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Exploring the Mechanism Underlying the Covering behavior of *Anthopleura sola*

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ABSTRACT

Some rocky intertidal organisms cover themselves with shells, shell fragments and gravel to protect against biotic and abiotic stressors (Peura et. al 2013, Dumont et. al 2007, Hart and Crowe 1977). What are the mechanisms that underlie this covering behavior? How do these fragments attach to the bodies of intertidal invertebrates that exhibit this behavior? Results of preliminary studies calculating attachment tenacities from fragment area and detachment forces for *Anthopleura sola* found in the rocky shores of the Hopkins Marine Station are presented in this paper as a first step towards investigating suction as one of two potential mechanisms underlying covering behavior in *A. sola*. Average tenacity measured in the field was lower than atmospheric pressure, as consistent with suction. Attachment tenacities measured in muscle-relaxed *A. sola* in the lab were above zero Pa and thus, inconsistent with a suction mechanism. Studies were proposed to further investigate the feasibility of suction as the attachment mechanism and to explore *A. sola* mucus as an adhesive secretion for fragment attachment.

INTRODUCTION

The rocky intertidal zone is a physically stressful environment. Organisms living in this habitat need to have mechanisms to adapt to changing physical conditions as the tides rise and fall. Adaptations to protect against desiccation, overheating, harmful levels of solar radiation, being washed away, and other environmental stressors, as well as biotic factors, are requirements for survival in this constantly changing physical environment. One interspecific adaptation that is relatively common in the rocky intertidal zone is covering behavior. For the purpose of this proposal, covering is defined as the adornment of an organism with any material that is not made by the organism itself. Under this definition of covering, bacterial and algal epibiotic biofilms, which form on most intertidal organisms including mussels, barnacles and macroalgae, would be the most common example of covering (Wahl et. al 2012). These biofilms can be protective against desiccation stress, UV radiation and overheating (de Carvalho 2018). Biofilms form without active input on the part of the macro organism. Conversely, some organisms have evolved mechanisms or adaptations that allow them to adorn themselves with some material cover. For example, hermit crabs adorn themselves with gastropod shells. This covering behavior is so integral to hermit crab survival that Peura et. al (2013) have suggested that "hermit crabs (*Paguridae*) cannot exist without shells produced by gastropods". Some species of sea urchins including members of the *Strongylocentrotus* genus, which live in the intertidal zone, are found covered in gravel, shell, leaves and other materials. This covering behavior has been hypothesized to be protective against predation, wave surge and UV radiation (Dumont et. al 2007). Some anemones, including some members of the genus *Anthopleura,* are found to cover themselves with shell fragments and gravel.

The reasons for covering behavior are often attributed to some sort of protection, and in organisms like intertidal hermit crabs, some species of sea urchins, and sea anemones, this behavior has been investigated (Peura et. al 2013, Dumont et. al 2007, Hart and Crowe 1977). The "whys" of covering behavior have been explored for all three organisms mentioned above, but the "hows" have not yet been elucidated for either sea urchins or sea anemones. It is important to investigate how these organisms have evolved mechanisms to protect themselves in the harsh intertidal environment. Understanding the physiology of these organisms, especially in the face of such harsh, fluctuating conditions is relevant as humans seek to understand the world around us. Climate change, currently of global concern, has impacts on abiotic stressors relevant to the intertidal habitat such as temperature, sunlight intensity, and amount of incident ultraviolet radiation among others. To understand how the impacts of climate change may affect intertidal organisms, it is important to understand how these organisms deal with, and protect themselves from fluctuations in these stressors on a daily basis. Thus, investigating the mechanisms underlying covering behavior in the rocky intertidal will be relevant in trying to understand the potential resilience of these organisms in the face of climate change, as well as how this resilience will affect changes in the composition and structure of intertidal communities due to the impacts of climate change.

Study Species

The rocky intertidal zone of the Pacific coast serves as habitat for multiple species of *Anthopleura*. *Anthopleura xanthogrammica*, *Anthopleura sola* and *Anthopleura elegantissima* are found in the rocky intertidal zone of the Hopkins Marine Station in Monterey Bay. *A. elegantissima* and *A. sola* are recognized as sibling species and occur together within similar regions of the rocky intertidal on rocks, in crevices and in tidepools (Pearson and Francis 2000).

Previous studies along the Pacific coast have suggested that *A. xanthogrammica* is found in the wave-exposed subtidal to low intertidal zone, whilst *A. elegantissima* and *A. sola* are found in the wave-protected middle intertidal zone (Pearson and Francis 2000, Jen 2010). The differential zonation of these three *Anthopleura* spp. is relevant in considering the amount of wind, sunlight and desiccation stress individuals of each species are subjected to. *A. elegantissima* and *A. sola* are likely to be more vulnerable to the above-mentioned abiotic stressors due to both vertical zonation as well as preference for wave-protected areas.

Previous studies relevant to *Anthopleura sola* covering behavior

Studies that discuss the covering behavior of *A. sola* and *A. elegantissima* attribute the behavior to protection against the increased vulnerability to desiccation stress and UV exposure (relative to *A. xanthogrammica*). In a series of experiments, Hart and Crowe (1977) found that gravel covering of *A. elegantissima* body column was protective against desiccation stress. Since then, Dykens and Shick (1984) have also attributed the covering behavior of *A. elegantissima* to protection against sunlight exposure to prevent the expulsion of zooxanthellae– the photosynthetic symbionts that live in *Anthopleura* species.

