© 2014. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2014) 217, 2548-2554 doi:10.1242/jeb.100149

RESEARCH ARTICLE

Attachment to challenging substrates – fouling, roughness and limits of adhesion in the northern clingfish (*Gobiesox maeandricus*)

Petra Ditsche[1,2,*], Dylan K. Wainwright[1,3] and Adam P. Summers[1]

ABSTRACT

Northern clingfish use a ventral suction disc to stick to rough substrates in the intertidal zone. Bacteria, algae and invertebrates grow on these surfaces (fouling) and change the surface properties of the primary substrate, and therefore the attachment conditions for benthic organisms. In this study, we investigate the influence of fouling and surface roughness on the adhesive strength of northern clingfish, *Gobiesox maeandricus*. We measured clingfish tenacity on unfouled and fouled substrates over four surface roughnesses. We exposed surfaces for 6 weeks in the Pacific Ocean, until they were covered with periphyton. Clingfish tenacity is equivalent on both fouled and unfouled smooth substrates; however, tenacity on fouled rough surfaces is less compared with tenacity on unfouled ones. We hypothesize that parts of biofilm may act as a lubricant and decrease friction of the disc margin, thereby making disc margins slip inwards and fail at lower tenacities. Nevertheless, even on fouled surfaces the adhesive forces are approximately 150 times the body weight of the fish. To identify the upper threshold of surface roughness the fish can cling to, we tested seven unfouled substrates of increasing surface roughness. The threshold roughness at which northern clingfish failed increased with specimen size. We hypothesize that because of the elastic properties of the disc margin, a larger disc can adapt to larger surface irregularities. The largest specimens (length 10–12 cm) were able to cling to surfaces with 2–4 mm grain size. The fish can attach to surfaces with roughness between 2 and 9% of the suction disc width.

KEY WORDS: Biofilm, Periphyton, Surface roughness, Suction disc, Attachment, Functional morphology, Gobiesocidae

INTRODUCTION

Fishes, even intertidal fishes, typically swim freely in the water column or rest on the substrate, but there have been more than a dozen independent evolutions of adhesive mechanisms that allow fishes to attach to a substrate. For example, many clingfishes (Gobiesocidae) have a suction disc that allows them to adhere to intertidal rocks with sufficient strength to resist dislodgement amidst crashing waves. The high-speed flows in stream environments have presumably shaped the attachment organs of gobies, balitorid loaches and loricariid catfishes (Gerstner, 2007; Maie et al., 2012; Roberts, 1998). Suction attachment has also allowed fishes to exploit entirely new habitats; for example, shark suckers (Echiniidae) attach themselves to larger fishes and live off the leavings of their host (Fulcher and Motta, 2006). Both marine and freshwater environments have examples of suction adhesion, with snailfish and lumpsuckers being prominent marine examples that have a dedicated suctorial disc (Arita, 1962; Budney and Hall, 2010). The process of adhesion can be quite dynamic, with some gobies able to climb waterfalls using a pelvic-fin-derived suction disc, and lampreys climbing waterfalls with an oral sucker (Reinhardt et al., 2008; Schoenfuss and Blob, 2003). The variety of substrates to which fishes can adhere is impressive, and the methods used for adhesion are diverse.

The northern clingfish [*Gobiesox maeandricus* (Girard 1858)] lives in the rocky intertidal of the Pacific Northwest (Fig. 1A). Here the clingfish lives among the wave-swept boulders, using an adhesive suction disc to prevent being washed away. The substrates that clingfish adhere to have a variety of surface topographies, from nearly smooth to very rough, and *G. maeandricus* sticks so well that it can launch predatory attacks on the archetypal attached marine invertebrate – the limpet. In fact, clingfish are able to stick better to rough surfaces than to smooth ones (Wainwright et al., 2013). This is counter to our expectations for suction cups, which adhere only to smooth surfaces (Pennisi, 2012). This ability to stick to rough surfaces seems to be connected with a specialized epithelial microstructure on the ventral surface of the clingfish adhesive disc (Fig. 1B) (Wainwright et al., 2013). Epidermal papillae made of tiny hair-like rods, which are subdivided at the tips into tiny filaments, cover the margin of the disc and we expect they play a role in adhering to rough surfaces (Arita, 1962; Green and Barber, 1988). However, intertidal surfaces are not simply rough, they are fouled by microbes, algae and invertebrates, which change the original substrate by forming a surface with different frictional and adhesive properties (Denny and Gaines, 2007; Ditsche et al., 2014).

