Attachment forces and the role of suction in the sea anemone *Metridium farcimen*

Abby van Hemmen[1], Petra Ditsche[2]

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[1]Department of Biology, University of Washington, Seattle, WA, 98105
[2]Friday Harbor Laboratories, University of Washington, Friday Harbor WA, 98250

Contact Information:
Abby van Hemmen
Biology Department
University of Washington
5021 Brooklyn Avenue NE
Seattle WA, 98105
arvh@uw.edu

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Abstract

This paper looks at the forces involved in the attachment of *M. farcimen* to substrate. Many sessile and transitory, aquatic animals rely on the secretion of an adhesive glue for attachment to substrate, like most sea anemones. However, other attachment mechanisms exist. For example, limpets and clingfish rely on suction. The pull off forces of *M. farcimen* attached to substrate were tested, and then used to calculate tenacity. Two groups were compared. One group was tested with *M. farcimen* attached to acrylic glass with 1.5mm holes drilled throughout, and the other was tested with *M. farcimen* attached to acrylic glass without any holes. Tenacity calculations revealed that specimens were more strongly attached to the acrylic glass without holes, suggesting that suction is involved in *M. farcimen* attachment to substrate.

Attachment site selection was also explored. When *M. farcimen* were given the choice between substrates with holes and substrates without holes, there was no significant difference in the frequency of selection for attachment.

Introduction

Organisms living in an intertidal environment require mechanisms and structures to assist in combating various wave activity. At high tide many of these organisms, especially sessile/transitory organisms, are submerged in water. At low tide, these same organisms must face drier conditions and wave action. To cope with the forces of wave activity, adaptations that attach an organism strongly to a substrate or reduce drag become beneficial.

Metridium farcimen living in the intertidal, have benefited from such an adaptation. *M. farcimen* are capable of withstanding wave action by strongly attaching to solid substrates. Without this ability to resist dislodgement, the organism would be washed out to sea with each tidal cycle. Other sea anemone species like *Metridium senile* have been documented possessing tenacities of 0.16 +/- 0.04 x 10^5 N m^{-2} when attached to acrylic glass (Young, Yule, and Walker 2009).

M. farcimen are considered transitory organisms because they are capable of moving while remaining attached to substrate (Sherman and Sherman 1976). Another organism that moves in a similar way are limpets. When given more than a few hours to attach, limpets have been observed forming an attachment to substrate based on an adhesive glue. This is a different mechanism of attachment than they normally form, which isn't as strong and is completely reliant on suction. Limpets appear to be capable of changing their method of attachment depending on their intention of mobility (Smith 1991).

While many organisms of the phylum Mollusca inhabit crevices, live under boulders, or actively bore into the rock itself, a soft bodied, sometimes large sea anemone like *M. farcimen,* which can grow to be larger than 50cm in length, cannot (Wilber and Yonge 1964). An adhesive glue, much like limpets, might be a more effective attachment mechanism for an animal of this size. Most aquatic organisms that rely on adhesive glue secrete a substance that is composed of modified amino acid sidechains with high substrate affinity. These sidechains form bonds with substrate complexes, attaching the organism to the substrate (Stewart, Ransom and Hlady 2011). A glue, perhaps operating in a fashion similar to the one explained, is secreted from the basal foot of many sea anemone species, *M. farcimen* included (Robson 1976).

Suction is another mechanism adopted by many intertidal organisms like limpets, octopus, chitons, and some fish. Suction occurs when air or water is forced out of a concealed space and forms an area of sub-ambient pressure. This pressure differential locks the animal to substrate. Therefore, suction is not possible on a substrate with holes which would prevent the formation of an area of lower pressure in comparison to the environment. Suction cups in aquatic animals come in many specialized forms dependent on the function they are required for, be it attachment to substrate in high speed currents, climbing waterfalls, etc. (Ditsche and Summers 2014). It has yet to be determined if suction plays a role in *M. farcimen* attachment, however the possibility does not seem unlikely, given the wide range of attachment methods utilized by other sea anemones. Actinaria not only attach to rock, shell, coral, bone, sponge, trash, and other animals, but have also been observed staying in place by clenching their basal foot around a ball of mud, or burrowing with a modified basal foot (Ammons 2008).