Little is known of the mechanism underlying the covering behavior of *A. sola* and *A. elegantissima*. However, two hypotheses have been suggested by previous studies. Parker (1917) suggested that the covering behavior of some sea anemones could depend on suction due to muscular contraction. This hypothesis was rejected by Harte and Crowe (1977) when tests revealed that relaxation of *A. elegantissima* muscles when placed in 4% magnesium chloride ($MgCl_2$) solution did not result in the loss of attached articles. Following the rejection of this hypothesis, Hart and Crowe (1977) suggested an alternative hypothesis, stating that attachment was mediated by an extracellular secretory product. No evidence has yet been provided in support of this theory. These two mechanisms are not mutually exclusive and may both play roles in the covering behavior of *A. sola.* The question of the mechanism underlying the covering behavior of *A. elegantissima* and *A. sola* is still unanswered. The intent of this project is to investigate these two hypotheses: the suction mechanism (based on muscular contraction) proposed by Parker (1917) and the adhesive mechanism proposed by Hart and Crowe (1977) to find the model that best explains and describes how *A. sola* cover themselves in shell fragments and gravel.

Suction hypothesis

The body column of *A. sola* bears protuberances called verrucae to which gravel and shell fragments attach (Hart and Crowe 1977). Parker's suction mechanism suggests that muscles within the verrucae contract to form a cavity between the shell fragment/ gravel and the *A. sola* body column. The pressure within this cavity would be less than ambient pressure— in this case surrounding atmospheric pressure — and thus the difference in pressure would generate a force acting over the area of the attached fragment to hold it onto the verrucae. Attachment force acting per unit area of an attached body is measured as tenacity. Tenacities equal to or greater than atmospheric pressure are considered evidence against a suction mechanism (Smith 1991). Thus, to be consistent with a suction mechanism, tenacities of fragment attachment to *A. Sola* must be less than the ambient pressure.

PRELIMINARY INVESTIGATION

Methods.

Field studies and lab experiments were performed to investigate the suction/ muscular contraction mechanism.

For both lab and field studies, anemones were selected by sampling along a 30m long transect, one transect for the anemones collected for lab experiments, and one for field experiments. Each transect was parallel to the shoreline in a section of the wave protected mid-lower intertidal zone of the Hopkins Marine Station. For field studies, a random number generator was used to generate random numbers between 0 and 30 to find the distance along the transect at which to place a $0.25m^2$ quadrat. All anemones within each quadrat were sampled till 40 observations were made. Sampling of anemones for lab experiments was determined by ease of anemone removal. 7 anemones distributed along the transect were removed from their habitat — by chiseling them off rocks — and relocated to a tank in the lab. The atmospheric pressure at sea level was obtained from the Integrated Ocean Observing System Database for the Monterey Bay Aquarium shore station as 100,900Pa.

In both field and lab studies, the force needed to pull off attached material, and the dimensions of the attached fragments were measured with a spring balance and a pair of Vernier calipers respectively. For simplicity, each fragment was measured as an ellipsoid.

Thus, the area of each attached fragment was found as:

$$Area = \frac{\pi}{4} \cdot (length) \cdot (width)$$

For both lab experiments as well as field observations, tenacities were found to follow a logarithmically normal distribution. All data analysis was thus performed on log-transformed data to meet normality assumptions for the statistical tests ran. Relevant statistics were then back-transformed into tenacities. Transformations were performed as follows:

Log Transformation

$$\textit{Log-transformed tenacity} = \log_e(\textit{tenacity}+1),$$

(tenacity+1) allows log-transformation when tenacity=0

Back Transformation

Log-transformed tenacity is referred to as log(tenacity) for simplicity

$$\textit{tenacity} = e^{\log(tenacity)} - 1$$

For simplicity, any values transformed specifically as described above will henceforth be denoted log(value).

Sample size calculation

Data from field observations provided an estimate of the standard deviation for tenacities and this was used to estimate the minimum number of samples needed to achieve power of 80% at the 5% significance level for a two-sample t-test by the following formula.

$$n \approx 16\left(\frac{\sigma}{D}\right)^2$$

$$\begin{aligned}&where\ n\\&= estimated\ numberof\ samples\ per\ group,\\&D = |mean_1 - mean_2|,\\&\sigma\\&= population\ standard\ deviation\end{aligned}$$

The suggested sample size for each group was found to be 35 observations. 40 observations from 4 anemones, were sampled for the $MgCl_2$ treated group. 30 observations from 3 anemones, were sampled for the control group.

Field observations

For each anemone sampled in the field, 5 attached fragments were pulled off. 40 observations were made by sampling 8 anemones in 6 randomly selected quadrats along the 30m transect.

Analysis

Log(tenacities), calculated from field measurements, were compared to the log(atmospheric pressure), as the null value, by a one-sided student's *t*-test. Log(tenacities) were expected to be less than log(atmospheric pressure)— the null value— to be consistent with a suction mechanism.

