and visual stimuli. The purpose of this study is to compare the responses of tactile and visual sensory systems of Christmas tree worms against one another to understand which disturbance produces a longer reemergence time and if flow rate has an additional effect on reemergence time. Because the worms are sacrificing their ability to eat and breathe when they are retracted into their tubes, a prolonged reemergence time will reflect either a greater fear response, a reduced need to feed and breathe due to higher nutrient circulation, or a combination of the two (Pezner *et al.* 2017).

I produced three types of disturbances to potentially elicit hiding behavior: tactile disturbances inside and outside of the calcareous tube's opening, visual disturbances from passing and lingering shadows, and visual disturbances from the appearance and disappearance of different colors of light over the worms. I expected tactile disturbances to produce longer reemergence times than visual disturbances since the former would simulate a direct predatory attack, and the threatening tactile disturbances that contact the calcareous tube to produce the longest reemergence times. Passing shadows were expected to produce longer reemergence times compared to lingering shadows, since they more accurately replicate the danger of a passing predator over the coral. For the visual light tests, yellow light was expected to produce the longest

reemergence times when compared with blue and red light because pigment in Christmas tree worm eyes absorbs maximally at 494nm and effectively screens light less than 550nm to adjust for the natural visual pigment of marine environments (Bok *et al.* 2017). Finally, I expected that reemergence times would be greater in high flow environments than in low flow environments for all field tests, potentially due to increased nutrient circulation and thus a decreased cost to hiding during stressful situations.

I cross examined the collected reemergence times against different factors like the number of surrounding worms and tube size to determine if there are other variables potentially altering the reemergence times. In addition, I conducted a survey of each site to measure the density of worms in each area along with fish, coral, and invertebrate populations at the different sites.

## METHODS

### *Study sites*

Three field sites were selected for this study on Mo'orea, French Polynesia: the reef of Temae Beach, the reef of Public Beach, and a coral head on the fringing reef of Pao Pao Bay. Data was collected between one and five meters deep from October 22<sup>nd</sup> to November 14<sup>th</sup> in 2018.

Temae Beach was selected to serve as a site with a relatively high flow rate with a high density of Christmas tree worms. Public Beach's reef and Pao Pao Bay's fringing reef were selected to represent sites with a relatively low flow rate. Two low flow rate sites were selected because each had a relatively lower density of Christmas tree worms than Temae Beach, the high flow site. Sites with differing flow rates were selected based on a study conducted on Mo'orea (Pezner *et al.* 2017) that highlighted how Christmas tree worms' reemergence times following a physical disturbance are linked to the amount of available nutrients. While the

worms are hidden in their tube, they are also foregoing time that they can eat and breathe (Strathmann *et al.* 1984). When the cost of this loss exceeds the threat of potential predators, the worms reemerge (Pezner *et al.* 2017). Because a higher flow rate would most likely indicate a higher nutrient flow, I was interested in how reemergence times would differ between high and low flow rate sites.

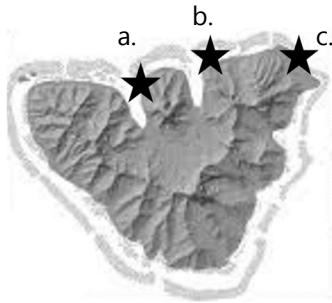


FIG. 3. a. Public Beach, b. Pao Pao fringing reef, c. Temae Beach.

Around twenty data points were taken from both high and low flow rate areas for each field-based test, with each data point signifying a different worm's reemergence time. For the purposes of this study, reemergence time was measured from the moment the worm disappeared into its tube until the moment it returned to its original fully extended state. Transects measuring 30m in length were used at Temae and Public Beach to measure Christmas tree worm density for site selection. For these transects, observations were recorded for one square meter every three meters perpendicular to the shore. Since only one coral head was used for data and observations on the fringing reef of Pao Pao Bay, the surface area of the coral head was calculated and the number of worm on it was recorded instead of conducting a transect measurement.

