

These Dead Fish Really Suck:  
Adhesion Performance of the Northern Clingfish

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A Blinks/REU project

Summer 2011

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## Introduction:

The ray-finned fish family Gobiesocidae includes over 100 species of fish that are commonly known as clingfish (Fishbase). These aptly named clingfish possess modified pelvic fins that are fused into a biological suction cup, allowing the fish to adhere to surfaces in their environments. The northern clingfish, *Gobiesox maeandricus*, is a common inhabitant of the rocky intertidal shores along the Pacific Northwest coast of North America. The northern clingfish can reach sizes up to 16cm but are much more common around 8-10cm (Fishbase). These clingfish use their suction discs to adhere to rocks in their intertidal home, providing a way to remain stationary and stable in the turbulent waters close to shore. Although intertidal rocks differ greatly in surface roughness, we have observed that the northern clingfish does not appear to be limited to only smooth rocks. While manufactured suction cups function only on smooth surfaces, clingfish appear able to stick on the myriad types of surfaces present in the intertidal. This study seeks to compare how well clingfish are able to adhere to surfaces of different roughness.

While suction-adhesion mechanisms are not especially rare in either nature or ray-finned fish, little has been described about how such mechanisms function in regard to surface roughness. Despite this lack of information, the ability to function on rugose surfaces is key for the success of biological suction cups. For example, an animal that could only use its suction disc on glass would be painfully limited in substrate choice, yet animals such as bats, octopi, remoras, and clingfish depend on these adhesive structures in a natural environment for movement, place-holding, and catching prey (Kier 1991, Smith 1991, Fulcher 2006, Arita 1967, Thewissen 1995). Expanding our knowledge of biological suction mechanisms may also prove to be directly useful in manufacturing suction cups.

Man-made suction cups stick to surfaces using a mechanism referred to as suction. Suction adhesion works by maintaining a sealed compartment of fluid against a surface and then increasing the volume of the compartment to generate negative pressure; the external atmospheric pressure will then press the organism to the surface as long as a seal is maintained (Vogel 2003). Furthermore, many natural suction cups also use mucus to help maintain a seal and provide further adhesion (Vogel 2003). While the clingfish suction disc may superficially resemble a suction cup, biological adhesion does not always limit itself to one mechanism for generating adhesive forces.

A second mechanism for generating adhesion is to take advantage of Van der Waals forces. These forces are long-range molecular attractions that are individually weak (Vogel 2003, Autumn, et.al. 2002). Despite their individual weakness, by using structures to increase surface area contact between surfaces, these forces can accomplish feats like allowing geckos to climb vertically up glass (Autumn et.al. 2002). A third type of adhesive mechanism, Stefan adhesion, creates adhesive forces by taking advantage of the viscous properties of fluids. In Stefan adhesion, a thin layer of fluid (mucus) separates two surfaces so when one surface is moved parallel to the other, the fluid will have to shear at a high rate, which is resisted by the viscous properties of the fluid (Vogel 2003). Furthermore, when the surfaces are moved apart from one another, the ambient fluid (water) must begin to insert itself between the two surfaces. To do this, the water

must also shear at a high rate because of the thin layer it must begin to form. Again, viscous forces fight the shear and keep the organism from detaching.

Arita (1967) has hypothesized that clingfish use mainly suction to adhere to surfaces. However, in order for clingfish to stick to the gritty surfaces found in nature it is likely these fish are using specialized morphology and alternative adhesion mechanisms to generate adhesive forces. In this study our purpose is twofold: we explore how well the northern clingfish adheres to surfaces of different roughness and we seek to explain our results using morphology of the suction disc.

#### Methods:

To test how well clingfish can stick to surfaces of different rugosities, eight surfaces were made by creating molds with dental wax. Coltene President light body dental wax was prepared and a rectangle of sandpaper taped to a piece of acrylic was pushed into the dental wax to make a mold of the sandpaper surface. Sandpapers were from Buehler and their Carbimet 2 assorted grits package were used. The assorted grits package included the following grit sandpapers, listed from roughest to smoothest using grit sizes determined by ANSI first and then FEPA in parenthesis: 60 (P60), 120 (P120), 180 (P180), 240 (P280), 320 (P400), 400 (P800), 600 (P1200). In the same order, these correspond to micrometer grit sizes of 269, 127, 78, 52.2, 35, 21.8, and 15.3. One surface was also made using glass to imprint the mold making a total of eight surfaces. After molds were made, 100% silicon was used to create a wall around the part of the mold that was to be used for casting the surfaces. The silicon wall was allowed to dry for 24 hrs and then the mold was attached to a piece of glass, again using 100% silicone, to prevent bending of the mold due to silicone shrinkage in the oven. All of this is illustrated in Figure 1.

Spurs resin was mixed and then cast in the molds. Eight surfaces were made in total – seven from the sandpapers listed above and then one from a mold of glass. The resin casts were baked at 70°C for up to 24 hours whereupon the mold and cast were removed and separated.

Eight small watertight aquaria were built and each surface was glued into the bottom of its own aquaria using ACE brand Quick Set Epoxy. The aquariums were built to have internal dimensions of 12.5cm long, 9cm wide, and 7cm deep. A 12-15cm 1/4inch strip of acrylic was glued onto the bottom of the aquariums to provide a hold. ACE brand Quick Set epoxy and ACE brand Plastic Repair epoxy were used to glue the aquariums together.