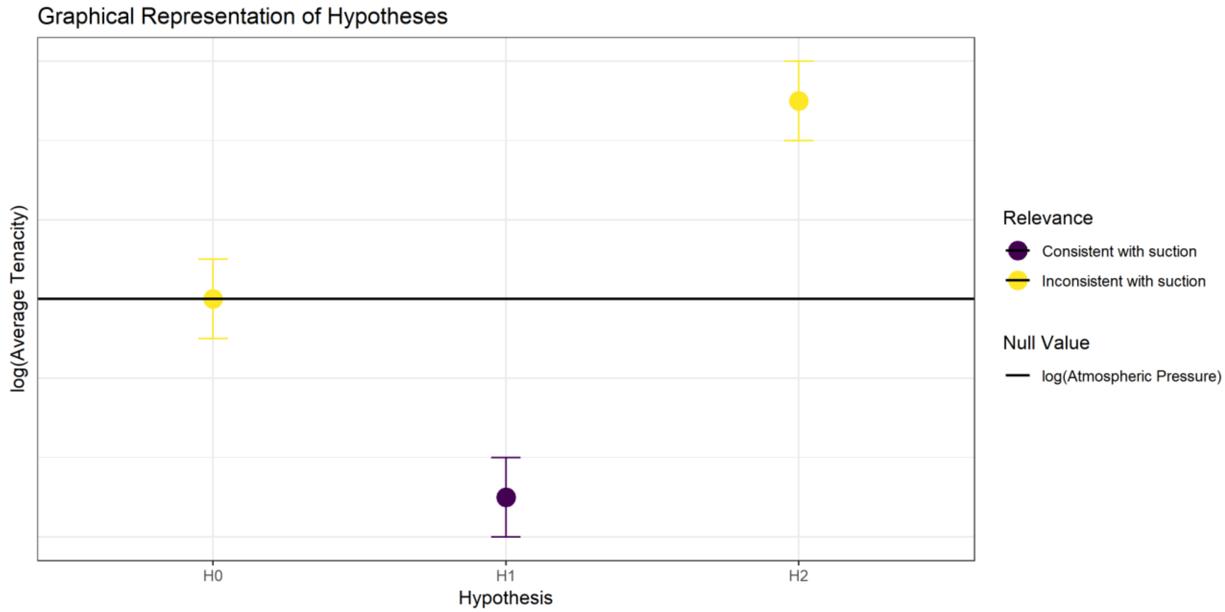

Figure 1: Graphical representation of hypotheses for one-tailed student's t-test to compare log(average tenacity) from field data to log(atmospheric pressure). H0 refers to the null hypothesis of no difference between log(average tenacity) and log(atmospheric pressure). H1 refers to the alternative hypothesis of interest, that log(average tenacity) is less than log(atmospheric pressure). H2 is the other possible hypothesis but was not tested.

Lab experiments

The 7 *A. sola* collected for lab experiments were randomly divided into a $MgCl_2$ treatment group and a control group. The $MgCl_2$ treatment group anemones were placed in a 1:1 solution of isotonic $MgCl_2$ solution to sea water, to cause muscle relaxation. The control group was only exposed to sea water. 10 attached fragments were pulled off each anemone in either group. The observations were pseudo replicated since the anemones in each group (treatment and control) were kept in one tank each during the experiment period.

Analysis

$MgCl_2$ treated anemones

The proposed suction mechanism is mediated by muscular contraction, thus if an anemone is rendered incapable of muscular contraction, suction cannot occur. If indeed suction is responsible for fragment attachment to *A. sola*, then the $MgCl_2$ treated, muscle-relaxed anemones would be unable to generate attachment forces by suction. With no attachment forces generated, tenacities would be expected to be zero. Since there would be no force attaching fragments to the anemone, the fragments would be unattached and expected to fall off.

Tenacities for the $MgCl_2$ treated anemones were thus expected to be equal to zero to be consistent with a suction mechanism. In the same vein, log(tenacities) for the $MgCl_2$ treated anemones were expected to be equal to the log(expected tenacity =0Pa) — the null value— to be consistent with a suction mechanism. Log(tenacities), calculated from the $MgCl_2$ treated anemones, were compared to a null value of 0— $\log_e$(tenacity=0+1) — by a one-tailed student's *t*-test. A one-tailed *t*-test was performed because data collection methods preclude negative tenacities.

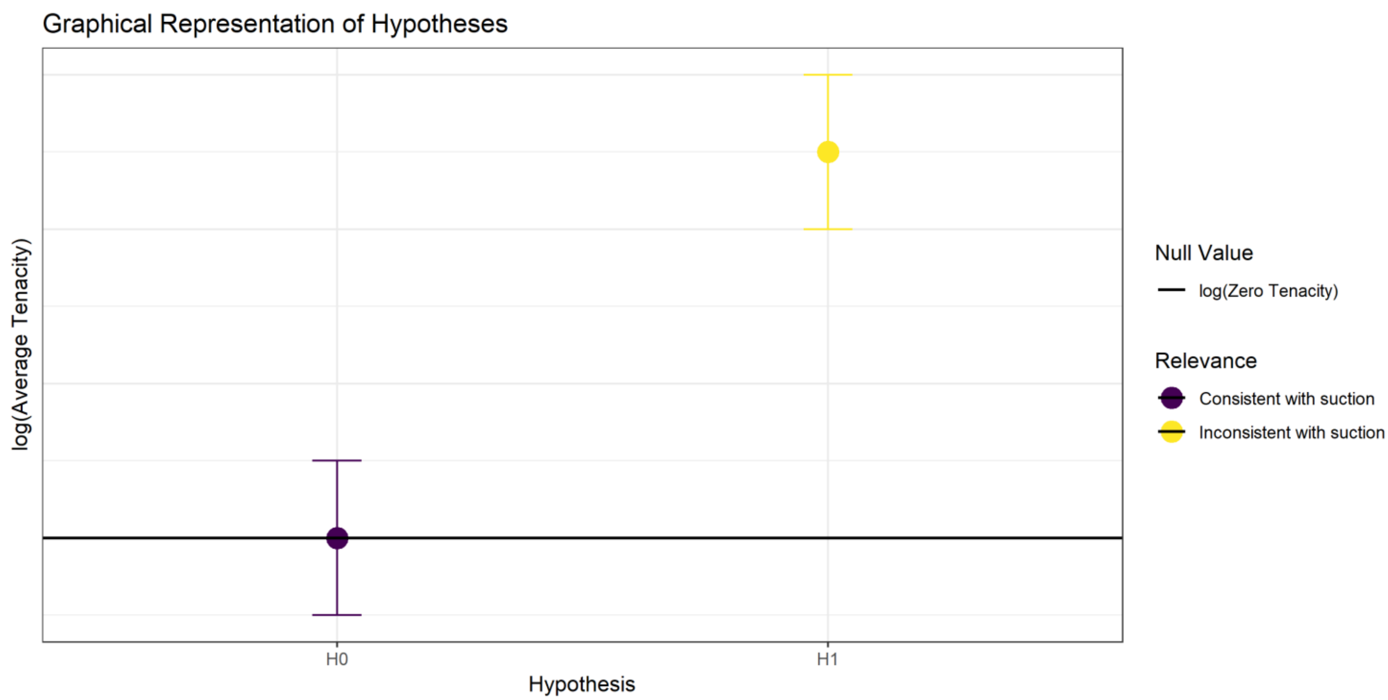

Figure 2: Graphical representation of hypotheses for one-tailed student's t-test to compare log(average tenacity) of $MgCl_2$ treated anemones to the suction-consistent expected log(average tenacity=0). H0, the null hypothesis, is that log(average tenacities) of $MgCl_2$ treated anemones = log(zero tenacity). H1 is the alternative hypothesis of interest, stating that log(average tenacities) of $MgCl_2$ treated anemones > log(zero tenacity).