#### *Tactile field tests*

Two different field tests were conducted to measure the Christmas tree worms' reemergence times in response to a tactile

threat. For both, a metal pipe measuring 17 cm in length, 0.6 cm in diameter, and 0.1 cm in thickness was used to prod the subjects in or near their tube. For the first test, the sampling pipe was inserted into the opening of the calcareous tube and held there for three seconds. While the worm was being sampled, the recorder was at least 60 cm away, and once the worm was sampled, the recorder retreated to at least one meter away. Care was taken not to swim over the worm or propel water in its direction so as not to create any additional disturbance. A stopwatch was used by the same recorder to measure the subject worm's reemergence time following the stressor.

Finally, the number of neighboring Christmas tree worms within 30cm<sup>2</sup> was counted and a ruler was used to measure the diameter of the opening of the calcareous tube. The width of the calcareous tube was measured to determine the age of the worm according to a study whose findings indicated that Christmas tree worms grow 0.2-1.0 mm each year in tube size, correlating tube diameter rather than branchial crown size to age (Nishi and Nishihira 1995). The number of surrounding worms in 30cm<sup>2</sup> was recorded due to a study on Mo'orea which found that Christmas tree worms exhibit longer reemergence time when aggregated in large groups due to a correlated increase in predation levels (Pezner *et al.* 2017). Once a worm was tested, no worms within 30cm<sup>2</sup> were tested for at least five minutes to avoid skewing the results by compounding disturbances.

For the benign tactile test, the sampling and measurements were conducted the same way as the threatening tactile test, except the metal sampling pipe was used to tap the substrate encasing the worm's tube three times three centimeters away from the calcareous tube's opening, rather than prodding inside the tube's opening.

#### *Shadow field tests*

Two additional tests were conducted in the field, but their goal was to measure Christmas

tree worms' reactions to disturbances to their visual sensory system rather than their tactile sensory system. The first test studied the worms' response to passing shadows. To do so, a rectangular, opaque piece of plastic 18 cm in length and 9 cm in width was secured to a clear plastic rod 16 cm in length which was used as a handle. The opaque rectangle was passed forwards and backwards over the worm to create a passing shadow over the subject. The recorder remained at least 60 cm away from the subject and took care never to cast any other shadow over the worm or create any other additional disturbances. If the test disturbance induced hiding behavior, the reemergence time of the subject worm was measured with a stopwatch by the same recorder from at least one meter away. After reemergence time was measured, the tube width and number of worms within 30cm<sup>2</sup> were measured and recorded, just as with the tactile tests.

The second visual field test focused on measuring the effect of lingering shadows on the Christmas tree worms. This was done with the same apparatus utilized to create the passing shadows, but for this test the recorder held the prop casting a shadow over the subject worm for five seconds, taking care to remain 60 cm or more away from the subject. Reemergence time was recorded, if necessary, with a stopwatch by the same recorder from at least one meter away. The same supplementary data listed above were recorded for this test as well. Just as with the tactile tests, no worms within 30cm<sup>2</sup> of the previously sampled worm were tested for at least five minutes to prevent collecting inaccurate data.

#### *Light Laboratory Tests*

Three supplementary tests were conducted in the lab to analyze the visual system of Christmas tree worms. Instead of focusing on shadows, these tests focused on studying the worms' reaction to the presence, and then sudden absence, of light. To do so, two worms embedded in a fragmented section of Porites coral were collected from Temae Beach on

September 22<sup>nd</sup>, 2018. They were taken back to the wet lab on UC Berkeley's Gump Station in Pao Pao Bay and kept alone in a tank with constant water flow directly from the ocean to mimic their previous environmental conditions.