All 22 fish were caught under rocks either in Dead Man's Cove or beside the Friday Harbor Labs dock. Both locations are on San Juan Island, Washington. Fish were killed using a lethal dose of MS-222 in seawater directly prior to testing and photographs were taken of the dorsal, ventral, front, and lateral sides of the fish using a ruler in the pictures for scale. These pictures were later loaded into ImageJ to measure length and surface area of the cling. The aquaria were always filled 5cm deep with seawater to ensure equivalent water pressures. Clingfish were attached to the materials testing machine by threading suturing thread under the vertebral column near the anterior end of the suction disc and then back again through opercular gill openings of the fish. The

suture was tied to itself where it began so that there was a loop on either side of the fish. This harness was then attached to the materials testing machine by hanging the suturing thread on a hook that was secured by a clamp.

All trials were completed on an MTS Synergie 100 materials testing machine and data was collected using the program Testworks. A 500 Newton load cell was used and the machine pulled at a speed of one meter per minute in order to minimize any leakage effects on the suction generated. A simple tensile test was performed to measure the maximum load that each fish generated on each surface. Experimental setup is shown in Figure 2.

22 fish were tested with four trials per surface. The order of testing was selected randomly for each fish using a random number generator designed in the computing language Octave. Fish were pre-sampled by completing three trials on the first randomly selected surface. These three trials were not used in the data set or analysis. After the pre-sampling, the fish completed one trial on each surface using the randomly generated order. After one trial, each fish went again through the random order of surfaces three more times for a total of four trials per surface. New random orders were used for new fish. If a fish did not stick for a particular trial, the trial was repeated as normal. Furthermore, eight suction cups of different sizes and materials were purchased and tested in the MTS using the same settings and testing method as the clingfish. If a suction cup or a fish was repeatedly pushed against a surface but would never stick down, data was recorded as a zero force for that surface.

Morphology of the cling was viewed using CT image data of a clingfish loaded into the program Amira. This was done in order to better understand how the clingfish suction disc actually works and how the morphology is modified to support the suction disc structure. The bones associated with the sucker disc structure were sectioned using Amira to better visualize the gross morphology of the cling.

Scanning electron microscopy was performed to view the epithelial microstructure of the suction disc to better determine suction mechanisms and understand the role of surface structure on the cling. A NeoScope JCM 5000 SEM was used. Three fish were prepared for SEM by bringing them up to 100% ethanol through a 50%, 70%, 90%, 95%, series with at least 2 hours in each bath. Then the clings were dissected out and prepared using HMDS starting with 50% HMDS with 50% ethanol, increasing to 70% HMDS, 90% HMDS, 95% HMDS, and then 100% HMDS. Specimens were then sputter coated with a gold palladium coater. Scanning electron microscopy was also used to characterize the surfaces. Two pictures were taken of each surface – one picture with no angle and another picture with a 15° tilt forward. Images were loaded into the program Stereoworks and then ImageJ with a plugin to generate a height map. By generating height maps of the surfaces, we were able calculate our own values of roughness for the surfaces and compare how they matched with the sandpaper values. This method proved to have problems with the roughest surfaces however, because fewer grains could be seen in pictures taken by the SEM, even at the lowest magnification. The calculated data is presented in Figure 3.

Suction adhesion depends both on surface area of the suction cup and the ambient pressure of the fluid outside the suction cup. The equation is organized as follows:

Equation [1]:

$$F=P*A$$

Where  $F$  represents force generated by suction,  $P$  is the ambient pressure, and  $A$  represents surface area of the suction cup. Knowledge of this simple relationship was used to represent data in a size corrected format and calculate the maximum physically possible suction force for clingfish and suction cups.

Animals in this study were approved for use by the IACUC protocol held by Dr. Adam Summers at Friday Harbor Labs.

## Results:

Figure 4 shows a table of the fish tested with various relevant measurements. For example, fish ranged in length from 7.04cm to 11.96cm. Also included in Figure 3 are the maximum possible suction forces that each clingfish could achieve based on surface area of their cling. These maximum possible forces were calculated using the surface area of the suction discs and the ambient pressure that was experienced in the experimental setup. The ambient pressure value was determined to be 101828Pa. This value was calculated using the depth of the water and the density of seawater added to the average atmospheric pressure at sea level. These maximum possible suction forces were calculated using Equation [1].

Figure 5 shows surface areas and maximum possible suction forces for the eight manufactured suction cups we tested. Suction cups were numbered starting with 100 in order to prevent overlap with fish tested. Suction cups ranged in size from ones with a surface area of 3.62cm<sup>2</sup> to the largest with a surface area of 15.17cm<sup>2</sup>. Note that some suction cups were larger than the biggest clingfish suction discs, although the ranges overlap considerably.

After all 22 fish were tested, we end up with four trials per surface per fish. Only the highest pull per surface per fish was used in data analysis because we were interested in how hard the fish can stick to the different surfaces. Suction cups were analyzed in the same way. This method then gives us eight data points for each fish, one data point for each surface.

By using the mass of each fish as a metric of size, we can analyze how hard each fish was able to stick with respect to its own body weight. Figure 6 shows the relationship between fish mass and how many times its body weight it was able to stick. The highest values are over 250 times fish body weight. For example, say we have a fish with a 10 gram mass. This fish then stuck to a surface with a force of 20N. We can transform 10 grams into a force by first dividing by 1000 to get 0.01kg. Then we multiply 0.001kg by gravity, 9.81 m/s<sup>2</sup> to get about 0.1N. The 20N force that the fish was able to stick with is then 200 times greater than the 0.1N force that is equal to the mass of the fish. To put this into perspective, the author Dylan Wainwright has a mass of over 80kg and if I was able to support 200 times my body weight, I could support 12 Honda Civics.

