EFFECTS OF OCEAN ACIDIFICATION ON THE FECUNDITY AND CHEMORECEPTION OF THE CHROMODORIS LOCHI NUDIBRANCH

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Abstract. Ocean acidification is no longer just about calcification. All marine organisms are prone to experience adverse effects from an acidic ocean directly or indirectly, regardless of their ability to calcify. A change in pH can affect different biochemical processes. Since chemoreception depends on the presence of chemical stimuli, a change in pH can affect the ability of an organism to detect these cues. I investigated the effects that projected acidic conditions would have on *Chromodoris lochi*, a species of nudibranch found on Moorea, French Polynesia. These effects were measured by testing for changes in fecundity as an indicator of the overall well being of the organisms and by testing their ability to detect chemical stimuli. Nudibranchs were held in two types of seawater: a.) acidified enriched with CO_2 (pCO_2 = 1200 ppm, pH = 7.60) or b.) untreated (pH = 8.02) as a control. Overall, the group of nudibranchs held in acidic seawater showed a higher fecundity, indicating higher stress. They were also less successful at detecting chemical stimuli. Thus, my results indicate that ocean conditions projected for the year 2100 can lead to reproductive challenges for this species of nudibranch.

Key words: Chromodoris lochi; nudibranch; ocean acidification; chemoreception; fecundity; Moorea, French Polynesia

INTRODUCTION

Anthropogenic carbon dioxide (CO_2) levels have been increasing since the Industrial Revolution changing chemical systems on the planet. The ocean, covering roughly 70% of the planet, absorbs about 30% of released anthropogenic CO_2 (Cooley and Doney 2009, Denman et al. 2007, Sabine et al. 2004). When CO_2 dissolves in water it produces a weak carbonic acid (H_2CO_3) lowering the pH. Ocean acidification is the drop of seawater's pH as a consequence of increased uptake of atmospheric CO_2. So far, the pH of seawater has dropped by about 0.1 units since pre-industrial times and it is projected to drop about another 0.3-0.4 units by the year 2100 (IPCC 2007), more than double the current ocean acidity. This change in the ocean's chemistry leads to changes in other systems including the biochemistry of organisms. Unless the levels of CO_2, and thus the pH, are stabilized, marine life will face unknown consequences, possibly serious (Hoegh-Guldberg et al. 2007, Guinotte and Fabry 2008).

The biota of the world's oceans has already undergone significant changes as a consequence of the increase in acidity, most noticeably regarding calcareous organisms like pteropods whose shells dissolve under these conditions (Orr 2005). Reefs, which are some of the most biodiverse ecosystems on the planet (Odum and Odum 1955), provide goods and services for humans, such as seafood or coastal protection (Moberg and Folke 1999), yet they have continued to deteriorate with increasing rates as a result of human influence (Hoegh-Guldberg et al. 2007). Other organisms, such as echinoderms and forams, are also highly susceptible because of their decreasing ability to calcify under acidic conditions (Dupont et al. 2010). Changes in pH along with the changes in temperature and carbonate saturation are occurring fast; while some organisms readily adapt, many others do not have a chance to adapt to the combined stress factors (Byrne and Przeslawski 2013). Among the calcifying organisms, some species of algae are also subject to the effects of ocean acidification. Calcareous algae, such as *Halimeda*, have often been neglected in past ocean acidification studies even though they play significant roles in reef ecosystems–they contribute greatly to calcification and productivity rates due to their rapid growth and turnover rates (Price et al. 2011).

While much attention has been paid to calcareous organisms, studies showing the effects of ocean acidification on non-calcifying organisms are not as common. Focus is starting to change with the realization that ocean acidification can affect all organisms at different stages in their development. A study by Trevor Allen (2012) concluded that under projected temperatures and acidity for the year 2100, there was reduced larval development of the opisthobranch gastropod, *Stylocheilus striatus.* While its adult form is non-calcareous, the larval stage forms a protective calcareous shell. Species of opisthobranchs could potentially decline in numbers since the shell, a common larval feature, is negatively impacted by the increased acidity. Studies on opisthobranchs in the context of ocean acidification have primarily focused on the larval stage, but adult opisthobranchs are also likely to show adverse effects in acidic seawater

Because acidity depends on the amount of H^+ ions, low pH conditions can disrupt chemical systems that are essential for marine organisms. Chemoreception is the ability of an organism to detect chemical stimuli in the environment, which aid in several functions like locating food or mates. Munday et al. (2008) discovered that larval clownfish were unable to respond to olfactory cues when pH dropped to a critical low level. There have been other studies that produced similar results among other fish and different species of crabs. Nudibranchs, a type of opisthobranch, are marine gastropods known to use chemoreception to locate food and mates due to their specialized diets and poor eyesight (Croll 1982). In 2005, Hampton Smith showed that nudibranchs are more likely to orient themselves toward food in the presence of a current. Based on the chemical compounds that nudibranchs use as chemical cues (Karuso 1987), one can infer that chemoreception might be compromised for the nudibranchs under acidified conditions.

