Characterizing and defining the optimal conditions for select protist photoprotein activity and testing for photoprotein activity in doliolid tunicates

Cheyenne Payne, Scripps Institution of Oceanography, UCSD

Mentor: Steven H. Haddock

Summer 2016

Keywords: bioluminescence, photoprotein, regeneration, radiolarian, phaeodarian, doliolid

ABSTRACT

We present the optimal pH, salinity, and Ca^{2+} concentration necessary for Collozoum photoprotein-activation, as well as the optimal pH for two genera of deep-water phaeodarians. Successful regeneration of radiolarian and phaeodarian photoproteins was achieved. Results on bioluminescence assays with the doliolid tunicate genus, Doliolula, are also presented.

INTRODUCTION

Bioluminescence and the biochemistry responsible for it have been the subject of research for the past 230 years, since Dubois first discovered the luciferin-luciferase system responsible for bioluminescence in the West Indies Beetle in 1885. The term luciferin refers to an organic compound that releases photons when oxidized, and the luciferase is an enzyme that catalyzes the luciferin's oxidative light-emitting reaction (Shimomura 2006). For 77 years, the luciferin-luciferase reaction was believed to be the sole source of bioluminescence, until 1962 when the first photoprotein system was described by Shimomura in the jelly *Aequorea aequorea* (Shimomura et al 1962).

Photoproteins are stable protein-compound complexes that emit light when they conformationally change due to a reaction with a cofactor, which is a chemical or molecule that binds to a photoprotein and causes its components to dissociate (Shimomura 2006). Many

photoproteins are Ca^{2+}-sensitive, such as those found in coelenterates, and are complexes containing the compound coelenterazine (Shimomura 2006). After a photoprotein has conformationally changed, the protein component is called apo-photoprotein. When incubated under the right conditions with the dissociated compound, apo-photoprotein will revert back to its photoprotein state such that it can once again react with its cofactor in a light-emitting reaction. This incubation process is termed "regeneration" (Shimomura 2006).

Since their discovery, photoproteins have been identified in several phylogenetic groups, including Protozoa. Among the protists are two classes with bioluminescent members: Polycystinea and Cercozoa. Within Polycystinea in the order Collodaria is the genus Collozoum, which is known to have calcium-activated photoproteins (Haddock et al 2010). In Cercozoa, the deep-sea phaeodarians are also reported to have calcium-activated photoproteins (Haddock et al 2010). Aside from cofactor sensitivity, little is known about Collozoum and phaeodarian photoproteins.

Even less is known about the bioluminescent members of a group of tunicates called the doliolids. While bioluminescence has been observed in genera such as Doliolula and Paradoliopsis from this group, nothing is known about their bioluminescence biochemistry, including whether or not the system responsible is luciferin or photoprotein-based.

To elucidate the biochemistry responsible for bioluminescence in the shallow-water radiolarian Collozoum, the deep-water phaeodarian genera Tuscaretta and Tuscarilla, and the doliolid genus Doliolula, the purpose of this summer's research was to determine the optimal conditions for photoprotein activity in the aforementioned protists, and to determine whether or not Doliolula bioluminescence is based on calcium-activated photoproteins.

METHODS

Doliolid and deep-water phaeodarian samples were collected by MBARI's ROV Doc Ricketts aboard the R/V Western Flyer in Monterey Bay, California, USA, and shallow-water radiolarians were collected by bluewater divers in the same location. Individuals were flash-frozen in liquid nitrogen shortly after collection, and transferred to a -80°C freezer. Samples were prepared for photoprotein assays by homogenizing single individuals in 100 mM Tris-HCl buffer with 50 mM EDTA (pH dependent on assay). To regenerate photoprotein from homogenized samples, aliquots of homogenate were incubated for 2 hours at 4°C in equal