Plastic sheets either left blank or colored red or blue were secured over a dive light to produce blue, red, or yellow light of the same intensity. The collected worms were sampled by holding the light at least 10 cm away and shining one of the three colors of light on the subject for 10 seconds every five minutes. Hour breaks were taken every ten samples to prevent the worms from habituating to the repeated stimulus (Pezner *et al.* 2017). One color light was used for all ten samples in a data collecting period, and only one worm was tested at a time. Measurements of the worms' calcareous tube diameter were also noted.

#### *Statistical Analyses*

To compare the resulting reemergence times from the three different types of tests, the Kruskal-Wallis test was used to determine if the type of stimulus or the flow rate environment had a significant effect on the reemergence times. The Dunn's Post-Hoc test was employed to determine where in the data set the significant differences were found. The Kruskal-Wallis test was also used to compare the means of sub-tests that stimulated the same sensory system to determine if one variation of the test produced a more extreme reaction than another. Lastly, the Kruskal-Wallis test was used to compare the reemergence times for the same test in different flow rate environments. For example, the means of the benign and threatening tactile tests from the same flow rate environment were compared to determine if there was a significant difference between the reaction times produced, and the means from the same tactile sub-test in high or low flow rate environments were compared using the Kruskal-Wallis test.

I was also interested in how natural factors (tube diameter and proximity to others)

influenced the worms' reemergence times. To analyze this, a regression analysis was utilized for these continuous data sets to attempt to uncover any underlying factors affecting reemergence time.

## RESULTS

To determine if perturbations of different sensory organs produced different reemergence times in Christmas tree worms, three tests were carried out and the results were compared against each other. The Kruskal-Wallis test was used to compare the reemergence times for the three tests against one another, and since there was a significant difference in reemergence times discovered ( $p=4.591E-07$ ), the Dunn's Post-Hoc test was used to identify where this significant difference occurred. According to Dunn's Post-Hoc test, the tactile tests had significantly different reemergence times from the visual shadow tests ( $p=3.191E-07$ ), the visual shadow tests had significantly different reemergence times from the visual light tests ( $p=0.0001649$ ), different reemergence times from the visual light tests ( $p=0.9132$ ).

The proportion of sampled worms that hid for each of the sub-tests is shown in Figure 4. Tactile tests induced more hiding than visual tests and the proportion of worms that hid did not vary between different flow rate

environments for threatening or benign tactile tests. Flow rate did alter the proportion of worms that hid for the visual shadow tests, with only 30% of sampled worms hiding due to the induced stress in low flow environments, and up to 45% of sampled worms hiding in high flow environments. The passing shadow test also induced 10% more hiding than lingering shadow tests. The light tests induced the lowest amount of hiding, between 25% and 30%, with very little variation in the proportion of hiding between the different light tests. A chi squared goodness of fit test was used to analyze the proportion of hiding within each of the three tests. No significant difference in percent hiding was found between sub-tests of the tactile tests, shadow tests, or light tests.

When the median reemergence times from the three different tests were compared using boxplots, the tactile tests were shown to have induced the longest reemergence times over the shadow and light visual tests. The median reemergence times produced by the tactile tests were on average the largest and the distributions were the widest of the three different tests. The threatening tactile test in the high flow environment exhibited the longest reemergence times of the four tactical sub-tests, and the benign test in the low flow environments exhibited the shortest reemergence times out of the four tactile sub-tests. Tactile tests performed in the high flow

