# How speed of separation affects suction adhesion of the limpet, *Lottia scutum*

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## ABSTRACT

The intertidal limpet, *Lottia scutum,* is a key grazer keeping algal levels in check. Because limpets rely heavily on suction to prevent dislodgment during foraging, it is important to understand the tenacity of suction adhesion. Through use of a clamping measurement apparatus, underwater limpet tenacity was measured at various pulling speeds to quantify the speed and force required for limpet dislodgement. The purpose of this measurement was to determine the maximum tenacity that a limpet can achieve at varying pulling speeds. Instead of seeing one speed at which tenacity was maximized, I saw a trend of increasing tenacity over time. There was no effect of speed of separation on suction attachment. Stefan adhesion does not play a large, at least under my experimental conditions.

Key Words: Limpet, *Lottia scutum,* suction, adhesion, separation speed, tenacity, Stefan equation, Friday Harbor Labs

## INTRODUCTION

Suction adhesion is used for both short and long term attachment in a diversity of marine animals, ranging across cephalopods, echinoderms and fish (Smith et al. 1993). Suction adhesion allows organisms to attach to substrata on shores and prevent dislodgment from environmental forces (waves, wind, currents, gravity), and as protection from predation. Limpets are prime examples of mollusks that demonstrate powerful suction adhesion (Grenon & Walker 1981).

Limpets demonstrate two types of adhesion: When constant moving is required, suction adhesion is used. If they have ample time (on the order of a few hours), they will form an even stronger attachment using glue. When compared to suction adhesion, glue adhesion allows limpets to remain stationary for extended periods of time at a potentially lower metabolic cost. Although suction is not as strong as glue, it is a compromise which allows the limpet mobility. Glue adhesion is used when limpets are exposed at low tides; this long term adhesion provides extra protection from predators and desiccation when mobility is not needed. During high tide, limpets are often foraging, and can actively decide when to generate suction to prevent dislodgement from environmental forces or predation. This suggests that they alternate adhesion mechanisms daily (Denny et al. 1985, Smith 1991). The limpet forms suction by decreasing the pressure of the water it encloses; the difference in pressure between the outside and inside of the sucker is what causes the sucker to attach to the substratum (Smith et al. 1993).

Stefan tack is another proposed adhesion as described by Grenon & Walker (1981). In Stefan adhesion, viscosity is the tack preventing separation of two adhering surfaces. This adhesion is described by the equation:

$$F = \frac{3\pi n R^4}{2h^3} \frac{\mathrm{d}h}{\mathrm{d}t} . \quad (1)$$

F is the force of separation, n is the viscosity, R is the radius of the limpet foot, h is the separation distance, and dh/dt is the speed of separation. This mathematical approach implies that the force required for separation will be proportional to the separation speed; the higher the pull speed, the higher the force required for separation.

It is important to understand the tenacity of suction adhesion over various time scales because limpets rely heavily on suction for survival. The ideal experiment to quantify and simulate the work required by a predator or environmental force to dislodge a limpet would be to measure the force of suction adhesion across various separation speeds. Grenon & Walker (1981) investigated the effect of speed of separation by pulling limpets at various constant speeds. They measured limpets on two different dry surfaces, to see how the normal tenacity was affected by varying pulling speeds. They gave a speed at which tenacity is maximized for each surface, but did not provide statistical analysis of peak loads for each pulling speed; they only analyzed peak load differences between surfaces. During their discussion, they also noted that tenacity is significantly higher when immersed in water even though they only tested on dry surfaces (Grenon & Walker 1981). With these uncertainties from Grenon & Walker's (1981) laboratory approach, my goal was to use more refined methods to test the effects of speed of separation. Through use of a clamping measurement apparatus, underwater limpet tenacity was be measured across various pulling speeds to quantify the speed and force required for limpet dislodgement. I predict that there would be overall greater tenacity underwater, as well as a plateau of maximum tenacity at greater pulling speeds rather than a decrease as presented by Grenon & Walker (1981).