volume of 50 mM Tris-HCl buffer pH 8.5 with 450 mM NaCl, 5 mM EDTA, 0.1% gelatin, and 2.5 µl of 2 mM coelenterazine in methanol (modified protocol from Powers et al 2012). All steps of the regeneration process were done in the dark. After regeneration, samples were immediately assayed or transferred to a -80°C freezer. All photoprotein activity assays were run in a custom-built photomultiplier-tube-based integrating sphere with accompanying LabView software that recorded the reaction light intensity as a function of time, as described in Powers et al. (2012). For each assay, aliquots of regenerated crude photoprotein samples were diluted 10x with 100mM Tris-HCl buffer with 50mM EDTA (pH dependent on assay) and placed in the photomultiplier, and calcium chloride was injected into the sample.

Optimal pH assays were conducted by diluting regenerated samples with Tris-HCl-EDTA buffer ranging in pH from 7.0-10.0 in 0.5 increments for Collozoum samples (Figure 1), and 7.5-9.5 in 0.5 increments for Tuscaretta samples (Figure 4). Optimal cofactor-concentration assays were conducted by diluting regenerated Collozoum samples with pH 8.5 Tris-HCl-EDTA buffer and injecting 1 M $CaCl_2$ for a final solution concentration of 20mM-450mM $CaCl_2$ (Figure 2). Optimal salinity assays were conducted by diluting regenerated Collozoum samples with pH 8.5 Tris-HCl-EDTA buffer with 0.2-1.6 M NaCl (Figure 3).

RESULTS

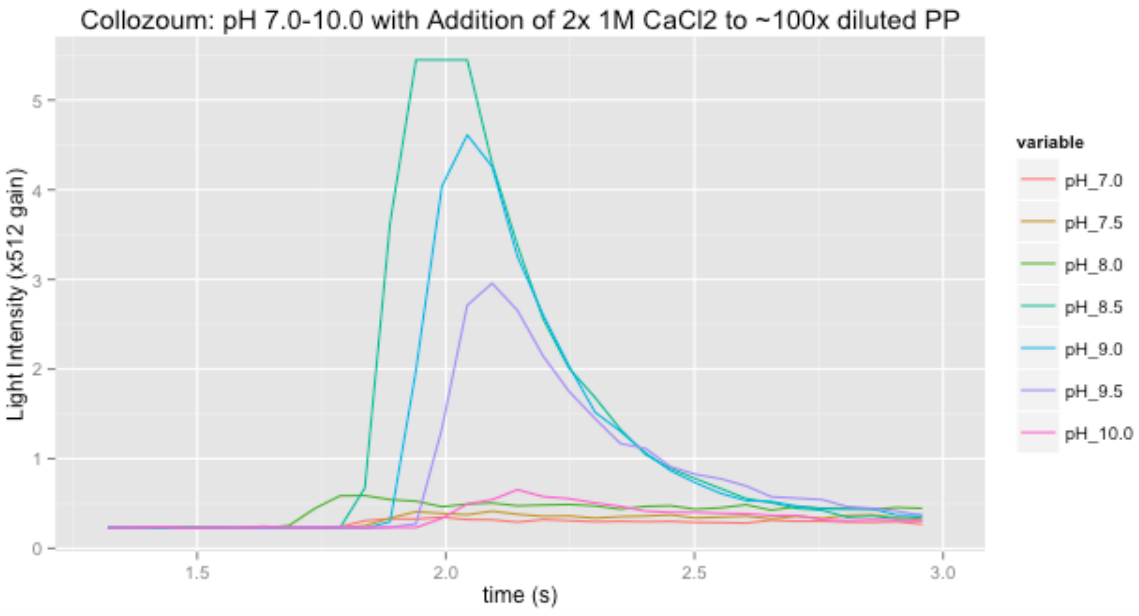

Figure 1. Using ~100x diluted regenerated crude photoprotein extract from the shallow-water radiolarian genus Collozoum, photoprotein-activation assays with Ca^{2+} were run at pH 7.0-10.0 with 0.5 increments.