This research will explore the adhesive forces that assist in intertidal *M. farcimen* attachment to substrate and the role of suction in *M. farcimen*. We will look at *M. farcimen* attachment site selection behaviors to determine if an avoidance for holed substrates, where suction is not feasible, exists. Using a mechanical testing machine, the force of *M. farcimen* attachment to artificial substrates with holes will be compared to attachment to substrates without holes, to determine if suction plays a role in attachment.

Methods

Animals

28 specimens were used for the choice experiment. 20 specimens were used for the attachment experiment. Animals were collected in the San Juan Islands of Washington State, at both the Friday Harbor docks in town, and the Friday Harbor Labs docks. Specimens were removed from the docks by hand and were immediately transported to the Friday Harbor Labs where they were kept in a sea-table. The weight of each specimen was taken.

Choice Experiment

To test if *M. farcimen* preferentially attach to substrates that permit attachment by suction, we set up a randomized array (4 by 5) of 10 holed, and 10 solid, square, artificial substrates in a sea-table. Each substrate was placed on clay feet to guarantee that no suction could take place through the holes in the substrates to the bottom of the sea-table. We then made a wire cage to surround the array to make sure that anemones did not leave the desired testing area (figure 1). Once set up, we placed the specimens into the cage. Each specimen was placed (basal foot down) on the border of two plates, one with holes and one without. We did this to ensure that no specimens would be introduced to the system upside down, and unable to attach. We gave the specimens 24 hours to attach, and then an additional 24 hours to see if they moved. Upon attachment after 48 hours, we recorded how many specimens were found attached to holed substrates and how many we found attached to solid substrates. We also counted the number of specimens that were attached on the edge of two plates of different types.

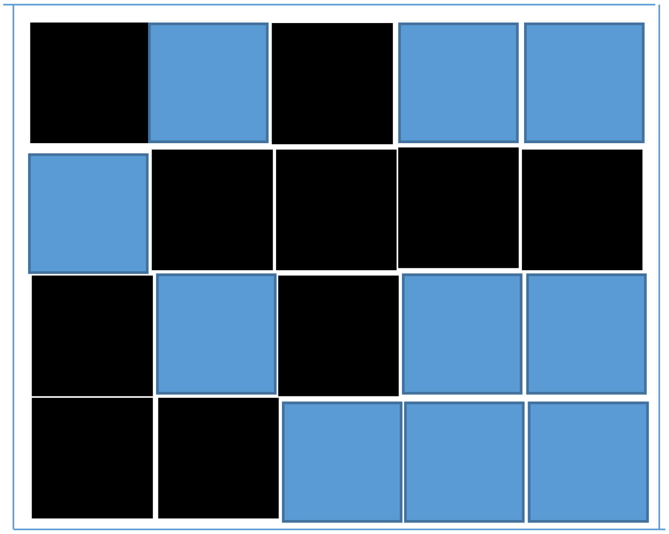

Figure 1. Example of the experimental set up for the substrate choice experiment. Black squares show the substrates with holes, blue squares show the substrates without holes and the blue outline marks the wire cage

Force Measurements

In order to determine the impact of suction (in *M. farcimen*) to overall attachment we measured attachment forces using a mechanical testing machine. Smooth substrates plates and substrates with drilled holes were made. Anemones were manipulated to attach to these plates which were placed in the sea-table where the anemones were kept. To ensure attachment to the desired area, the specimen was placed on a substrate and then covered by a wire cage. Each specimen was given 24 hours to attach. Using a mechanical testing machine (MTS Synergie 100, Cary, NC, USA). The pull off force of anemone to substrate was tested. The specimen (attached to substrate) was attached to the machine using a clamp (figure 2). Each specimen was tested one time on either a substrate without holes or a substrate with holes. Data were collected as pull off forces in Newtons. Pull off forces and measurements of the attached surface area of the basal foot of each specimen, were then used to calculate the tenacity.

