

ONTOGENETIC AND PHYSIOLOGICAL VARIATION OF IRIDESCENCE IN *DIADEMA SAVIGNYI*

KAREN V. GALLARDO

Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. Iridescence, a type of structural coloration, has a variety of functions and is found in many different taxa. The echinoid family, Diadematidae, is known for possessing members with iridescent spines and tests. The iridophores of the genus *Diadema* have been particularly studied, but no studies have characterized variation of iridescence within this genus. Past studies have also found depressions on the genital plates matching the location of iridophores, but they have not reported them on other iridescent areas of the test. The principal objective of this study was to explain and characterize the variation of iridescence in *Diadema savignyi* resulting from changes in development and from physiological color change. The second objective was to describe the morphology of depressions on the test of *D. savignyi* corresponding to the location of iridophores. Urchin specimens were collected from various fringing reefs on the island of Moorea, French Polynesia, were photographed and measured for test and spine size, and the amount of iridescence around the apical system was determined with photographic analysis. The tests of five individuals were also bleached and analyzed for indentations matching the location of iridophores. The present study found that the amount of iridescence around the apical system decreases with age, but mid-sized individuals appear to have the most iridescence on the entire test. Around the apical system, the amount of iridescence is the same between dark- and light-adapted phases, but it seems to be higher in the interambulacrum in dark-adapted phases. Finally, there are grooves on the genital plates and around the madreporite that match the location of iridophores, but there are no abnormal grooves on the interambulacrum or surrounding the tubercles.

Key words: *marine invertebrates; Diadema; iridescence; ontogenetic variation; French Polynesia; Echinoidea*

INTRODUCTION

Animals and plants vary widely in color patterns and color serves many biological functions in nature. Many animals, including some birds (Keyser and Hill 2000), fishes (Houde 1987), and reptiles (Weiss 2006), choose their mates based, in part, on their coloration. Angiosperm flower color evolves to attract pollinators (Weiss 1991) and cephalopods are known to expand and contract their chromatophores for crypsis (Messenger 2001). Hence, producing color can provide many evolutionary benefits, from mate choice to predator avoidance.

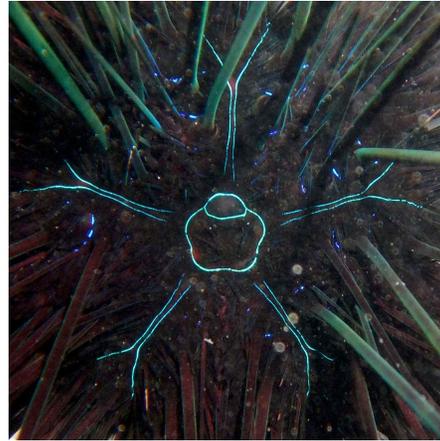
Pigmentary and structural coloration are two primary mechanisms for color production (Kinoshita et al. 2008). Pigmentary coloration occurs when pigments interact with light to absorb certain wavelengths. This is how color occurs in pigments, dyes, and metals. Structural coloration is produced by nanostructures on the organism that physically interact with light (Doucet and Meadows 2009). Such structural colors are produced

purely through the reflection, diffraction, and refraction of light on the iridescent structures of animals (Kinoshita et al. 2008). For example, there is no pigment in peacock feathers; rather, it is structural coloration that causes the feathers to shift between blue, green, and purple. This type of structural color is referred to as iridescence, a visual characteristic of surfaces that appear to change in color with viewing angle or angle of light (Land 1972).

Iridescence has been found in many taxa, including plants (Glover and Whitney 2010) and animals like arthropods, comb jellies, polychaetes, and mollusks (Doucet and Meadows 2009). Studies on cephalopods (Mathger et al. 2009) and diadematid echinoids (Millot and Manly 1961) have found structures called iridophores, changeable iridescent cells in the skin that underlie chromatophores. Some proposed biotic explanations for the presence of iridescence in animals are age differentiation (Lim and Li 2007), mate choice (Kodric-Brown and Johnson 2002), and predator deterrence (Fabricant et al. 2014). However, Parker (1998)

revealed that the evolution of iridescence predates the evolution of eyes, suggesting that not all iridescence functions for communication. Other potential functions of iridescence include thermoregulation (Kobelt and Linsenmair 1992), water repellency (Gower 2003), and photoreception enhancement (Douglas and Marshall 1999).

