

**Fluorescence in an Inclusive Community and Inducible Fluorescence in the Polychaete
Species *Dodecaceria concharum* and *Abarenicola pacifica***

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Abstract

Fluorescence in marine organisms is a common phenomenon, yet the anatomical structures from which it originates and the behavioral function behind it are not well understood. This study had two distinct purposes; to survey fluorescence in a marine fouling community and to describe inducible fluorescence in two Polychaete species. In the first part of the study, organisms were collected, the fluorescence was photographed, the morphological feature(s) that fluoresced was identified and the wavelength was characterized when possible. The functional morphology of the fluorescent structures was then considered to determine if the fluorescence might have a behavioral purpose, although no clear correlations were found. Eight species in four phyla fluoresced in green, orange and red wavelengths. The second part of the study identified the anatomical source of inducible fluorescence and the stimulus which caused its release in two Polychaete worms, *Abarenicola pacifica* and *Dodecaceria concharum*. The fluorescence in *A. pacifica* was found to be mucus secreted from a pore dorsal to the parapodia during dissection and tactile stimulation. In both *A. pacifica* and *D. concharum*, the density of the fluorescence coupled with its secretion without puncture of coelomic cavities or blood vessels suggested it was mucus, although due to optical constraints the anatomical origin in *D. concharum* could not be determined. Chemosensory cues did not induce either species to release fluorescent material, but tactile stimuli did, including human fingers, shells and predators, suggesting a potential defense mechanism.

Introduction

Fluorescence is found in multiple taxa across various marine ecosystems, yet the anatomical origins are not well categorized and its behavioral function is largely unknown (Mazel *et. al.*

2003). Many Anthozoa have been found to fluoresce (Mazel 1995, Matz *et. al.* 1999, Carter *et. al.* 2004), as well as certain species of Cephalopoda (Mathger and Denton 2001). Fluorescence is common in shallow water, as emitted fluorescence is dependent on the excitation wavelength of light and shallow habitats receive more incident light before long wavelengths attenuate (Yentsch 1962). However, fluorescence has also been found in deep-sea environments, such as red fluorescent organisms including Siphonophora, although their fluorescence is coupled with red bioluminescence (Haddock *et. al.* 2005). In corals, the function of fluorescence has been suggested as photoprotection and/or an enhancement of photosynthesis, but these claims are still somewhat controversial (Mazel and Fuchs 2003). A behavioral function of fluorescence has only been shown in one marine organism, *Lysiosquillina gabriuscula*, in which fluorescence is used during threat displays between males (Mazel *et. al.* 2003a).

Fluorescence in communities may act as an interspecific or intraspecific signal during activities such as prey luring, predation, sexual selection, mating, or antagonistic encounters. This hypothesis is dependent on the visual capability of the intended receiver and requires that the fluorescence be exhibited on an anatomical structure that is visible. Communities on docks and pilings have been well studied and their diversity is often limited by effective predators, which can result in spatial and temporal heterogeneity (Karlson 1978, Peterson 1979). The first goal of this project was to categorize the anatomical origin of fluorescence in organisms collected on a single tire at the dock of the Friday Harbor Labs in San Juan Island, WA, and establish any trends that may exist between the anatomical location of fluorescence and behavior. As fluorescence has not been examined in the context of an inclusive community, the diversity of fluorescence in a single tire and the potential for fluorescent signaling is of interest. Analogous

morphological fluorescence would be expected in organisms that employ similar feeding methods, such as filter feeders versus mobile predators. If no correlation was found, it is possible that the fluorescence is simply a structural characteristic of inherent proteins, and does not serve a behavioral purpose across taxa. The second goal of this project concerned two species of Polychaete worms. The first is *D. concharum* and is known to expel clouds of fluorescent material when disturbed (M. Matz, unpub. obs.). The stimulus that causes the release of fluorescence has not yet been characterized, nor has its anatomical source. The second species is *A. pacifica*, which secretes a fluorescent fluid when punctured or prodded (pers. obs.). In order to test whether this behavior in the two species is a result of disturbance, both were tested in the presence of chemosensory and tactile cues.