Because of their heavy reliance on chemoreception, I hypothesize that nudibranchs are especially prone to suffer consequences under projected conditions. Specifically, due to the effects of ocean acidification on chemoreception, the nudibranch, *Chromodoris lochi*, is likely to be less successful at finding mates. Furthermore, since reproduction can serve as an indicator of an animal's well being, I also hypothesized that nudibranchs subject to the effects of ocean acidification would be under increased stress and therefore show an increase in fecundity (Moberg 1985). This study used lab studies to test these hypotheses.

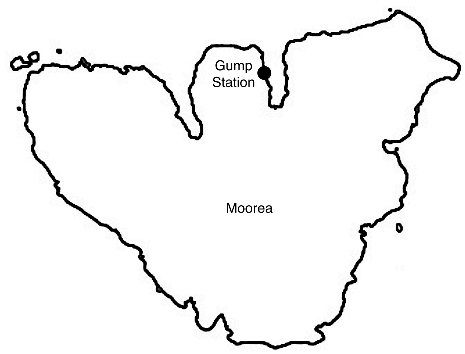

FIG. 1. Map depicting the location of the University of California Gump Field Research Station and the location of the collection site for *C. lochi* on Moorea, French Polynesia.

METHODS

Site description, organism collection and maintenance

Adult *Chromodoris lochi* (N=120; Appendix A and B) were collected from the degraded fringing reef off the coast of the University of California Gump Field Research Station on Moorea, French Polynesia (-17.489781, -149.825462; Figure 1) (Gosliner 1996). The first collections occurred on the week of October 7th, 2013, with weekly collections occurring from October 28th, 2013 until November 11th, 2013. Each week, 40 individuals were collected and divided into two batches of 20, one for each seawater treatment (see below). All batches were kept for 2 weeks in order to allow the nudibranchs to adapt to the new environment during the first week with tests occurring on the second week. The nudibranchs were starved during the duration of the experiment because their specialized food was not found on the reef, which may be due to overgrazing by the nudibranchs.

Following collection, nudibranchs were kept in a gallon-sized Ziploc bag with seawater during transport to the laboratory where they were transferred to individual cups of varying volumes within five hours of harvest. Half of the specimens were randomly assigned to one of two seawater treatments: a.) acidified seawater enriched with CO_2 (pCO_2 = 1200 ppm, pH = 7.60) or b.) untreated seawater (pH = 8.02) as a control supplied from Cook's Bay by a constant flow through system. Water changes occurred daily in order to maintain constant pH levels and oxygen concentrations.

Description of egg masses

In the laboratory, *C. lochi* egg clutches were oviposited on either the inner lining of the cups housing the nudibranchs or were free floating in the seawater of the cups. Egg clutches were mostly produced as continuous egg ribbon spirals averaging 89.61 cm^2 in area (Appendix C) but occasionally the integrity of the spiral was compromised and the egg ribbons were either tangled or the spiral shape was skewed. The egg spirals were laid flat and the ova were cream in color, both typical characteristics for this particular species of nudibranch (Wilson 2002).

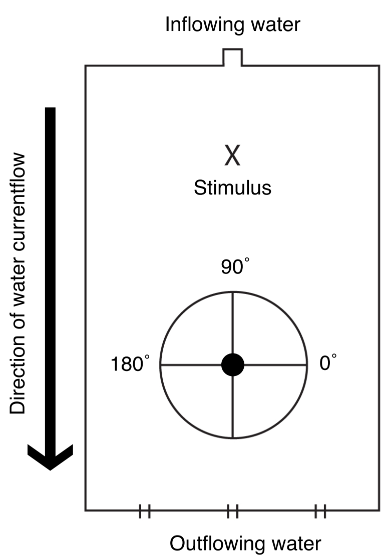

FIG. 2. Diagram of experimental setup. The test subject–represented by the black circle–was placed in the center of the circle facing 180˚ in order to avoid directional influence. A chemical stimulus was placed at the opposite end of the container and was carried by a current, creating a plume in the process. The test subject was placed 30cm away from the stimulus in a circle with a radius of 15cm.