The main question of our study, however, was how well clingfish can stick to different roughness. Figure 7 illustrates the gradient of surfaces used with photos taken with a SEM. All pictures are at 40x magnification except the flat surface, which is at 300x magnification. In Figure 8 we represent the data from all of the clingfish pulls. The x-axis is surface roughness and the y-axis is stress in kilopascals. Stress is simply

size corrected force. It is obtained by dividing the sticking force of the fish by the area of the suction disc. Remember that suction depends upon area, so calculating stress is a reasonable way to correct for size.

Figure 8 shows how well the fish performed on each surface, representing the data as a series of box and whisker plots. The stresses fall out into two statistically significant groups: the flat surface and all surfaces with roughness. For the statistics, we had the computing language R run a two-way ANOVA to analyze the stress data with surface roughness and fish number as effects. Tukey honest significant different test showed the significant differences. Figure 8 denotes this statistical difference by coloring the flat data differently and also providing bars under the boxplots to denote the two statistical groups. Notice that the clingfish performed better on the rough surfaces than on the flat surface.

In Figure 9, we represent the clingfish stress data with the suction cup data on the same graph. Again, statistically significant groups are denoted by bars under the clingfish boxplots and bars above the suction cup boxplots. Clingfish data was found to be significantly different from suction cup data and suction cup data also fell into two statistically significant groups. All suction cups tested were able to stick to only the flat surface and the two smoothest surfaces cast with sandpaper molds – this became one statistical grouping. On the rest of the surfaces, the suction cups were unable to stick and therefore have stress values of zero. These made up the remaining statistical group.

Using the maximum possible stresses possible, we can calculate how well the clingfish and the suction cups performed with respect to their maximum possible value. To do this we used percents by dividing measured maximum forces by the calculated possible maximum force for each specimen. This data is shown in Figure 10.

To gain insight into the microstructure of the clingfish cling, we used the SEM to take photos. Clingfish suction discs are covered in raised bumps call papillae (Green 1988). Figure 11 shows these papillae that are only on the edges of the cling. Figure 12 shows a papillae that has been magnified. The papillae are made of small hair-like structures called microvilli. These structures have been described before by DM Green (1988) although the complete function of them is not known. Green (1988) did not believe these structures do anything but provide additional friction for the cling so that clingfish do not slide on surfaces. Figure 13 shows these microvilli with a photo taken from the SEM.

## Discussion:

To find that clingfish stick better on rough surfaces than flat surfaces is the complete opposite one would expect based on how suction cups work. Although this makes functional sense because these fish will hardly ever encounter glass-like surfaces in nature, these fish outperform all manufactured suction cups tested on the five roughest surfaces. Furthermore, to find the clingfish able to stick just as well to the roughest surface as the smoothest (but not flat) surface shows a functional adaptation of being able to handle surfaces equally well, something that manufactured suction cups are also unable to do.

By morphological inspection of the cling, papillae and microvilli are likely responsible for clingfish being able to outperform suction cups on rough surfaces. Having pads of hair will provide a much better seal on rough surfaces because hairs will conform to the surface texture, allowing the suction disc to remain sealed.

Additionally, the microvilli may increase Van der Waals forces by diversification of surface area. Suction cups often fail by buckling, where the edges will slip inward when under stress. This slippage can then create a leak, leading to failure of the suction cup. Microvilli using Van der Waals forces would increase the amount of friction between the cling and the surface, making it more difficult for the edges of the cling to slip inward and buckle.

Clingfish are unable to stick as well to the flat surface because of a few reasons. The raised nature of the papillae may hinder suction on flat surfaces because spaces in-between papillae create small leaks, leading to earlier failure. Also, it was observed that clingfish on the flat surface slid around very easily. This is because the flat surface provides no surface texture or diversification for the microvilli to increase the amount of Van der Waals forces.

Moreover, it was seen through SEM photos like Figure 14 that the papillae and microvilli are sometimes covered in mucus. Having mucus secreted between the cling and the substrate would provide a situation for Stefan adhesion to come into play. Stefan adhesion would both increase the friction between the cling and the surface, therefore increasing the force needed for the edges to slip and buckle. More so, Stefan adhesion will resist forces that move the cling away from the substrate because both mucus and water are viscous enough to have an effect.

In this experiment, we were able to design a method to create surfaces that eliminate all differences, chemical and physical, except for roughness. With this method we were able to show that clingfish stick to rough surfaces with higher forces than on flat surfaces and also clingfish are able to stick onto much rougher surfaces than manufactured suction cups. By investigating the microstructure of the cling, we have gathered morphological evidence that can be paired with the known adhesive mechanisms. It is likely clingfish use both Van der Waals forces and Stefan adhesion in concert with physical suction to stick to rough surfaces.

#### Acknowledgements:

I would like to thank Adam Summers, Thomas Kleinteich, Stephanie Crofts, and Anja Kleinteich for their immeasurable help and advice. I would like to also thank Kiki Contreras, Kelsey Gaessner, Thomas Kleinteich, Anja Kleinteich, Lea Kleinteich, Stephanie Crofts, and Phillip for their help collecting the organisms used in the study. My thanks also go out to Friday Harbor Marine lab, Sophie George, and the REU program for providing me with this opportunity, IACUC for their support our research protocol, and especially the National Science Foundation for providing funding for this research.

