

RESEARCH ARTICLE

Adhesive plasticity among populations of purple sea urchin (*Strongylocentrotus purpuratus*)

Alyssa Y. Stark*, Carla A. Narvaez and Michael P. Russell

ABSTRACT

Sea urchins native to the nearshore open coast experience periods of high, repeated wave forces that can result in dislodgement. To remain attached while clinging and locomoting across rocky substrates, sea urchins use adhesive tube feet. Purple sea urchins (*Strongylocentrotus purpuratus*) adhere to a variety of rock substrates (e.g. sandstone, mudstone, granite), and display morphological plasticity (skeletal morphology) to native substrate. We tested the hypothesis that their adhesive system is also plastic and varies as a function of native population and substrate. The results of our study support our hypothesis. Sea urchins from sandstone adhere less strongly to most substrates than those native to mudstone and granite rock. Sandstone produced the lowest whole animal adhesive force values across all populations, suggesting that this rock type is particularly challenging for sea urchins to adhere to. The number of adhesive tube feet that failed during experimental trials and the area used by sea urchins to attach, matches closely with whole animal adhesive force values: higher forces resulted in more tube foot failure and larger attachment area. On artificial substrates (glass and Plexiglass), differences in adhesion among populations was consistent with differences in adhesion on rock substrates except on glass, where sea urchins native to sandstone adhered more strongly to glass than any other substrate tested. To our knowledge, this study is the first to describe population-level plasticity in a biological adhesive system related to native substrate, and has significant implications for sea urchin ecology, behavior and functional morphology.

KEY WORDS: Echinoid, Hydrodynamic forces, Surface roughness, Tenacity, Tube feet

INTRODUCTION

In the wave-dominated nearshore open coast, hydrodynamic forces dictate key ecological parameters such as species distribution, richness and community structure (Denny, 1995; Denny et al., 1998). Specifically, water velocities of breaking waves in the intertidal zone can reach 10 m s^{-1} , making resistance to these forces key to avoiding damage and dislodgement, which often results in death (Denny et al., 1998; Flammang et al., 2005; Santos and Flammang, 2007; Gutiérrez et al., 2018). Shallow benthic organisms in particular must also resist high drag and lift forces of sea water flowing over them repeatedly during wave events (Denny et al., 1998; Cohen-Rengifo et al., 2017). The combination

of periodic breaking waves, drag and lift forces that these organisms experience has led to impressive adaptations, principally the ability to remain attached in variable and extreme environmental conditions.

One group of shallow benthic marine organisms that use an attachment system to combat high hydrodynamic forces is echinoderms (Flammang et al., 2005). Echinoderms, like sea stars and sea urchins, use an adhesive (glue) secreted by specialized cells located within a flattened disc-shaped structure at the distal end of a long, highly extensible soft stalk (i.e. tube foot; see figs 1A and 3A in Hennebert et al., 2012 for tube foot images; Flammang et al., 1994, 1998; Flammang, 1996). To detach, a second set of adjacent, specialized cells secretes a chemical that breaks the adhesive bond at the interface between the disc and the glue (i.e. outer layer of cuticle), leaving behind an invisible, sponge-like adhesive footprint (100–230 nm thick depending on the substrate) made primarily of proteins and carbohydrates (Flammang et al., 1994, 1998, 2005; Flammang, 1996; Hennebert et al., 2008; Santos et al., 2009; Lebesgue et al., 2016). Adhesion is strong, and detachment often results in material failure of the tube foot stalk, rather than the adhesive (Santos and Flammang, 2005; Hennebert et al., 2010; Cohen-Rengifo et al., 2017). Indeed, sea star and sea urchin adhesive performance is a function of the glue, the material properties of the tube foot stalk, and adhesive area (often measured as the area of adhesive footprints left behind on a substrate; Sharp and Gray, 1962; Santos and Flammang, 2005, 2007, 2008). Although sea stars and sea urchins share some glue protein homologies, sea urchin tube feet are stronger, stiffer and tougher than sea star tube feet, and have a terminal connective tissue plate that encloses a calcified skeleton (Santos and Flammang, 2005; Pjeta et al., 2020), suggesting that sea urchins are subjected to particularly extreme conditions that require firm attachment.

Local environmental conditions shape sea urchin morphology, which is remarkably plastic. For instance, body size and shape can change in relation to wave exposure, food availability and/or quality, and habitat (Levitan, 1991; Minor and Scheibling, 1997; Russell, 1998; Hernández and Russell, 2010; Haag et al., 2016; Cohen-Rengifo et al., 2017; Connolly et al., 2017). Sea urchin adhesion is also plastic. In exposed sites, sea urchins tend to be smaller (although this may be due to other factors like growth rate or age; Jacinto and Cruz, 2012; Cohen-Rengifo et al., 2017), and tube foot density, extensibility and toughness is higher (Cohen-Rengifo et al., 2017). These parameters likely result in higher resistance to flow velocities by the whole animal (Cohen-Rengifo et al., 2017). Sea urchin adhesive plasticity can also occur over short time scales. For instance, sea urchin adhesion and adhesive protein expression decreased significantly when sea urchins were removed from the field and kept in the laboratory for several weeks (Toubarro et al., 2016; Cohen-Rengifo et al., 2018). In the field, adhesion was significantly higher when sea urchins were exposed to high wave height up to 3 days before sampling (Santos and Flammang, 2007).

Department of Biology, Villanova University, 800 E. Lancaster Avenue, Villanova, Pennsylvania 19085, USA.

*Author for correspondence (alyssa.stark@villanova.edu)

 A.Y.S., 0000-0002-4217-2850

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Thus, spatial and temporal variation in environmental conditions can have significant consequences for the adhesive plasticity and performance of nearshore open coast sea urchins.

Purple sea urchins (*Strongylocentrotus purpuratus* Stimpson, 1857), found on the west coast of North America between Mexico and Alaska, experience a wide range of environmental conditions that influence plasticity (Russell, 1987). Some of the morphological plasticity that purple sea urchins exhibit is related to pit-boring behavior on substrates with different hardness and friability. Specifically, purple sea urchins from hard, less friable granite rock create shallower pits and their test (skeleton) is flatter than those from soft, friable mudstone and sandstone substrates, where pits are deeper (Hernández and Russell, 2010; Russell et al., 2018). Although the hydrodynamics associated with life within and outside of a pit have not been quantified, it is likely that pits protect sea urchins from wave-associated forces (e.g. drag, lift; Jacinto and Cruz, 2012; Cohen-Rengifo et al., 2017). This hypothesis is further supported by the flat shape of sea urchins from shallow pit-forming granite, which is the prevalent shape of sea urchins in high flows (Lewis and Storey, 1984; Cohen-Rengifo et al., 2018). Therefore, we hypothesize that low-profile granite pits result not only in population-level morphological plasticity of the test, but also plasticity of adhesive performance, where sea urchins native to shallow-pitted granite substrates are more adhesive than those from the deep-pitted mudstone and sandstone substrates. To our knowledge, this is the first study to directly test the hypothesis that native substrate lithology drives adhesive plasticity in sea urchins.