Fecundity

Changes in the fecundity of the nudibranchs were measured by tracking the deposited egg clutches during the daily water changes. The date the egg clutches were deposited and the seawater type were both noted. Egg mass dimensions were measured in order to calculate the area in cm^2 using this as a proxy for total egg count assuming there is a constant egg density. Egg clutches were marked to avoid double counting. Data measuring the effect of the seawater treatment on the area of the egg clutches was analyzed using a Mann-Whitney U test. The total egg clutches per seawater treatment was compared using a chi-squared test. All data analyses were conducted in R (R Development Core Team 2013).

Chemoreception

The effect of acidic seawater on the chemoreception of *C. lochi* was tested in a tub with inflowing seawater that exited on the opposite side creating a current. A mate acting as a stimulus was placed in a cage on one side of the table and the individual being tested was placed 30 cm away. A circle with a 15 cm radius was drawn around the test subject in order to measure the angle of its position upon exiting in relation to the stimulus. Test subjects were placed perpendicular to the stimulus facing 180˚ in order to avoid directional influence. Hampton Smith's experimental design from 2005 served as a model for this experimental setup. Figure 2 shows a diagram of the experimental set up.

Three minutes were allowed between trials in order to create a chemical stimulus plume in the seawater and each individual was allowed five minutes to exit the circle. An individual organism was considered successful if it exited the circle during the allotted five minutes. The angle of the exit point was measured using a protractor and the time was recorded with a stopwatch. Unsuccessful individuals were noted as having a time of 301s and their orientation at the end of the allotted time was recorded. Before each trial, the pH of the seawater in the individual's cup was measured to two decimal places in order to check for consistency between individuals and between water treatments. In between trials, the table was cleared of debris and leftover chemical cues from the previous individual.

Circular statistical tests were applied to the data measuring the exit angles of the nudibranchs. Rao's spacing test was used to determine whether the angles were uniformly distributed. Then, the means of each group was tested for similarity using the Watson-Williams test. Finally, the effect of the seawater on the time it took for each test subject to complete the experiment was tested using the Mann-Whitney U test. Data involving circular statistical tests were analyzed using PAST (Hammer and Harper 2001). All other analyses were conducted in R (R Development Core Team 2013).

Seawater acidification

Seawater was obtained from Cook's Bay and passed through a sand filter. The pCO_2 treatment was maintained using the Neptune Systems Aquacontroller Apex Lite aquarium control system at a diel range of 1200 - 1400 ppm with a corresponding pH range of 7.6-7.5 depending on the time of day (Diurnal: 1200 ppm, pH = 7.6; Nocturnal: 1400 ppm, pH = 7.5). Pure CO_2 was bubbled in order to decrease pH. A solenoid controlled the addition of CO_2 by opening and closing when the pH was elevated or depressed respectively. Addition of ambient air bubbled through a column of soda lime was used to elevate the pH. This was controlled by an aquarium air pump (Fluval Q202) activated by the aquacontroller when the pH was below the set level. Desired CO_2 levels were maintained within 50 ppm with the combined use of pure CO_2 and scrubbed air (C. Lantz, personal communication).

RESULTS

Ocean acidification effects on egg masses

Seawater type did not have a significant effect on the area of the egg masses. Although area from acidic seawater egg clutches trended to be larger overall and have a larger spread than the normal seawater batches, differences between the two were not statistically significant (Figure 3; P = 0.9703). Although seawater treatment did not have an effect on the area of the egg masses it did have an effect on the total egg clutches laid. The cumulative number of egg clutches from nudibranchs kept in acidic seawater (N = 38) was borderline significant (P=0.0548) when compared to clutches reared in normal seawater (N = 23). Data focusing on the first group of *C. lochi* collected (N = 40) shows a different distribution between egg masses laid in acidic seawater (N = 10) and the ones laid in normal seawater (N = 1). This 10:1 ratio was more significant (P = 0.0067).