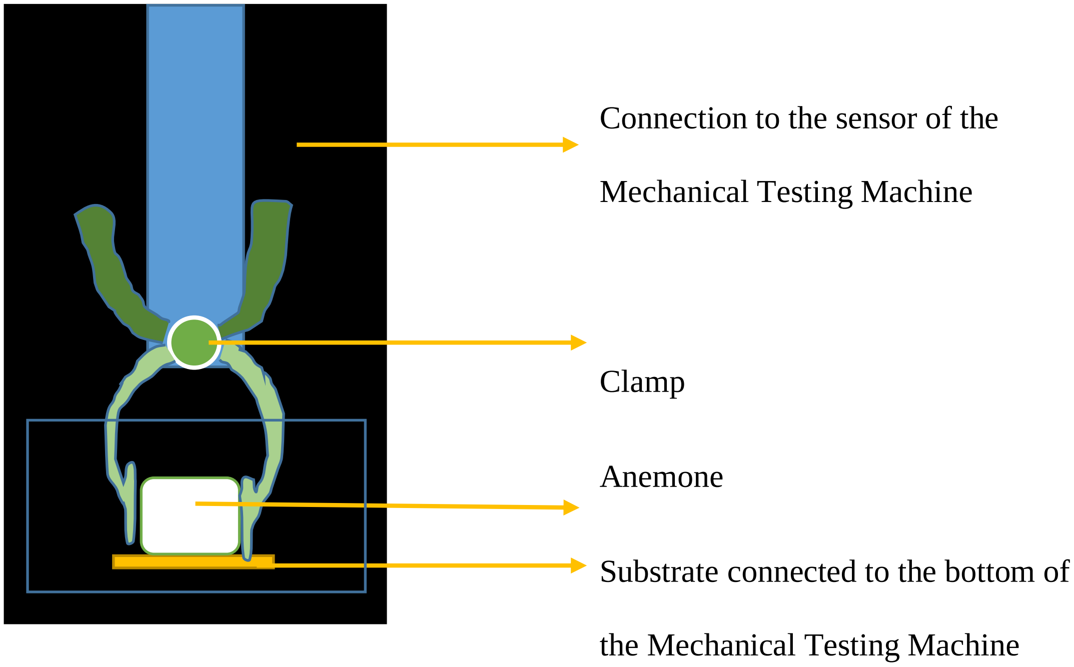

Figure 2. Mechanical testing machine set up. Blue rectangle represents the small water tank holding the specimen and substrate. Tank was connected to the machine so it did not move. Tank was modified to hold down the substrate while the specimen was being detached.

Preparation of Substrates

Substrates were made out of 0.25 in. thick acrylic glass. Using a laser cutter (Ivan Category 2, Hurricane Lasers, Las Vegas) 10cm by 10cm squares were cut. 10 solid substrates, and 10 holed substrates were made for the choice experiment. The substrates with holes had 1.5mm thick holes made in a grid array across the entire substrate, each hole 4mm apart from another. The 20 substrates for the attachment experiment (10 with holes, 10 without) were smaller (8x7.5 cm.) and made using the same method.

Results

Force Experiment

The average weight of *M. farcimen* used in the attachment force experiment was 26.95g with a standard deviation of 12.31. Basal foot area ranged from 3.74cm^2 to 15.056cm^2. Pull off force was dependent on surface area for the specimens attached to both substrates, with holes and without (figures 3 and 4).

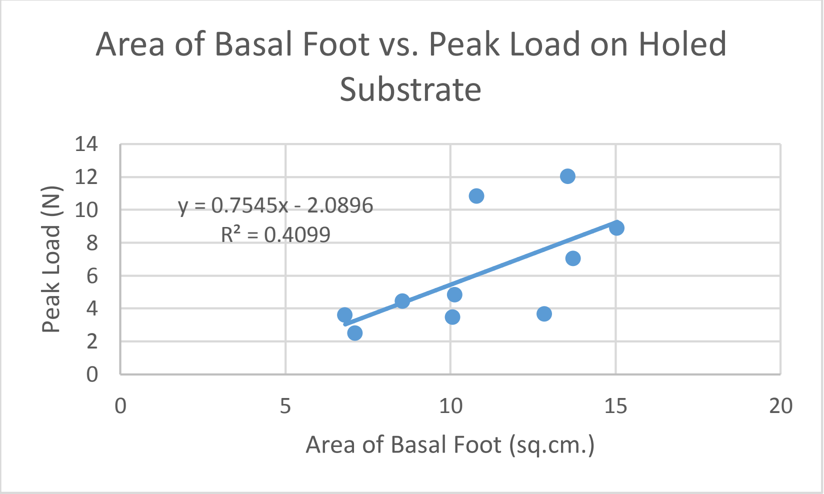

Figure 3. Demonstration of a weak positive correlation between area of basal foot and peak load of *M. farcimen* when detached from a substrate with holes.