To determine if purple sea urchins exhibit adhesive plasticity and which morphological parameter(s) drives this plasticity, we measured: (i) whole animal adhesive force, (ii) attachment area, (iii) number of broken tube feet and (iv) tenacity (whole animal adhesive force divided by attachment area; Flammang and Walker, 1997; Santos and Flammang, 2007; Cohen-Rengifo et al., 2017). We tested sea urchins on native and foreign natural substrates (i.e. granite, sandstone and mudstone), and on high and low surface energy artificial substrates that were not friable and generally hard (i.e. glass and Plexiglass). We predicted that sea urchins native to low pit-profile granite adhere more strongly to natural and artificial substrates than sea urchins from deep pit-profile sandstone and mudstone substrates. We expected this result to be driven by three key features of the sea urchin adhesive system: attachment area, material properties of the tube foot stalk, and the glue. Specifically, we predicted that sea urchins native to granite would have larger attachment area, fewer broken tube feet, and/or higher tenacity. The latter two predictions would imply differences in tube foot stalk material properties and glue, respectively. On artificial substrates we also expected adhesion across all populations to be low on the high surface energy substrate (glass), as found previously (Santos and Flammang, 2006). The results of this work have important implications for understanding sea urchin adhesive plasticity, performance and ecology in the dynamic, wave-swept intertidal zone.

MATERIALS AND METHODS

We conducted experiments at the low tides of two sampling trips. On the first trip (2–4 November 2017), we measured whole animal adhesion of each population to all three natural rock substrates (see below) and an artificial substrate (Plexiglass). After measuring adhesion, we photographed the substrate and counted the number of tube feet that had broken off and remained attached to the substrate (Fig. S1A). On the second trip (12–18 June 2018), we measured

whole animal adhesion of each population to smooth, artificial substrates that varied in surface energy measured as wettability (moderately hydrophobic Plexiglass, hydrophilic glass). After measuring adhesion, we stained the surface with Crystal Violet (MilliporeSigma; Burlington, MA, USA), photographed the substrate, and counted the number of tube feet that had broken off and remained attached to the substrate (replicating procedure from the first sampling date; Fig. S1B). In addition, we quantified the area of footprints left behind on the artificial substrates (invisible prior to staining and impossible to image on natural substrates; Flammang et al., 1994; Flammang and Walker, 1997; Santos and Flammang, 2007). We analysed the results of each trip independently, except for a comparison of whole animal adhesive force on Plexiglass between the two sampling periods to test for temporal differences in adhesion within populations.

Field sites

Field sites were the source of rock substrates and of sea urchin populations. Granite rock substrate was collected from Bodega Bay, CA, USA (38° 19' 08.28" N, 123° 04' 27.85" W), mudstone was collected from Palomarin Beach, CA, USA (37° 55' 48.81" N, 122° 44' 44.09" W), and medium-grain sandstone was collected from Bean Hollow Beach, CA, USA (37° 13' 36.08" N, 122° 24' 41.70" W). Russell et al. (2018) reviewed the geology of the sites and provided detailed descriptions of the lithologies of the substrata. Briefly, the sedimentary rocks (mudstone and sandstone) at Palomarin Beach and Bean Hollow Beach are less dense and softer than the metamorphic substrate at Bodega Bay (granite). However, because the grain size of the sandstone at Bean Hollow Beach (1.00–0.25 mm diameter) was much coarser than the fine mud/silt particles comprising Palomarin Beach mudstone (<0.06 mm diameter), the surface rugosity and friability of the sandstone was greater than mudstone. The difference in particle sizes of the sedimentary rocks made the surface of the mudstone more similar to granite (i.e. granite and mudstone had smoother surfaces than the large grain sandstone).

Sea urchin collection

We collected sea urchins directly from pits in the field and tested adhesion within ~2 h of collection on the beach of origin. Sea urchins were hand collected from exposed tide pools, and only sea urchins that had minimal damage to the test and tube feet during collection were used. When collecting, we attempted to match the size ranges across the three different populations. Sea urchins ranged in test diameter from 13.8 to 70.1 mm (see Table S1 for specific population and year information regarding sizes).

Experimental substrates

Prior to the field trips, we extracted large pieces of granite and sandstone from bedrock with a rock hammer to make experimental substrates. We cut these irregular blocks into similar sized units (approximately 9 cm×9 cm×2 cm) with a wet masonry saw (14-inch diamond blade). Standard mudstone units were impossible to make the same way because mudstone shattered when cut with the wet masonry saw. Instead, flattened cobbles of mudstone derived from the mudstone bedrock were collected and the surfaces were hand sanded, first with coarse (50 grit) and then fine (100 grit) sandpaper, to smooth and even the surface. Cut and sanded rock samples were then imbedded in ~1.5–3.0 cm marine epoxy (105; West Systems, Bay City, MI, USA) so that 0.5–1.0 cm of the flattened, exposed rock surface was above the epoxy. Rock substrate preparation is further detailed in Russell et al. (2018). Glass and Plexiglass units

were cut into 12 cm×12 cm pieces. A PVC pipe (1.5–3.0 cm long; 1.27 cm diameter) was fixed with epoxy to the bottom of the rock and artificial (glass and Plexiglass) units to serve as a post. This post was fitted tightly over a PVC pipe (0.64 cm diameter) fixed with epoxy to the bottom of a small, clear 4.7-liter plastic bucket (18 cm×18 cm×18 cm) used as our experimental chamber (Fig. 1). The buckets and substrates with posts were interchangeable, such that a new substrate and new bucket could be prepared while others were in use. The tight fit of the substrate unit post and the PVC pipe secured firmly to the bottom of the plastic bucket ensured that the rock samples did not move during adhesion tests. The experimental bucket was held by hand during adhesion testing to prevent movement of the experimental chamber (see Fig. 1).

Measurement of whole animal adhesive force

We measured whole animal adhesive force in the field by pulling sea urchins vertically off the substrate units that were fully submerged in the experimental chambers. Immediately prior to a trial, we positioned substrates in the experimental chamber and filled the chamber with seawater from an aerated tide pool. Sea urchins were then harnessed using monofilament fishing line (12 lb Shakespere Omniflex, Columbia, SC, USA) tied into a cross that was attached to a force gauge (either 20N, 100N or 200N FGE-XY digital force gauge depending on substrate type; Nidec-Shimpo Instruments, Glendale Heights, IL, USA). The cross-harness ensured that the sea urchin was pulled from the substrate evenly when the intersection of the cross was positioned directly on the peristome. Trials where the harness was improperly positioned, and caused the sea urchin to peel, rather than be pulled from the substrate, were not used in analysis. The four-line harness also distributed the force associated with sea urchin adhesion among all single lines, increasing the breaking strength. We randomly used more than one harness per experiment day and across all experiment days. We did not notice any changes related to wear in the harnesses across experiments. Once harnessed, we positioned sea urchins on the experimental unit and induced them to cling to the substrate by agitating them. This agitation caused most sea urchins to cling to the substrate with their oral and ambital tube feet within a few minutes. After attachment, we pulled sea urchins by hand to the point of detachment. The maximum force recorded during the pull was reported as maximum normal (vertical) whole animal adhesive force. Sea urchins were pulled from the substrate at 2.54 cm s⁻¹. To ensure uniformity of strain rate across trials, only one experimenter

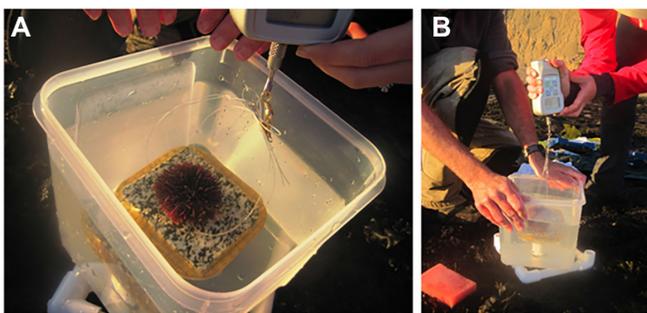


Fig. 1. Whole animal adhesive force was measured in the field using a custom designed experimental chamber. *Strongylocentrotus purpuratus* sea urchins were harnessed and placed on the experimental substrate submerged in fresh seawater (pictured: granite rock). Sea urchins were prompted to secure themselves to the substrate (A), then pulled vertically until they were removed from the substrate (B). The maximum force recorded by the force sensor during the vertical pull was recorded.

conducted whole animal adhesion trials and substrate was randomized across all field days.