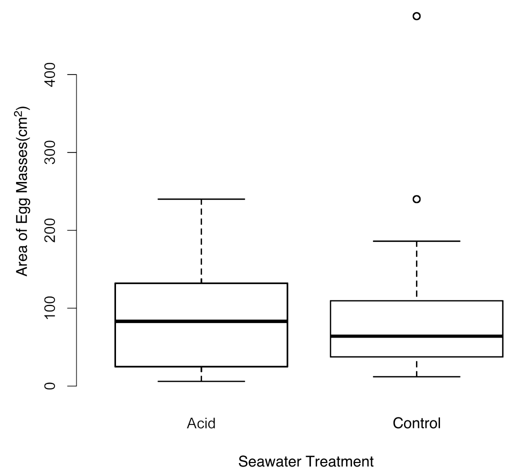

FIG. 3. *Chromodoris lochi*. The area of egg masses deposited by nudibranchs held in control (pH=8.02) and acidic (pH=7.60) seawater treatments. There was no significant difference observed between the two populations (P = 0.9703).

Orientation under the presence of a stimulus

Directionality could be associated to the exit points of the test subjects. Recorded exit angles did not follow a random distribution around the circle for either the normal or acidic seawater groups. Test subjects from both groups significantly (P < 0.05 for both) favored exiting toward a certain direction and in both cases quadrant II seemed to be the preferred general direction. The normal seawater group had a mean angle–which takes circularity into account–of 121.4˚ while the acidic seawater group had a mean angle of 151.9˚ (Figure 4).

When compared, these means were significantly different from each other (P = 0.0256). Simply put, nudibranchs reared in normal seawater were on average moving in a different direction than their acidic seawater

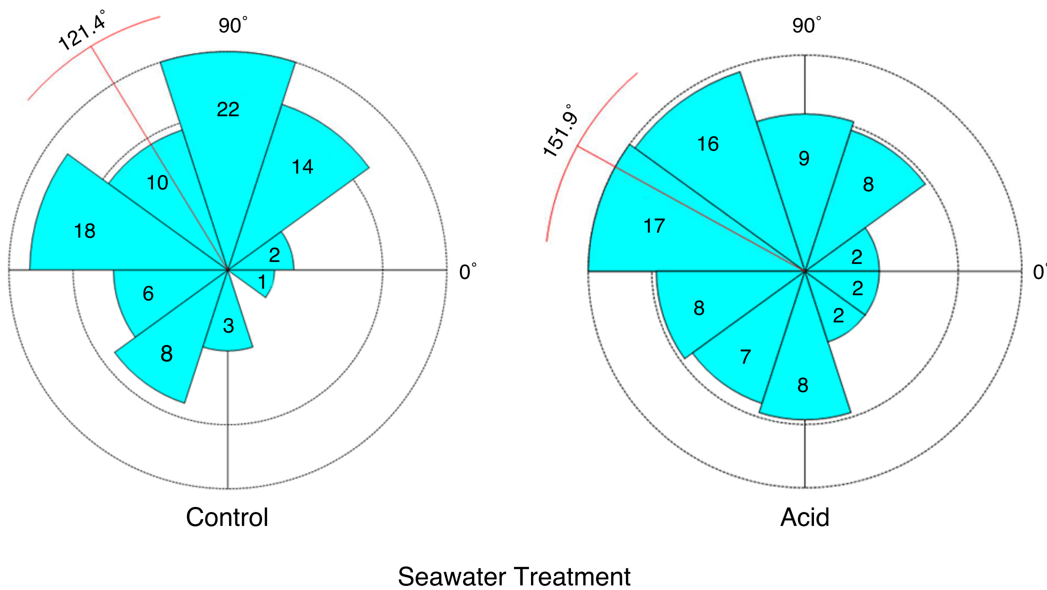

FIG. 4. *Chromodoris lochi*. The distribution of exit angles from nudibranchs held in control seawater (pH = 8.02) and acidic seawater (pH = 7.60) along a circle in relation to a stimulus at 90˚. The circles are divided into 10 segments each with a range of 36˚. Numbers indicate the total count of individuals that exited through that particular range. The mean angles of the control batch (121.4˚) and the acidic batch (151.1˚) take circularity into account and are shown by the red line. Rao's spacing test shows that neither the distribution of the control group ($U = 170.8$, $P < 0.05$) nor the distribution of the acidic group ($U = 158.9$, $P = 0.0048$) is randomly distributed among the circle.