Methods

Tire Community

Eight different fluorescent organisms were collected from a single tire on the Friday Harbor Dock on San Juan Island, WA, at 10:00PM on 7/2/12, 7/5/12 and 7/16/12. The tire was selected randomly and was located on the southeast corner of the main north dock. The same tire was used for each of the three collections. Two light sources were used when examining organisms for fluorescence; a UV light and a blue LED light with a yellow filter. The yellow filter was used to decrease the amount of reflected blue light in order to look for fluorescence. The tire was first examined while still in the water, and any fluorescent organisms that were swimming within the tire were collected. The tire was then flipped up onto the dock in order to search for organisms. The collected organisms were kept in separate tubs based on predator/prey interactions in a large tank that was supplied with local seawater through a PVC piping system. The unfiltered water

continually flowed through the tank and was pumped directly from the sea, maintaining temperature. The eight collected organisms were identified according to Koslov (1987). The organisms were examined either without magnification or under a dissecting microscope at 6x-8x magnification (depending on their size) to identify the morphological feature(s) from which the fluorescence originated. Each species was photographed for fluorescent display using either a light microscope and a Canon PowerShot G6 with a conversion lens adapter LA-DC58D and scope attachment (*Balanus glandulus* and *Pandalus platyceros*), or without a scope using an EF-S 60mm Canon macro lens on a Canon 60D body (all others). Both camera setups utilized a blue light and a yellow filter. Two species were examined using an Ocean Optics USB2000 Spectrometer attached to the light microscope in order to characterize the wavelength of the emitted photons (*Tonicella lineata* and *Mitrocoma cellularia*). The software used to identify the spectrum was OOIBase32™ Spectrometer Operating Software from Ocean Optics, version 2.0.6.5. The morphological feature that fluoresced, the wavelength of the fluorescence, the feeding method and any encrusting algae were then catalogued.

Polychaete Fluorescence

In order to test the reaction to olfactory or chemosensory cues and whether they caused a release of fluorescent material, *D. concharum* was exposed to water taken from two potential predators; the seastar *Pycnopodia helianthoides* and the snail *Ceratostoma foliatum*. The water was taken from the surface of the predators and pipetted directly on the *D. concharum*. This was repeated twice for each predator. The *A. pacifica* were exposed to water that contained predatory Nemertea over the course of several weeks to determine if fluorescent material was expelled. To examine reactions to tactile cues on *D. concharum*, the Corraline algae in which the colony was

living was broken using a small shovel, and eight individual live worms were extracted. The live, in-tact worms were prodded using a probe, and worms that had been cut by the shovel were also examined. Magnifying lenses were used to attempt to determine the anatomical source of the fluorescence. To examine reactions to tactile cues in *A. pacifica*, four species were examined. Two species were prodded using a probe or forceps without puncturing the body cavity. The anatomical location of the fluorescence that was secreted was identified using a magnifying lens, as neither the dissecting scope nor the light microscope can be used with a yellow filter. Two additional species were punctured with a scalpel near the parapodia to determine if fluorescent fluid was secreted. Fluorescence was photographed using an EF-S 60mm Canon macro lens on a Canon 60D body, a blue light and a yellow filter. The fluorescence was also measured using a UV light and the Ocean Optics USB2000 Spectrometer attached to the light microscope with OOIBase32™ Spectrometer Operating Software from Ocean Optics, version 2.0.6.5

Results

Tire Community

There were eight species collected on 7/2/12, 7/5/12 and 7/16/12 that exhibited fluorescence. The fluorescence was photographed (Figure 1) for each of the eight species, with the exception of *Mitrocoma cellularia*, which died before photos could be taken. The wide variation of fluorescence in various species of the genus *Tonicella* was also catalogued photographically, although only one of those species was collected from the tire (Figure 2). Spectrometer readings were taken for the fluorescence of two of the eight tire species, *Tonicella lineata* and *Mitrocoma cellularia* (Figure 3, Figure 4). A table was made comparing the anatomical source of

fluorescence, the wavelength of fluorescence, the feeding behavior of the organism and any encrusting algae (Table 1). A detailed list of the organisms collected from the tire is as follows:

1. *Mutilus trossulus*: 3.8cm. This mussel exhibited green fluorescence from both valves and the byssal threads. It was attached to the tire through byssal threads, is a filter feeder, and was not found to have any encrusting algae.

2. *Pododesmus macroschisma*: 3.5cm. This filter-feeding bivalve exhibited green fluorescence from the calcified byssus which was used to attach to the tire. It also exhibited minimal red fluorescence which was likely from encrusting algae inside the ventral valve hole.

3. *Metandrocarpa tayleri*: tunic 0.9cm, stalk 1.2cm. This filter-feeding tunicate exhibited orange fluorescence from tunic and red fluorescence from the stalk, which was likely from encrusting algae. It attached to the tire using a holdfast in the stalk.

4. *Halocynthia igaboja*: 0.6cm. This filter-feeding tunicate exhibited yellow fluorescence in the tunic and red fluorescence on the siphons, which was likely from encrusting algae. It was attached to the tire using a holdfast at the base of the tunic.