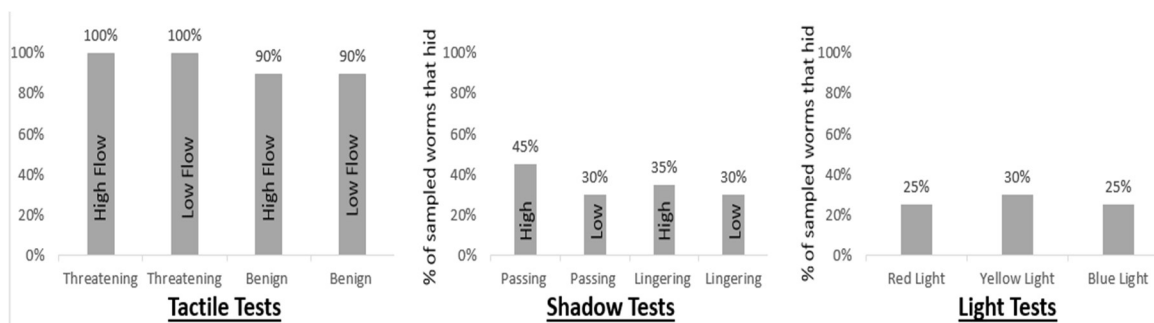


FIG. 4. Flow rate did not alter the proportion of hiding for tactile tests because the threat from the induced stress outweighed the cost of lost nutrients. For shadow tests, flow rate altered the proportion of hiding worms more than the difference in stress level between tests. In the light tests, the difference in light color had minimal effect on the proportion of hiding.

environment had longer reemergence times than tactile tests performed in the low flow environment. The visual shadow tests exhibited a similar pattern in reemergence times, with longer reemergence times in the high flow environment and shorter reemergence times in the low flow environments. However, the visual shadow tests had much lower median reemergence times and much smaller distributions of reemergence times than the tactile tests did. The visual light tests had no clear patterns for the distribution of reemergence times or the median reemergence times for the three light tests.

#### *Tactile field tests*

The Kruskal-Wallis test was also employed to analyze the reemergence times for the sub-tests within each field or laboratory test. The reemergence times for the two tactile sub-tests, prodding either inside (threatening) or next to (benign) the calcareous tube, were found to be significantly different from one another in the low flow environments ( $p=0.0002039$ ) but not in the high flow environment ( $p=0.5917$ ). When the same sub-test was performed in

different flow rate environments, no significant difference in reemergence times between high and low flow sites was found for the threatening tactile test ( $p=0.3569$ ), but for the benign tactile test the reemergence times at high and low flow rate sites were found to be significantly different from one another ( $p=0.0001264$ ).

#### *Shadow field tests*

The reemergence times for the two visual shadow sub-tests, passing versus lingering shadows, were not found to be significantly different from one another for neither high nor low flow rate environments ( $p=0.728$  for high flow environments,  $p=0.6274$  for low flow environments). However, while there were no significant differences found in reemergence times between the two shadow tests when performed in the same flow rate environment, there were significant differences in reemergence times when the same test was performed in different flow rate environments. When reemergence times from the same shadow test in different flow environments were compared, a significant difference was

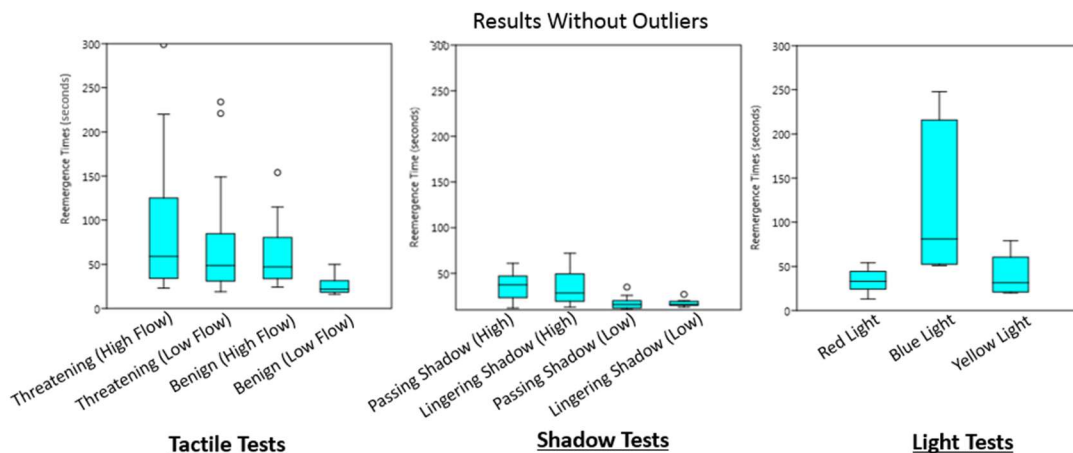


FIG. 5. The tactile tests had larger distributions and relatively higher median reemergence times than the shadow tests. The light tests had variable distributions and no clear trend in median reemergence times.

found for both passing shadow ( $p= 0.01163$ ) and lingering shadow ( $p= 0.005163$ ) visual tests.