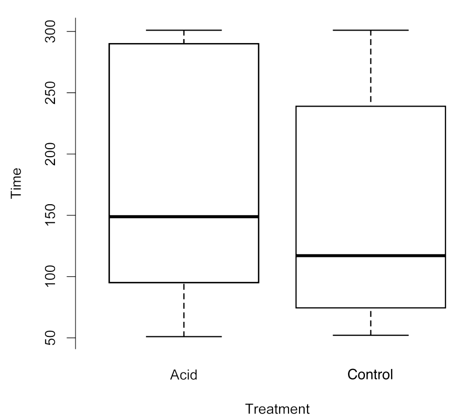

FIG. 5. *Chromodoris lochi*. The amount of time nudibranchs held in acidic seawater (pH = 7.60) and control seawater (pH = 8.02) spent completing the experiment. A Mann-Whitney U test showed that there was a statistically significant difference in amount of time taken by each population ($P = 0.0171$).

counterparts. In this case, the control group was about 30˚ closer to the direction of the stimulus.

Ocean acidification effects on the amount of time to detect a stimulus

Seawater type had an effect on the time nudibranchs spent completing the experiment. *C. lochi* housed in acidic seawater had a higher median and overall larger spread (Figure 5). They spent a significantly longer amount of time in the circle ($P = 0.0171$).

DISCUSSION

Ocean acidification effects on egg masses

Seawater type did not significantly affect the area of the egg masses laid by *C. lochi*, but it did affect the amount of egg clutches laid. Assuming egg density does not vary among clutches, the greater number of egg clutches deposited by the acidic seawater group indicates that this it was more fecund. The original group of nudibranchs (N = 40) exemplified this even better. Since they were kept and starved the longest (six weeks) the ratio of 10:1 greatly favors the acidic seawater group. This supports previous studies showing that stressed individuals, like some species of flies and watersriders, release more eggs (Kaitala 1991, Wang 2001). Sensing the increasing stress, the nudibranchs from acidic seawater likely made considerable physiological sacrifices in order to lay eggs (Moberg 1985). Since they were kept isolated it is probable the laid eggs were not viable, unless the nudibranchs stored sperm from previous sexual encounters. This phenomenon warrants further research.

Ocean acidification effects on chemoreception

Nudibranchs from both seawater treatments showed a significant preferred direction of travel. While neither group actually traveled directly toward the stimulus, the nudibranchs held in normal seawater were about 30˚ away and the acidic seawater nudibranchs were about 60˚ away. Since both mean angles fell within quadrant II, there is a possibility that the current of the water traveled at an angle instead of straight, affecting the nudibranchs' orientation. However, taking these angles into account, the nudibranchs from normal seawater did come closer to orienting themselves toward the stimulus. This and the fact that nudibranchs held in acidic seawater significantly took longer to complete the experiment indicates that the acidic group was less successful at detecting a chemical stimulus, supporting previous studies showing that a decrease in pH hampers the chemoreception of individuals (Munday et al. 2008).

Conclusion

Since acidic nudibranchs were less successful at detecting a chemical stimulus from a mate, it is like that they would exhibit similar results when presented with a stimulus from a food source. In a natural setting, a nudibranch that could not detect stimuli from either a food source or a mate would be in trouble. If finding food becomes the limiting factor for the organisms' well being, then this increase in stress should be followed by an increase in egg deposition according to this study's results. However, this would only be beneficial if deposited eggs are viable, which also depends on the ability to locate a mate. Under projected conditions a drop in the population of *C. lochi* may occur if increasingly stressed nudibranchs cannot locate food or mates and only lay poor quality eggs, unless they are able to adapt to these changes.

The capacity for acclimation or adaptation of taxa are important factors to consider when assessing the susceptibility of organisms to climate change (Munday *et al*. 2009). This capacity to adapt has not been tested or measured for many marine organisms, especially the non-calcifying. It is likely that many of taxa do not have the genetic variation to adapt to low pH, given that ocean pH has remained relatively stable for the past 650,000 yeas (Kleypas et al. 2006, Munday et al. 2009). Since anthopogenically-induced ocean acidification is causing pH to drop at a rate more than 100 times faster than ever before, it is very likely that most populations do not have the capacity to genetically adapt rapidly and maintain their viability, aside from those that have very rapid generation times (Hoegh-Guldberg et al. 2007, Munday et al. 2009, Byrne and Przeslawski 2013).

Thus, results from this study further show that non-calcifying organisms experience stress under low pH conditions. With growing concern over marine ecosystems due to increasing anthropogenic CO_2 and decreasing seawater pH, studies should become more inclusive regarding non-calcifying organisms and seek to further understand the potential effects of climate change at the ecosystem level. As knowledge increases, it should be applied to environmental policy limiting and stabilizing CO_2 emissions and in the process also limiting the extent of ocean acidification and its effects on marine biota.