Control group anemones

The control group served as a negative control for comparison with $MgCl_2$ treated anemones. Analysis performed on control group data alone was identical to analysis for field observations (see figure 1 above for analysis hypotheses).

Comparison of tenacities for $MgCl_2$ treated and control groups

The log(tenacities) for treatment and control groups were compared by an unpaired two-sample *t*-test. Log(tenacities) for control group anemones ranging from zero to any value less than log (atmospheric pressure) are consistent with the suction mechanism. However, log(tenacities) for $MgCl_2$ treated anemones are only consistent with a suction mechanism if they are equal to zero. Thus, log(tenacities) for $MgCl_2$ treated *A. sola* are expected to be less than log(tenacities) for control group *A. sola* to support a suction mechanism. If the suction mechanism is invalid, $MgCl_2$ treatment would be expected to have no effect on the tenacity of fragment attachment to *A. sola;* log(tenacities) would not be expected to differ between the treatment groups.

Thus, a nested ANOVA was performed to compare variation of log(tenacities) among anemones to variation of log(tenacities) between treatment groups. To be consistent with a suction mechanism, variation of log(tenacities) between treatment groups would be expected to be significantly different from the variation of log(tenacities) among anemones. That is, no significant difference between log(tenacities) of $MgCl_2$ treated versus control group *A. sola* is evidence against a suction model.

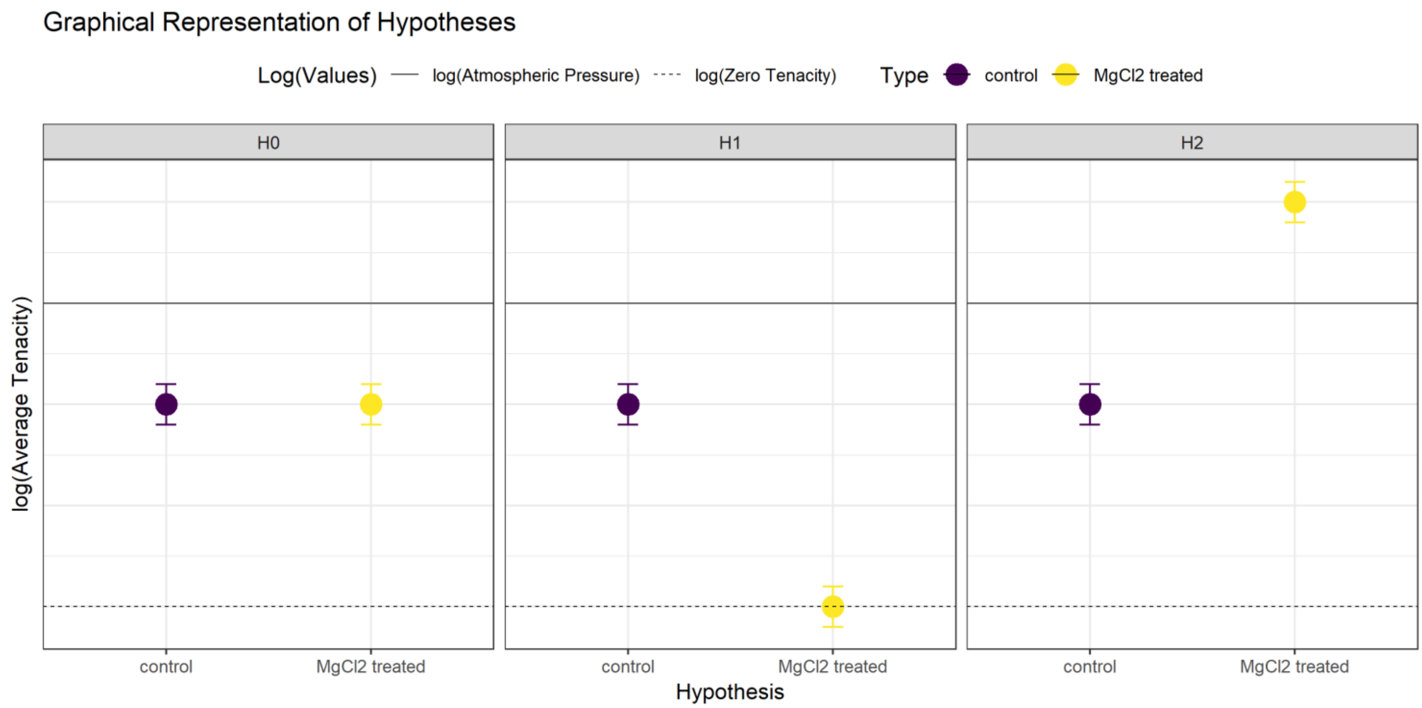

Figure 3: Graphical representation of hypotheses for comparison of log (average tenacity) between $MgCl_2$ treated and control groups. The null hypothesis (H0) is one of no difference in log(tenacities) between treatment groups. Both alternative hypothesis (H1) and (H2) are represented by the alternative hypothesis of the ANOVA (hypothesis of difference between treatment groups). It is important to note that only H1 (log(tenacities) $MgCl_2$ treated < log(tenacities) control group) is fully consistent with a suction mechanism.

RESULTS

Field Data.