5. *Mitrocoma cellularia*: 6.3cm. This planktonivore hydrozoan was found swimming inside the tire and exhibited green fluorescence on the bell margin.

6. *Balanus glandulus*: 1.3cm. The entire interior portion of this barnacle exhibited a green fluorescence, most notably from the cirri and the mantle. It was attached to the tire through cement glands. There was encrusting algae with red fluorescence on the tergum and scutum.

7. *Tonicella lineata*: 1.6cm. This chiton exhibited orange and red fluorescence from the plates and girdle. It is a grazer and was attached to the tire through its suction foot.

8. *Pandalus platyceros*: 6.3cm. This prawn exhibited green fluorescence from portions of the pereopods, antennae and various spots covering the carapace and thorax. It is an omnivore and was settled in the bottom portion of the tire walking on its pereopods.

Polychaete Fluorescence

Two species of Polychaete worms, *D. concharum* and *A. pacifica*, were examined under a dissecting microscope at 6x -10x. Their fluorescence was photographed (*D. concharum* Figure 5, *A. pacifica* Figure 6), and spectrometer readings of their fluorescence were taken (Figure 7, Figure 8). When *D. concharum* was twice exposed to water taken from two potential predators; the seastar *Pycnopodia helianthoides* and the snail *Ceratostoma foliatum*, the colony did not release fluorescent material. Similarly, *A. pacifica* did not release fluorescence when exposed to water that housed the potential predators Nemertea for several weeks. The only time fluorescence was noted in the tank was when the actual body cavity was perforated (tactile disturbance). The eight individual live *D. concharum* that were extracted from the Corraline algae did expel fluorescent material when prodded using a probe, and worms that had been cut by the shovel also released fluorescent material. The two *A. pacifica*, that were prodded using a probe or forceps without puncturing the body cavity expelled fluorescent material from a pore near the dorsal area of the parapodia, and the two *A. pacifica* that were cut using a scalpel also released fluorescent material. The fluorescent material was denser than the surrounding water, further suggesting it was mucus rather than coelomic fluid or blood. The fluorescent material taken from *A. pacifica* bleached within 30 seconds per drop under blue light during 5 trials, but the fluorescent material taken from *D. concharum* did not.