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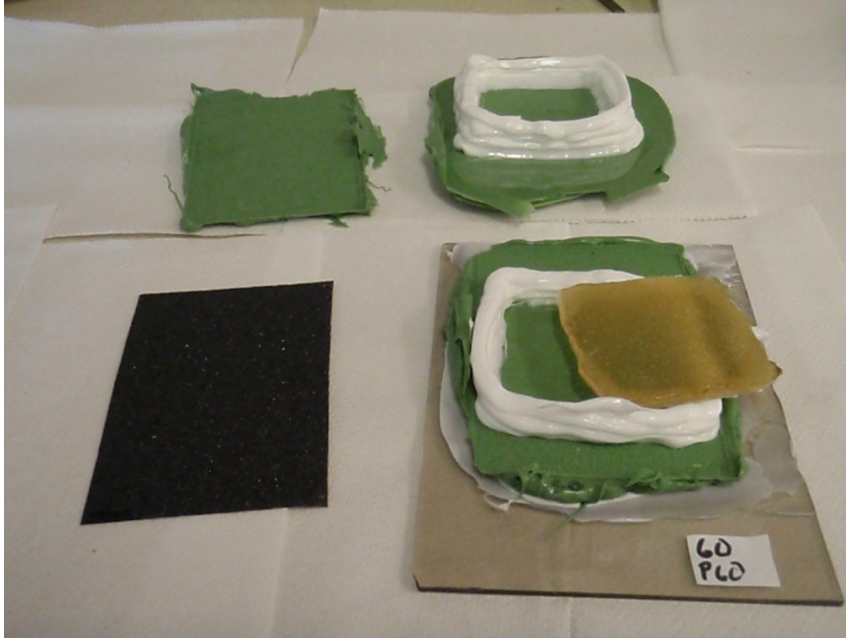
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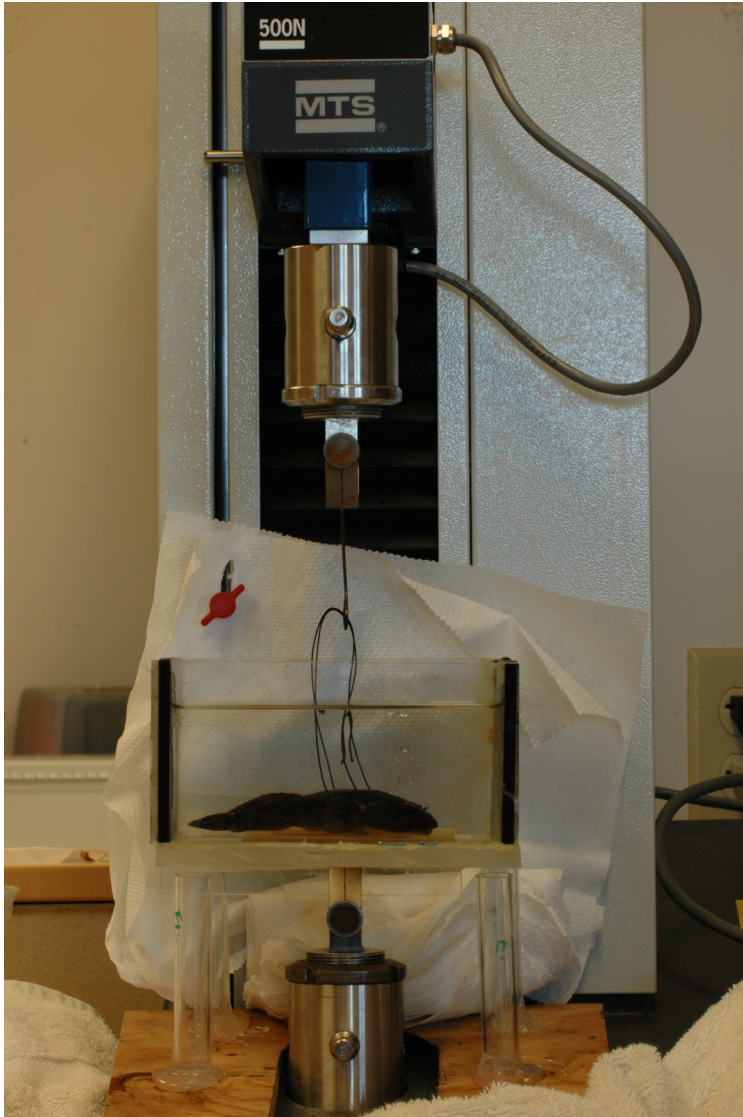
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**Figure 1: Molds for surfaces. Black rectangle in bottom left is sandpaper. Green is dental wax molds and white is silicone used as a wall on the molds. At the bottom right you can see a surface that has been made using Spurs resin.**



**Figure 2: Experimental Setup.** Clamp at bottom holds the tank steady. Tank has a surface glued onto the bottom, which the fish rests on. Fish is attached to upper clamp using a harness and a fish hook hung from the clamp. Upper clamp moves up to increase tension and measures force with a 500N load cell.



**Figure 3: Calculated roughness for surfaces. All units in micrometers. Both Rq and Ra are measures of roughness.**

Sandpaper	Rq	Ra
15.3 $\mu\text{m}$	10.1422	8.7663
21.8 $\mu\text{m}$	17.1348	14.7652
35.0 $\mu\text{m}$	31.4828	27.1545
52.2 $\mu\text{m}$	49.1787	42.4651
78.0 $\mu\text{m}$	129.9215	112.3969
127.0 $\mu\text{m}$	100.7224	87.5636
269.0 $\mu\text{m}$	160.3200	141.5148

**Figure 4: Table of specimens used with length, area of suction disc, Mass, and calculated expected suction force. GM in the Note column denotes the fish *Gobiesox maeandricus*.**