Iridescence of echinoids in the family Diadematidae has been recorded in at least five of its nine genera (Appendix A). However, it has been particularly studied in the genus *Diadema* (Millot 1950, 1952, 1953, 1954; Millot and Manly 1961; Randall 1964; Coppard and Campbell 2004; Coppard and Campbell 2006; Rodriguez et al. 2013). *Diadema savignyi*, the focus of this study, has brightly colored lines on the test around the apical system, around the tubercles, and lining the central part of the interambulacrum. These bright lines, or sometimes 'spots', as in other *Diadema* were at first thought to be eyes (Sarasin and Sarasin 1887), but when examined in *D. antillarum*, they were correctly identified as iridophores (Millot and Manly 1961). *D. savignyi* also has iridescent spines. Both spines and iridophores have short-wavelength iridescence, changing from violet, to blue, to green depending on the angle of light. After preliminary observations of this species, I noticed variation between individuals regarding the amount of iridophores covering the test, but no previous work has quantified and established explanations for variation of iridescence in this species or genus. Studies by Millot (1954) and Millot and Manly (1961) found that *D. antillarum* undergoes a physiological color change in which melanophores contract with decreased light intensity, revealing white coloration underneath. This occurs particularly in juveniles, most noticeably in the central part of the interambulacrum and around the apical system, where iridophores are also located. The dark pigment disperses again over the test when this species is exposed to bright light. Thus, these urchins have two phases: a light-adapted phase, and a dark-adapted phase (Fig. 1). This physiological color change has been noted in other members of the genus, as well (Coppard and Campbell 2006, Appendix A). In addition, differences in spine coloration between juveniles and adults suggest that coloration may be linked to development in *Diadema savignyi*.



a)



b)

FIG. 1. Aboral view of the same *D. savignyi* individual in the a) light-adapted phase and b) dark-adapted phase.

Previous studies have found depressions on the naked genital plates of *D. savignyi* that match the location of iridophores (Coppard and Campbell 2006, Rodriguez et al. 2013), but not much detail has been provided.

One objective of this study was to describe the morphology of depressions on the test of *D. savignyi* corresponding to the location of iridophores, but the primary goal of this study was to characterize variation of iridescence in different life stages of *D. savignyi*. Specifically, I asked the following questions: (a) Does test iridescence decrease as *D. savignyi* grows? (b) Is there more test iridescence in the dark-adapted phase of *D. savignyi* than in the light-adapted phase? I hypothesized that (a) Test iridescence would decrease as *D. savignyi*

grows and (b) the amount of iridescence would be higher in dark-adapted individuals of *D. savignyi*.

METHODS

Collection methods

This study was performed during October and November 2015 at the UC Berkeley Gump Research Station located on the island of Moorea, French Polynesia. Collection sites for *D. savignyi* were in the fringing reef of Temae Public Beach, the fringing reef of Motu Ahi, and the fringing reef in between Cook's Bay and Opunohu Bay (Fig. 2). However, most specimens were collected primarily from the fringing reef and rock wall near the dock of the UC Berkeley Gump Research Station (17°29'26.10"S, 149°49'33.39"W). These sites were chosen due to their accessibility and the presence of *Diadema savignyi* there.

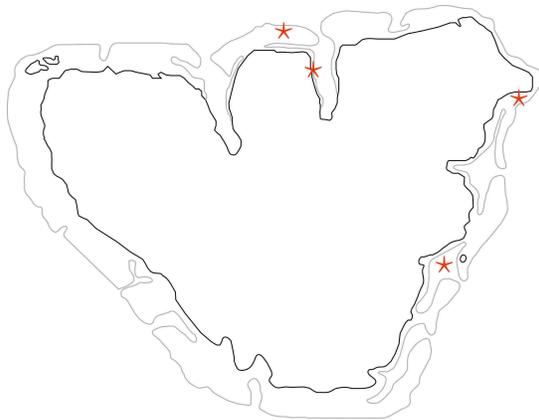


FIG. 2. Map of Moorea, French Polynesia. Stars indicate collection sites of *D. savignyi*.

Individuals were collected by using long forceps to gently prod them off the substrate, allowing them to land into a plastic container held below. Care was taken to capture them oral side down to prevent spine fracture. They were then transported to saltwater flow tanks. To facilitate differentiation between urchins, those of similar size were kept separate from each other. Urchins were kept for a maximum of five weeks and were fed *Sargassum pacificum* and *Padina pavonica* (Coppard and Campbell 2007). Presence or absence of iridescence was noted for each specimen collected.

Size measurements

Spine length and horizontal diameter (h.d.) was measured for all specimens. Using calipers, the horizontal diameter of each individual was measured at the ambitus, from the center of the ambulacrum to the center of the opposite interambulacrum. The jaws of the calipers were placed on the test, between the spines, being careful to maintain the urchin upright.

Ten of the most intact primary spines were then randomly chosen from various parts of the test and measured to acquire the average spine length. The bottom and sides of a thin plastic ruler were cut and rounded so the zero line was at the base and so it would fit better between the spines of the urchin. This ruler was placed next to a primary tubercle, touching the test, and the spine was pressed against the ruler to obtain its length.

Photography of iridescence

A Nikon COOLPIX AW110 camera was used to photograph all urchins with and without light to catalogue their natural and iridescent color. A ruler was placed next to the urchin for scale. For species with spine iridescence, spines were photographed using the 'fill flash' setting to reveal their iridescent colors.