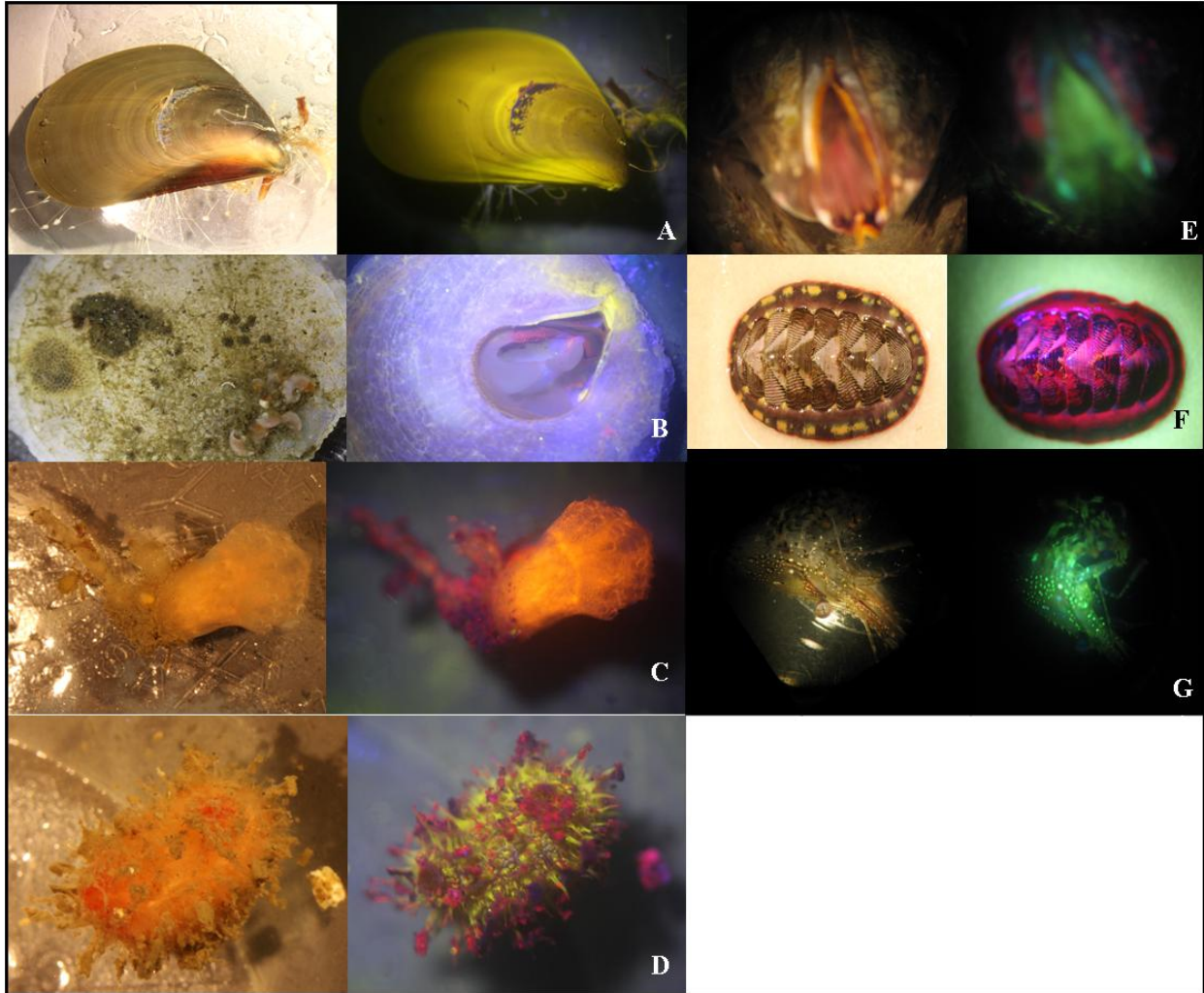


Figure 1: Fluorescence photographs of seven of the eight barnacle species (excluding *Mitrocoma cellularia*). [A] *Mutilus trossulus* [B] *Pododesmus macroschisma* [C] *Metandrocarpa tayleri* [D] *Halocynthia igaboja* [E] *Balanus glandulus* [F] *Tonicella lineata* [G] *Pandalus platyceros*.



Figure 2: Six variations of fluorescent variation in *Tonicella* chitons.

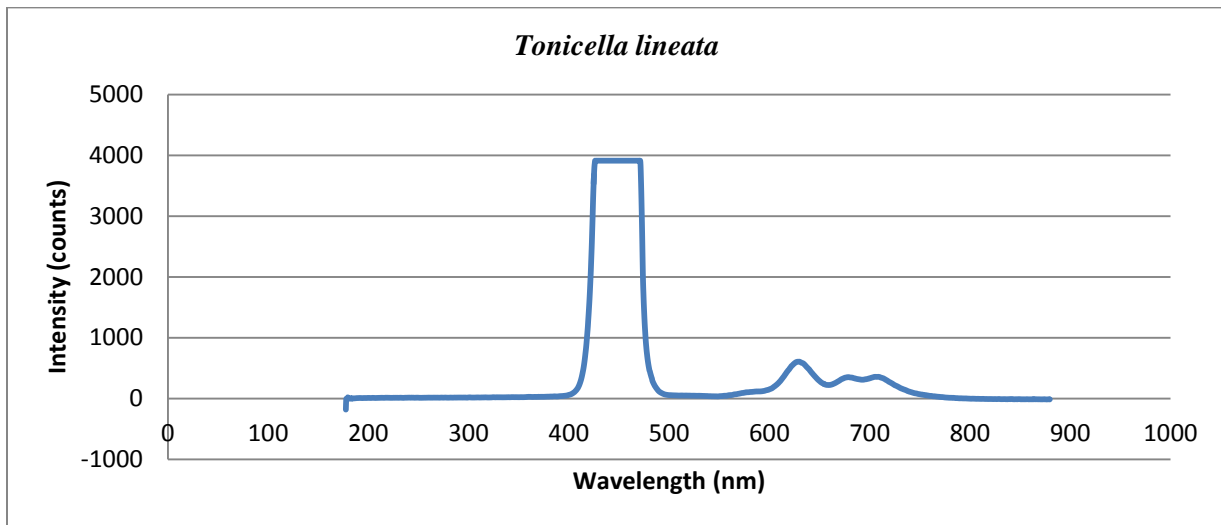


Figure 3: Spectrometer reading for *Tonicella lineata*. The peak at ~400nm is from the blue light, and the peaks in the ~630-750nm range represent the red and orange fluorescence.