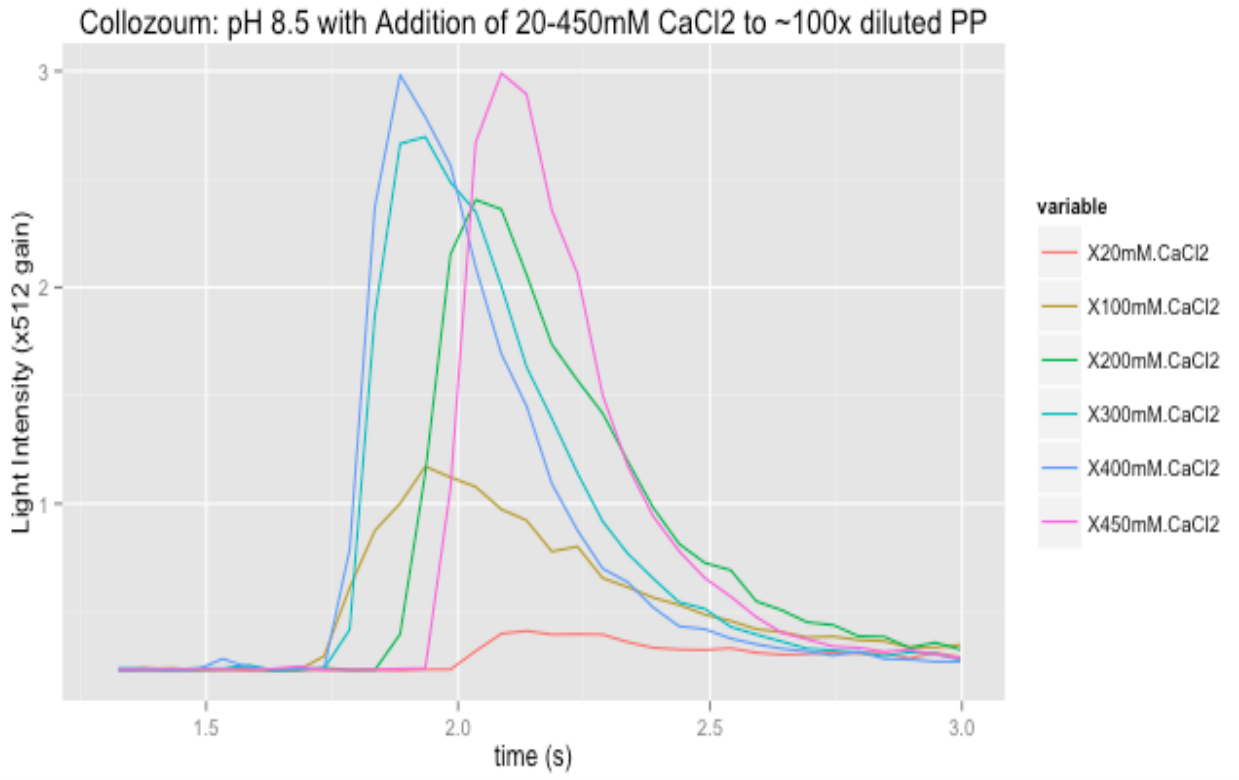

Figure 2. Using ~100x diluted regenerated crude photoprotein extract from the shallow-water radiolarian genus Collozoum, photoprotein-activation assays with final Ca^{2+}-concentrations of 20mM-450mM were run at pH 8.5.