After measuring adhesive performance, we measured sea urchin height, diameter and weight. We also photographed the experimental unit to count the number of broken tube feet. Adhesion measurements on artificial substrates made during the second trip were followed by substrate staining (0.05% aqueous solution of Crystal Violet; Santos and Flammang, 2006) and imaging, so that broken tube feet and adhesive footprints that remained on the substrate could be counted and measured using ImageJ software (Abràmoff et al., 2004). After imaging, we cleaned all substrates with a sponge and fresh seawater before re-use. There was no effect of trial order on whole animal adhesive force among any substrates (linear regression glass: $t=0.125$, $r^2=0.0005$, $P=0.9022$; linear regression Plexiglass: $t=1.788$, $r^2=0.1025$, $P=0.0850$), suggesting that cleaning of the substrates did not increase the roughness and consequently whole animal adhesive force of sea urchins (Santos et al., 2005). After removing trials that did not fit our criteria (e.g. harness moved), we tested approximately 10 sea urchins per experimental substrate (exact sample sizes are detailed in Tables S2 and S3). No sea urchin was measured more than once, thus we tested a total of 118 and 60 individuals in the first and second trips, respectively.

To account for sea urchin shape, which varies in both height and diameter depending on the local environment (Hernández and Russell, 2010), we standardized size by modeling the sea urchins as oblate spheroids (Ebert, 1988; Middleton et al., 1998; Elliott et al., 2012). First, we calculated the surface area of an oblate spheroid (S_{oblate} ; Eqn 1) using the equatorial radius (a ; one half of the measured diameter) and the polar radius (c ; one half of the measured height) of the sea urchin, then divided this area by 2, to account for the surface area available for adhesion (i.e. the surface from the ambitus to the oral pole):

$$S_{\text{oblate}} = 2\pi a^2 + \pi \frac{c^2}{e} \ln\left(\frac{1+e}{1-e}\right), \text{ where } e^2 = 1 - \frac{c^2}{a^2}. \quad (1)$$

We then divided the force we measured in the field by this value, to account for sea urchin size on force output. Thus, whole animal adhesive force is reported as N cm⁻² (i.e. measured force divided by one half of the surface area of an oblate spheroid sea urchin model). We also used this size normalization factor to standardize the number of tube feet lost after detachment and number of footprints left on substrate surfaces. Number of tube feet, disc surface area and number of footprints increase with body size, so controlling for body size is critical (Connolly et al., 2017).

We estimated attachment area from images of broken tube feet and footprints left behind on artificial substrates after adhesion measurements. Attachment area was not collected on natural substrates because the footprints are not distinguishable from the rock substrate, which uniformly stains purple. For each individual, we counted the total number of footprints ($N_{\text{footprints}}$) and measured the area of all footprints ($A_{\text{footprints}} = \text{sum of all footprint areas}$). In instances where the tube feet broke, the attached discs from the broken tube feet covered the footprint, making the footprint area impossible to measure. Removing the attached discs before staining was not possible because of field constraints. Measuring the area of the attached disc would have over-estimated the area of the footprint, as attached discs include both the footprint (glue) and the peripheral ring of the disc. To account for the additional attachment area attributed to broken tube feet, we calculated the mean attachment area of a single tube foot from the footprints

($\bar{A}_{\text{footprint}} = A_{\text{footprints}} / N_{\text{footprints}}$) for each individual. We used $\bar{A}_{\text{footprint}}$ to calculate the area from all the broken tube feet, so total attachment area A_{total} is:

$$A_{\text{total}} = (\bar{A}_{\text{footprint}} \times N_{\text{brokentubefect}}) + A_{\text{footprints}}. \quad (2)$$

Attachment area was also used to estimate tenacity (whole animal adhesive force divided by attachment area).

To standardize attachment area measurements, we outlined tube footprints with the color threshold tool in ImageJ to objectively eliminate very faint footprints. We then evaluated the largest continuous curve of each outlined tube footprint as an arc of a perfect circle. If the arc was greater than or equal to half the circumference of the circle, the stain was counted as a tube footprint and area of the circle measured. If the arc was not one half of the circumference of a circle, but the stain was present in three-quarters of the area of the circle, the stain was also counted as a tube footprint and area of the circle was measured. If a footprint stain did not meet these criteria, we did not count this stain as a tube footprint or measure area. We assume these stains represent footprints that either did not contribute strongly to adhesion (i.e. small area, inconsistent contact) or they were small staining heterogeneities.

Statistical analysis

Statistical analyses and graphs were executed in R (<https://www.r-project.org/>). To test for an effect of source population and natural rock substrate on whole animal adhesive force (N cm^{-2}) and tube foot failure (number cm^{-2} ; November 2017 sampling), we used a 3×3 factorial analysis of variance [ANOVA; i.e. three source populations (Bodega Bay, Palomarin Beach, Bean Hollow Beach) tested on three substrates (granite, mudstone, sandstone)]. A separate analysis was conducted for the artificial substrate Plexiglass with only source population as a factor. To test for an effect of source population and artificial substrate on whole animal adhesive force, attachment area and tenacity (June 2018 sampling), we used a 3×2 factorial ANOVA [i.e. three source populations (Bodega Bay, Palomarin Beach, Bean Hollow Beach) tested on two artificial substrates that vary in wettability (glass and Plexiglass)]. To test for temporal differences in whole animal adhesive force on the artificial Plexiglass substrate, we used a 3×2 factorial ANOVA [i.e. three source populations (Bodega Bay, Palomarin Beach, Bean Hollow Beach) and two years (2017 and 2018)]. Validation of the model assumptions was conducted graphically. Normality of the model residuals was assessed with a histogram and was achieved in all cases, except tube foot failure on the Plexiglass substrate during the 2017 sampling. We ignored this because the homogeneity of variances assumption was satisfied. Homogeneity of the variances was assessed with a box plot of the model residuals against each explanatory variable, and in some cases the variances of levels of the model factors (substrate and source population) were found to be heterogeneous. In this case, general linear least square model (GLS) with the package *nlme* was used (Pinheiro et al., 2012). When all factors are categorical, as in our study, a GLS is similar to an ANOVA that accounts for deviations of homoscedasticity of variances with the function *VarIdent* (Zuur et al., 2009). When appropriate, a Tukey's honestly significant difference (HSD) test was conducted to reveal differences among treatment groups with the *emmeans* package (Lenth, 2019). Details on data transformations, factors that did not meet homoscedasticity of variances assumptions (which were included in the GLS) and results of the Tukey's tests are in Table S5. We also include a power analysis for every significant pairwise comparison in Table S4. To

test for outliers we used the *outlierTest* of the package *car* (Fox and Weisberg, 2018) in every analysis. If an outlier was identified, we circled it in the associated figure and re-analysed the data with the outlier removed. When applicable, the results of the re-analysed data with the outlier removed are noted in the figures and main text, and are available in Table S4 and Table S5. Graphical visualization of the results was completed using the package *ggplot2* (Wickham, 2016).