The process of biofouling starts with adsorption of dissolved organic molecules, followed by colonization of prokaryotes and eukaryotes, and finally settlement of algal spores and invertebrate larvae (Maki and Mitchell, 2003). However, these three stages can also overlap or occur in parallel (Dobretsov, 2002). Biofilms are usually very heterogeneous with respect to both space and time (Donlan, 2002). In the marine environment, biofilms consist mainly of various bacteria and diatoms that secrete extracellular polymer substances (EPS), embedding microbial cells and non-cellular materials in an organic sublayer of primary polysaccharides (Donlan, 2002; Railkin, 2004). In addition, different species of macro-organisms can settle on substrates, adding to the heterogeneity of fouled surfaces (Donlan, 2002). Fouling influences surface properties such as roughness, material stiffness, wettability

---

[1]University of Washington, Friday Harbor Laboratories, WA 98250, USA. [2]Department of Functional Morphology and Biomechanics, Zoological Institute of the University of Kiel, 24098 Kiel, Germany. [3]Harvard University, Cambridge, MA 02138, USA.

*Author for correspondence (pditsche@uw.edu)

Received 26 November 2013; 7 April 2014

List of symbols and abbreviations

A	surface area of the adhesive disc
E	Young's modulus of elasticity
EPS	extracellular polymer substances
F_{ad}	pull-off force
P_{ad}	tenacity

and surface chemistry, which can then affect adhesive strength and friction (Ditsche-Kuru et al., 2010; Ditsche et al., 2014; Scherge and Gorb, 2001). A biofilm can lead to an increase in attachment force in the face of biofouling or a decrease, depending on many different factors (Ditsche et al., 2014; Hadfield, 2011).

The high energy and well-fouled environment of the northern clingfish make this an interesting system in which to investigate the effects of roughness and surface fouling on adhesive ability. Here we have four questions. (1) Is there a maximum roughness beyond which a clingfish cannot stick? (2) Does the size of the fish determine the maximum roughness to which it can adhere? (3) Does surface fouling influence the attachment strength of *G. maeandricus*? (4) Is there an interaction between surface roughness and biofouling that determines attachment strength?

RESULTS

The fish used in our study were between 5 and 10 cm long and 1.5–15 g in mass. The disc area varied between 1 and 9 cm^2 and correlated with the size of the fish (Fig. 2A). The pull-off force depends on the area of the suction disc (Fig. 2B), as shown for a substrate of grain size 35 µm. To account for the impact of disc size, we compared tenacity (stress) for the tested substrates.

The influence of fouling on suction adhesion

The surface structure changed considerably after fouling on all surfaces (Fig. 3). Qualitatively, the biofilm developed more quickly and macroalgae growth appeared sooner on rougher substrates. To create comparable experimental conditions, we chose substrates for the attachment experiments that showed more or less similar periphyton growth across different types of surface roughness. Invertebrates did not attach firmly to the surfaces during our time span of exposure.

After exposure in the Pacific Ocean, diatoms covered all tested substrates in unicellular and multicellular layers. Macroalgae grew over the diatom layers. On the smooth surfaces, some green algae of the genus *Kormania* grew up to 5 mm in length (Fig. 3A,E). On the finest rough surface (grain size 35 µm), we found filamentous diatoms, brown algae, *Ulva linza*, *Chaetomorpha* sp. and *Urospora* sp., in addition to cellular diatom layers (Fig. 3B,F). Filamentous algae reached up to 6 mm above the substrate. On the medium surface roughness (grain size 78 µm), growth was seen by filamentous diatoms, *Navicula* sp., *U. linza*, *Cladophora* sp., *Ectocarpus* sp. and *Urospora* sp., all growing 3–5 mm over the diatom layer(s) (Fig. 3C,G). On the roughest tested surface (grain size 269 µm), the diatoms were accompanied by *U. linza*, *Ulva intestinalis* and *Polysinphonia hendryi*, most reaching a height up to 5 mm, but in rare cases up to 15 mm (Fig. 3D,H).

The primary surface roughness influenced the attachment strength of northern clingfish on fouled surfaces (Fig. 4). On smooth surfaces, fouling did not influence tenacity (t-test, d.f.=26, $t=-0.21$, $P>0.05$). In contrast, on all rough surfaces there was a decrease in attachment strength on fouled surfaces compared with their unfouled counterparts. On substrates of grain sizes 35 and 78 µm, the tenacities decreased by 26% and 21%, respectively (t-test, grain size 35 µm: d.f.=26, $t=-6.85$, $P<0.001$; grain size 78 µm: d.f.=26, $t=-5.66$, $P<0.001$). On the roughest of the tested substrates (grain size 269 µm), tenacity decreased by only 6% on average (t-test, d.f.=25, $t=-2.13$, $P=0.045$). After Holm–Bonferroni correction, all cases were still significant ($\alpha=0.015$; 35 µm: $P<0.017$; 78 µm: $P<0.034$; 269 µm: $P<0.05$). Surface roughness caused significant differences in tenacity on unfouled substrates (ANOVA, d.f.=59, $F=14.18$, $P<0.001$), but fouled substrates showed no differences in tenacity over different roughnesses (ANOVA, d.f.=59, $F=1.79$, $P>0.05$). Pull-off forces were 7–26 N on unfouled surfaces and 7–18 N on fouled surfaces, which is approximately 200 and 150 times the body weight of the fish.