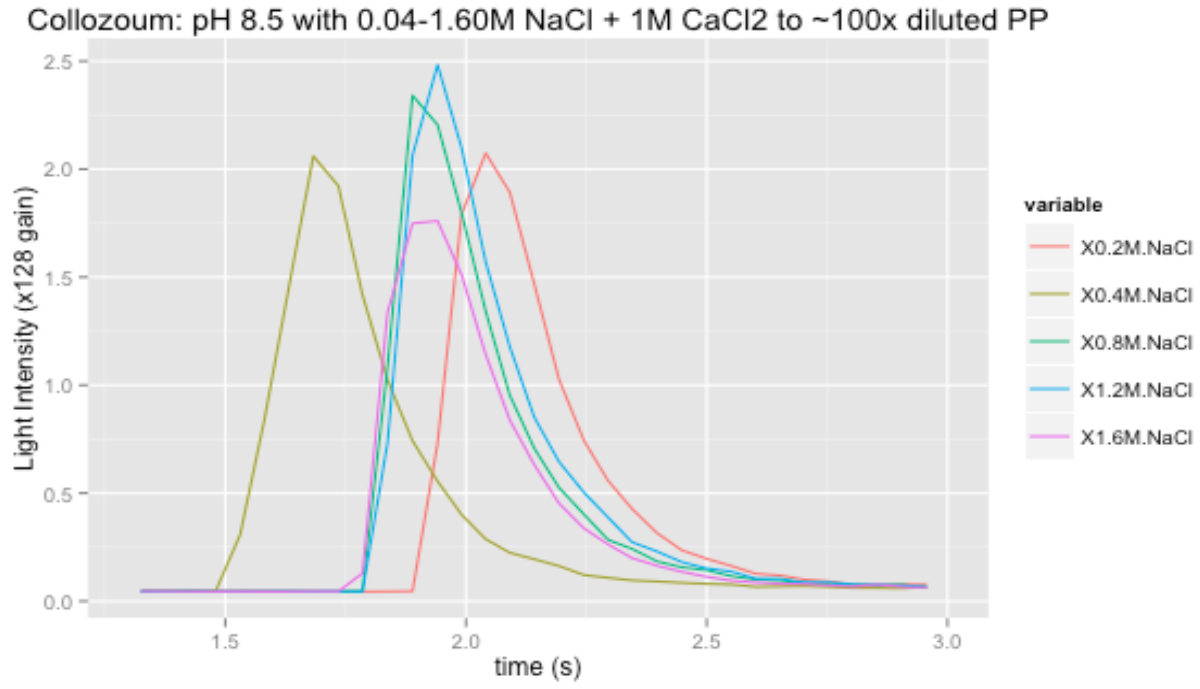

Figure 3. Using ~100x diluted regenerated crude photoprotein extract from the shallow-water radiolarian genus Collozoum in buffer with a sodium chloride concentration of 0.2M-1.6M, photoprotein-activation assays with Ca^{2+} were run at pH 8.5.