RESULTS

Our data show considerable variance in all the measurements we quantified. Despite this natural variation, there were clear patterns in both population-level adhesive plasticity and adhesive substrate specificity. In some cases, the outliers shifted the significance levels of some pairwise comparisons (noted below and in figure captions when relevant). The mean power of the experiment was 0.8 ± 0.02 (mean \pm s.e.m.). Out of the 56 significant pairwise comparisons in all analyses, 75% had a power >0.7 and 59% had a power >0.8 . The lowest power was calculated for tube foot failure on Plexiglass in the Palomarin Beach and Bodega Bay samples (0.29), tube foot failure on natural substrates in the Palomarin Beach and Bean Hollow Beach samples (0.46), and whole animal adhesive force in the 2018 trip for Palomarin Beach and Bean Hollow Beach Plexiglass samples (0.48; see Table S4 for power analysis on all pairwise comparisons).

Whole animal adhesive force on natural rock substrates and Plexiglass

Sea urchin adhesion on the first trip (2–4 November 2017) was tested on natural substrates (granite, mudstone, sandstone) and Plexiglass. Whole animal adhesive force differed in response to the interaction of source population (Bodega Bay, Palomarin Beach, Bean Hollow Beach) and natural substrate (granite, mudstone, sandstone; $F_{4,79} = 7.78$, $P < 0.0001$). This interaction was driven by low adhesion of all populations to sandstone, and consistently low adhesion of sea urchins from Bean Hollow Beach (native to sandstone). Overall, adhesion to granite and mudstone substrates was generally high and populations from these substrates (Bodega Bay and Palomarin Beach) adhered more strongly (Fig. 2A). Whole animal adhesive force to the artificial substrate (Plexiglass) followed a similar pattern to the natural substrates ($F_{2,27} = 26.40$, $P < 0.0001$), where sea urchins from Palomarin Beach (native to mudstone) adhered more strongly than those from Bodega Bay and Bean Hollow Beach (Fig. 2A).

The number of tube feet broken after being pulled from natural rock substrates in the field differed in response to the independent effects of source population ($F_{2,77} = 6.63$, $P = 0.0022$) and substrate ($F_{2,77} = 381.80$, $P < 0.0001$). Specifically, independent of the substrate used, sea urchins from Bodega Bay and Palomarin Beach experienced more tube foot failure than those from Bean Hollow Beach. With respect to substrate, sea urchins tested on granite experienced more tube foot failure than those on mudstone, which experienced more tube foot failure than those on sandstone (Fig. 2B). Tube foot failure on the artificial Plexiglass substrate showed that sea urchins from Palomarin Beach experienced more tube foot failure than those from Bodega Bay and Bean Hollow Beach ($F_{2,25} = 10.93$, $P = 0.0004$; Fig. 2B).

Whole animal adhesive force on artificial substrates

Sea urchin adhesion on the second trip (12–18 June 2018) was tested on artificial substrates (Plexiglass and glass). Whole animal adhesive force differed in response to the interaction of source population and artificial substrate (glass, Plexiglass; $F_{2,55} = 3.60$,

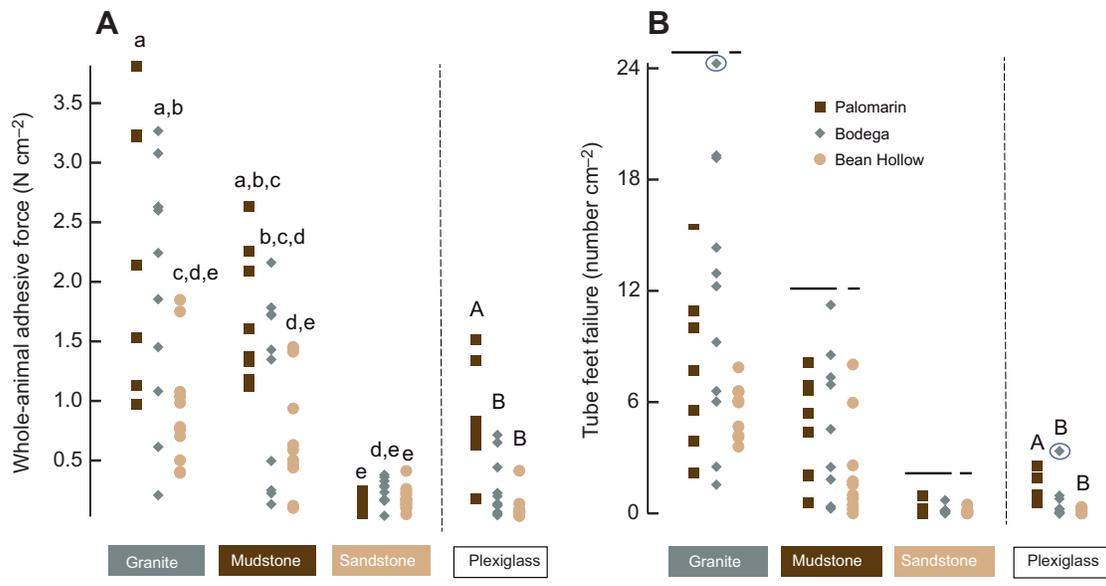


Fig. 2. Whole animal adhesive force and tube foot failure of three populations of sea urchins tested on three natural substrates (granite, mudstone, sandstone) and one artificial substrate (Plexiglass). Whole animal adhesive force (A) was measured as the maximum force required to pull a sea urchin off the substrate, and tube foot failure (B) was measured as the number of broken tube feet left behind after a sea urchin was pulled from the substrate. Both measures were divided by 1/2 sea urchin body surface area (N cm^{-2} ; calculated as an oblate spheroid). Natural rock substrates were analysed separately from the artificial Plexiglass substrate (vertical dotted line separates the two analyses). Population and rock substrate are color coded to match the native source habitat: Palomarin Beach, dark brown mudstone; Bodega Bay, gray granite; Bean Hollow Beach, tan sandstone. Treatment groups with the same letter or sharing the same bar are not significantly different. When appropriate, letters were used to denote all pairwise comparisons (upper and lower case letters are used to show differences within two independent analyses that are displayed on the same graph, separated by the dotted line), and bars were used to denote all single-effect differences. Letters and bars depict statistical results of analyses using the full dataset, without removal of outliers (analyses with the outliers removed are available in Table S5). Outlier analyses identified the two circled data points in panel B as outliers. Removing these points did not change the statistical results.

$P=0.0339$). Each population had higher whole animal adhesive force on glass than Plexiglass, and sea urchins from Bean Hollow Beach had remarkably high adhesion on glass (Fig. 3A). Upon removal of one outlier in the Bean Hollow Beach population tested on glass, the interaction of source population and artificial substrate was no longer significant, and instead both single effects showed significant differences among groups (population: $F_{2,54}=24.11$, $P<0.0001$; substrate: $F_{1,54}=101.97$, $P<0.0001$). Specifically, sea urchins native to Palomarin Beach adhered more strongly than those from Bean Hollow Beach, followed by those from Bodega Bay. All urchins adhered more strongly to glass than Plexiglass.