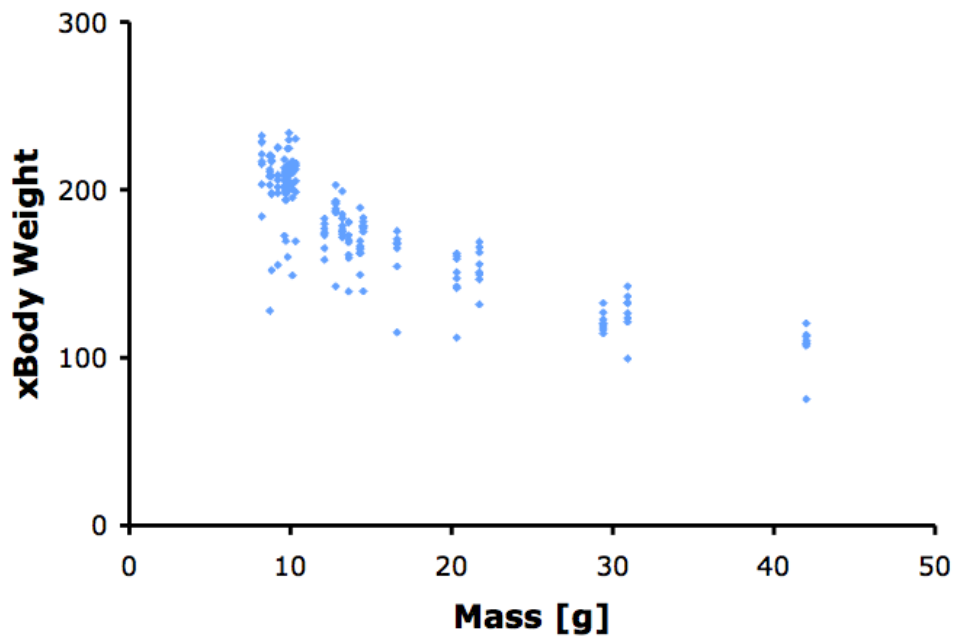
Number	Note	Length [cm]	Cling SA [cm <sup>2</sup> ]	Mass	Expected Force [N]
1	GM	7.9	5.05	9.8	51.39
2	GM	8.8	6.34	14.3	64.54
3	GM	8.38	7.35	14.5	74.89
4	GM	10.26	9.27	21.7	94.35
5	GM	7.34	5.26	9.6	53.58
6	GM	10.9	11.16	29.4	113.59
7	GM	7.47	4.51	8.7	45.9
8	GM	7.7	6.94	12.1	70.71
9	GM	7.11	5.62	9.7	57.2
10	GM	8.93	8.6	16.6	87.59
11	GM	8.26	5.3	13.2	53.96
12	GM	7.41	4.28	10.3	43.62
13	GM	7.73	4.83	12.8	49.19
14	GM	9.02	6.99	20.3	71.23
15	GM	8.34	5.05	13.6	51.38
16	GM	7.23	4.7	8.8	47.87
17	GM	10.81	10.78	30.9	109.76
18	GM	11.96	12.29	42	125.11
19	GM	7.46	4.69	9.9	47.74
20	GM	7.27	4.19	9.2	42.66
21	GM	7.07	4.32	10.1	43.99
22	GM	7.04	3.91	8.2	39.81

**Figure 5: Suction cups with relevant surface area and possible force. SC in the Note column denotes suction cup.**

Number	Note	Cup SA [cm <sup>2</sup> ]	Expected Force [N]
100	SC	15.17	154.43
101	SC	6.52	66.35

102	SC	9.72	98.94
103	SC	11.15	113.52
104	SC	12.34	125.65
105	SC	3.62	36.82
106	SC	14.49	147.55
107	SC	6.21	63.25

**Figure 6: Clingfish mass vs how many times their body mass they were able to stick.**



**Figure 7: Different surfaces. All shown at 40x magnification in the SEM except the flat surface, which is at 300x. Roughness increases from left to right, top row is smoother than bottom row.**

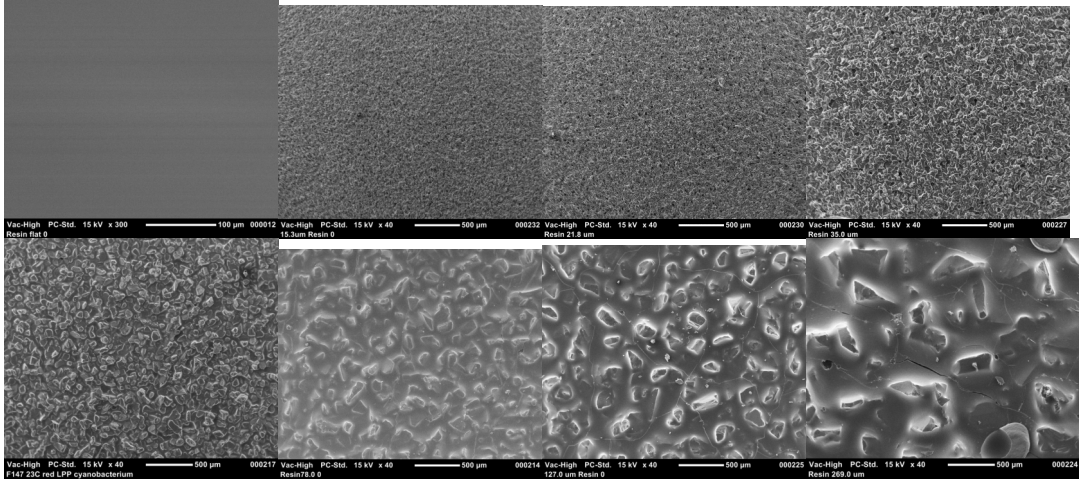


Figure 8: Stress vs Surface roughness for clingfish only.