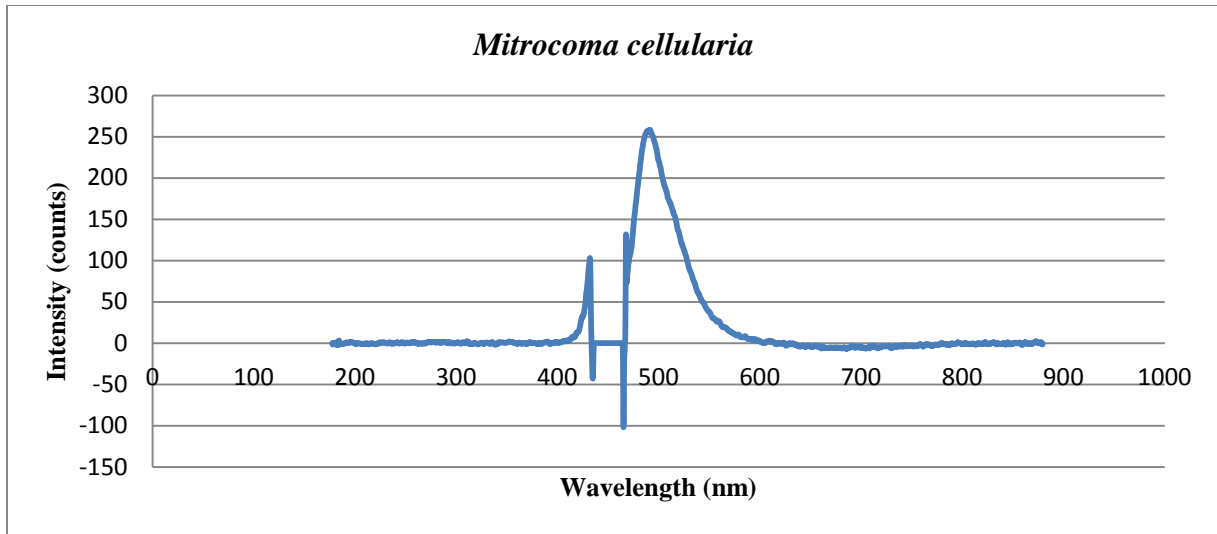


Figure 4: Spectrometer reading for *Mitrocoma cellularia*. The peak at 400nm is from the blue light, and the peak in the ~480-600nm range represents the green fluorescence.

