

Comparative study of sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis* adhesive performance

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Abstract

The marine intertidal is subject to challenging hydrodynamic forces. Organisms that reside within these zones; such as purple (*Strongylocentrotus purpuratus*) and green (*Strongylocentrotus droebachiensis*) sea urchins, must have strong attachment to prevent dislodgement, ambulate, and feed in intense environmental conditions. Sea urchins attach to the substrate via their podia (tube feet). The disc found in the distal part of tube feet has a duo-gland that releases specialized adhesive compounds and de-adhesive enzymes to achieve adhesion. Adhesive performance was evaluated to determine if purple sea urchins, that are predominantly found in high wave exposure areas, have better adhesive performance than green sea urchins, which are abundantly found in areas protected from wave action. We evaluated the following adhesive performance variables, stem mechanical properties, tube foot disc tenacity, attachment area, speed of detachment and whole animal adhesive force. We found that for most adhesive performance metrics, purple urchins performed better than greens, except for detachment speed. These results suggest that, in the field, behavioral changes may play an important role in determining risk of detachment for sea urchins. Understanding how morphology, behavior and mechanical properties of tube feet work in conjunction to provide strong attachment, is critical to predict sea urchin fitness in challenging environmental conditions.

Introduction

The rocky intertidal zone of the Pacific Northwest is a wave-swept home to a wide array of intertidal invertebrates from sessile mollusks to highly mobile cephalopods (Ray, 1991). This diverse zone is subjected to extreme hydrodynamic forces that can reach up to 2 m/s (Yang et al., 2021). These forces vary depending on temporal changes such as tide fluctuations; during high tides large wave breaks will produce drag and lift on the rocky shores as velocity, the combined effect of drag and lift, is highest near the surface (Denny, 2014). In high velocity environments, organisms can become dislodged or experience breakage as the hydrodynamic force that is being produced exceeds the mechanical limit of the organism (Denny et al., 1985). There can be other factors that affect dislodgement risk, in the field such as organism energetic state (Carrington et al. 2015), presence of disease (Shimizu et al. 1994), and substrate to which they attach (Stark et al. 2020).

Benthic intertidal organisms such as mussels and echinoderms adhere to the substrate in the intertidal zone so that they may withstand the acute hydrodynamic forces. Mussels use permanent byssal threads to attach themselves to substrate. These threads are secreted from a gland in the mussels' foot and once attached, the byssal thread is removed only when mechanical failure, such as breakage, occurs (Moeser et al., 2006). In contrast, echinoderms such as sea stars and sea urchins utilize specialized cells within the flattened disc structure at the distal end of their podia (tube feet) to create semi-permanent adhesion (Narvaez et al., 2022). These specialized cells secrete an organic glue made up of multiple amino acids and proteins that allows them to adhere to various substrata. Detachment occurs when these cells produce and secrete an enzyme that breaks

the adhesive bond (Santos et al., 2013). There are hundreds of podia all over a sea urchin's test or a sea star's arms, which are not only used for attachment, but also for light detection, respiration, chemoreception, feeding and locomotion (Santos et al., 2013; Hennebert et al., 2010).

Sea urchins ambulate and attach using podia on the oral side as it is the part of the urchin that is closest to the substratum (Narvaez et al.2022). A sea urchin's typical, round shape with its spines sticking straight out, isn't conducive to a streamlined form to avoid detachment (Stewart and Britton-Simmons, 2011), therefore the hydrodynamic flow surrounding the urchin will determine their spatial distribution (Siddon and Witman, 2003). This is observed in *Mesocentrotus franciscanus* when in response to flow as low as 10 cm/s *M. franciscanus* begins to lower the angle of its spines in order to flatten itself closer to the substratum and achieve a more streamlined form (Stewart and Britton-Simmons, 2011). Thus, assessing adhesive performance by including biomechanical measurements of the stem and disc together with behavioral components (i.e., number of tube feet used attachment, volume changes with spine movements) is critical to fully understand how the environment influences adhesion in sea urchins.

In the Salish Sea, multiple species of urchins can be found. The purple sea urchin, *Strongylocentrotus purpuratus*, inhabits the intertidal and subtidal habitats of wave-battered sites and the green sea urchins, *Strongylocentrotus droebachiensis*, are more commonly found in subtidal habitat of wave-protected sites like bays (Allee et al 2000) Previous studies in *Paracentrotus lividus*, have shown that wave exposure influences biomechanical properties of sea urchins tube feet (Cohen-Rengifo et al. 2017). In this study, we tested the hypothesis that purple sea urchins have higher adhesive performance

than green sea urchins, which could explain their ability to inhabit high energy environments.