#### *Light laboratory tests*

For the visual light laboratory tests, only one of the two collected and sampled worm's reemergence times were statistically significant difference between red, yellow, and blue lights ( $p= 0.04416$ ). The Dunn's Post-Hoc test determined that the significant difference between reemergence times for that worm occurred between the red and blue light tests ( $p= 0.01268$ ). The other worm that was collected and sampled did not exhibit significant differences in reemergence times when presented with the three different colors of light ( $p=0.06029$ ). The visual light laboratory tests therefore were insufficient to determine if red, yellow, and blue light created a significant difference in the reemergence times of Christmas tree worms.

#### *Supplementary data*

Additional data to the reemergence time was collected for each worm studied for both the tactile and shadow visual field tests. The diameter of the subject's tube opening and the number of worms within  $30\text{cm}^2$  of the subject was recorded and analyzed using a linear regression analysis to test for correlations between these factors and reemergence time. No significant p-values were found for reemergence times from the tactile or shadow visual field tests and tube diameter, indicating that there was no correlation between tube diameter and reemergence time under either of these two types of stress. The sample size was too small (merely the two collected worms) to perform this analysis for the visual light laboratory tests. The linear regression analysis for the number of neighboring worms only found a significant correlation for the lingering shadow sub-test in low flow environments and the number of neighboring worms out of all the sub-tests performed for the tactile and visual

shadow field tests ( $p= 0.0034978$ ). The correlation between reemergence time and the number of neighboring worms was positive, showing that as the number of neighboring worms increased, so did the reemergence time. This points to a significant correlation, but there was insufficient data to confirm the correlation entirely.

#### DISCUSSION

The results from the Kruskal-Wallis tests performed on reemergence times for the three tests in this study showed that Christmas tree worms exhibited significantly longer reemergence times when presented with a tactile perturbation over a visual shadow perturbation. This is potentially because a tactile stress, especially one directly to the opening of the calcareous tube, would most closely resemble a direct predatory attack on the worm whereas a shadow cast over the worm, whether passing or lingering, would likely not induce the same degree of fear response because it would resemble an organism merely passing over the worm instead of predating on or near it. Consequently, the worms sampled for the shadow visual field tests hid less often and had significantly lower reemergence times compared to the tactile field tests (Fig. 4 and 5).

The visual shadow field tests also had significantly different reemergence times from the visual light laboratory tests. These results are potentially because a change in light availability would not indicate the presence of a potential predator, whereas a shadow might. Surprisingly, the Dunn's Post-Hoc test did not find a significant difference between reemergence times for the tactile field tests and the visual light laboratory tests, but this is most likely because the visual light laboratory tests were lacking in adequate sample size and a constant light environment as the wet lab was outside and exposed to varying natural light conditions.

Additionally, the results indicated that worms in areas with relatively higher flow



rates hid for longer periods of time than worms in areas with relatively lower flow rates (Fig. 5). In areas with low flow rates, a significant difference in reemergence times was visible between tests that differed in the amount of stress they created, as with the threatening and benign tactile tests, but no such difference was visible between tests that did not create widely varying degrees of stress, as with the passing versus lingering shadow field tests (Appendix A).