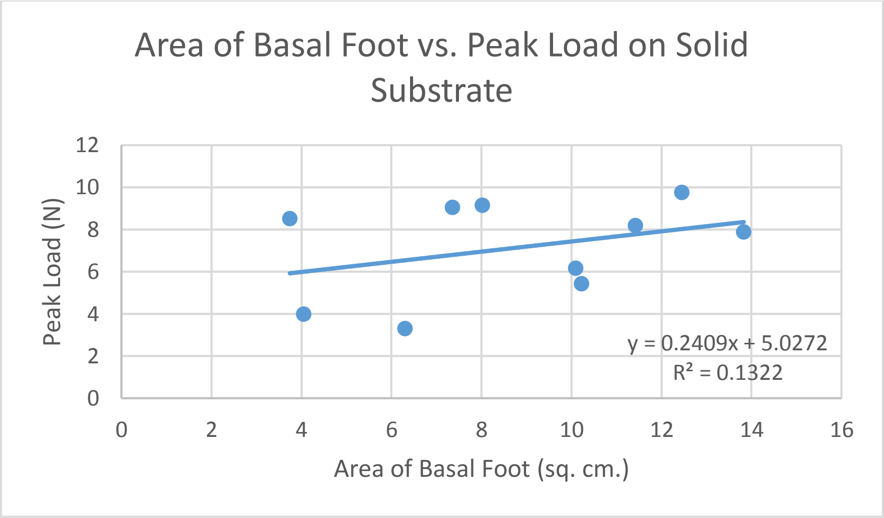

Figure 4. Demonstration of a weak positive correlation between area of basal foot and peak load of *M. farcimen* when detached from a substrate without holes.

The mean peak load for the specimens attached to the substrates with holes was 6.11 N ± 3.38. The mean peak load for substrates without holes was 7.14 N ± 2.28. There was no significant difference between force of attachment in the two groups (figure 5; t test assuming equal variances, t = -0.79, P = 0.44).

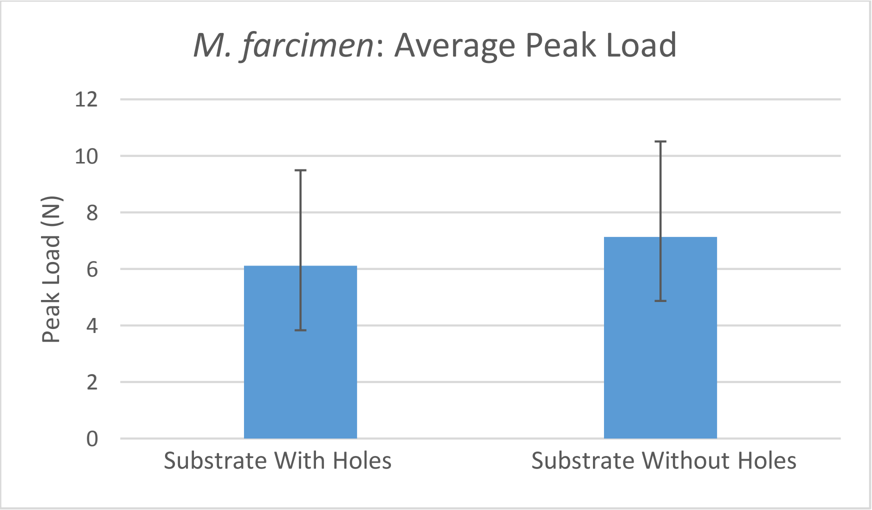

Figure 5. Average peak load of 10 *M. farcimen* attached to substrates with holes and average peak load of 10 *M. farcimen* attached to substrates without holes. The mean peak load for the specimens attached to the substrates with holes was 6.11 N ± 3.38. The mean peak load for substrates without holes was 7.14 N ± 2.28.