Each individual of *D. savignyi* was photographed in its light-adapted phase after being exposed to light for at least an hour. Each individual was also photographed in its dark-adapted phase by being placed in a matte, covered tank for at least two hours before photography. Photographs were taken individually, placing the urchin in a rectangular plastic bin filled with salt water. A bright dive light was used to examine the iridescence and take note of its presence before photographing the urchin. Photographs were taken from above, capturing the apical system, madreporite, and central part of the interambulacra. The side of each individual was also photographed, capturing the ambulacra, the primary tubercles, and the central part of the interambulacra. Photographs were taken using the 'macro' mode and 'fill flash' settings without zoom. Photographs can be found on Figshare.com under the title of this paper.

Photographic analysis

Photographic analysis of iridophores was conducted using Adobe® Photoshop CC®

software. The apical system most noticeably demonstrated variation within the species and its photographic analysis was the easiest to standardize for all specimens examined. Therefore, the percent cover of iridophores in this area was chosen as a proxy for iridescence on the entire test. The brightness and contrast of the image were first adjusted to maximize visibility of the iridophores. The image was then cropped just outside of the gonopores to create a square around the apical system. Because individuals smaller than 10 mm in horizontal diameter had not yet developed noticeable apical plates or gonopores, the photographs were cropped immediate to the iridophores surrounding the apical system, where gonopores seem to be usually located in larger *D. savignyi*. After cropping the photograph, the 'color range' selection tool was used to automatically select pixels containing iridophore colors, and this selection was refined with the 'quick selection' tool. Finally, the number of selected pixels was divided by the total number of pixels in the cropped image to obtain the percent cover of iridophores in the apical system.

The anus of some individuals obstructed half of the apical system. For these photographs, the image was cropped further to bisect the apical system at the madreporite. I then used this cropped image to obtain the percent cover of iridescence. To determine if this was a reliable proxy for percent cover of iridescence around the apical system, photographs with a completely unobstructed apical system were cropped in the same fashion. Their percent iridescence values were then compared to those of the full apical system. The amount of iridescence did not vary between photographs of the whole apical system and half of the apical system (t-test, $p > 0.05$). Therefore, only half was used to determine percent cover of iridophores around this area for all individuals measured.

Identifying grooves on test

I bleached the tests of five individuals of *D. savignyi* whose iridescence I had previously photographed. I used urchins of differing sizes (hd = 42.5 to 90.2 mm) and examined their tests under a dissecting microscope. I also photographed them at the same angle as when they were live specimens to better compare location of iridophores with location of depressions on the naked test. The test of

one individual (h.d. = 65.8) has been deposited in the University of California Museum of Paleontology (UCMP123403, Appendix B).

Statistical analysis

All statistical analysis was conducted with the computer program, R (R Development Core Team, 2015). Percent iridescence between light- and dark-adapted phases was tested for normality with the Shapiro-Wilk Normality Test. Because the data was not normal, A Wilcoxon Rank Sum was run to assess the difference in quantity of iridescence between light and dark-adapted phases of *D. savignyi*.

A regression analysis was performed to compare spine length growth with horizontal diameter in *D. savignyi*. Another regression analysis was run to assess the relationship between horizontal diameter and percent cover of iridophores in the apical system. For this regression, I used the measurements obtained from photographic analysis of the dark-adapted phase of specimens.

RESULTS

Statistical analysis

The dark-adapted phase of *D. savignyi* does not have more iridescence around the apical system than the light-adapted phase (Wilcoxon Rank Sum, $W = 85.5$, $p > 0.05$).

Spine length increased with horizontal diameter in *D. savignyi*, signifying that the largest individuals had the largest spines, and vice versa (regression, $F = 80.04$, $p < 0.001$, $R^2 = 0.7768$, Fig. 3).

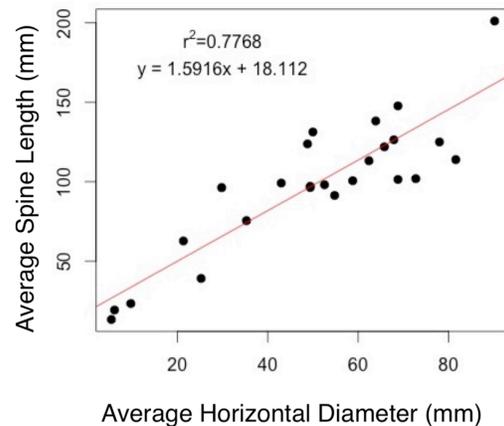


FIG. 3. Spine length increases as *D. savignyi* grows.

The smallest individual (h.d. = 5.44 mm) had 13.39% more iridescence than the largest individual (h.d. = 90.2). The amount of iridophores present on the apical system decreases as *D. savignyi* grows (regression, $F = 35.73$, $p < 0.001$, $R^2 = 0.6776$, Fig. 4).