The number of tube feet broken after being pulled from an artificial substrate differed in response to the interaction of source population and substrate ($F_{2,54}=10.39$, $P=0.0002$). Similar to the whole animal adhesive force results, more tube feet were broken on glass than Plexiglass for each population, and sea urchins from Bean Hollow Beach had remarkably high tube foot failure on glass (Fig. 3B).

Attachment area differed in response to the interaction of source population and substrate ($F_{2,54}=5.02$, $P=0.0100$). Specifically, attachment area did not differ among populations on Plexiglass, but did on glass (i.e. sea urchins from Bean Hollow Beach had higher attachment area than sea urchins from Bodega Bay; Fig. 3C). Upon removal of one outlier in the Bean Hollow Beach population tested on glass, one pairwise comparison changed: attachment area on Plexiglass of sea urchins from Palomarin Beach was significantly larger than attachment area of sea urchins from Bodega Bay. Although whole animal adhesive force and attachment area varied among populations and substrates, we found no difference in tenacity among populations ($F_{2,54}=2.77$, $P=0.0717$) or across artificial substrate types ($F_{1,54}=1.85$, $P=0.1667$; Fig. 4).

Seasonal differences

Whole animal adhesive force on Plexiglass did not differ as a function of year (November 2017 and June 2018; $F_{1,54}=0.42$, $P=0.5145$) within each of the three source populations (Bodega Bay, Palomarin Beach, Bean Hollow Beach; Fig. S2), indicating there was no seasonal difference (at least for the two time points we sampled). Upon removal of one outlier in the Palomarin Beach population tested in 2018, whole animal adhesive force was significantly higher in 2017 than 2018 in the Palomarin Beach population only.

DISCUSSION

Our work represents one of only a few examples of population-level differences in adhesive performance of a biological adhesive system. Unlike our prediction, population-level adhesive plasticity was not driven by the depth of pits available to sea urchin populations; instead, sea urchins native to sandstone tended to perform worse than those native to granite and mudstone across all natural substrates. On smooth, hard, non-friable Plexiglass, sea urchins native to mudstone (Palomarin Beach) adhered more strongly than those native to granite (Bodega Bay) and sandstone (Bean Hollow Beach), rejecting our general prediction that sea urchins native to granite would have superior whole animal adhesive force compared with the other populations. Indeed, on glass, sea urchins native to granite were the least adhesive population of those tested. Thus, while purple sea urchins exhibit clear population-level adhesive plasticity, it is probably not driven by pit-boring behavior in hard and soft friable rocks.

Mechanistically, in the first sampling period we found that sea urchins native to mudstone generally experienced more tube foot failure on natural substrates and Plexiglass than the other two

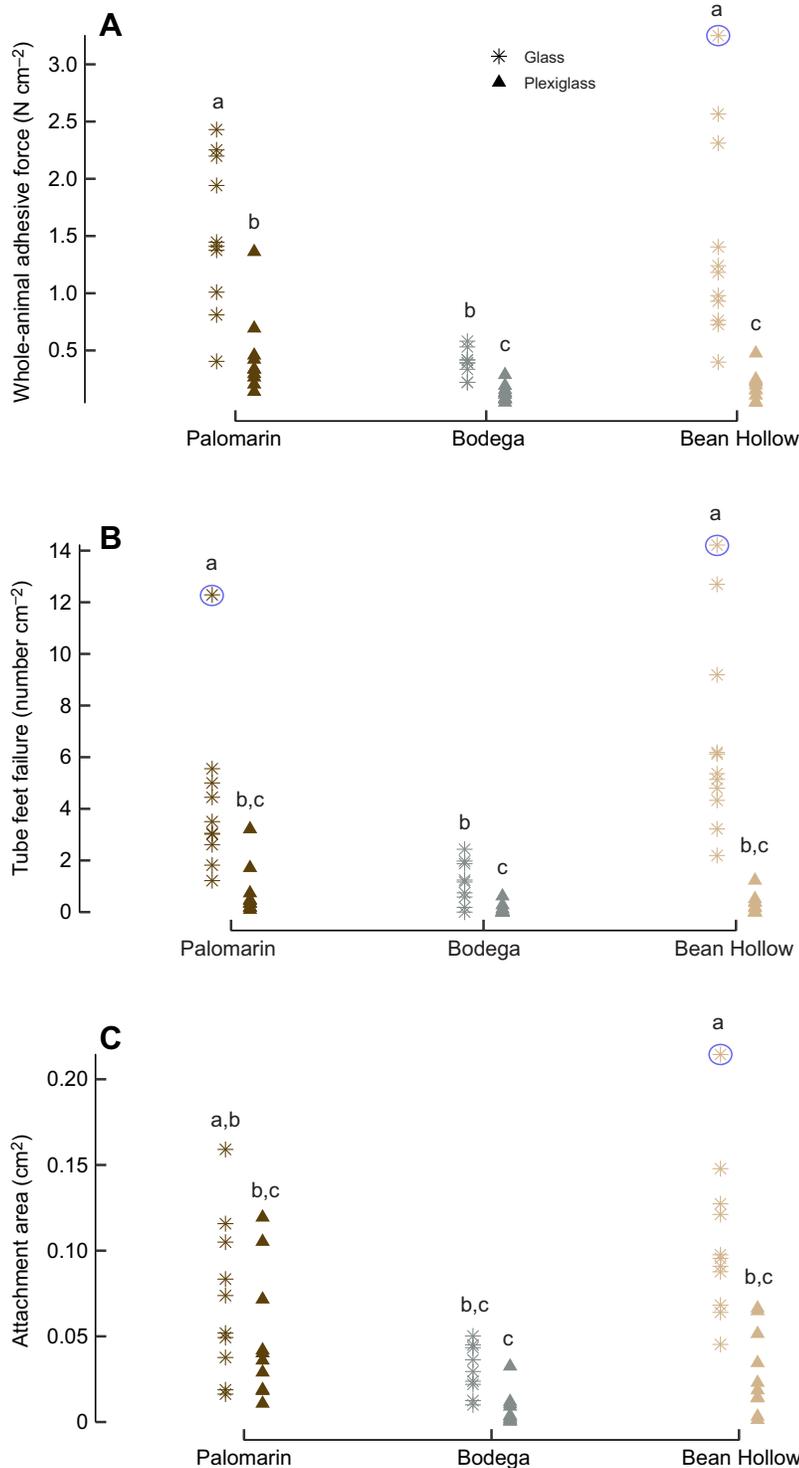


Fig. 3. Whole animal adhesive force, tube foot failure and attachment area of three populations of sea urchins tested on two artificial substrates (glass, Plexiglass). Whole animal adhesive force (A) was measured as the maximum force required to pull a sea urchin off the substrate, tube foot failure (B) was measured as the number of broken tube feet left behind after a sea urchin was pulled from the substrate, and total attachment area (C) was calculated as the mean attachment area multiplied by total number of broken tube feet plus the sum of all footprint areas (see Eqn 2). Whole animal adhesion and tube foot failure measures were divided by 1/2 sea urchin body surface area (calculated as an oblate spheroid). Population and substrate are color coded to match source population and native habitat (see Fig. 2 for details). Treatment groups with the same letter are not significantly different. Letters depict statistical results of the analyses using the full dataset, without removal of outliers (analyses with the outliers removed are available in Table S5). Outlier analyses identified the four circled data points as outliers. Removing the single outlier in panel A resulted in no interaction between substrate and population, and significant differences in whole animal adhesion among the single effects of population and substrate (i.e. Palomarin Beach>Bean Hollow Beach>Bodega Bay; glass>Plexiglass). Removing the two outliers in panel B did not change the statistical results. Removing the single outlier in panel C changed one pairwise comparison (attachment area on Plexiglass of sea urchins from Palomarin Beach is significantly larger than attachment area of sea urchins from Bodega Bay).