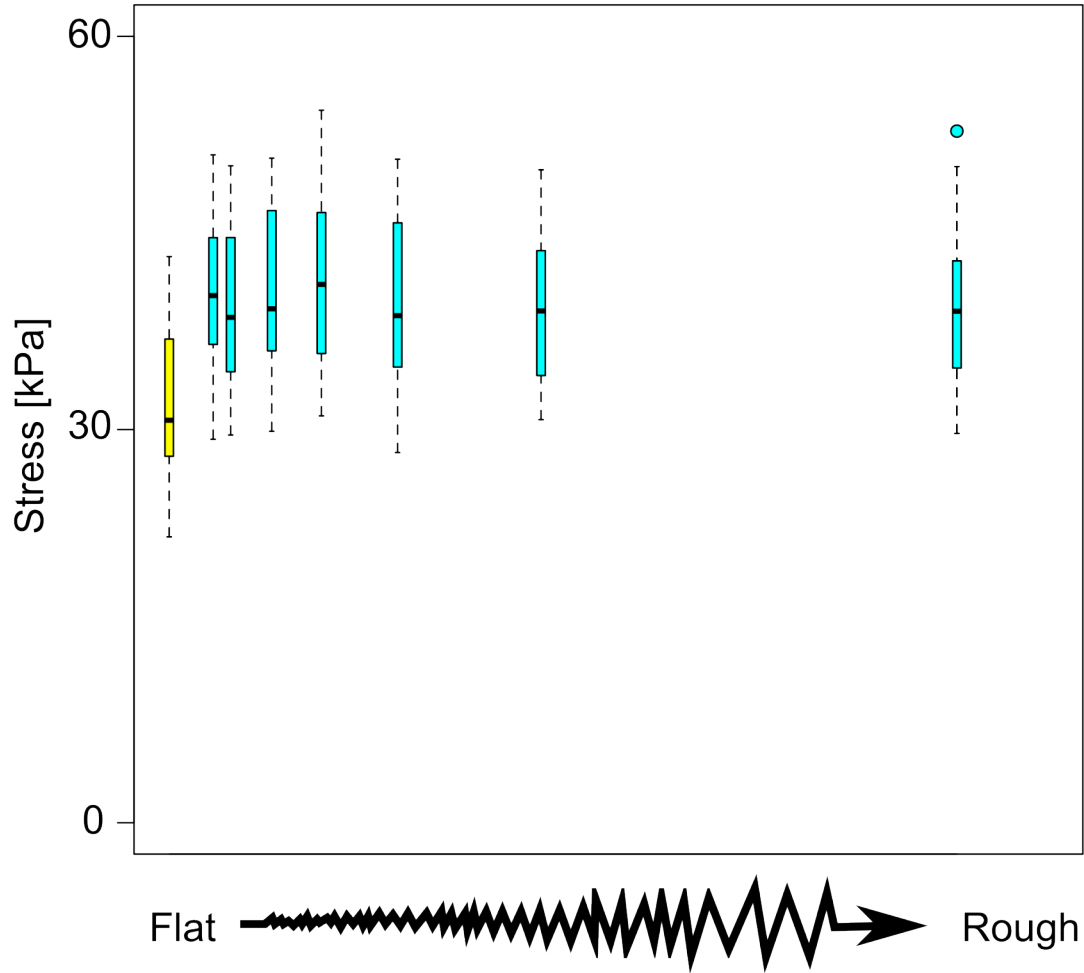
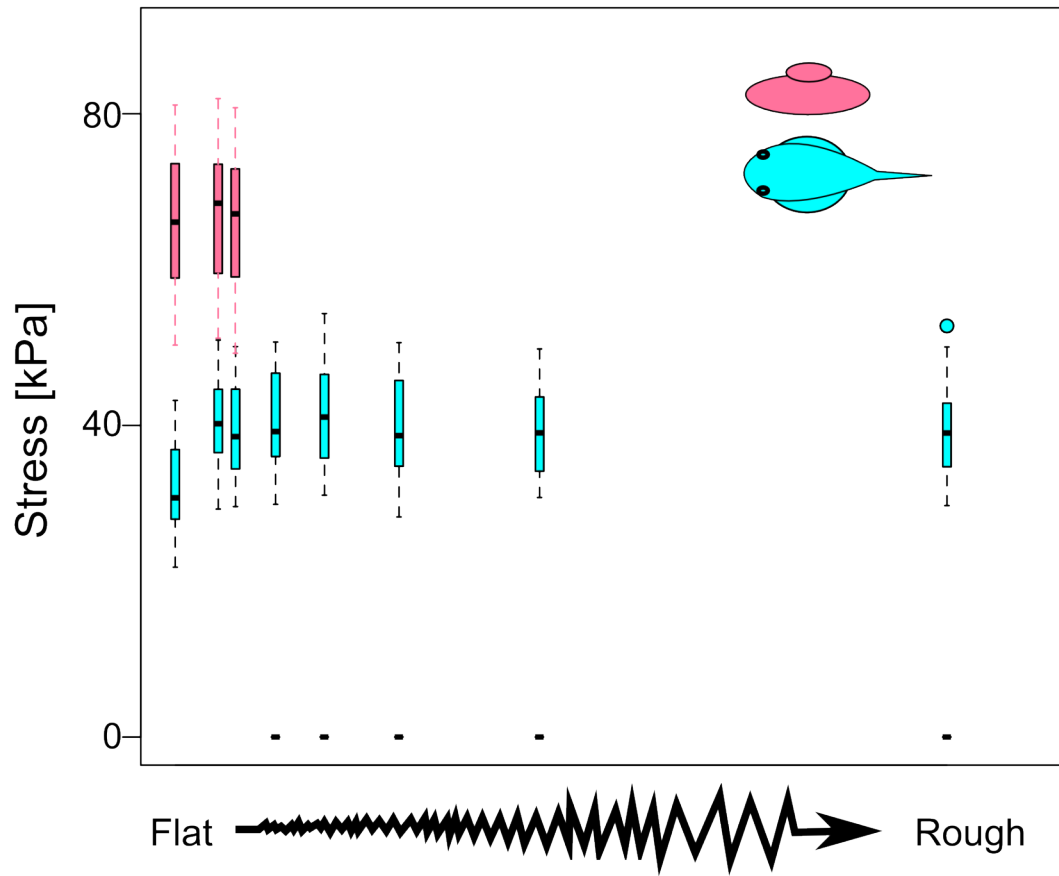