## METHODS

### *Limpet collection and maintenance*

Fifty specimens of *Lottia scutum* between 20-22 mm lengths were collected from various rocky shores at Friday Harbor Laboratories, Friday Harbor, Washington, USA. A slow twisting of a knife under the leading edge of the shell was used to carefully remove limpets from the substratum as described by Ellem et al. (2002). When surfaces were dry, a horizontal sheer force was enough to safely dislodge limpets. Limpets with signs of damage on the underside of the foot most likely had adhered with glue and were discarded on site. Limpets were transported to the laboratory and placed in tanks of circulating sea water of roughly 10°C. Twenty-four hours after collection, laboratory tanks were full of spawned eggs and sperm. The subsequent collection showed that the limpets at the site had spawned as well; smaller spawning events occurred throughout the experimental period.

### *Measurement of force of adhesion*

Force of adhesion was measured to determine the maximum tenacity that a limpet can achieve at varying pulling speeds. The shells of limpets were first carefully cleaned of algae and debris with a coarse sponge (Smith et al. 1993, Ellem et al. 2002). To ensure epoxy adhesion, the shell apex was lightly wiped down with a q-tip dipped in acetone. By placing the limpets on a sheet of glass, the shells were dry when a harness was attached. With Z-Spar Splash Zone Epoxy, a harness was made from 19 gauge stainless steel wire and was attached as close to the central axis as possible. A harness at the central axis is desired because shear forces require much less force to detach a limpet than lift forces alone (Grenon & Walker 1981, Smith 1991, Smith et al. 1993, Ellem et al. 2002). Epoxy was left to dry for a minimum of 24 hours, at which point individual harnesses could be tagged with tape flags. To limit stress on specimens, each limpet was not tested more than once within eight hours. Using a monofilament fishing line to attach the harness to the clamping measurement apparatus, the apparatus was programmed to pull at constant speeds (0.03, 0.10, 0.60, 1.00 and 1.50 cm * s$^{-}$1) until detachment; the clamping measurement apparatus used was a MTS Synergie 100 with a 500 N load cell. Using a small aquarium lined with Lucite substrata, limpets were tested while immersed in 10°C seawater. For standardization, limpets were allowed 15 minutes to settle and were then tapped repeatedly to induce a suction adhesion prior to detachment. It was then found that this was too short of an adjustment period for the limpets and hours of settlement would be required for best results. With sample specimens so close in size (20-22 mm), the area of the limpet's foot is effectively the same in all replicates. As confirmation, measurements were estimated with calipers through the transparent surface prior to experiments. With 50 samples and five pulling speeds, a total of 172+ data points were collected.

Equations of regression for the best fit curves define the relationship between the force of suction adhesion and the surface area of the foot. I used a single factor ANOVA, to compare tenacity across different separation speeds. To validate previous theories of limpet suction, I tested my data against Stefan's equation of tack, rearranging his equation:

$$h = \left( \frac{3\pi n R^4 \frac{\mathrm{d}h}{\mathrm{d}t}}{2F} \right)^{\frac{1}{3}} \qquad (2)$$

The separation distance can be found by substituting in known variables.

## RESULTS

Pulling forces of less than 1-2 N were seen with *Lottia scutum* when they were placed in the testing tanks for less than 15 minutes prior to testing. After their initial reattachment in the testing tank, no clamping activity was observed despite application of a stimulus. Trials after 30 minutes of reattachment gave similar results, so further testing gave limpets a minimum of eight hours to adapt; this allowed the limpets to attach more firmly. The limpets adhere to a Lucite substrate and maintain much of their attachment force even after sliding short distances, which is consistent with observation of Smith (1991) and confirms that glue was not used. Solid residues on the substrate and a damaged foot would be indicators of glue being used.

After a short sliding of the limpet and harassment from a finger/forceps/pencil, the mean tenacity of *L. scutum* across five pulling speeds ranged from 7.37 to 8.92 N with a standard error of less than 0.53 N. Regression equations for force versus foot area of 20-22 mm limpets had slopes close to zero for all pulling speeds (Table 1), and the regression P-values were all well above 0.291 indicating that the model does not explain the variations. Linear regression models for force required for separation at all pulling speeds versus foot size again showed a slope close to zero ($Y = -0.0091x + 9.01091$) and little explained variation ($R^2 = 0.0225$), but a p-value that was almost significant ($P = 0.068$).