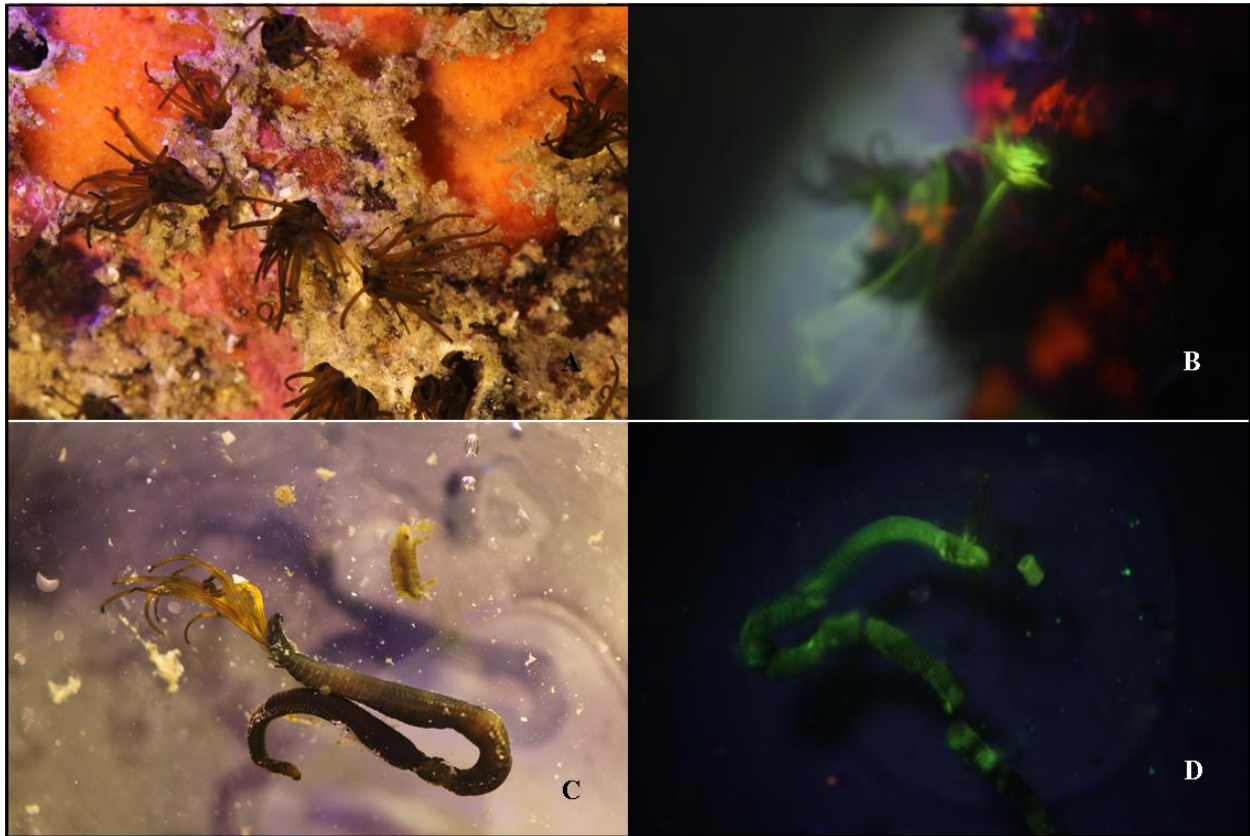


Figure 5: *D. concharum* [A] burrowed in Corraline Algae, [B] exhibiting fluorescence when disturbed with a tactile cue (human finger), [C] single worm removed from Corraline algae [D] single worm fluorescing when removed from Corraline algae.

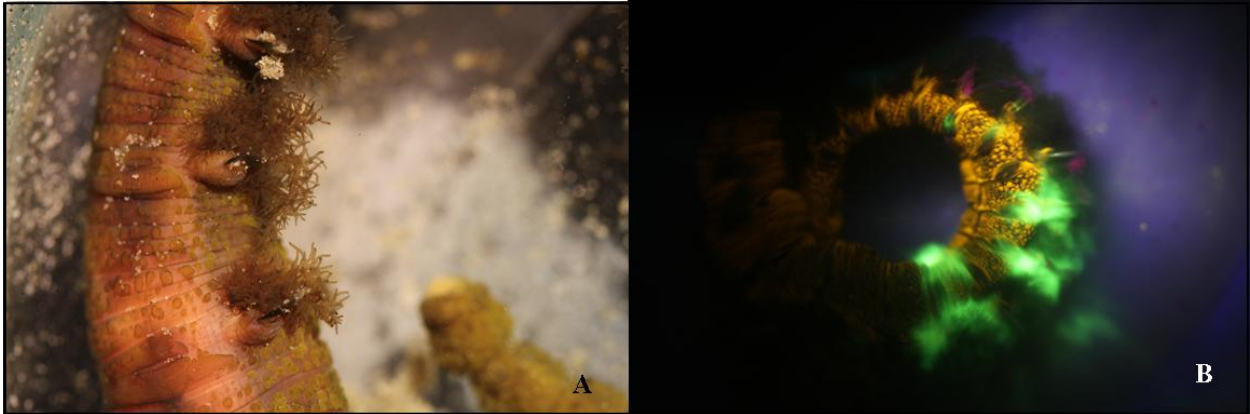


Figure 6: *A. pacifica* [A] under white light and [B] showing yellow fluorescence on skin and secretion of green fluorescent mucus from pores near parapodia.

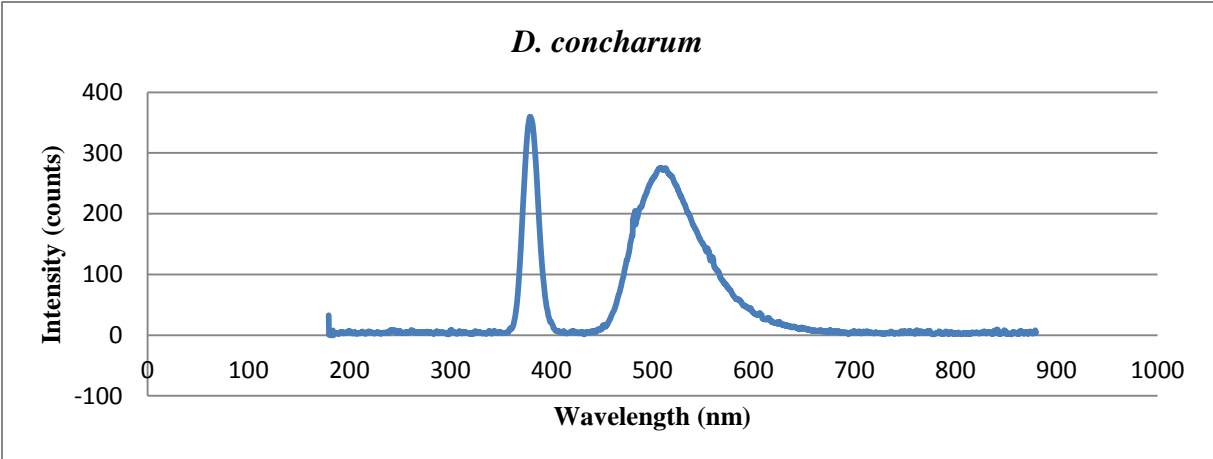


Figure 7: Spectrometer reading for *D. concharum*. The peak below 400nm is from the UV light, and the peak in the ~450-600nm range represents the green fluorescence.