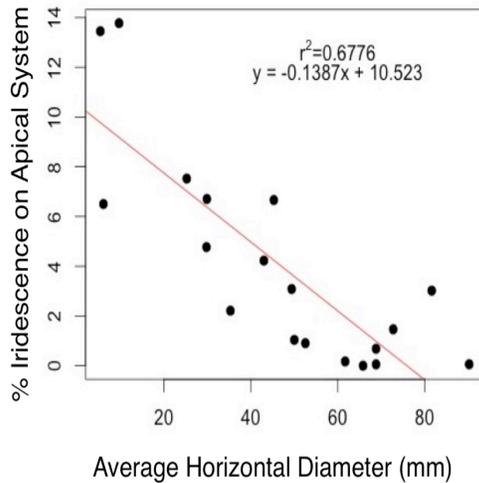
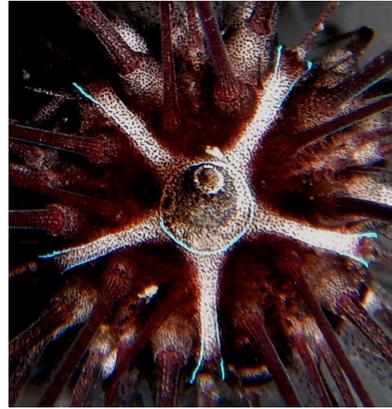


FIG. 4. Iridescence around the apical system decreases as *D. savignyi* grows.

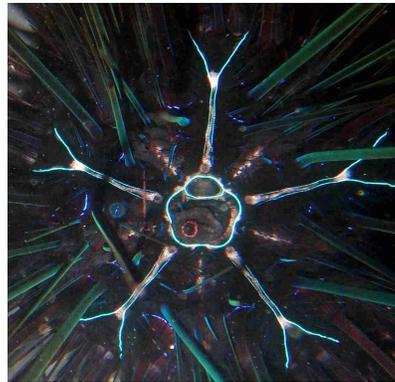
Iridescence of D. savignyi

The youngest urchins (h.d. = 5.4 to 9.7 mm) do not have strong iridescence on their spines, but in all individuals, spines are uniformly iridescent from the base to tip. Iridophores are found at the base of the triangular apical plates proximal to the anus, surrounding the madreporite, around the primary tubercles of the interambulacrum, and as a double row and fork in the center of the interambulacrum (Fig 5b). The collective shape of iridophores on the test changes as urchins grow in test diameter (Fig 5a, b, c).

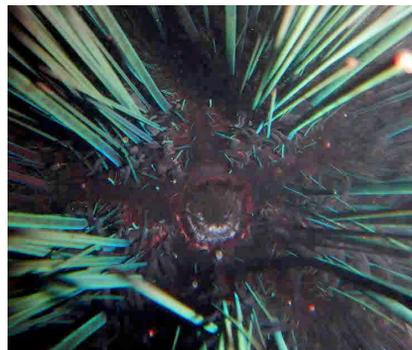
Apical system and madreporite: The smallest urchins, which have not developed a mature apical system yet, have a circular shape of iridophores around the periproct (Fig. 5a), but as they grow, the shape of the iridophores surrounding the periproct changes from round to increasingly more pentagonal, following the triangular base of the genital plates and the sides of the madreporite (Fig. 5b). A line of iridophores also develops at the base of the madreporite. However, individuals gradually lose this iridescence as they grow.



a)



b)



c)

FIG. 5. Aboral view of iridophores and spine iridescence in dark-adapted *D. savignyi* of varying horizontal diameters: a) 6.1 mm, b) 43 mm, c) 90.2 mm.

Tubercles: Iridophores brightly surround the primary interambulacral tubercles of medium-sized individuals, but these iridophores are not seen in small nor large individuals. Only one, medium-sized, individual possessed

iridophores around the primary tubercles of the ambulacra.

Interambulacrum: In medium, small, and large individuals, a parallel row of iridophores lines the center of the interambulacrum, radiating out from the gonopores. These rows of iridophores are parallel in small individuals, but in medium-sized individuals, they bifurcate into an arch-shaped fork near the ambitus at the apex of the interambulacrum, where a white dot appears in dark-adapted individuals. This fork of iridophores passes around the base of some primary and secondary tubercles in the interambulacrum, creating a wavy pattern (Fig. 6). Large individuals lose this iridescence, beginning with the parallel lines, and losing the fork of iridophores last.

There was no statistical difference in the amount of iridescence found around the apical system between dark-adapted and light-adapted phases of *Diadema savignyi*. However, there is a visual difference between these two

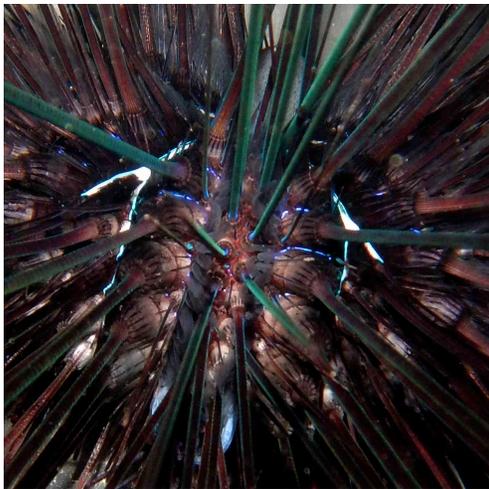


FIG. 6. Side view of individual of *D. savignyi* with iridescence around primary tubercles of ambulacrum (h.d. = 43 mm).

phases in some individuals, especially in the iridophores appearing in the central part of the interambulacrum (Fig. 7a, b).