populations, rejecting our prediction that more adhesive populations will have tougher, more extensible tube feet that resisted failure (although direct mechanical measurements of toughness and extensibility need to be made to confirm this conclusion; Fig. 2). In the second sampling period we did not detect a significant difference in tube foot failure or attachment area on Plexiglass among populations, but did detect a strong difference on glass, where sea urchins native to sandstone broke many tube feet and had higher attachment area than those native to granite (Fig. 3). While the differences in sampling year may be a result of low sample size

(i.e. mean number of tube foot failures on Plexiglass is higher in the mudstone population than the other two in the second sampling period, although not statistically significant), it is unclear why glass and Plexiglass produce such different results (see Discussion below). Regardless, our results show that counter to our prediction, high whole animal adhesive force is related not only to high attachment area, but also more tube foot failure.

Sea urchins native to the sandstone substrate produced generally lower whole animal adhesive force, used less attachment area, and experienced less tube foot failure than populations from mudstone

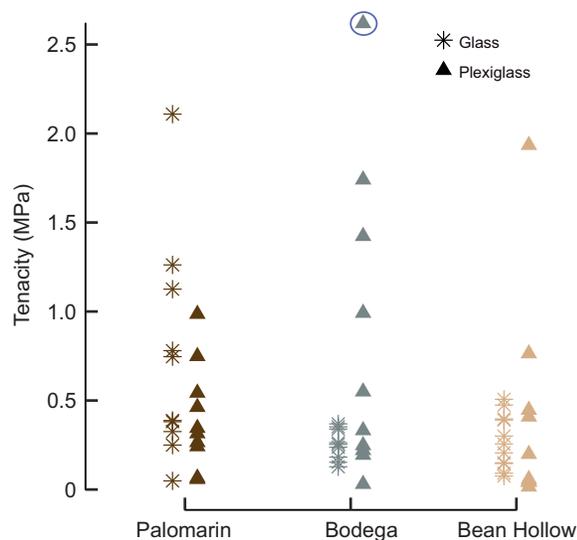


Fig. 4. Tenacity of three populations of sea urchins tested on glass and Plexiglas artificial substrates. Tenacity was measured as whole animal adhesive force divided by the attachment area. Population and substrate are color coded to match source population and native habitat (see Fig. 2 for details). There was no significant difference among populations or substrates. Outlier analyses identified the circled data point as an outlier. Removing this point did not change the statistical results.

and granite, except on glass. Specifically, sea urchins native to sandstone adhered as strongly, broke as many tube feet, and had the same attachment area as those from the consistently high-performing mudstone population on glass, but on no other substrate. It is unclear what is driving this result, but it may be due to the similarity of sandstone and glass substrates (i.e. both have a high silica component). The comparability between glass and sandstone suggests an adhesive affinity of sea urchins native to sandstone to silica-dominated substrates that is not evident in other substrates or populations. In contrast, sea urchins native to mudstone adhered equally well to glass as those from sandstone, and in general performed better on all substrates than the other populations, regardless of substrate. Future work should focus on characterizing morphological and chemical plasticity among populations of sea urchins, such as single tube foot mechanical properties, tube foot disc size, tube foot adhesion, glue chemistry, and adhesive thickness left behind on glass and Plexiglass substrates. This would significantly improve our understanding of how sea urchins from the mudstone substrate population retain whole animal adhesive force across a variety of substrates, and why sea urchins from the sandstone substrate population perform best on smooth artificial glass.

We found a significant effect of substrate on whole animal adhesive force, where all sea urchins generally adhered poorly to the soft, friable sandstone substrate. Attachment of mussels and algae to friable rock is weaker (i.e. high likelihood of dislodgement) due to failure of the substrate, rather than the organism (Thomsen et al., 2004; Gutiérrez et al., 2018). Indeed, in our study very few tube feet were left behind on the sandstone substrate, supporting substrate failure rather than organismal failure. In contrast, the soft, friable mudstone substrate produced high whole animal adhesive force and more total tube foot failure than sandstone. The primary difference between the two substrates is grain size. Specifically, sandstone grain size is much larger than mudstone (0.25–1.00 mm particle diameter versus <0.06 mm particle diameter, respectively), making the sandstone substrate both rougher and more friable (Russell et al.,

2018). Because surface roughness increases sea urchin adhesion (Santos et al., 2005), we believe that friability, rather than surface roughness, is the key factor dictating the low whole animal adhesive force of sea urchins on sandstone. Unfortunately, it was impossible to see obvious substrate failure in the field, which probably occurred at the millimeter grain-size scale. We suggest that future work should focus on imaging the adhesive tube foot discs after detachment from these substrates to assess potential differences in substrate failure post-adhesion testing (i.e. count the number of substrate grains attached to the tube foot post-adhesion test).

We also found differences in whole animal adhesive force among artificial substrates, where purple sea urchins adhered more strongly to glass than Plexiglass. Our finding contrasts with a different sea urchin species (*Paracentrotus lividus*) tested on glass and Plexiglass. Single tube feet from this European species adhered better and left larger adhesive footprints on Plexiglass than glass (Santos and Flammang, 2006). Santos and Flammang (2006) also noticed significant reluctance of one species (*Arbacia lixula*) to adhere to glass, which was also opposite to our observations – the purple sea urchins in our study were more reluctant to attach to Plexiglass than glass (authors' personal observation). Taken together, these results suggest that there may be species-level differences in adhesion and behavior among sea urchins that have not been fully characterized.

Despite differences in whole animal adhesive force among populations and substrates, tenacity of the whole animal did not differ among artificial substrates and populations (Fig. 4). This means that when whole animal adhesive force is normalized by attachment area, a sea urchin's ability to stick is the same across all populations and artificial substrates, unlike previous results that showed variation in whole animal tenacity among species (Santos and Flammang, 2007). Because area is controlled in this measure and there was no difference in tenacity among populations, we believe that differences in adhesive plasticity among populations are driven by the tube feet (e.g. single tube foot area, single tube foot tenacity and/or number of tube feet used to adhere). Recent work suggests that at least one source of plasticity in whole animal adhesive force is related to differences in tube foot area among two of the three populations (Narvaez et al., 2020). It is also possible that while whole animal tenacity does not differ, single tube foot tenacity does. Likewise, we cannot discount the possibility that sea urchins may actively choose to use different attachment area when experiencing detachment forces (i.e. adhere more or less tube feet). As noted by other studies, footprint staining may not accurately measure total number of tube feet used or attachment area (Flammang and Walker, 1997; Cohen-Rengifo et al., 2017). Tenacity measurements using adhesive tube footprint staining likely under-estimates whole animal tenacity because some footprints were probably placed prior to adhesion pulls, inflating total adhesion area. In this case, counts of total number of tube feet used to resist detachment would also be inflated. It is also possible that failure at the adhesive interface may result in the full detachment of the footprint and tube foot, which would lower the total attachment area measured by staining (i.e. no footprint to stain) and thus reduce total attachment area measured and over-estimate whole animal tenacity (Santos and Flammang, 2006). Failure at the interface would also reduce counts of total number of tube feet used to resist detachment. Future work is needed to compare attachment behavior (i.e. number of contacts made) of sea urchin populations on artificial substrates during adhesion tests, and the effectiveness of single tube feet (i.e. adhesive force of a single tube foot normalized by area of that tube foot) to resolve potential

over- and under-estimates of tenacity among populations and to address possible differences in adhesive behavior to resist detachment.