#### *Tactile field tests*

The flow rate of the test site and the varying threat level presented by the threatening versus the benign tactile test affected the Christmas tree worms' reemergence times. When the two tactile tests were performed in the same flow rate environment, the results showed that in a high flow rate environment with potentially excess nutrients, there was no significant difference in reemergence times, whereas in a low flow rate environment with potentially limited nutrients, there was a significant difference in reemergence times between threatening and benign tactile tests (Appendix A). I attribute the lack of difference in reemergence times in high flow rate environments to a higher nutrient quantity that lowers the cost of lost foraging time in favor of hiding from potential threats for a longer period, no matter the stress level produced by the tactile test. Contrarily, in low flow rate environments, there are potentially less available nutrients, highlighting a difference in the stress created by the two tactile tests since worms exhibited longer reemergence times for the threatening tactile test than for the benign tactile test. When there are potentially less available nutrients due to a lower flow rate, the worms must extend their branchial crown for eating and breathing more frequently (Strathmann *et al.* 1984), so hiding from predators for a longer time period is unfavorable, resulting in lower reemergence times. This concept is further supported by the lack of a significant difference

found between reemergence times for the threatening test in high versus low flow rate environments, where the threat created by the test outweighs the cost of lost foraging time in both cases. The presence of a significant difference between reemergence times for the benign tactile test in high versus low flow rate environments also indicates that the amount of available nutrients appears to be influencing the reemergence times when the threat level is decreased.

Other organisms like crickets and copepods also use disturbances to mechanoreceptors to detect and avoid predatory attacks (Steinmann and Casas 2017, Buskey *et al.* 2012). A study by Steinmann and Casas (2017) discussed how crickets utilize sensitive hairs to detect motion, helping them avoid predators. The air disturbance caused by predators moving towards their cricket prey was also found to be comparable to the hydrodynamic disturbance of fish as they approach their prey (Steinmann and Casas 2017). The filaments that make up the branchial crown of Christmas tree worms can be compared to the sensitive hairs on the crickets, as both sense alterations to their environment to avoid predators.

Copepods possess mechanoreceptors that also aide them in detecting hydrodynamic disturbances, allowing them to more easily evade predation (Buskey *et al.* 2012). Buskey *et al.* (2012) also noted that turbulence plays an essential role in how effectively copepods can sense and escape predators, with relatively lower turbulence levels favoring the predator. This is potentially because it becomes more difficult for the prey to detect the difference between the turbulence and the water movement caused by predatory advances. Alternatively, larger turbulence levels were found to favor they prey by making feeding more difficult for the predator (Buskey *et al.* 2012).

This same logic can be applied to the predator-prey dynamic between Christmas tree worms and reef fish, with more turbulent areas and areas with relatively higher flow

rates reducing the worms' ability to sense prey over the static disturbances from water flow. This can also serve as an additional explanation for why worms in higher flow rates hide for longer periods of time than worms in lower flow rates. If it is more difficult for the worms to decipher the nature of the disturbance, they may hide for longer to ensure that any potential predator has moved on.

#### *Shadow field tests*

The results indicate that flow rate, rather than the different tests performed, had a larger effect on the proportion of hiding and the length of the reemergence time in sampled Christmas tree worms. The worms hid more often when presented with passing shadows versus lingering shadows in high flow rate environments, but in low flow rate environments the proportion of worms that hid was identical (Fig. 4). The flow rate therefore had a greater effect on the degree of stress response observed in Christmas tree worms than the type of shadow test did since the low flow environments with potentially lower nutrient values had an identical proportion of hiding worms.

Comparisons of reemergence times between the visual shadow field tests and the two flow rate environments also indicated that flow rate had a larger influence on reemergence times than the type of visual shadow test performed. For both tests, reemergence times increased in high flow environments and decreased in low flow environments. There was no significant difference in reemergence time between the two shadow sub-tests in the same flow rate environment, implying that there was no perceived difference in stress level between the two sub-tests (Appendix A). However, there was a significant difference in reemergence time between the two flow rate environments each test was performed in, implying that the flow rate and potentially the nutrient availability of each environment controlled reemergence time instead of the sub-test being performed. Supposedly, neither the

passing nor lingering shadow sub-tests caused enough stress to shift the cost of hiding versus obtaining nutrients more than the flow rate did.