Materials and Methods

Field site, sea urchin collection and laboratory conditions

We collected 25 sea urchins of each species (*S. purpuratus* and *S. droebachiensis*) from Pile Point (48.48214°N, -123.09439°W), a rocky marine intertidal zone located at the southwestern point of San Juan Island (Washington, United States). Pile Point is near Kanaka Bay and is fed into by the Haro Strait. On the day of collection, tides were at a yearly low of -3.9ft. All sea urchins were placed into a cooler and immediately transported to Friday Harbor Laboratories. Once at the laboratory, individual sea urchins were kept separately on a sea table with air stones and seawater pumped in from Friday Harbor Bay to ensure adequate flow, aeration, temperature, and salinity. Measurement of test height, test width and whole animal wet weight was collected immediately before beginning trials. All measurements were conducted on the same 25 individuals of each species except for flume detachment speed that was estimated for 10 of each species.

Disc maximum tenacity and area

Maximum disc tenacity (maximum force required to detach a tube foot per unit area; MPa or N/m²) was calculated by dividing the disc maximum adhesive force (N) required to detach one tube foot by the average disc surface area of that individual (m²).

Tube foot disc maximum adhesive force (N) was measured by having a single tube foot disc attach to a glass bead of approximately 1 cm in diameter connected to a digital force logger (IMADA ZTA-0.4) with a monofilament fishing line (4 lb.

Shakespeare Omniflex, Columbia, SC, USA). Once attached, a custom-built force rig pulled (Fig. 1a) the force logger at a constant speed of 1 cm/s until the disc detached from the bead. To standardize the measurement, the same person conducted all trials. This procedure was repeated three times on different tube feet and the highest value was used to determine maximum tenacity to account for behavioral variability.

Tube foot disc area (m²) was measured by photographing tube feet discs attached to a glass Petri dish with a 1 mm scale. A picture was taken after at least 10 tube feet discs were visibly attached to the glass. Surface area was measured, using Image J software we were able to calculate the mean disc surface area of each sea urchin (Narvaez et al 2020).

Stem breaking force

Tube feet stem breaking force was calculated as the maximum force (N) required until mechanical failure was reached. Sea urchins were placed oral side up, in a sponge and PVC pipe in order to immobilize them underwater (Narvaez et al 2020). The stem breaking force was measured by clamping a metal alligator clip (BlastCase Steel Toothless Alligator Clips; John Miller, Inc.) to an extended oral tube foot at approximately half its length. The metal clip was connected to a digital force logger (IMADA ZTA-0.4) with a monofilament fishing line (4 lb. Shakespeare Omniflex, Columbia, SC, USA). Once the stem was clipped, the force logger was pulled at a constant speed of 1 cm/s by a custom-built force rig (Fig 1a) until the stem broke. We recorded the breaking force of three tube feet stem per individual and used the mean.

Whole animal adhesive force

We measured the whole animal adhesive force by pulling the sea urchins vertically off a fully submerged glass substrate (Fig 1b). Immediately prior to a trial, the chamber was filled with the same sea water that was in their holding tank to ensure proper salinity and temperature. The sea urchins were harnessed using monofilament fishing line (12 lb. Shakespeare Omniflex, Columbia, SC, USA) tied into a cross that was attached to a force gauge (OMEGA DFG35-20). The cross-harness ensures that the sea urchin was pulled from the substrate evenly when the intersection of the cross was positioned directly on the mouth. Once harnessed, we positioned sea urchins on the glass substrate and induced them to attach to the substrate by touching them with a cable tie for 25 seconds. The force gauge was attached to a custom-built force rig (Fig 1b) that pulled up the animal at a constant speed of 8 cm/sec.

Flume detachment speed

We measured the water speed at which both species detached in a flume box (Fig 2). We placed the animal in the center of the test chamber (Fig 2) and allowed it to acclimate for 2 minutes before increasing the flow speed in increments of 5 (i.e., 0, 5, 10, 15, 20, 25, 30, 35, 40). Speed was increased every 2 minutes until the animal detached and was simultaneously recorded from the side and bottom view from outside the flume through the clear Plexiglas® wall, using a video camera (Olympus tough TG-6) for the bottom view and a camera (GoPro Hero 4) from the side. Every animal was only tested once.