Though mean values were similar, Figure 1 shows the large range and variance in adhesion strength within each pulling speed category. Pulling speeds with the greatest number of data points, 0.01 and 1.0, showed the greatest range in force; for these pulling speeds the majority of the data collected laid between the median and the upper extreme. All other pulling speeds exhibited smaller ranges, with most data points between upper and lower quartiles. Overall, there was no difference in mean peak detachment force across the five pulling speeds (One-Way ANOVA, $F = 1.565$, $P = 0.187$).

Having noticed a mass spawning event 48 hours prior to the start of experiments, time of day was recorded during testing due to the possibility that spawning had caused fatigue. Despite smaller spawning events during later testing, limpet strength improved after the first test, regardless of pulling speed. A linear regression model (Figure 2) shows a small increase in attachment strength with date ($y = 0.0283x + 7.37$) over the duration of the experiment; this relationship was significant ($P = 0.0152$) and explains much of the observed variation ($R^2 = 0.8054$).

Using the Stefan equation expressed in differential form (as described by Grenon & Walker 1981) the separation distances can be solved for using other known variables using Equation 2. With viscosity of 10°C seawater (n = 0.001346 kg/m*s), average radius of limpet foot (0.00675 m), mean forces (Table I) and separations speeds (0.0003, 0.001, 0.006, 0.01, 0.015 m/s), I calculated separation distances of 0.0076, 0.0114, 0.022, 0.0252 and 0.0289 mm for respective separation speeds. Thus even with a pulling speed 50 times that of the slowest, separation distances were still extremely small with minimal increase.

Nine individual limpets were tested at all five separation speeds (Figure 3). A One Way Repeated Measures analysis of variance tested if there were differences in attachment strength amongst these individuals at these separation speeds. All assumptions of the ANOVA were met (Shapiro-Wilk normality, equal variance), and the test showed there were no differences among individuals regardless of separation speed ($F(4, 32) = 1.50$, $P = 0.2$).

## DISCUSSION AND CONCLUSIONS

My results showed clearly that pulling speed has no effect on *L. scutum* tenacity. This is in complete contrast with results of Grenon & Walker (1981) who found a maximum tenacity at speeds between 0.10 and 1.00 cm/s for an acrylic surface and 0.01 cm/s for a slate surface. Grenon & Walker (1981) presented a statistical analysis between maximum tenacities on the two substrates, however they did not include a statistical contrast of pulling speeds.

Other authors (Grenon & Walker 1981, Smith 1991, Ellem et al. 2002) have found a linear relationship between maximum tenacity and foot size. All the individuals in my experiments were very similar in size, so little variation in attachment with foot size was seen. However when all the trials were pooled (because pulling speed was not a significant factor), my data also suggest that larger foot size leads to stronger attachment

Although Stefan adhesion has been proposed for limpets, my evidence does not support that hypothesis. The tenacity of a Stefan adhesive is proportional to the strain rate (Smith 1991), however this was not seen in my experiments. The Stefan equation also states that the attachment force of a Stefan adhesive is proportional to the fourth power of the foot radius (Smith 1991), however my data as well as others' (Grenon & Walker 1981, Ellem et al. 2002) have shown that the attachment force is linearly proportional to the foot area. My data also suggest that tack makes up a very small portion of what the limpet uses during adhesion, which implies that the majority of the adhesion acquired by the limpet is due to other known adhesions such as suction or glue.

The method developed for measuring how tenacity is affected by varying pulling speeds required removing limpets from their natural environment and thus may cause artifacts in behavior. As with methods of previous experiments (Grenon & Walker 1981, Smith 1991, Smith et al. 1993, Ellem et al. 2002), the pulling method does not quite simulate a peeling dislodgment which a predator might actually use. Despite these weaknesses, a laboratory approach allows isolation of the many known variables (temperature, surface roughness, angles of attachment, etc.) which affect limpet tenacity.

Another potential source of error arose from the difficulty of centering the harness directly above the apex of the shell. Being attached off center or at an angle would create a slight peeling force, which is known to detach an animal more readily than purely lifting. Error in interpretation may also come from collecting samples during a spawning period. Recently spawned limpets detached unusually readily, with forces less than 1-2 N.

In summary, we have demonstrated that Stefan adhesion does not play a large role in adhesion in *L. scutum* and that based on our data and that there is no particular correlation between speed of separation and maximum tenacity. In future studies one may consider revising harness methods, adding staining for residues of glue like adhesives that adheres to the substrate (Smith 1992), and increasing samples to run a more effective One Way Repeated Measures ANOVA.