Further research

While this study has shown that ocean acidification increases stress and fecundity while decreasing chemoreception ability of *C. lochi*, many questions remain about the mechanisms behind these physiological changes. How acidic conditions interact with chemoreceptors and the chemical stimuli would contribute to knowledge about the chemical ecology of nudibranchs. I have some suggestions that would further contribute to this study: 1) Because of time constraints, the eggs or larva were not studied. It would prove useful to show whether rearing of eggs under acidic conditions have an effect on adult chemoreception. 2) For this study, nudibranchs were housed in acidic seawater but the chemoreception tests were done under normal seawater. It would be interesting to see how running the tests under acidic seawater affects the stimulus and the chemoreception.

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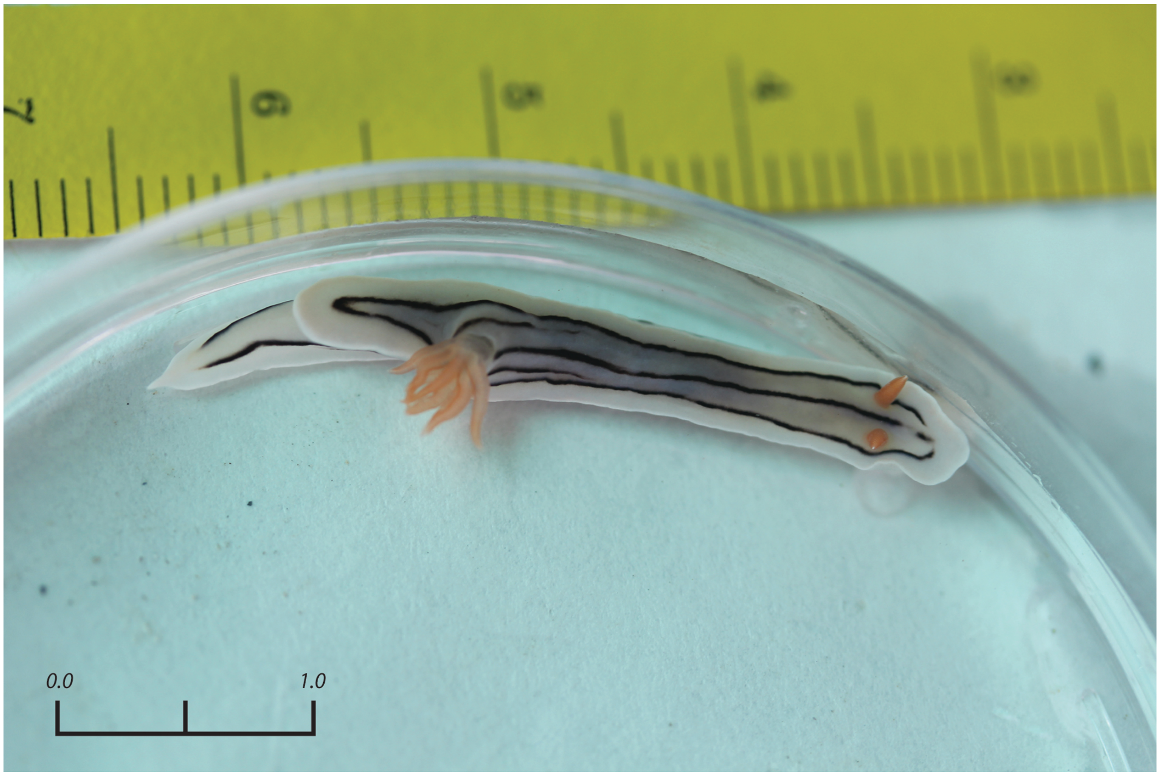
APPENDIX A

Adult *Chromodoris lochi* on degraded coral head, its natural habitat on Moorea, French Polynesia. Its translucent white or bluish-white color and three distinct black or dark blue lines characterize it. Also, note the colorful rhinophores on its dorsal side.

APPENDIX B



Adult *Chromodoris lochi* collected on November 11, 2013. Individual pictured has not begun the week of acclimation. Scale bar = 1.0 cm

APPENDIX C

Diagram representing a spiral egg mass, typical of *C. lochi*. The masses were usually laid flat on a surface, but on occasion, they were free floating on water. Area was always calculated by taking the length from point A to B, and multiplied by the measured perpendicular length.