Statistical analysis

The effect of urchin species (*S. purpuratus* and *S. droebachiensis*) on adhesive performance metrics (disc surface area, maximum disc tenacity, stem breaking force,

detachment speed) was assessed with a one-way Anova on *R*. Assumptions test of normality and homogeneity of variance was assessed with a Shapiro-Wilk and a Bartlett test respectively. Maximum disc tenacity and stem breaking force were log 10 transformed to meet the assumptions.

Results

Disc maximum tenacity and area

Disc area was not statistically different among both species ($F_{1,47}=3.8819$, $p=0.0547$), but *S. purpuratus* had discs that were 20% larger than *S. droebachiensis* (Fig 3a). Disc maximum tenacity was comparable among both species ($F_{1,47}=2.2329$, $p=0.1418$), but *S. purpuratus* showed more variability (Fig 3b)

Stem breaking force

Stem breaking force was significantly different among species ($F_{1,48}=23.3380$, $p<0.0001$), with *S. purpuratus* having stems that required 50% more force to cause material failure (Fig 4).

Whole animal adhesive force

The was significantly higher in *S. purpuratus* ($F_{1,48}=25.4540$, $p<0.0001$), with this species requiring 30% more force to be removed from a glass substrate (Fig 5)

Flume Detachment Speed

Flume detachment speed showed no significant difference both species ($F_{1,27}=1.2948$, $p=0.2652$), but there was a wider range of results for purple urchins.

Discussion

Sea urchin adhesion has been observed to be biologically vital for fitness components such as growth, reproduction, and overall survivorship (Cohen-Rengifo et al. 2018, Santos et al. 2007, Santos et al. 2005). Our results showed that purple sea urchins have larger disc areas, albeit not significant, than greens. Previous studies have suggested that the more surface area in a disc, the higher the adhesive performance in the intertidal. In previous studies (Santos 2005, Narvaez 2022) differences between tube feet morphology has been found in different urchins from different hydrodynamic environments. It was found that sea urchins from the intertidal have oral tube feet with significantly larger areas which correlated to their overall tenacity. This, combined with the mechanical strength of the stem is equally as important in adhesive performance as it must be able to withstand hydrodynamic force applied to the organism.

Purple urchins were found to have a higher stem breaking force than greens. As aforementioned, the stem bears the tensions from wave action therefore playing a critical role in the urchin's ability to adhere in dynamic environments. Previous studies (Santos 2005) looking at the mechanical properties of tube foot tissue suggested that tube feet need an increased strain rate in order to improve their adherence performance. We may then conclude that if tube feet have a positive strain rate dependence, urchins found in the intertidal where they are constantly more wave exposed, will mechanically have stronger stem tissue and be able to outperform those species found in the subtidal, a less high energy environment.

When looking at the results of all the biological and mechanical components measurements support our hypothesis that *S. purpuratus* adheres with more strength than

S. droebachiensis. However, tenacity and disc area were found to not have a significant difference between the two urchin species. This could be in part due to there being no change in the glue amount and consistency in which it is put down, between the species. More studies need to be conducted to corroborate this claim. Additionally, our flume measurements do not match up with the rest of our data, this can be attributed to behavioral components such as when urchins will put their spines down, in order to flatten their overall shape when experiencing high wave velocity. It is dependent on the individual as to when their spines will go down, therefore making for high variability; this behavioral phenomenon was observed in other urchin species as well (Cohen-Rengifo et al. 2018, Stewart, Britton-Simmons 2011). This behavior reduces the individuals drag and lift by effectively streamlining their body shape similar to that of a limpet (Warburton 1997). Though the behavior is predictable as it occurred in every tested individual amongst both species, there was variability amongst when each individual would begin this streamlining behavior and even higher variable difference between the two species. We found that green urchins would more quickly and frequently assume a streamlined position which resulted in an ability to better adhere within the flume (Oceguera, personal observation). This may explain why results showed greens and purples having equal speed of detachment in the flume despite purples biomechanically measurements suggesting that they should be the stronger species. More studies need to be conducted to conclusively say why this is, if we were to conduct more experiments looking at the number of tube feet that they are using for attachment, it would give us more insight as to why this difference is occurring.