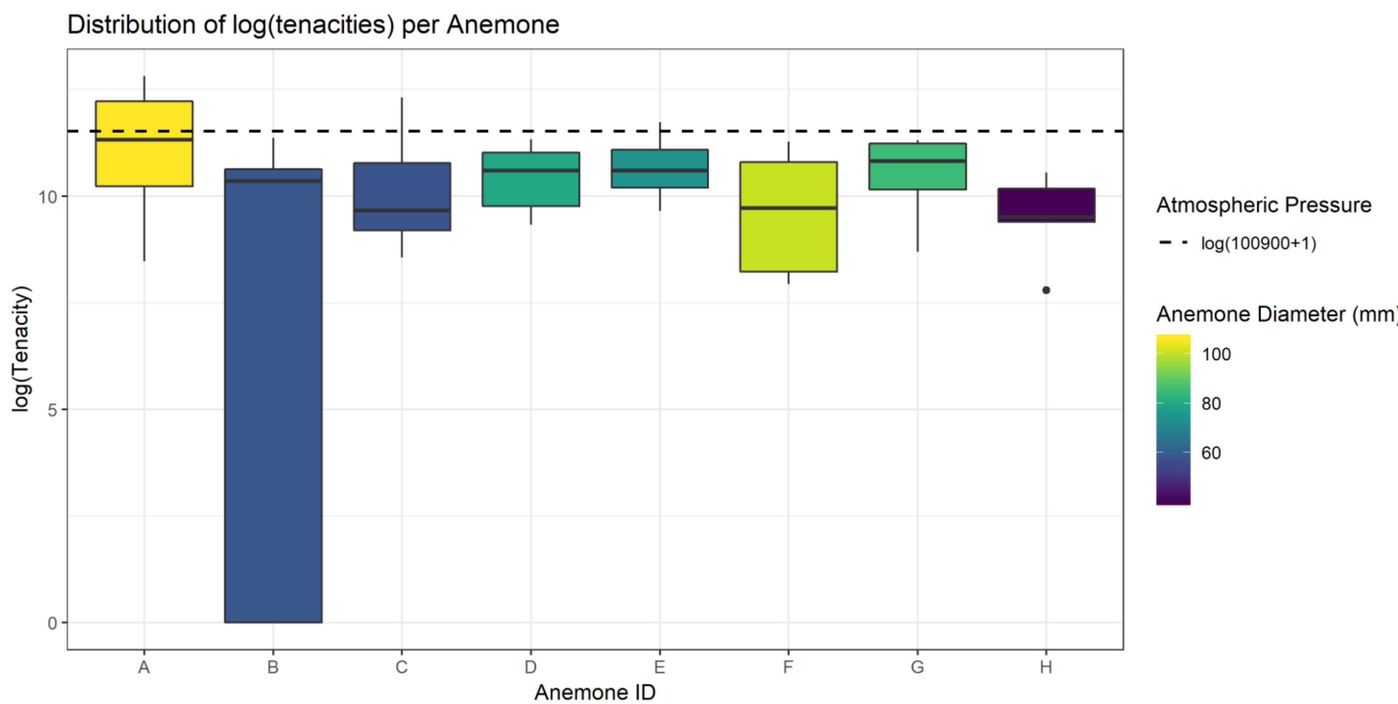

Figure 4: Distribution of log(tenacities) per anemone.

The mean tenacity of fragment attachment to *A. sola* was 17552.89Pa (95% CI: 7737.959 - 39815.61 Pa). One-tailed student's *t*-test analysis showed that mean tenacity was less than the atmospheric pressure (100,900Pa) recorded on the day of sampling (df= 39, t_{39} = -4.319, p= 5.213×10^{-5}). Tenacities less than atmospheric pressure are consistent with a suction mechanism (Smith 1991).

Lab Data

Control

Mean tenacity for control group *A. sola* was 39893.82Pa (see table 1 for 95% CI). Mean tenacity was less than atmospheric pressure, consistent with a suction mechanism (df= 29, t_{29} = -4.7382, p=2.6243x10^{-5}).

Treatment group	**Mean Tenacity (Pa)**	**95% CI (Pa)**
$MgCl_2$ treated	34266.41	25262.13 - 46480
control	39893.82	26727.42 - 59545.99

Table 1: Mean tenacities and 95%CI for $MgCl_2$ treated and control *A. sola.*

$MgCl_2$ treated

Muscle-relaxation in *A. sola by* $MgCl_2$ treatment did not eliminate attachment forces holding fragments onto the anemone body column. Fragments did not fall off the anemones and were still attached. No zero tenacities (0Pa) were recorded for $MgCl_2$ treated *A. Sola.* The mean tenacity—34,266.41Pa — was found to be significantly different from the expected zero tenacity consistent with a suction mechanism (df=39, t_{39} = 69.28301, p= 9.4854x10^{-43}) (see table 1 for 95% CI).