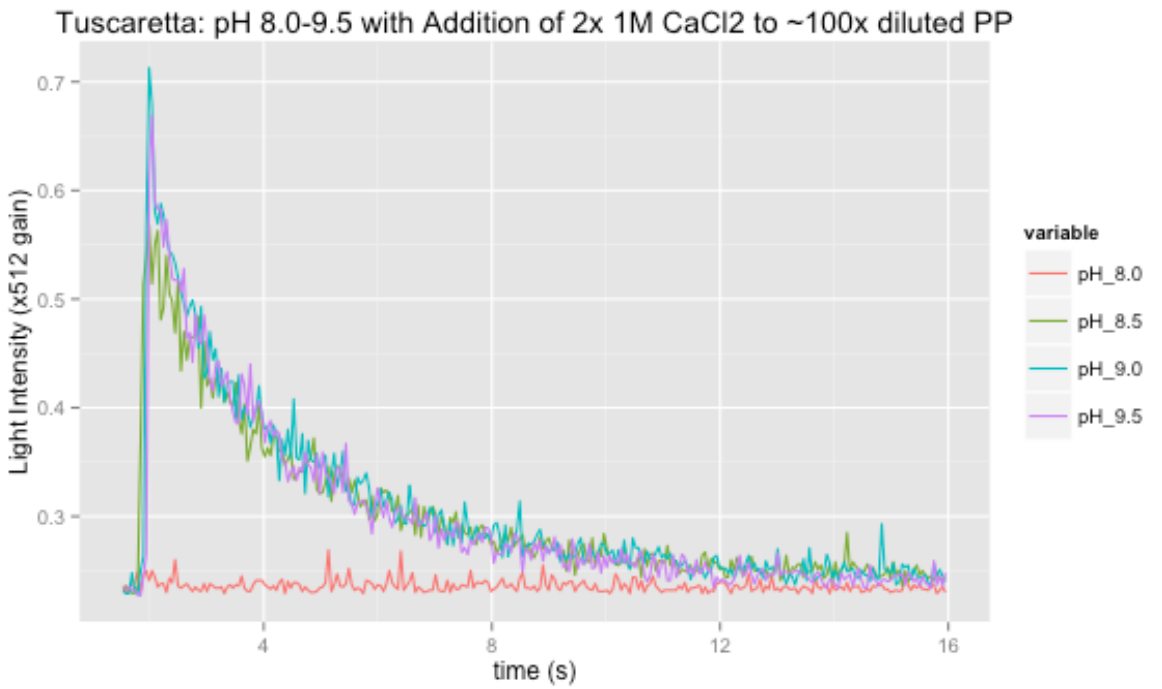

Figure 4. Using ~100x diluted regenerated crude photoprotein extract from the deep-water phaeodarian genus Tuscaretta, photoprotein-activation assays with Ca^{2+} were run at pH 8.0-9.5 in 0.5 increments.

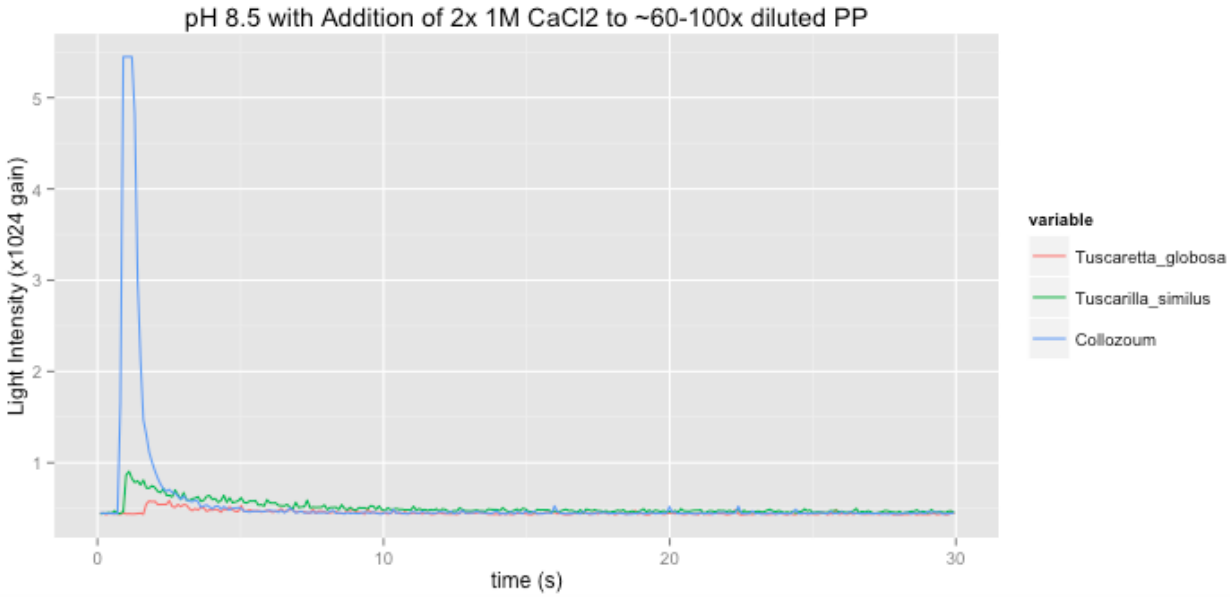

Figure 5. Comparison of the activity of Collozoum, Tuscaretta, and Tuscarilla photoproteins at pH 8.5 when activated with Ca^{2+}.