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Figures



Fig. 1: (A) Whole animal adhesive force was measured using a custom designed force rig. Sea urchins were harnessed and placed on glass substrate in the experimental chamber. Sea urchins were prompted to secure themselves to the substrate and then pulled vertically at 8 cm/s until they were removed from the substrate. The maximum force recorded by the force logger. (B) Sea urchin disc adhesive force and stem breaking force was measured a different custom designed force rig. Sea urchins were immobilized in a custom experimental chamber, oral side up and a glass bead (1 cm in diameter) or metal clip was used to attach the disc or stem to the filament and pulled at a constant rate of 1 cm/s until the disc detached or stem mechanical failure occurred. The force was recorded by the force logger.

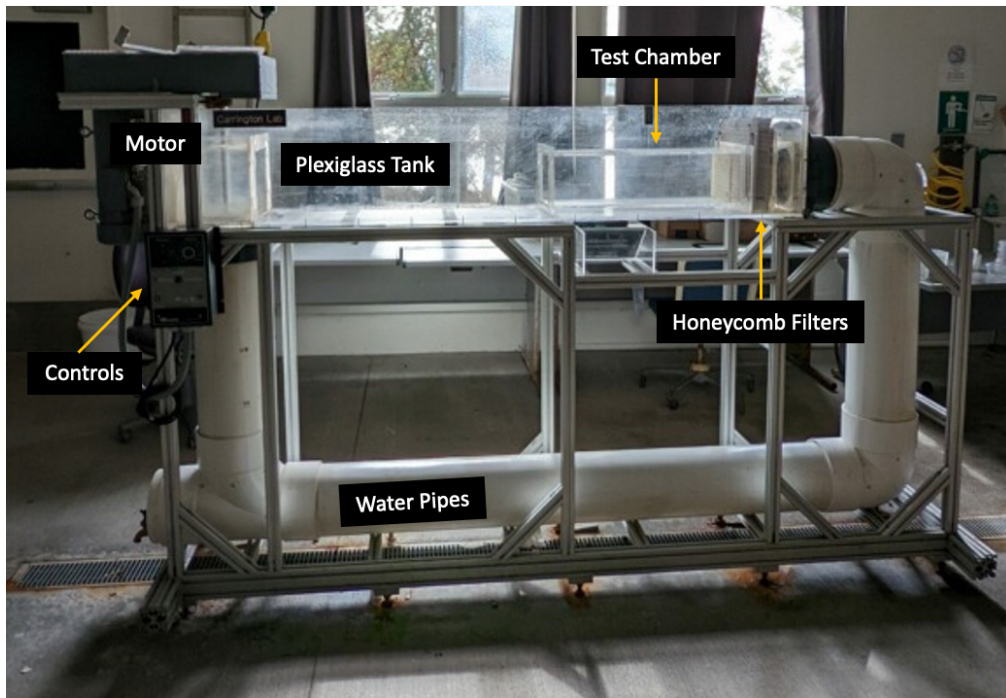


Fig. 2: Individual speed of detachment was determined for *S. droebachiensis* and *S. purpuratus* ($n = 25$ per species) using a flume. The sea urchin was placed in the center of the test chamber within the plexiglass tank. It was allowed to acclimate for 2 minutes before increasing the flow speed in increments of 5 (i.e., 0, 5, 10, 15, 20, 25, 30, 35, 40). Speed was increased every 2 minutes until the animal detached.