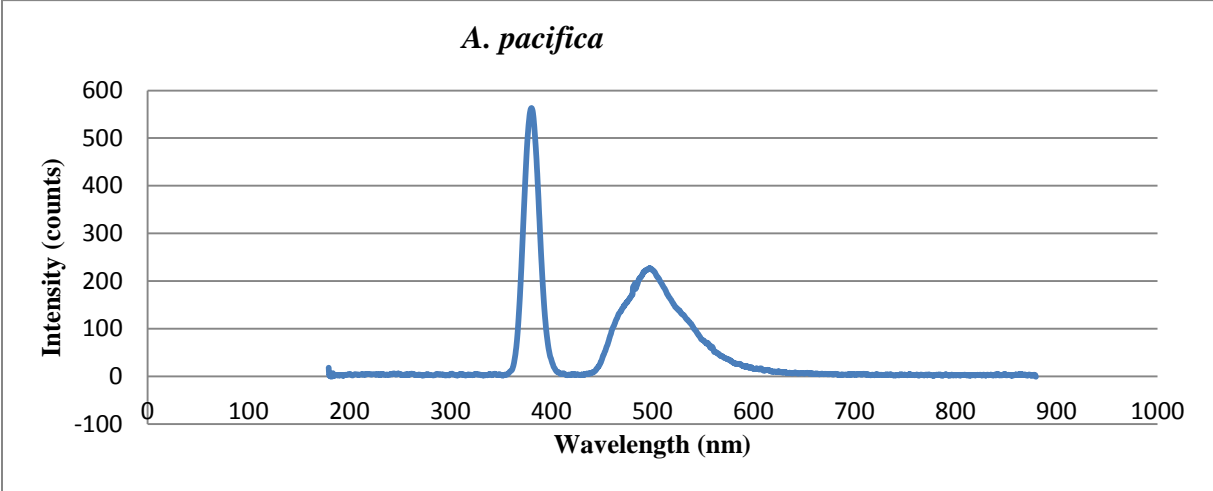


Figure 8: Spectrometer reading for *A. pacifica*. The peak below 400nm is from the UV light, and the peak in the ~450-600nm range represents the green fluorescence.

Table 1: Species collected from the tire listing the anatomical origin of fluorescence, its wavelength, their primary feeding method and whether or not they housed encrusting algae.

Organism	Morphological Feature	Fluorescence Wavelength	Feeding Method	Encrusting Algae
<i>Mutilus trossulus</i>	valves and byssal threads	green	filter feeder	no
<i>Pododesmus macroschisma</i>	byssal threads	green	filter feeder	red algae
<i>Metandrocarpa tayleri</i>	tunic and stalk	orange tunic, red stalk	filter feeder	stalk with red algae
<i>Halocynthia igaboja</i>	tunic and encrusting material	yellow tunic, red encrusting	filter feeder	red algae
<i>Mitrocoma cellularia</i>	bell margin	green	planktonivores	no
<i>Balanus glandulus</i>	cirri, mantle cavity	green	planktonivores	plates with red algae
<i>Tonicella lineata</i>	plates, girdle	orange, red	grazers	red algae
<i>Pandalus platyceros</i>	pereopods, carapace, antennae	green	omnivores	no

Discussion

Tire Community

The tire community contained a wide variety of fluorescent organisms that exist in separate trophic cascades, including filter feeders, grazers, omnivores and planktonivores. Eight species representing four phyla were collected, including Mollusca, Arthropoda, Cnidaria and Chordata. There was a wide variety of fluorescent wavelengths, including green, yellow, orange and red, although most of the red fluorescence likely originated from encrusting algae and not from the organisms themselves. The anatomical structures from which the fluorescence originated ranged widely based on trophic groups, and were considered based on the hypothesis that fluorescence might lure prey. In the filter feeders, the valves and the byssal threads used for attachment in *Mutilus trossulus* and *Pododesmus macroschisma* fluoresced, neither of which are involved in feeding, and the complete tunic in *Metandrocarpa tayleri* and *Halocynthia igaboja* fluoresced, rather than the incurrent siphon which might be hypothesized to attract phototaxic prey. In the planktonivores, the bell margin above the tentacles in *Mitrocoma cellularia* fluoresced, and the entire inner cavity and feeding cirri in *Balanus glandulus* fluoresced. Their fluorescence was