Comparing treatment groups

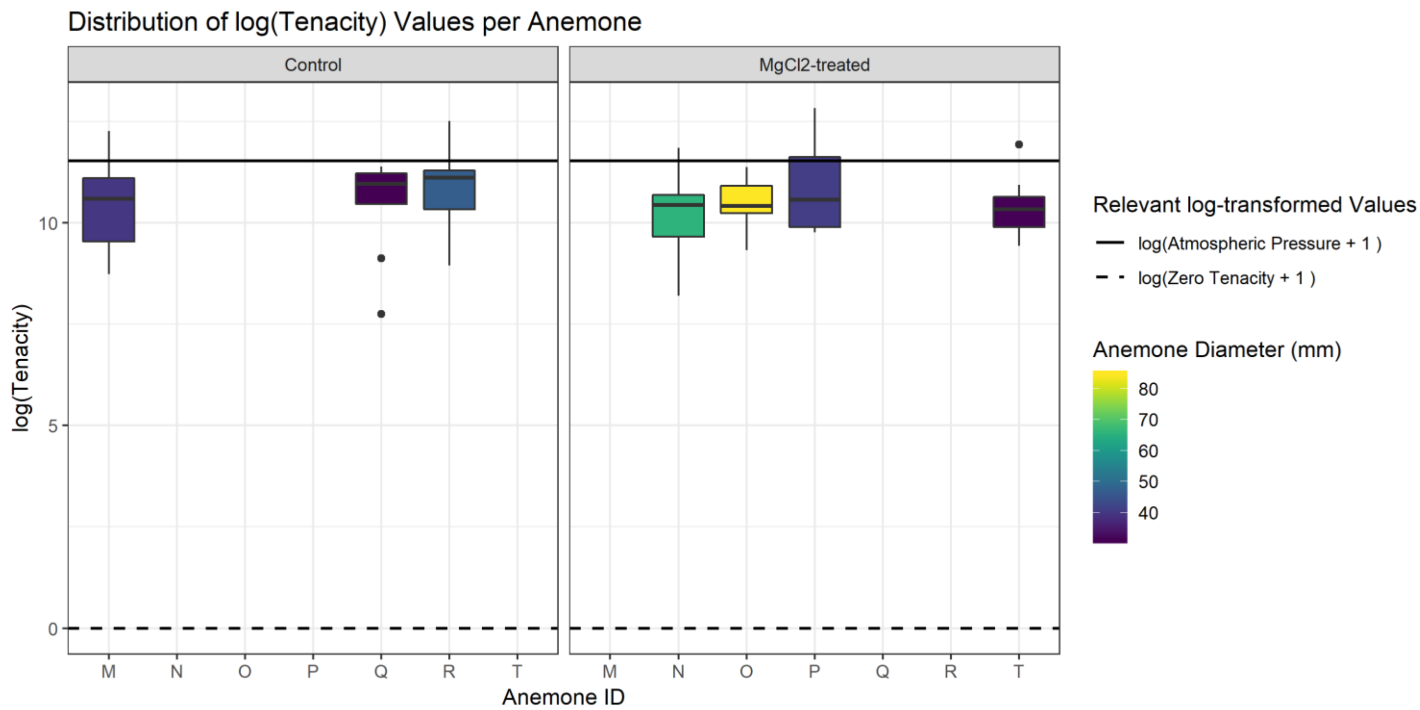

Figure 5: Distribution of log(tenacities) per anemone for $MgCl_2$ treated versus control *A. sola*.

Muscle–relaxation by $MgCl_2$ treatment of *A. sola* was found to have no significant effect on fragment attachment tenacities. Average tenacities for both $MgCl_2$ treated and control *A. sola we*re much larger than zero and did not differ significantly between $MgCl_2$ treated and control *A. sola*. Variations in tenacities between $MgCl_2$ treated and control *A. sola* did not differ significantly from variation in tenacities among different *A. sola* sampled ($F_{1,5} = 0.6878$, p= 0.4447).

DISCUSSION

The analysis of tenacities from field data and lab control group data provide the first line of evidence in support of a suction mechanism of attachment of fragments to *A. sola*. These results show that the average tenacity for fragment attachment to *A. sola* that have not been exposed to any treatment is less than atmospheric pressure. Unless tenacity outliers greater than atmospheric pressure are due to sampling error, these outliers serve as the first challenge to a suction mechanism.

Thus far, the strongest evidence against a suction mechanism is the lack of detectable change in tenacity of fragment attachment upon muscle-relaxation ($MgCl_2$ treatment). Muscular contraction-mediated suction must be affected (inhibited) by inhibition of muscular contraction.

PROPOSED WORK

To explore the mechanism by which shell and gravel attach to the body column of *Anthopleura sola*, both the suction and adhesive secretion mechanisms must be explored as feasible options. The data from preliminary investigations is inconclusive on the suction mechanism. Further tests are needed to investigate suction as the mechanism of attachment. Additionally, attachment by adhesive secretion must be investigated. These two mechanisms are not necessarily mutually exclusive since some marine invertebrates such as limpets, use both suction and adhesive secretion as mechanisms of attachment (Smith 1992). Both mechanisms have been explored for attachment of different species of intertidal organisms (including limpet species, snail species, anemone species) to substrate. Though the context is different, experimental methods can be adapted from such studies to explore the mechanism behind covering behavior in *A. sola*.

Proposed Suction Investigation

Further investigations of the suction mechanism will be done in a 2-experiment study. Methods described below have been adapted from studies exploring attachment mechanisms of organisms to substrate in the rocky intertidal zone (Smith 1991, van Hemmen and Ditsche2014).

Experiments

Effect of lowering ambient pressure on tenacities of fragment attachment

One experiment will investigate the effect of changing ambient pressure on the tenacity of fragment attachment to *A. sola*. The proposed suction mechanism assumes that muscles within the verrucae of *A. sola* contract to form a cavity between the shell fragment/ gravel and the anemone body column, with lower pressure within this cavity than surrounding ambient pressure. Tenacities are generated by this pressure differential. The proposed suction mechanism is thus dependent on the magnitude of ambient pressure because tenacities equal to and/ or greater than ambient pressure would be considered evidence against a suction mechanism. Under a valid suction mechanism, lower tenacities would be expected under lower pressure conditions relative to atmospheric pressure conditions.

Effect of holes in attached fragments on tenacities of fragment attachment

The second experiment will investigate the effect of holes in attached fragments, on tenacities of attachment. Holes in the fragment would be expected to interfere with suction because the anemone would be unable to form a sealed cavity between its verrucae and the attached fragment; the cavity would be open to the surrounding environment and would thus have the same pressure as the ambient region (in this case the atmosphere). To allow for suction, smaller cavities of lower pressure would have to be formed excluding the holes and thus tenacities would be expected to be less for *A. sola* attached to holed fragments versus whole fragments.