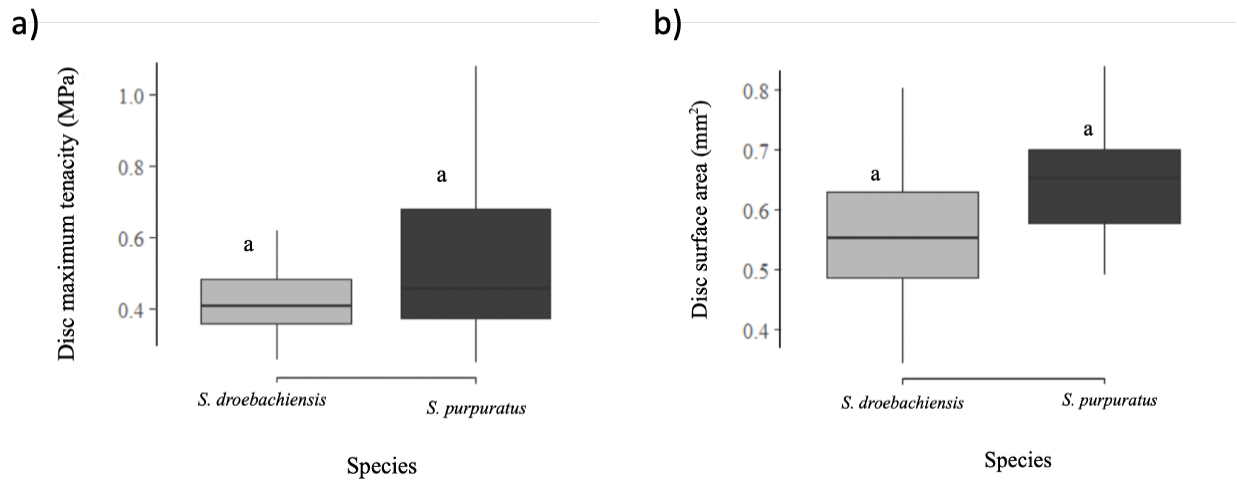


Fig 3: Disc maximum tenacity (a; MPa) and disc surface area (b; mm²) and were obtained from *S. droebachiensis* and *S. purpuratus* (n = 25 per species). A one-way Anova showed that there were no significant differences found for disc surface area ($F_{1,47}=3.8819$, $p=0.0547$) or disc maximum tenacity ($F_{1,47}=2.2329$, $p=0.1418$) among species.

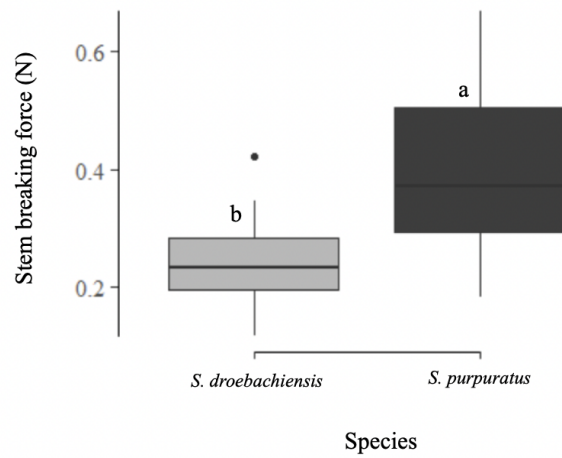


Fig. 4: Stem breaking force (N) data was obtained from *S. droebachiensis* and *S. purpuratus* (n = 25 per species). A one-way Anova showed significant differences among both species ($F_{1,48}=23.3380$, $p<0.0001$).

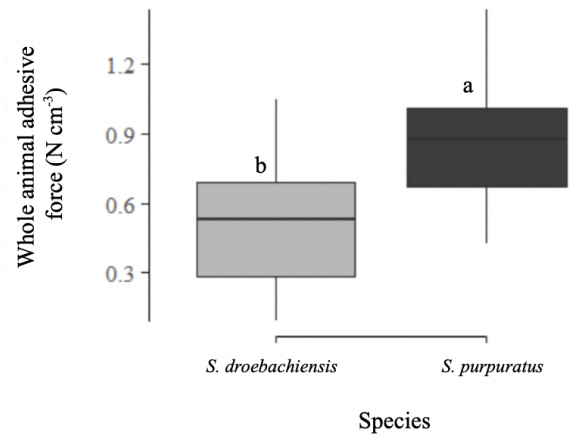


Fig. 5: Whole animal pull (N cm⁻³) data was obtained from *S. droebachiensis* and *S. purpuratus* (n = 25 per species). A one-way Anova showed significant differences among both species ($F_{1,48}=25.4540$, $p<0.0001$).

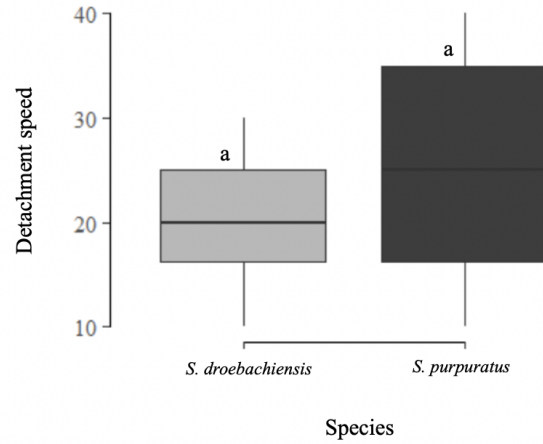


Fig. 6: The detachment speed data was obtained from *S. droebachiensis* and *S. purpuratus* (n = 10 per species). A one-way Anova showed that there were not significant differences among both species ($F_{1,27}=23.1.2948$, $p=<0.2652$).

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