Our work has significant implications for understanding the plasticity and adaptive significance of the sea urchin adhesive system in the nearshore marine zone, where sea urchins are key ecological players. Unexpectedly, the results of our work contrast with previous studies in several ways. First, previous work on European species showed no difference in adhesion on rock and artificial substrates, or among populations in some, but not all species (Sharp and Gray, 1962; Santos and Flammang, 2007). Second, our results differ with respect to the role surface energy plays on adhesive performance, and are more similar to sea stars (i.e. sea star adhesion is higher on glass; Thomas and Hermans, 1985; Flammang and Walker, 1997; Santos and Flammang, 2006). Third, the general maintenance of whole animal adhesive force across years on Plexiglass (except when one outlier is removed in the Palomarin Beach population) suggests that our results are not a function of temporal differences in wave height and temperature, at least in two populations, which have been shown to be important determinants of adhesion in other species (Santos and Flammang, 2007). It is unclear why the Palomarin Beach population was slightly less adhesive (shown in the adjusted analysis removing the outlier) and did not experience significantly more tube foot failure than the other two populations at the later sampling date. Future work investigating spatial and temporal variation in the environments of each population is needed to clarify this result. Although our results do not support our hypothesis that low-profile, less protective pits made in granite result in higher whole animal adhesive force of granite-native sea urchins, it is clear that purple sea urchin adhesion is a function of both native population and substrate. Future work should focus on morphological, behavioral and developmental plasticity among purple sea urchin populations to clarify our results. This work also serves as an important example of biological adhesive plasticity and challenges the way we think about biological adhesive systems as static units of performance. We believe that future studies of adhesive plasticity in sea urchins and other biological adhesive systems will improve our understanding of adhesion in natural environments and expand potential design parameters for bio-inspired synthetic adhesives that are more adaptable to variable environmental conditions.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.Y.S., C.A.N., M.P.R.; Methodology: A.Y.S., C.A.N., M.P.R.; Formal analysis: C.A.N.; Investigation: A.Y.S., C.A.N., M.P.R.; Resources: M.P.R.; Data curation: C.A.N.; Writing - original draft: A.Y.S.; Writing - review & editing: A.Y.S., C.A.N., M.P.R.; Supervision: A.Y.S., C.A.N., M.P.R.; Project administration: A.Y.S., C.A.N.; Funding acquisition: M.P.R.

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Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.228544.supplemental>

References

- Abràmoff, M. D., Magalhães, P. J. and Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International* **11**, 36-42.
- Cohen-Rengifo, M., Agüera, A., Detrain, C., Bouma, T. J., Dubois, P. and Flammang, P. (2018). Biomechanics and behaviour in the sea urchin *Paracentrotus lividus* (Lamarck, 1816) when facing gradually increasing water flows. *J. Exp. Mar. Biol. Ecol.* **506**, 61-71. doi:10.1016/j.jembe.2018.05.010
- Cohen-Rengifo, M., Moureaux, C., Dubois, P. and Flammang, P. (2017). Attachment capacity of the sea urchin *Paracentrotus lividus* in a range of seawater velocities in relation to test morphology and tube foot mechanical properties. *Mar. Biol.* **164**, 79. doi:10.1007/s00227-017-3114-0
- Connolly, D. M., Desvignes, T. and Williamson, J. E. (2017). Influence of body size on tube feet morphology and attachment capacity in the sea urchin *Holopneustes purpurascens* (Temnopleuridae). *Mar. Biol.* **164**, 223. doi:10.1007/s00227-017-3270-2
- Denny, M. (1995). Predicting physical disturbance: mechanistic approaches to the study of survivorship on wave-swept shores. *Ecol. Monogr.* **65**, 371-418. doi:10.2307/2963496
- Denny, M., Gaylord, B., Helmuth, B. and Daniel, T. (1998). The menace of momentum: dynamic forces on flexible organisms. *Limnol. Oceanogr.* **43**, 955-968. doi:10.4319/lo.1998.43.5.0955
- Ebert, T. A. (1988). Allometry, design and constraint of body components and of shape in sea urchins. *J. Nat. Hist.* **22**, 1407-1425. doi:10.1080/00222938800770841
- Elliott, L., Russell, M. and Hernandez, J. (2012). Estimating echinoid test volume from height and diameter measurements. In *Echinoderms in a Changing World: Proceedings of the 13th International Echinoderm Conference, 5-9 January 2009, University of Tasmania, Hobart, Tasmania, Australia*, pp. 105: CRC Press.
- Flammang, P. (1996). Adhesion in echinoderms. *Echinoderm Stud.* **5**, 1-60.
- Flammang, P. and Walker, G. (1997). Measurement of the adhesion of the podia in the asteroid *Asterias rubens* (Echinodermata). *J. Mar. Biol. Assoc. U. K.* **77**, 1251-1254. doi:10.1017/S0025315400038807
- Flammang, P., Demeulenaere, S. and Jangoux, M. (1994). The role of podial secretions in adhesion in two species of sea stars (Echinodermata). *Biol. Bull.* **187**, 35-47. doi:10.2307/1542163
- Flammang, P., Michel, A., Cauwenberge, A., Alexandre, H. and Jangoux, M. (1998). A study of the temporary adhesion of the podia in the sea star *Asterias rubens* (Echinodermata, Asteroidea) through their footprints. *J. Exp. Biol.* **201**, 2383-2395.
- Flammang, P., Santos, R., Haesaerts, D. (2005). Echinoderm adhesive secretions: from experimental characterization to biotechnological applications. In *Echinodermata* (ed. V. Matranga), pp. 201-220. Springer.
- Fox, J. and Weisberg, S. (2018). *An R Companion to Applied Regression*. Sage Publications.
- Gutiérrez, J. L., Bagur, M., Arribas, L. P. and Palomo, M. G. (2018). Does rock type account for variation in mussel attachment strength? A test with *Brachidontes rodriguezii* in the southwestern Atlantic. *Helgol. Mar. Res.* **72**, 10. doi:10.1186/s10152-018-0514-6
- Haag, N., Russell, M. P. and Hernandez, J. C. (2016). Effects of spine damage and microhabitat on resource allocation of the purple sea urchin *Strongylocentrotus purpuratus* (Stimpson 1857). *J. Exp. Mar. Biol. Ecol.* **482**, 106-117. doi:10.1016/j.jembe.2016.05.005
- Hennebert, E., Haesaerts, D., Dubois, P. and Flammang, P. (2010). Evaluation of the different forces brought into play during tube foot activities in sea stars. *J. Exp. Biol.* **213**, 1162-1174. doi:10.1242/jeb.037903
- Hennebert, E., Santos, R. and Flammang, P. (2012). Echinoderms don't suck: evidence against the involvement of suction in tube foot attachment. *Zoosymposia* **7**, 25-32. doi:10.11646/zoosymposia.7.1.3
- Hennebert, E., Viville, P., Lazzaroni, R. and Flammang, P. (2008). Micro- and nanostructure of the adhesive material secreted by the tube feet of the sea star *Asterias rubens*. *J. Struct. Biol.* **164**, 108-118. doi:10.1016/j.jsb.2008.06.007
- Hernández, J. and Russell, M. (2010). Substratum cavities affect growth-plasticity, allometry, movement and feeding rates in the sea urchin *Strongylocentrotus purpuratus*. *J. Exp. Biol.* **213**, 520-525. doi:10.1242/jeb.029959
- Jacinto, D. and Cruz, T. (2012). *Paracentrotus lividus* (Echinodermata: Echinoidea) attachment force and burrowing behavior in rocky shores of SW Portugal. *Zoosymposia* **7**, 231-240. doi:10.11646/zoosymposia.7.1.21
- Lebesgue, N., Da Costa, G., Ribeiro, R. M., Ribeiro-Silva, C., Martins, G. G., Matranga, V., Scholten, A., Cordeiro, C., Heck, A. J. and Santos, R. (2016). Deciphering the molecular mechanisms underlying sea urchin reversible adhesion: a quantitative proteomics approach. *J. Proteomics* **138**, 61-71. doi:10.1016/j.jprot.2016.02.026
- Lenth, R. (2019). Emmeans package: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.3.