Previous studies have concluded that the eyes of Christmas tree worms are best suited for detecting shadows rather than shapes or colors (Bok *et al.* 2017). Also, Christmas tree worms' eyes are oriented upwards from their tube, most likely to further the detection of shadows passing over them (Bok *et al.* 2017). These two findings help explain why the worms in this study were more sensitive to, i.e. had longer reemergence times for, the shadow experiments over the light experiments. From personal observations, the Christmas tree worms were seen hiding when a fish swam over them and cast a shadow over them, but if the shadow did not fall over the worm, often no hiding was induced.

Other animals, like the freshwater pond snail *Lymnaea stagnalis*, have also been known to use shadows as a method of predator detection. *L. stagnalis* uses dermal photoreceptors on its skin and mantle cavity to initiate a shadow-induced withdrawal response, similar to how Christmas tree worms use their eyes to induce hiding (Sunada *et al.* 2010). The withdrawal response of *L. stagnalis* was also found to vary when presented with stimuli of different intensities, paralleling how reemergence times for Christmas tree worms varied for tests of differing stress levels (Sunada *et al.* 2010). I expected that sudden shadows, especially passing ones, would induce longer reemergence times and more frequent hiding because shadows are usually indicative of passing predators, whereas lingering shadows are not. This theory is supported by the fact that in high flow environments with excess nutrients, 10% more sampled worms hid during the passing shadow tests than for the lingering shadow tests (Fig. 4) indicating that the passing shadow test induced a higher stress level.

### *Light laboratory tests*

There was no clear pattern in the data collected from the light tests in the proportion of worms that hid and the reemergence times for each of the three sub-tests. The Dunn's Post-Hoc test showed that the only significant difference in reemergence times occurred between the reemergence times recorded for red and blue lights in one of the two collected worms. Because of this significant difference, it is possible that the color of light used to disturb the worms significantly affects the reemergence time of that worm. However, because the data has very little significance and very few patterns for the other sub-tests, and there was only significant data for one of the two collected worms, further research is required to confirm the potential significance found between the red and blue tests. Ideally, a larger sample size and a more controlled light environment would be necessary to yield accurate results. Because the wet lab where the collected worms were sampled was outside, the ambient light conditions varied greatly, potentially altering the data collected.

### *Supplementary data*

The size of the worm's tube positively correlated with reemergence times for five out of eight of the field sub-tests (Appendix B), indicating the possibility of a trend where older and larger Christmas tree worms hid for longer periods of time than younger, smaller worms. Because smaller Christmas tree worms are younger (Nishi and Nishihira 1996), those organisms have not developed significant experience from potential predatory attacks or any other threats. A study conducted in 2007 found that predation on newly settled sessile invertebrates did not play a role in shaping the structure of these communities (Sams and Keough 2007), indicating that younger, smaller, and more recently settled organisms, like Christmas tree worms, may not attract enough predators to shape their behavior,

leading to shorter reemergence times for smaller worms.

Reemergence time was positively correlated with the number of additional worms within 30cm<sup>2</sup> of the subject worm for five out of the eight field sub-tests as well (Appendix C). Based on the same study from UCLA (Pezner *et al.* 2017), this is most likely because predation risk increases when Christmas tree worms form large aggregations. While the UCLA study found this to be true for tactile disturbances, I also found it to be true for visual disturbances with regards to the two visual shadow sub-tests. Because the predation risk increases when Christmas tree worms are in large aggregations, there must be a benefit to settling around large numbers of conspecific organisms since Christmas tree worms are often found in large aggregations. Because Christmas tree worms are broadcast spawners (Hagman *et al.* 1998), the aggregates may increase the chance of fertilization during spawning events. The risk of increased predation may then be overwhelmed by the increased fitness that comes with closer proximity to conspecific worms.