displayed near or on their feeding appendages, but in *Balanus glandulus* the cirri are only exposed to feed and are then quickly retracted, allowing little time for phototactic prey to react, and in *Mitrocoma cellularia*, the bell margin and not the tentacles fluoresced, yet the tentacles are what are used to capture prey. However, as the bell margin is constantly exposed, future experiments could be done to examine prey capture with and without fluorescence. In the grazer *Tonicella lineata*, bright fluorescence in orange and red wavelengths were exhibited on the plates and the girdle, which would likely make it either more attractive to predators or perhaps warn predators off as aposematic coloration depending on their optical capabilities. Fluorescence on the foot would not be visible to prey due to the lack of available light. Similarly, the randomly scattered green fluorescence on *Pandalus platyceros* might either attract prey or deter predators depending on the visual system of the predator or prey.

The anatomical locations of fluorescence did not show any strong correlations to feeding method based on trophic groups, creating a weak defense of fluorescence being used for prey luring. To date, the only study that showed that fluorescence acted in prey luring was the case of a deep-sea siphonophore that coupled fluorescence with bioluminescence (Haddock *et. al.* 2003). There haven't been studies to prove a similar mechanism for fluorescence in shallow and sessile marine organisms, which is a potential area for future study. The wide variation in fluorescent wavelengths and anatomical structures across phyla in this study reiterates the fact that trends in ecological and behavioral purposes behind fluorescence are difficult to unravel. Beliefs about the function of fluorescence are also frequently changed or considered controversial, as evidenced by the disproving of enhanced photosynthesis as a purpose behind fluorescence in reef corals (Mazel *et. al.* 2003b). A popular hypothesis remains that bright colors in sessile marine

organisms, including fluorescence, might serve an aposematic purpose (Wicksten 1989), but this hypothesis needs to undergo further study. The photoreceptive abilities and phototaxis of the intended receiver are a factor whenever fluorescence is considered as a signal, so there remains much future work to be done with fluorescence in a behavioral context.

Polychaete Fluorescence

D. concharum and *A. pacifica* both secrete fluorescence that is believed to be mucus. The density of the fluid along with its secretion without piercing of the body cavity or blood vessels supported this hypothesis. The secretion only occurred when the organisms were cued with a tactile stimulus, whether from a human finger, a shell, or a potential predator. The inference that the fluorescence is indeed mucus is still somewhat speculative, and will need to be investigated further through histology and dissection under a fluorescence scope, which was unavailable to this study. As neither organism secreted fluorescence when exposed to chemosensory cues of a predator, the release of fluorescence was limited to a tactile response, but this does not rule it out as a defense mechanism. Further tests on the chemical nature of the fluorescent secretion as well as tests as to whether or not it is noxious will be crucial to this determination. Although no reactions from potential predators were observed during these trials, it is possible that predators might react to the secretion during an attack (such as an attack on *A. pacifica* by Nemertea, which was never observed in the lab). A predatory attack on *D. concharum* would likely have to be witnessed under magnification to assess predator reaction, as the relatively large *Ceratostoma foliatum* showed no visible reaction. Prey reactions will also be difficult to observe directly, as *D. concharum* quickly retract into their Corraline algae burrows when disturbed. The amount of fluorescent material that remains in the water column from either species, as well as how much

of that reaches the predator attacking is unknown, and the chemosensory receptors on those predators may vary, which would also have to be considered in future experiments. The qualitative observations in this study can be used to shape methodology for future work in the potential of the secreted fluorescence in a behavioral context. Future tests could also be done in controlled trials where predators are exposed to varying concentrations of the fluorescent material.

The hypothesis that the fluorescent mucus in these two Polychaete species might be a defense mechanism is still speculative at this point, but it is an interesting possibility given the widespread use of noxious chemical secretion as a defense mechanism in the ocean. Animals that utilize this method include the Soleidae family of flatfish that secrete a known shark-repellant (Thompson *et. al.* 1986) and the noxious defenses secreted by nudibranchs that are acquired through food. The nudibranchs are particularly interesting examples, as they have adapted *de novo* noxious secretions that are independent of diet (Faulkner and Ghiselin 1983). Organisms in the class Cephalopoda often ink as a defense mechanism, and their ink includes noxious complexes that inhibit predators, including passive and active chemical defenses (Derby 2007). Despite numerous studies of noxious secretions, there have not been any publications about noxious secretions that are also fluorescent. The possibility that the fluorescence in the secretion is simply a byproduct of the material structure must be considered, but future behavioral tests to rule out the role fluorescence in defense would be worthwhile.

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