- Levitan, D.** (1991). Skeletal changes in the test and jaws of the sea urchin *Diadema antillarum* in response to food limitation. *Mar. Biol.* **111**, 431–435. doi:10.1007/BF01319415
- Lewis, J. B. and Storey, G. S.** (1984). Differences in morphology and life history traits of the echinoid *Echinometra lucunter* from different habitats. *Mar. Ecol. Prog. Ser.* **15**, 207–211. doi:10.3354/meps015207
- Middleton, D. A., Gurney, W. S. and Gage, J. D.** (1998). Growth and energy allocation in the deep-sea urchin *Echinus affinis*. *Biol. J. Linn. Soc.* **64**, 315–336. doi:10.1111/j.1095-8312.1998.tb00336.x
- Minor, M. and Scheibling, R. E.** (1997). Effects of food ration and feeding regime on growth and reproduction of the sea urchin *Strongylocentrotus droebachiensis*. *Mar. Biol.* **129**, 159–167. doi:10.1007/s002270050156
- Narvaez, C. A., Padovani, A. M., Stark, A. Y. and Russell, M. P.** (2020). Plasticity in the purple sea urchin (*Strongylocentrotus purpuratus*): tube feet regeneration and adhesive performance. *J. Exp. Mar. Biol. Ecol.* **528**, 151381. doi:10.1016/j.jembe.2020.151381
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and Team, R. C.** (2012). nlme: Linear and nonlinear mixed effects models. *R Package Version 3*, 1–89.
- Pjeta, R., Lindner, H., Kremser, L., Salvenmoser, W., Sobral, D., Ladurner, P. and Santos, R.** (2020). Integrative transcriptome and proteome analysis of the tube foot and adhesive secretions of the sea urchin *Paracentrotus lividus*. *Int. J. Mol. Sci.* **21**, 946. doi:10.3390/ijms21030946
- Russell, M. P.** (1987). Life history traits and resource allocation in the purple sea urchin *Strongylocentrotus purpuratus* (Stimpson). *J. Exp. Mar. Biol. Ecol.* **108**, 199–216. doi:10.1016/0022-0981(87)90085-2
- Russell, M. P.** (1998). Resource allocation plasticity in sea urchins: rapid, diet induced, phenotypic changes in the green sea urchin, *Strongylocentrotus droebachiensis* (Müller). *J. Exp. Mar. Biol. Ecol.* **220**, 1–14. doi:10.1016/S0022-0981(97)00079-8
- Russell, M. P., Gibbs, V. K. and Duwan, E.** (2018). Bioerosion by pit-forming, temperate-reef sea urchins: history, rates and broader implications. *PLoS ONE* **13**, e0191278. doi:10.1371/journal.pone.0191278
- Santos, R. and Flammang, P.** (2005). Morphometry and mechanical design of tube foot stems in sea urchins: a comparative study. *J. Exp. Mar. Biol. Ecol.* **315**, 211–223. doi:10.1016/j.jembe.2004.09.016
- Santos, R. and Flammang, P.** (2006). Morphology and tenacity of the tube foot disc of three common European sea urchin species: a comparative study. *Biofouling* **22**, 173–186. doi:10.1080/08927010600743449
- Santos, R. and Flammang, P.** (2007). Intra-and interspecific variation of attachment strength in sea urchins. *Mar. Ecol. Prog. Ser.* **332**, 129–142. doi:10.3354/meps332129
- Santos, R. and Flammang, P.** (2008). Estimation of the attachment strength of the shingle sea urchin, *Colobocentrotus atratus*, and comparison with three sympatric echinoids. *Marine Biology* **154**, 37–49. doi:10.1007/s00227-007-0895-6
- Santos, R., Da Costa, G., Franco, C., Gomes-Alves, P., Flammang, P. and Coelho, A.** (2009). First insights into the biochemistry of tube foot adhesive from the sea urchin *Paracentrotus lividus* (Echinoidea: Echinodermata). *Mar. Biotechnol.* **11**, 686. doi:10.1007/s10126-009-9182-5
- Santos, R., Gorb, S., Jamar, V. and Flammang, P.** (2005). Adhesion of echinoderm tube feet to rough surfaces. *J. Exp. Biol.* **208**, 2555–2567. doi:10.1242/jeb.01683
- Sharp, D. T. and Gray, I. E.** (1962). Studies on factors affecting the local distribution of two sea urchins, *Arbacia punctulata* and *Lytechinus variegatus*. *Ecology* **43**, 309–313. doi:10.2307/1931986
- Thomas, L. A. and Hermans, C. O.** (1985). Adhesive interactions between the tube feet of a starfish, *Leptasterias hexactis*, and substrata. *Biol. Bull.* **169**, 675–688. doi:10.2307/1541309
- Thomsen, M. S., Wernberg, T. and Kendrick, G. A.** (2004). The effect of thallus size, life stage, aggregation, wave exposure and substratum conditions on the forces required to break or dislodge the small kelp *Ecklonia radiata*. *Bot. Mar.* **47**, 454–460. doi:10.1515/BOT.2004.068
- Toubarro, D., Gouveia, A., Ribeiro, R. M., Simões, N., Da Costa, G., Cordeiro, C. and Santos, R.** (2016). Cloning, characterization, and expression levels of the nectin gene from the tube feet of the sea urchin *Paracentrotus lividus*. *Mar. Biotechnol.* **18**, 372–383. doi:10.1007/s10126-016-9698-4
- Wickham, H.** (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer.
- Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A. and Smith, G. M.** (2009). *Mixed Effects Models and Extensions in Ecology with R*. Springer Science & Business Media.