The comparative analysis of the Christmas tree worms' sensory systems revealed that they rely on shadow detection as preliminary detection of predators and mechanoreceptor stimulation as a secondary and final detection. The Christmas tree worms were found to react the most intensely and hide for the longest amount of time when presented with a tactile perturbation over a visual perturbation (Fig. 5). Because the worms possess rudimentary eyes, it was expected that mechanical disturbances would elicit more severe reactions than light disturbances (Bok *et al.* 2017). The visual light tests yielded inconclusive results, so to confirm that red and blue lights create a significant difference in reemergence times, a larger sample size and less variable experimental light environment would be necessary. For future research, I would suggest manipulating the amount of nutrients and oxygen available in their environment and

defining the correlation between their availability and the reemergence times of the Christmas tree worms.

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**APPENDIX A**

Threatening Tactile Test	High Flow Environment	NO SIGNIFICANT DIFFERENCE; P= 0.5917	PASSING SHADOW TEST	High Flow Environment	NO SIGNIFICANT DIFFERENCE; P= 0.728
Benign Tactile Test	High Flow Environment	NO SIGNIFICANT DIFFERENCE; P= 0.0002039	LINGERING SHADOW TEST	High Flow Environment	NO SIGNIFICANT DIFFERENCE; P= 0.6274
Threatening Tactile Test	Low Flow Environment	NO SIGNIFICANT DIFFERENCE; P= 0.3569	PASSING SHADOW TEST	High Flow Environment	SIGNIFICANT DIFFERENCE; P= 0.01163
Threatening Tactile Test	Low Flow Environment	SIGNIFICANT DIFFERENCE; P= 0.0001264	LINGERING SHADOW TEST	High Flow Environment	Significant difference; p= 0.005163
Benign Tactile Test	High Flow Environment	SIGNIFICANT DIFFERENCE; P= 0.0001264	LINGERING SHADOW TEST	Low Flow Environment	Significant difference; p= 0.005163
Benign Tactile Test	Low Flow Environment	SIGNIFICANT DIFFERENCE; P= 0.0001264	LINGERING SHADOW TEST	Low Flow Environment	Significant difference; p= 0.005163

P-values obtained using the Kruskal-Wallis test for tactile and shadow sub-tests. Different sub-tests in the same flow rate environment and the same sub-test in different flow rate environments were compared.

**APPENDIX B**

Sub-test	Flow Rate	Slope of linear regression line	p-value
Threatening Tactile Test	High Flow	260	0.20332
Threatening Tactile Test	Low Flow	45.691	0.23991
Benign Tactile Test	High Flow	101.2	0.1552
Benign Tactile Test	Low Flow	-49.895	0.14897
Passing Shadow Test	High Flow	46.892	0.10974
Passing Shadow Test	Low Flow	9.018	0.74678
Lingering Shadow Test	High Flow	-60.47	0.083209
Lingering Shadow Test	Low Flow	-17.029	0.35261

Table showing the sub-test, the flow rate, the slope of the linear regression line between the tube diameter and the reemergence time, and the p-value calculated for each linear regression test.

**APPENDIX C**

Sub-test	Flow rate	Slope of linear regression line	p-Value
Threatening Tactile Test	High Flow	-0.65425	0.94306
Threatening Tactile Test	Low Flow	2.4561	0.26447
Benign Tactile Test	High Flow	1.6587	0.68291
Benign Tactile Test	Low Flow	-1.7258	0.28455
Passing Shadow Test	High Flow	3.7812	0.27334
Passing Shadow Test	Low Flow	0.40971	0.65946
Lingering Shadow Test	High Flow	-1.9079	0.15909
Lingering Shadow Test	Low Flow	2.8799	0.003498

Table showing the sub-test, the flow rate, the slope of the linear regression line between the number of surrounding worms in 30cm<sup>2</sup> and the reemergence time, and the p-value calculated for each linear regression test.