Grooves on test of D. savignyi match location iridophores

The final part of the study found that, in *D. savignyi*, the location of iridophores around the tubercles and in the interambulacrum does not match any grooves on the test. However, there is a deep narrow groove outlining the

madreporite directly below the area where iridophores are located (Appendix C).



a)



b)

FIG. 7. Aboral view of the same *D. savignyi* individual (h.d. = 72.8 mm) in the a) light-adapted phase and b) dark-adapted phase. Iridophores are not present in the light-adapted phase, but become apparent in the dark-adapted phase.

There is also a groove on the apical plates, clearly outlining the shape of iridophores around the apical system. This groove is shaped like a bell curve and is approximately the same width as the groove of the madreporite. It is obvious between the gonopores and the small tubercles of the genital plates (Appendix C). The edges of this groove lead to an area between the small tubercles of the ocular plate and the periproct, but there is no noticeable second groove here. The areas described match exactly with the

location of iridophores in live specimens and they are present even after the urchin has lost iridescence in these areas (Appendix C).

DISCUSSION

This study has shown that test iridescence changes with age in *Diadema savignyi*. Although the smallest individuals have the greatest percent cover of iridescence on the apical system, it appears as though the medium sized individuals are the brightest overall because they have iridescence around the tubercles, under the madreporite, and on the interambulacral fork, in addition to the apical system. This may signify that the mid-sized urchins have a greater need for iridescence than small and large urchins, although the reason why is unclear. This study only quantified iridescence around the apical system, but a decrease in iridescence is visually noticeable from medium to large individuals in other parts of the test, beginning with the apical system and ending with the interambulacral fork. The present study also found that the shape of iridophores on the test changes with age, and adds to the description of iridophores of *D. savignyi* given by Coppard and Campbell (2006), which did not mention the presence of iridophores around the tubercles of the interambulacrum.

Coppard and Campbell (2006) also described 'clear arch-shaped depressions' on the naked genital plates of both juveniles and adults. The present study found grooves on the genital plates that agree with this description, and as Rodriguez et al. (2013) observed, their location match that of the iridophores on live specimens. A new finding of this study is that there is also a deep groove around the madreporite that matches the location of iridophores. Because these grooves are also present in other members of this genus and correspond with the location of iridophores, they may be used as a way to determine the presence of iridophores in fossil species of this genus. However, because *D. savignyi* retains these grooves even after losing iridescence, we can only determine if these iridophores were once present on the test, but not at the time of death.

The function of iridescence in diadematids remains unknown, but the results of this study may provide some clues. Iridophores are located on the aboral side of the test, so iridescence may function as a way to communicate with predators. One possibility is that iridescence functions as an aposematic

signal, but if this were true, it would be expected for the largest, most dangerous individuals to have the most iridescence, and this is not the case.

Alternatively, a recent study showed that iridescence in flies reduces the attack success of avian predators (Pike 2015). Although this study focused on terrestrial taxa, it may also apply to echinoids. The primary predators of *Diadema* are the balistid fishes, *Balistaphus undulatus* and *Rhinecanthus aculatus* (McClanahan and Shafir 1990), both species present on Moorea. These fish have the ability to differentiate between blue and purple (Pignatelli et al. 2010), which are colors present on the iridophores of the echinoids in the genus *Diadema*. This opens the possibility that the color of iridescence in *Diadema savignyi* corresponds to the color vision of their primary predators. Although *D. savignyi* hide in crevices during the daytime, they still face the risk of predation by diurnal balistid fishes and by nocturnal predators, like crabs, so we cannot deny the possibility that iridescence functions as a way to startle predators.

Finally, this species is sensitive to light and responds to shadows of potential predators on its test by waving its long, sharp spines in defense. Millot (1954) suggested that iridophores in *D. antillarum* may function as a way to enhance photosensitivity in the darkness by using their reflective properties to diffuse light over the surface of the photoreceptive test. The present study showed that the percent cover of iridescence around the apical system is the same between the dark- and light- adapted phase of *D. savignyi*. However, I did not measure iridescence over the entire test. Visually, there appears to be more iridescence in the dark-adapted phase, particularly in the interambulacrum, so we cannot deny the hypothesis that iridophores enhance photosensitivity. Shadows are much less defined in the darkness, so by exposing its iridophores in the dark, individuals may have a greater ability to perceive shadows in dark environments, thus allowing individuals to escape potential predators.