The mean tenacity for the specimens attached to substrates with holes was 5.48 kPa ± 2.31. The mean for substrates without holes was 9.36 kPa ± 5.35. We found that *M. farcimen* attach more strongly to substrates without holes (figure 6; t test assuming equal variances, t = -2.10, P = 0.0496*)*. *M. farcimen* attached to solid substrates displayed an average tenacity 70.75% higher than specimens attached to substrates with holes.

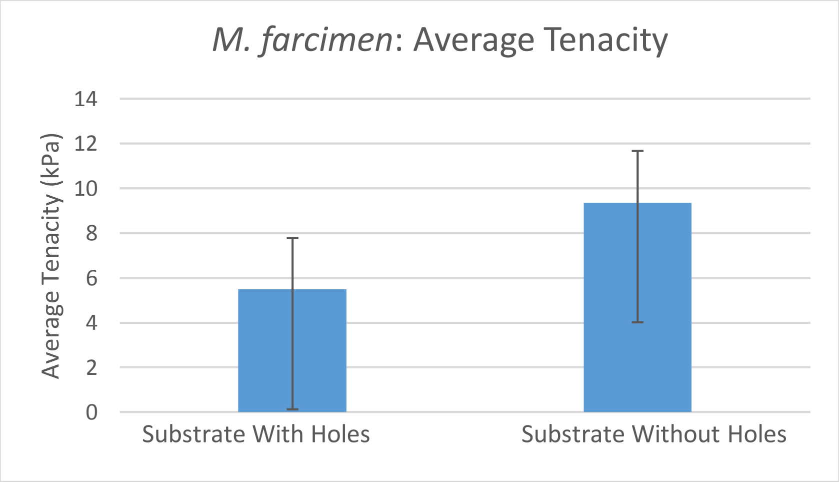

Figure 6. Average tenacity of 10 *M. farcimen* attached to substrates with holes and average tenacity of 10 *M. farcimen* attached to substrates without holes. The mean tenacity for the specimens attached to substrates with holes was 5.48 kPa ± 2.31. The mean for substrates without holes was 9.36 kPa ± 5.35.

Choice Experiment

M. farcimen used in the choice experiment ranged in volume from 9mL to 85mL, and mass from 6.09g to 75.51g. Of the 28 specimens used in each of the three trials, three did not attach at all. Mean percent of specimens attached to holed substrates over the three trials was 34.52% ± 5.46. Mean percent of specimens attached to substrates without holes was 26.19 ± 2.06. Mean percent of specimens attached to both substrate types was 28.57 ± 7.14. *M. farcimen* did not display a significant preference for solid or holed substrates for attachment sites (figure 7; t test assuming equal variances, t = 2.47, P = 0.069). There was also no significant difference between the averages of number of individuals attached to holed substrates, solid substrates, and specimens that attached to both (figure 7; ANOVA test; d.f = 2, F-value = 1.3, P = 0.37). Movement of some *M. farcimen* occurred in both the first and second 24 hours allotted for attachment.