Figure 6. Preliminary result showing the light emission intensity after Ca^{2+}-activation of some component of a crude Doliolula extract.

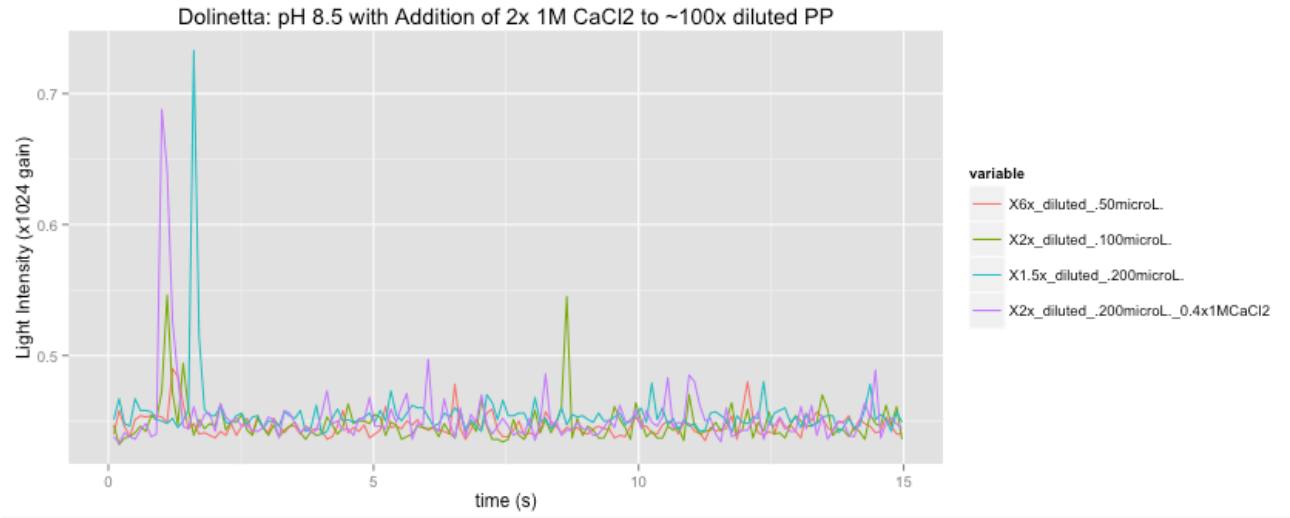

Figure 7. Results showing the light emission intensity after Ca^{2+}-activation of some photoprotein in a crude, regenerated Doliolinetta extract.

DISCUSSION

Crude photoprotein extract from frozen radiolarian and phaeodarian samples did not initially emit light upon addition of calcium, but did after undergoing successful regeneration. It is uncertain as to why the photoproteins dissociated into apo-photoprotein during specimen preservation, however possible explanations include cold-shock from freezing or the leakage of cofactor, i.e. calcium, to photoproteins during flash-freezing. Regeneration of Collozoum, Tuscaretta, and Tuscarilla samples was successful, and yielded the material used in these assays.

Maximum Collozoum photoprotein light emission for ~100x diluted sample was achieved at pH 8.5, salt (NaCl) concentration of 1.2 M, and with injected calcium chloride concentration of 400mM (Figures 1, 2, 3). Maximum Tuscaretta photoprotein light emission for ~100x diluted sample was achieved at pH 9.0. While these results were consistent over photoproteins from several specimen, they should be used only as approximate baseline conditions for future radiolarian and phaeodarian photoprotein assays.