Future Directions

I did not collect enough different color morphs to compare iridescence between them, but I suspect that iridescence may not be as bright in lighter-colored urchins based on the observations I made of the few I collected. Perhaps this could be a future study that may

provide further clues regarding the function of iridescence in *Diadema*. To determine if iridescence truly is a defense against predation, one can run predation trials with hermit crabs and balistid fish, which are known predators of these urchins. Additionally, one could possibly test the hypothesis that iridophores increase sensitivity to light by lightly scraping off iridophores from the test and following a methodology similar to that described in Millot and Manly (1961). Finally, Diadematidae is not the only echinoid family to have iridescence. I have also noticed it on the aboral central tubercles of *E. mathaei*, and have seen images of *Asthenosoma* spp. with what may be iridophores on the aboral test as well. Establishing which echinoid groups and species have iridescence and comparing these with a complete phylogeny of Echinoidea could give us information regarding the evolution of iridescence. However, no complete phylogeny of Echinoidea nor Diadematidae exists, so this could also be a future project.

ACKNOWLEDGEMENTS

I must first thank the UC Berkeley Undocumented Student Program and the NERDS Program for providing me with grants to be able to participate in this course. I appreciate all of the help I received from the professors, Brent Mishler, Stephanie Carlson, Patrick O'Grady, Jonathon Stillman, Cindy Looy, and Vince Resh in developing and organizing my project. The GSIs were truly the most amazing and dedicated GSIs I have ever had and always went beyond the line of duty. Thank you, Eric Armstrong for your help with R, Dave Kurz for your help with writing, and Camilla Souto, echinoderm queen, for helping me with my million questions on sea urchins. I must also thank Erica Ta, Elliot Steele, Blair Conklin, John Bobroskie, Numfah Vanitchanant, Morgan Ziegenhorn, and Yoon Woo for accompanying me on my night expeditions to collect sea urchins. Finally, my most sincere mauru'uru (thank you) to my Gump family, the class of 2015, for making this course the best experience of my life.

LITERATURE CITED

Coppard, S. E., and A. C. Campbell. 2004. Taxonomic significance of spine morphology in the echinoid genera

- Diadema* and *Echinothrix*. *Invertebrate Biology* **123**:357-371.
- Coppard, S. E., and A. C. Campbell. 2006. Taxonomic significance of test morphology in the echinoid genera *Diadema* Gray, 1825 and *Echinothrix* Peters, 1853 (Echinodermata). *Zoosystema* **28**:93-112.
- Coppard, S. E., and A. C. Campbell. 2007. Grazing preferences of diadematid echinoids in Fiji. *Aquatic Botany* **86**:204-212.
- Doucet, S. M., and M. G. Meadows. 2009. Iridescence: a functional perspective. *Journal of The Royal Society Interface* **6**:S115-S132.
- Douglas, R. H. and N. J. Marshall. 1999. A review of vertebrate and invertebrate ocular filters. *in* S. Archer, M.B. Djamgoz, E. Loew, J. C. Partridge, S. Vallerga, editors. *Adaptive Mechanisms in the Ecology of Vision*. Springer Netherlands, Kluwer, Dordrecht, The Netherlands.
- Fabricant, S. A., A. Exnerova, D. Jezova, and P. Stys. 2014. Scared by shiny? The value of iridescence in aposematic signalling of the hibiscus harlequin bug. *Animal Behaviour* **90**:315-325.
- Glover, B. J., and H. M., Whitney. 2010. Structural colour and iridescence in plants: the poorly studied relations of pigment colour. *Annals of Botany* **105**:505-511.
- Gower, D. J. 2003. Scale microornamentation of uropeltid snakes. *Journal of Morphology* **258**:249-268.
- Houde, A. E. 1987. Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution* **41**:1-10.
- Keyser, A. J., and G. E. Hill. 2000. Structurally based plumage coloration is an honest signal of quality in male blue grosbeaks. *Behavioral Ecology* **11**:202-209.
- Kinoshita, S., S. Yoshioka, and J. Miyazaki. 2008. Physics of structural colors. *Reports on Progress in Physics* **71**:076401.
- Kobelt, F., and K. E. Linsenmair. 1992. Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia: Anura: Hyperoliidae) to its arid environment - VI. The iridophores in the skin as radiation reflectors. *Journal of Comparative Physiology* **162**:314-326.
- Kodric-Brown, A., and S. C. Johnson. 2002. Ultraviolet reflectance patterns of male guppies enhance their attractiveness to females. *Animal Behaviour* **63**:391-396.