Figure 9: Clingfish and Suction cup Stress vs Surface roughness.



**Figure 10: Percent of maximum possible suction for both clingfish and cups. Cups in red, clingfish in blue.**

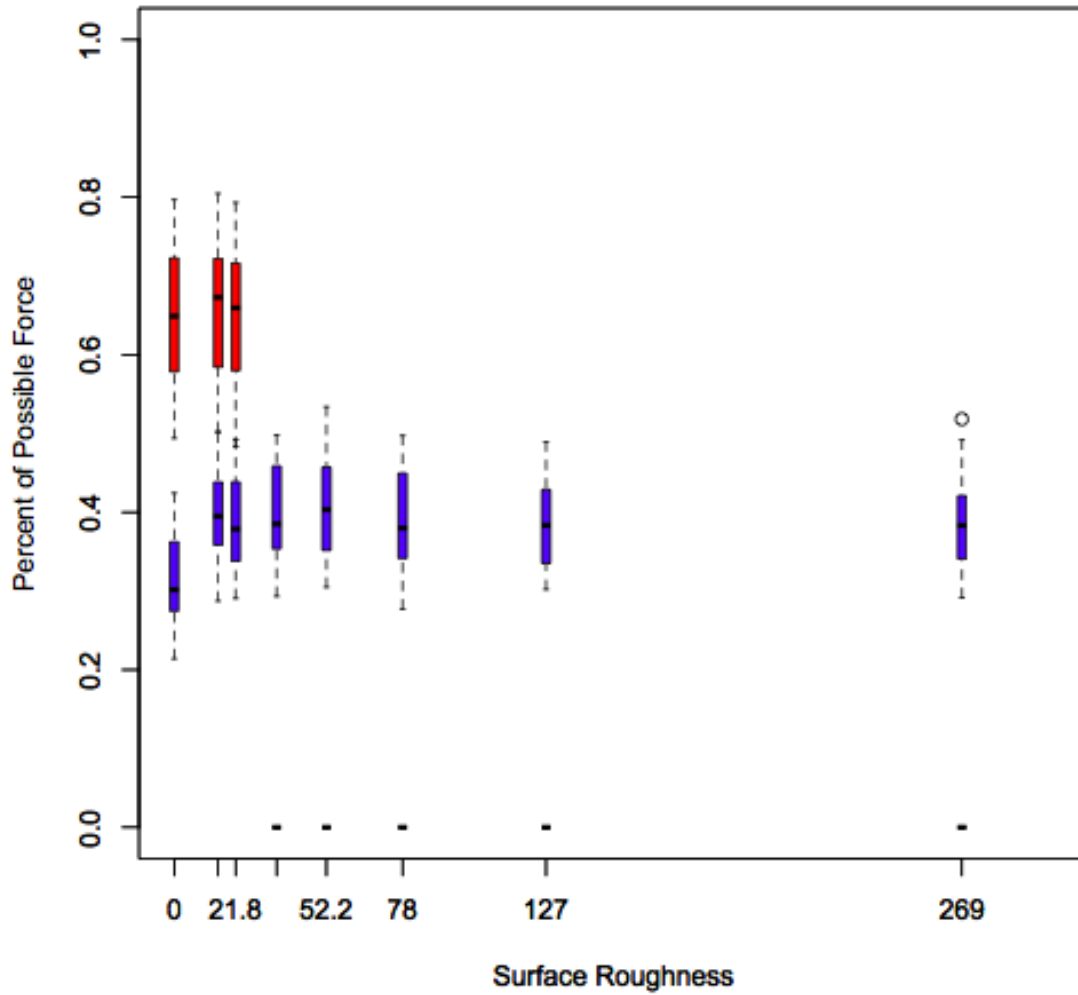
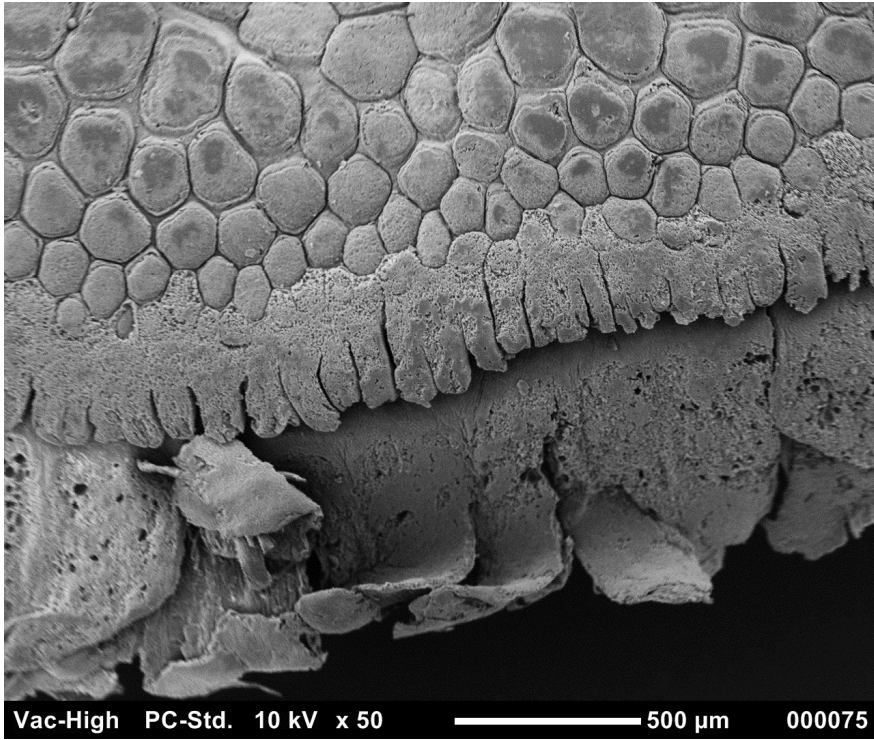
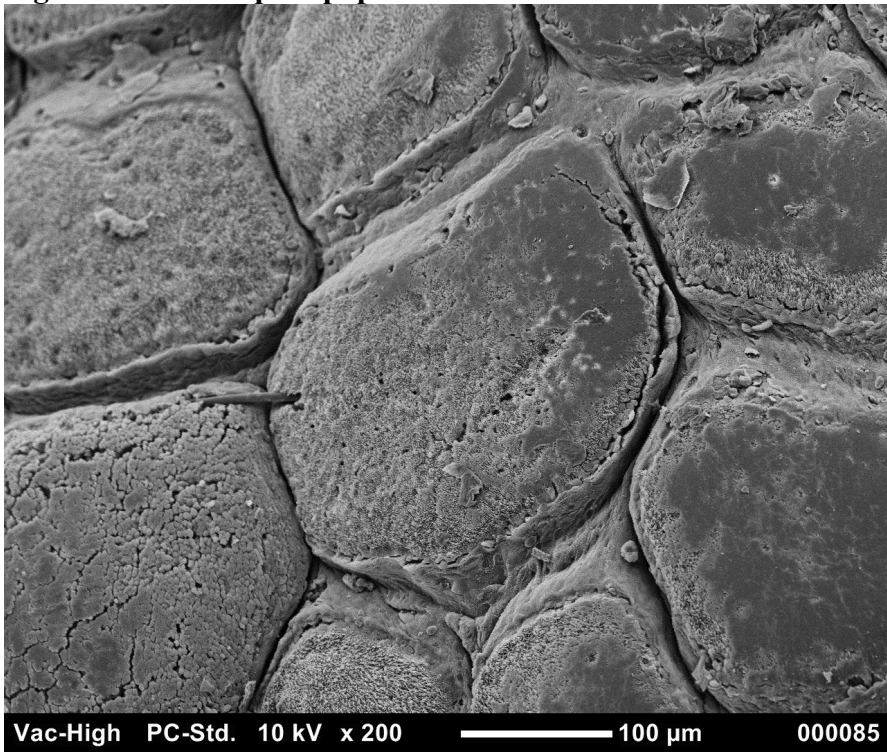