Photoprotein from the shallow-water radiolarian genus, Collozoum, emitted light at an intensity greater than 10x that of photoprotein from either deep-water phaeodarian genera Tuscaretta and Tuscarilla (Figure 5). Additionally, when activated with calcium, Collozoum photoproteins emitted a flash of light that died after less than three seconds, whereas Tuscaretta and Tuscarilla photoprotein light emission lasted over 10 seconds. This difference in photoprotein light emission properties suggests that shallow-water radiolarians and deep-water phaeodarians have different photoproteins.

Preliminary results from calcium-injection assays on fresh Doliolula zooid samples initially suggested that a photoprotein was responsible for Doliolula bioluminescence (Figure 6). However, repeated assays in the lab both before and after attempted photoprotein regeneration showed no light reaction. Because the light reaction was irreplicable with calcium injection, it is possible that the preliminary result on the ship was due to contamination in the fresh Doliolula samples. One potential contaminant could have been a bioluminescent hydroid with a photoprotein, as some species are known to associate with doliolid zooids (Raskoff & Robison 2005). The light reaction achieved from calcium injection to a sample of Doliolinetta (Figure 7), a doliolid that is not known to be bioluminescent, could also be attributed to contamination, however there are no known bioluminescent associates for this genus.

CONCLUSION

More samples and data are required to identify the source of light from Doliolula preliminary and Doliolinetta regenerated photoprotein assays, therefore no conclusions can be drawn from data collected by these genera.

The optimal conditions for ~100x diluted Collozoum photoprotein bioluminescence are roughly determined to be at pH 8.5 with a salt (NaCl) concentration of 1.2 M, activated with a

minimum of 400mM calcium chloride final concentration. The optimal pH for ~100x diluted Tuscaretta photoprotein bioluminescence is roughly determined to be at pH 9.0.

While results from this research are valuable measures to use as a baseline for future work on these photoproteins, they should be considered preliminary results. More samples and data must be collected in order to make overreaching conclusions on the photoprotein activity of these genera.

A table summarizing the conclusions from this research is provided below:

Genus	pH	Salinity	Relative [ ] CaCl2	Regenerated
Collozoum	8.5	400mM	1.2 M	yes
Tuscaretta	9.0	?	?	yes
Tuscarilla	8.5-9.0	?	?	yes
Doliolula	8.5 (?)	?	?	no
Doliolinetta	?	?	?	yes

ACKNOWLEDGEMENTS

Thank you to Steve Haddock at MBARI for providing the opportunity, resources, and guidance required to make this research both possible and a positive experience. Thank you to the Haddock lab (Lynne Christianson, Severine Martini, Darrin Schultz, and Manabu Bessho) for continued support and help throughout this experience. Thank you to MBARI internship coordinators George Matsumoto and Linda Kuhnz for their support and for managing all internship details that fostered intern research. Thank you to the 2016 MBARI interns for their support and encouragement during this experience. Finally, thank you to the Julie and Hewlett Packard Foundation and all MBARI personnel for allowing for the respectful collaboration between MBARI professionals and burgeoning young scientists.

References:

Haddock, S. H. D., Moline, M. A., Case, J. F. (2010). Bioluminescence in the Sea. Annu. Rev. Mar.Sci., 2: 443-493.

Powers, M. L., Mermott, A. G., Shaner, N. C., & Haddock, S. H. D. (2012). Expression and characterization of the calcium-activated photoprotein from the ctenophore Bathocyroe fosteri: Insights into light-sensitive photoproteins. Biochemical and biophysical research communications, 431: 360-366.

Raskoff, K. & Robison, B. (2005). A novel mutualistic relationship between a doliolid and cnidarian, Bythotiara dolioeques sp. nov. Journal of the Marine Biol. Assoc. of the UK, 85(3): 583-593.

Shimomura, O. (2006). Bioluminescence: Chemical Principles and Methods. World Scientific Publishing Co. Pte. Ltd., Singapore.

Shimomura, O., Johnson, F. H. & Saiga, Y. (1962). Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. J. Cell Comp. Physiol., 59: 223-239.