- Land, M. F. 1972. The physics and biology of animal reflectors. *Progress in Biophysics and Molecular Biology* **24**:75-106.
- Lim, M. L. M., and D. Li. 2007. Effects of age and feeding history on structure-based UV ornaments of a jumping spider (Araneae: Salticidae). *Proceedings of the Royal Society of London B: Biological Sciences* **274**:569-575.
- Mäthger, L. M., E. J. Denton, N. J. Marshall, and R. T. Hanlon. 2009. Mechanisms and behavioural functions of structural coloration in cephalopods. *Journal of The Royal Society Interface* **6**:S149-S163.
- McClanahan, T.R., and S. H. Shafir. 1989. Causes and consequences of sea urchin abundance and diversity in Kenyan coral reef lagoons. *Oceologia* **83**:362-370.
- Messenger, J. B. 2001. Cephalopod chromatophores: neurobiology and natural history. *Biological Reviews* **76**:473-528.
- Millot, N. 1954. Sensitivity to Light and the Reactions to Changes in Light Intensity of the Echinoid *Diadema antillarum* Philippi. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **238**:187-220.
- Millot, N. 1950. The sensitivity to light, reactions to shading, pigmentation, and colour change of the sea urchin *Diadema antillarum* Philippi. *The Biological Bulletin* **99**:329-330.
- Millot, N. 1952. Colour change in the echinoid *Diadema antillarum* Philippi. *Nature* **170**:325-326.
- Millot, N. 1953. Light Emission and Light Perception in Species of *Diadema*. *Nature* **171**:973.
- Millot, N. 1954. Sensitivity to light and the reactions to changes in light intensity of the echinoid *Diadema antillarum* Philippi. *Philosophical Transactions of the Royal Society* **238**:187-220.
- Millot, N. and B. M., Manly. 1961. The iridophores of the echinoid *Diadema antillarum*. *Quarterly Journal of Microscopical Science* **102**:181-194.
- Parker, A. R. 1998. Colour in Burgess Shale animals and the effect of light on evolution in the Cambrian. *Proceedings of the Royal Society of London B: Biological Sciences* **265**:967-972.
- Pignatelli, V., C. Champ, J. Marshall, and M. Vorobyev. 2010. Double cones are used for colour discrimination in the reef fish, *Rhinecanthus aculeatus*. *Biology Letters* **6**: 537-539.
- Pike, T. W. 2015. Interference coloration as an anti-predator defence. *Biology Letters* **11**: 20150159.
- Randall, J. E., R. E. Schroeder, and W. A. Starck. 1964. Notes on the biology of the echinoid *Diadema antillarum*. *Caribbean Journal of Science* **2**:421-433.
- Rodriguez A., J. C. Hernandez, S. Clemente, and S.E. Coppard. 2013. A new species of *Diadema* (Echinodermata: Echinoidea: Diadematidae) from the eastern Atlantic Ocean and a neotype designation of *Diadema antillarum* (Philippi, 1845). *Zootaxa* **3696**:144-170.
- Sarasin and Sarasin. 1887. *Ergebnisse Naturwissenschaftlicher Forschungen Auf Ceylon*. Wiesbaden: C.W. Kreidel.
- Weiss, M. R. 1991. Floral colour changes as cues for pollinators. *Nature* **354**:227-229.

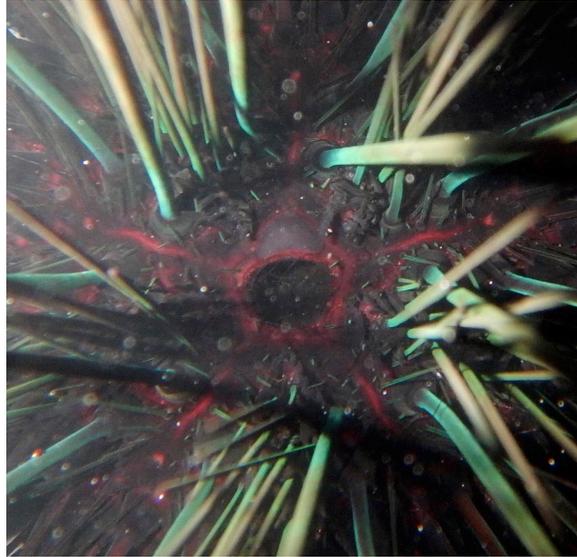
APPENDIX A

Diadematidae species and observed presence of spine and test iridescence, and of physiological color change.