Methods and Materials

Both experimental phases will require the collection of *A. sola* for lab testing. *Anthopleura sola* will be sampled along three 100m transects parallel to the shoreline. One transect will be set up along the rocky shore of the Hopkins Marine Station and the two other transects will be at two different sites along the Monterey Bay. All transects will be set-up in the mid-low intertidal zone of wave-protected rocky shore. $0.25m^2$ quadrats will be placed at randomly generated distances along the quadrat and all *A. sola* within each chosen quadrat that can be safely collected will be removed for relocation to the lab. From each sampling site, 30 anemones will be collected. Anemones will be randomly assigned into control versus treatment groups.

Experimental set-up to create lower ambient pressure conditions

Bell jars will be connected to vacuum pumps to create lowered pressure of known magnitude within the bell jars. Spring balances will be suspended through a hole in each bell jar with a seal around each spring balance. Anemones will be placed under the bell jar and the clip of the spring balance will be attached to one attached fragment. The vacuum pump will be used to create ambient pressure of known magnitude and the spring balance will be used to detach the fragment, with force measurements recorded.

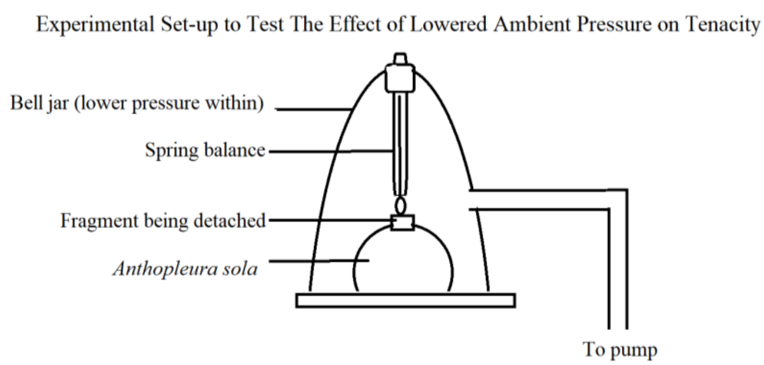

Figure 3: Experimental set-up to measure tenacity under lower surrounding pressure conditions

Experimental groups

There will be three experimental groups. 10 anemones from each sample site will be randomly assigned into each group. All organisms in each group will be exposed to light of intensity comparable to the average sunlight intensity from the days of sampling. All anemones will be kept in separate small tanks with wave pumps. Exposure to light intensity and wave motion are intended to simulate field conditions to result in covering behavior in the anemones (Hart and Crowe 1977).

Control group

This group will be the no-treatment control for the experiment investigating the effect of holes on tenacities. Force and area measurements will be taken in detaching shell fragments and gravel from *A. sola* in this group. 10 fragments will be detached per anemone under atmospheric pressure and lower ambient pressure conditions respectively. Tenacities will be calculated for both pressure conditions and compared by paired two- sample student's *t*-test. Lower tenacities under lower ambient pressure conditions would be consistent with suction.

Holed fragments group

Force and area measurements will be taken in detaching 50-100% of shell fragments and gravel from *A. sola* in this group. 10 fragments will be detached per anemone under lower ambient pressure conditions, and all others under atmospheric pressure conditions. The observations made under atmospheric pressure will be randomly sampled for 10 observations for comparison by paired *t*-test with observations made under lower ambient pressure conditions. Lower tenacities under lower ambient pressure conditions would be consistent with suction.

Once denuded of gravel, this group of anemones will be provided with 0.1cm thick, 1cm^2 acrylic pieces. Each acrylic piece will have 1, 2 or 3 holes— each hole covering an area of 0.25 cm^2. Anemones will be monitored in the lab for a week to allow for acrylic attachment. Force and area measurements will be made in detaching the acrylic pieces and tenacities will be recorded by acrylic piece area (which is dependent on the number of holes in the piece). Tenacities will be compared between groups of observations made for the different sized acrylic pieces by ANOVA.

Significant differences in tenacities by acrylic piece size (or number of holes) would be consistent with a suction mechanism. Tenacity would be expected to decrease as the number of holes increase and it is possible that adhesion would fail on holed acrylic pieces entirely. Failure of fragment attachment due to holes would also be evidence for a suction mechanism, as suction fails due to leaks caused by holes in attached material.

Holed and whole fragment group

Anthopleura sola may avoid attaching to holed fragments. None of the fragments detached off *A. sola* were found to have holes. To test whether *A. sola* do not attach at all to holed fragments, or attach preferentially to whole fragments, all the above procedures for the holed fragments group will be repeated for this treatment group. The only different conditions will be that only two types of acrylic pieces will be provided to these organisms in equal amounts: 0.1cm thick, 1cm^2 acrylic pieces with no holes, and acrylic pieces of the same size, with only one 0.25 cm^2 hole. The numbers of each type of acrylic pieces that attach to the anemones will be recorded and compared by paired student's *t*-test. This data will also be compared to the count of each type of acrylic piece attached to holed group *A. sola* by ANOVA. If the suction mechanism is valid, both tests would be expected to show significant differences between the counts by type of acrylic pieces attached per anemone. The mean count of each type of acrylic piece per anemone would be expected to be significantly higher for acrylic pieces with no holes to suggest *A. sola* preference for whole fragments. *A. sola* preference for whole fragments would support a suction mechanism.

The results of the two experiments described will provide more insight into the feasibility of a suction mechanism. Together with the results of the preliminary studies discussed, these experiments should provide enough evidence to support or invalidate the suction mechanism.

Regardless of the conclusion following these suction experiments, an adhesive gel mechanism will be investigated as a potential mechanism underlying *A. sola* covering behavior.