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Table 1. Equations of regression for the best fit curve of the relationship between the force of adhesion and the surface area of the foot of a *Lottia scutum* (20 – 22 mm) on Lucite plates, detached in 10°C seawater, at various pulling speeds.

| **Pulling Speed (cm/s)** | **Equation of Regression** | **R^2** | **n** | **Mean** | **SE** | **P-value** |
|---|---|---|---|---|---|---|
| 0.03 | Y = -0.0241x + 9.5184 | 0.0438 | 24 | 8.9268 | 0.3676 | 0.2909 |
| 0.10 | y = 0.0119x + 8.4615 | 0.0032 | 40 | 8.7881 | 0.5341 | 0.7357 |
| 0.60 | Y = 0.0331x + 6.7176 | 0.0384 | 27 | 7.3749 | 0.4326 | 0.5316 |
| 1.00 | y = -0.0269x + 8.8939 | 0.0206 | 30 | 8.2636 | 0.4808 | 0.3755 |
| 1.50 | y = -0.0245x + 8.8061 | 0.0312 | 28 | 8.1991 | 0.3941 | 0.3633 |

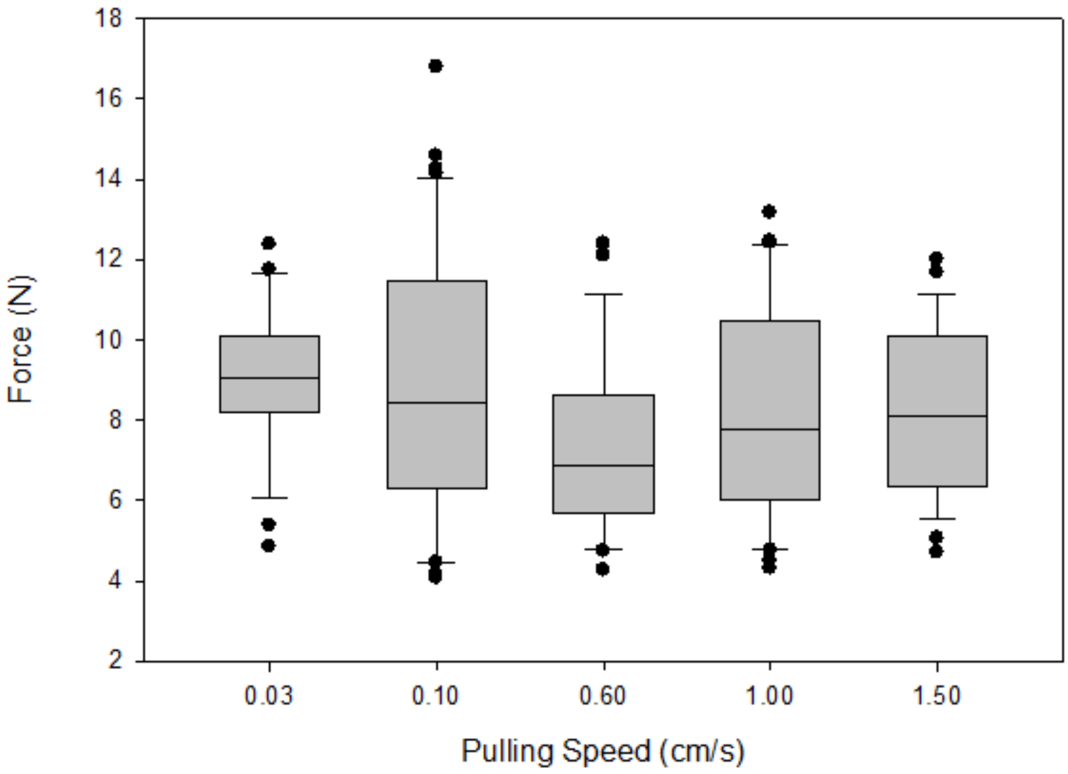

Figure 1. Box and whisker plot of 172 test of maximum tenacity across pulling speeds, detached in 10°C seawater, on Lucite plates.