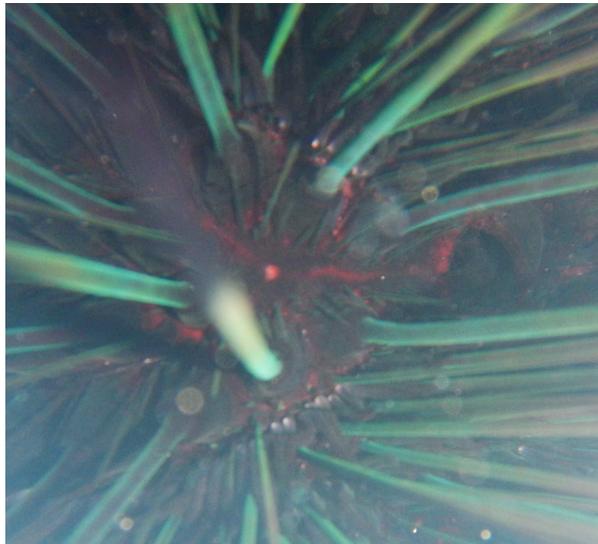
Genus	Species	Spine iridescence	Iridophores on test	No iridescence	No information	color change	citation
<i>Centrostephanus</i>	<i>coronatus</i>	x					Personal observation: google images
	<i>longispinus</i>	x					Personal observation: google images
	<i>rogersii</i>	x					Personal observation: google images
	<i>tenuispinus</i>	x					Personal observation: google images
	<i>asteriscus</i>				x		Personal observation: google images
	<i>besnardi</i>					x	Personal observation: google images
	<i>nitidus</i>					x	Personal observation: google images
	<i>sylviae</i>					x	Personal observation: google images
<i>Echinothrix</i>	<i>Calamaris</i> (light morph)			x			Personal observation
	<i>Calamaris</i> (dark morph)	x					Observed by Simon Coppard
	<i>diadema</i>	x	x				Personal observation: google images
<i>Diadema</i>	<i>africanum</i>	x	x				Personal observation
	<i>antillarum</i>	x	x			x	Coppard and Campbell 2006
	<i>ascencionis</i>	x	x			x	Coppard and Campbell 2006
	<i>mexicanum</i>	x	x			x	Coppard and Campbell 2006
	<i>paucispinum</i>	x	x			x	Coppard and Campbell 2006; personal observation: google images
	<i>savignyi</i>	x	x			x	Coppard and Campbell 2006
	<i>setosum</i>	x	x			x	Coppard and Campbell 2006
	<i>palmeri</i>		x				Personal observation: google images
<i>Astropyga</i>	<i>magnifica</i>		x				Personal observation: google images
	<i>pulvinata</i>		x				Personal observation: google images
	<i>radiata</i>		x				Personal observation: google images
	<i>nuptialis</i>					x	Personal observation: google images
<i>Chaetodiadema</i>	<i>granulatum</i>		x				Personal observation: google images
	<i>japonicum</i>		x				Personal observation: google images
	<i>pallidum</i>				x		Personal observation: google images
	<i>keiense</i>					x	Personal observation: google images
	<i>africanum</i>					x	Personal observation: google images
	<i>tuberculatum</i>					x	Personal observation: google images
<i>Lissodiadema</i>	<i>lorioli</i>			x			Observed by Rich Mooi
<i>Leptodiadema</i>	<i>purpureum</i>			x			Personal observation: google images
<i>Eremopyga</i>	spp.					x	Personal observation: Google images
<i>Goniodiadema</i>	spp.					x	Personal observation: Google images

APPENDIX B

Light-adapted phase of *D. savignyi* specimen deposited in University of California Museum of Paleontology. h.d. = 65.8. Specimen ID number: UCMP123403; a) aboral view of apical system and interambulacra; b) side view of interambulacrum and interambulacral fork.



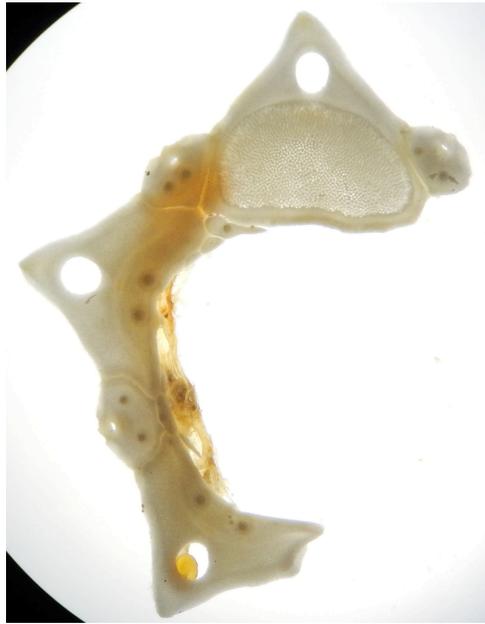
a)



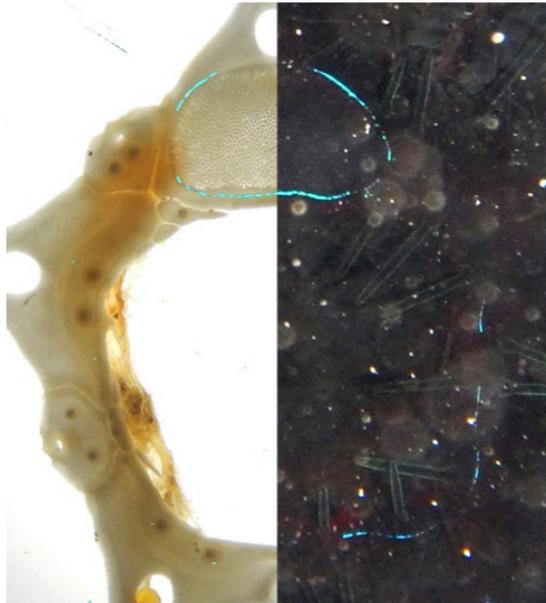
b)

APPENDIX C

Images depicting the grooves of the naked apical system of the same individual of *D. savignyi* (h.d. = 58.8 mm). a) half of the naked apical system, note the groove on the genital plates and surrounding the madreporite; b) image (a) placed alongside an image of the apical system of the live individual. Iridophores were cut and pasted above the image of the naked apical system to demonstrate how well they match the groove of the madreporite. Image was edited using Adobe® Photoshop CC®.



a)



b)