Proposed Adhesive Mechanism Investigations

Quite a few intertidal marine invertebrates such as limpets, periwinkles and some worms make use of adhesive gels in attaching to materials (particularly underlying substrate). Adhesive gels, in this context, are viscoelastic networks of polymers that take on partly solid form (Smith 2002). Adhesive gels secreted by invertebrates are usually described as mucous secretions however, the term mucus is generally used to describe a slimy secretion from an epithelial surface (Smith 2002). Most anemone species are known to secrete mucus. Mucus presence on *A. sola* was confirmed in field and lab preliminary investigations; a slimy, secretion was seen and felt on the body columns of *A. sola* sampled. In detaching fragments off *A. sola*, some fragments came off with some semi-solid material attached. This material did not appear to be part of the anemone body wall. According to Hart and Crowe, *A. sola* verrucae contain many secretory cells and this information on verrucae structure may be relevant for fragment attachment to the body column. Altogether, these observations present the possibility that *A. sola* may secrete a mucus-like gel that solidifies to adhere fragments to the *A. sola* body column.

The investigation of adhesive gels as a potential mechanism for fragment attachment to *A. sola* will focus on investigating the properties of *A. sola* mucus as a potential adhesive gel.

Presence of mucus/ adhesive- gel on detached fragments

If an adhesive gel is used in fragment attachment, it would be expected that residues of this gel would be found on the detached fragment. Fragments detached off *A. sola* in suction experiments will be stained with 1% crystal violet solution to search for the presence of adhesive gel/ mucus residues.

Characterizing *A. sola* mucus

Anthopleura sola mucus will be collected from the organisms used for suction investigations. The body column of *A. sola* will be gently scraped to collect mucous secretions. Half the volume of total secretion collected per anemone will then be analyzed biochemically to characterize the properties. The mucus viscosity relative to water will be measured using a viscometer. Protein content of *A. sola* mucus will be characterized by Bradford assay and the sizes of the proteins that make up the mucus will be determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). These assays will help to determine whether *A. sola* mucus is consistent with the currently characterized adhesive mucous secretions of other marine invertebrates.

Atomic force microscopy will be used to measure the mechanical properties of the mucus such as the elastic modulus of the mucus and the force required to stretch the monomers of the mucus (Smith 2002). The measured mechanical properties of *A. sola* mucus will be compared to the mechanical properties of a negative control, water- since water is not considered an adhesive, and two positive controls—limpet mucus and periwinkle mucus, both adhesive gels. An ANOVA will be run to compare the mechanical properties of mucus between the control groups and *A. sola*. Paired t-tests will be used to compare these mechanical adhesion properties between the A. sola mucus and each control group. Mechanical properties would be expected to be significantly different from the properties of water if *A. sola* mucus is an adhesive gel; if these properties are not significantly different from at least one type of mucus between limpet mucus and periwinkle mucus, this may suggest an adhesive nature of *A. sola* mucus.

Testing *A. sola* mucus as adhesive glue

The remaining half of the collected *A. sola* secretion will be tested as an adhesive. If *A. sola* fragment attachment is by adhesive mucus, it is reasonable to assume that this mucus can hold two fragments together. The adhesive properties of the *A. sola* mucus will be tested on both 0.1cm thick, $1cm^2$ acrylic pieces and on shell fragments sampled from the anemone sampling sites. Attachment will be done between acrylic pieces, between shell fragments and between pairs of acrylic pieces and shell fragments. The mucus will be applied on one of the surfaces to be attached together and the other surface will be placed onto the applied mucus somewhat asymmetrically to allow for ease of detachment. The number of attachment pairs possible will be dependent on the amount of mucus collected. The attached pairs will be randomly assigned into 5 groups. The first group of attached pairs will be detached 1minute following fragment positioning on the attaching mucus covered fragment, the second group after 10minutes. The third, fourth and fifth groups will be detached after 2 hours, 8 hours and 24hours respectively. Force and area measurements will be recorded, and tenacities will be calculated. The same experiment will be replicated in 2 control groups, with limpet mucus, and with water. Tenacity values will be compared among the *A. sola* mucus-attached pieces detached at different time points by ANOVA. Tenacity values will be compared among the three treatment groups per time point of detachment by ANOVA. To be consistent with an adhesive gel mechanism, tenacities recorded after longer periods following attachment are expected to be significantly higher than measurements made immediately following attachment since the gel will need to solidify. Tenacities recorded for *A. sola* mucus would also be expected to be significantly different from tenacities measured for the water control group. Limpet mucus tenacities would serve as positive control to which *A. sola* mucus tenacities can be compared. If *A. sola* mucus is an adhesive gel, the fragments attached together by the mucus will be expected to adhere after drying. This can be easily determined by observation and handling of the attached pairs.

The analysis of results from all the studies described, to investigate the adhesive gel mechanism as the underlying mechanism for *A. sola* should be conclusive as to whether *A. sola* mucus serves as an adhesive gel for fragment attachment.

CONCLUSION

The analysis of results from all the proposed studies are expected to provide valuable insight into the attachment mechanism of fragments to *Anthopleura sola*.

The effects of relocation to lab conditions on *A. sola* behavior — during preliminary studies, *A. sola* behavior in lab differed from observed field behavior for example, in terms of responsiveness to touch and retraction of tentacles — may pose a challenge in the proposed studies. Other potential challenges include anemone collection for relocation to the lab, and collection of sufficient amounts of mucus for tests.

Despite the foreseeable and unforeseeable challenges, this study is relevant because understanding the attachment mechanism underlying *A. sola* covering behavior is the first step in understanding the physiology of this behavior, including how *A. sola* modify attachment mechanisms to lead to fragment attachment or detachment in response to changing environmental conditions. The results of this study may be relevant for *A. elegantissima* covering behavior as well, and may provide insight into the mechanism underlying covering in other intertidal organisms. Perhaps most importantly, in the face of climate change and its impact on abiotic stressors in the intertidal zone, it is important to know how *Anthopleura sola* cover themselves with fragments as protection against environmental stressors daily. This knowledge will provide insight on the resilience of *A. sola* in the intertidal zone as abiotic conditions are affected by climate change. *A. sola* resilience will be relevant in understanding how intertidal community composition and structure may be affected by climate change.

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