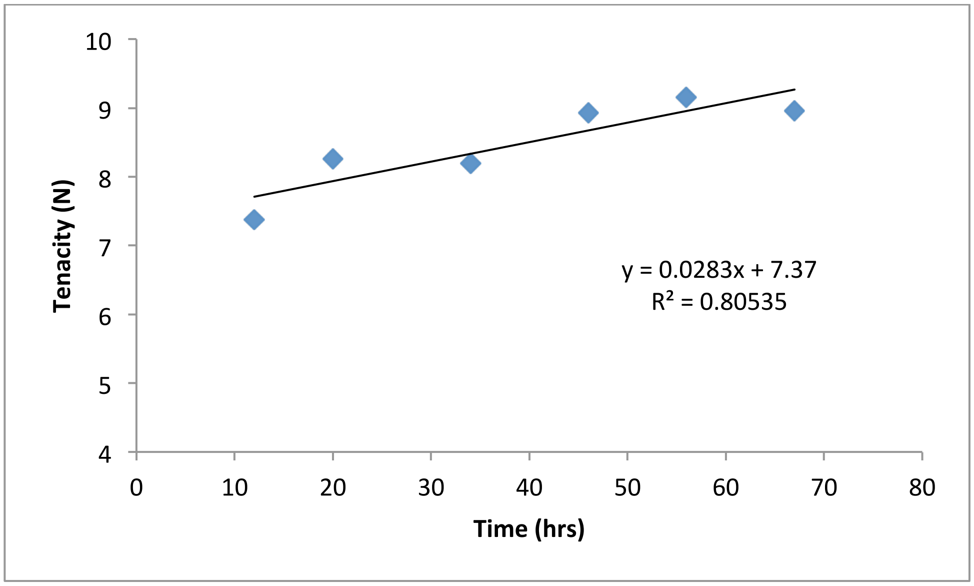

Figure 2. Relationship between the force of adhesion and time after the first experiment. t=0 is 48 hours after a spawning event. Tests were made on Lucite plates, detached in 10°C seawater, at different pulling speeds. P = 0.01525

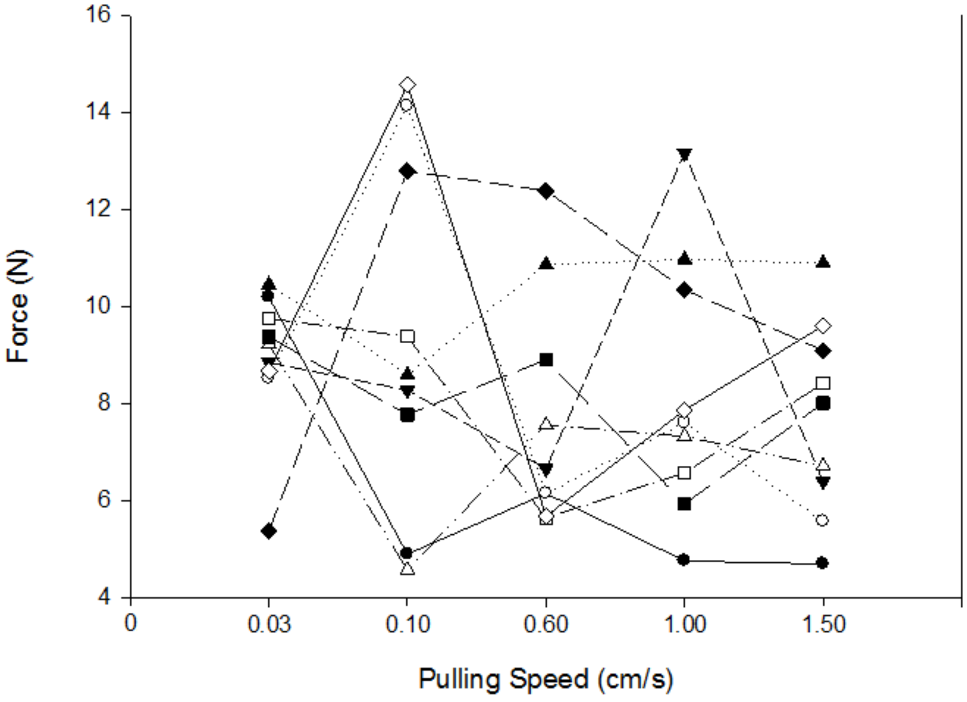

Figure 3. A comparison of individual limpets that were tested against all separation speeds. Tests were made on Lucite plates, detached in 10°C seawater, at different pulling speeds.