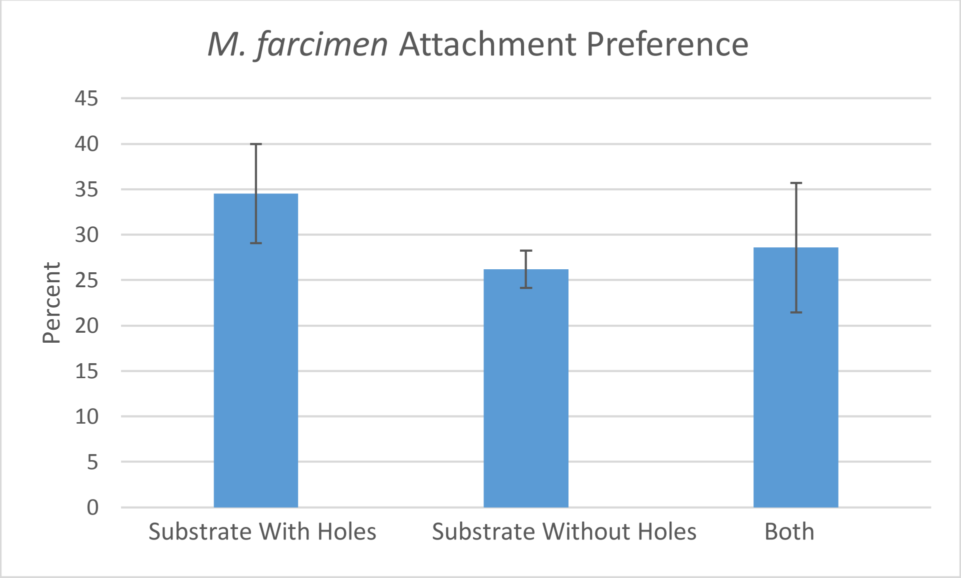

Figure 7. Mean percent of substrates chosen for attachment from the three trials. Specimens under the category “Both” attached on the border of two substrate plates (one with holes and one without). Mean percent of specimens attached to holed substrates was 34.52% ± 5.46. Mean percent of specimens attached to substrates without holes was 26.19 ± 2.06. Mean percent of specimens attached to both substrate types was 28.57 ± 7.14.

Discussion

Force Measurements

Tenacity was greatest when *M. farcimen* were attached to a surface without holes. The decrease in tenacity that correlates with attachment to a substrate with holes is most likely attributed to the inability to use suction on a holey surface. This provides strong support for our hypothesis that some degree of suction is involved in the attachment of *M. farcimen* to substrate. On a solid substrate suction is utilized to aid attachment. Nevertheless, glue seems to be the dominant mechanism, which could explain the indifference of many sea anemone species in attachment site selection.

Measured tenacity results on solid substrates was lower than *M. senile* and lower than limpets. When attached to acrylic glass, the average tenacity of *M. senile* was around 16,000 Pa (Young, Yule, and Walker 2009). This is higher than the average tenacity of *M. farcimen* on solid substrate which was around 9,300 Pa. This was surprising considering that *M. farcimen* living in the intertidal endure a more turbulent environment than sublittoral *M. senile*. This discrepancy might be explained by differences in methodology and specimen size used in each study. When attached to glass, limpets (*Tecura Scutum*) had and average tenacity of 130,000 kPa (Smith 1991).

It should also be noted that the design set up did not account for the effect that peel has on force measurements. When taking the force measurements, the clamp connecting the animal to the mechanical testing machine would sometimes slide slightly off center. This would result in the specimen being detached from the substrate with an uneven distribution of force on the basal foot. A peeling component would decrease the force required to detach the specimen from a substrate.

It is possible that the size of the holes in the substrate has an effect on attachment forces. In this experiment, holes were 1.5mm in diameter. A hole so small can easily be plugged by a soft bodied organism like *M. farcimen*. Filling the holes would reduce the maximum size of the area of reduced air pressure under the basal foot, but would not prevent suction entirely. Further testing with substrates drilled with larger holes could provide more insight on the degree to which *M. farcimen* rely on suction for attachment.

Choice Experiment

The presence of holes in substrate does not deter *M. farcimen* from attaching. This could be attributed to a number of possibilities including an indifference for maximum attachment tenacity, or an absence of advanced behaviors. There was very little current in the sea-tables where the specimens were kept. It is possible that under these sheltered conditions energy input for attachment is not necessary. Although some sea anemones have been documented displaying behavior and personality characteristics involved in space competition, it is unknown if *M. farcimen* have developed discretion behaviors used in selecting an attachment site (Ayre and Grosberg 1995).

Transitory movement was evident in this experiment. Given 48 hours, approximately 2/3 of the specimens moved from their initial placement on the border of two substrate plates to either one or the other. Some moved across multiple plates before finally settling. If given more time to attach and settle, it would be interesting to see if preference trends shift.

Conclusion

M. farcimen are able to attach more strongly to substrates without holes than substrates with holes, strongly suggesting that some degree of the attachment mechanism is dependent on suction. However, *M. farcimen* do not avoid substrates with holes where suction is not possible.

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