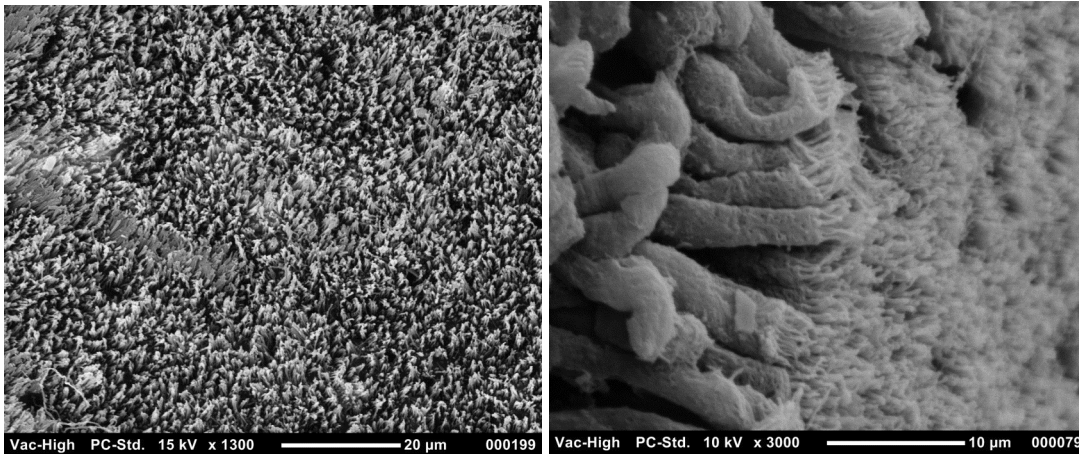
Figure 11: Papillae on the distal edges of the cling epithelium.



**Figure 12: Close up of papillae.**



**Figure 13: Microvilli. Hairs on papillae. Left is a top down view, right is a view from the side, showing the larger stalks that have a diversification of hairs at the end.**



**Figure 14: Presence of mucus on the surface of papillae. Likely a mucus pore in the center. Left side of the photo shows hair not covered by mucus, while the right side shows a layer of mucus on the hairs. Mucus represents the potential for Stefan adhesion.**