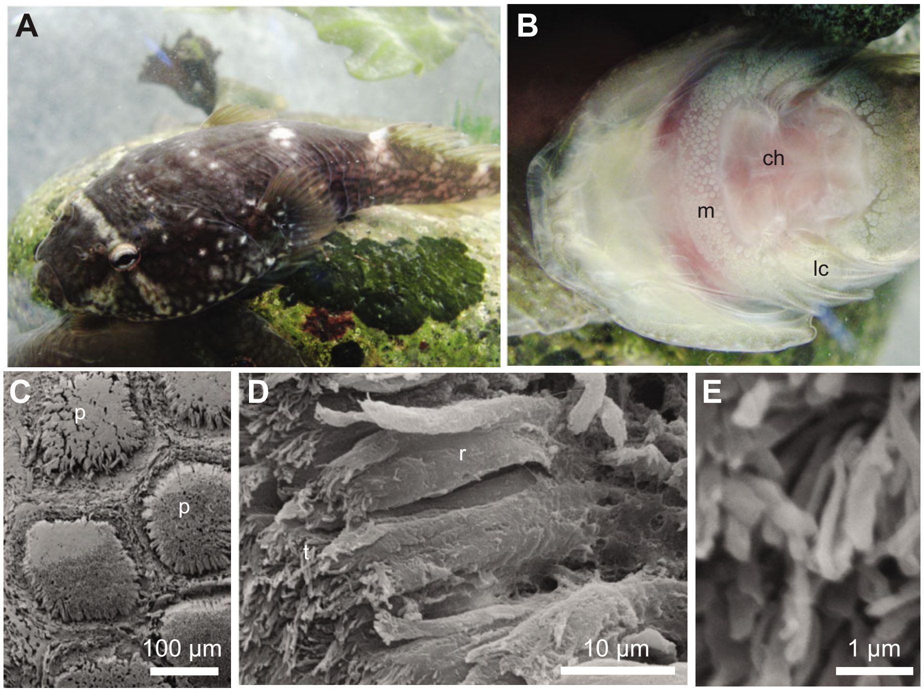

Fig. 1. Images of *Gobiesox maeandricus* and the hierarchical structures on its suction disc. (A) Northern clingfish *G. maeandricus* sticking to a fouled rock. (B) Ventral view of the adhesive disc with epidermal papillae. (C) Papillae cover the disc margin. (D) Papillae consist of tightly packed rods, which are divided into tiny filaments at their tips. (E) Filaments on the tips of the rods. lc, lateral cleft; ch, inner chamber of suction cup; m, disc margin; p, papillae; r, rods; t, tips.

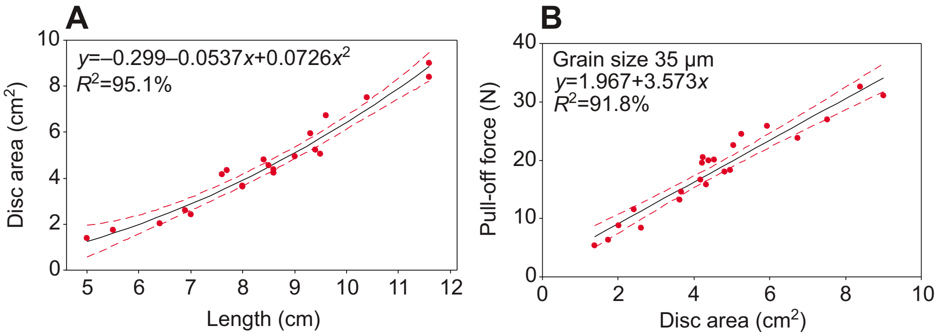

Fig. 2. Relationship between body length, disc area and pull-off force in *G. maeandricus*. (A) Area of the adhesive disc in relation to body length of *G. maeandricus*. Disc area increases with fish length. (B) Pull-off force of *G. maeandricus* in relation to area of the adhesive disc for unfouled substrate of grain size 35 µm. Pull-off force increases with the disc area. Regression lines are shown with 95% confidence intervals.

The influence of surface roughness on suction adhesion

Our results show a threshold roughness above which *G. maeandricus* is unable to adhere (Fig. 5), and we found that tenacity was significantly different over different roughnesses (ANOVA; P<0.001, F=9.5, d.f.=152; Tukey *post hoc*). Tenacity was significantly lower on smooth surfaces compared with the rough surfaces of 35, 78 and 269 µm grain size. There was a significant decrease of tenacity between substrates of grain size of 500–1000 µm and 1000–2000 µm. While most northern clingfish could adhere to the 1000–2000 µm grain surface, 86% failed on the 2000–4000 µm grain surface. The tenacity difference between substrates with a grain size of 1000–2000 µm and 2000–4000 µm was also significant. Clingfish adhered to the surface of the field-collected stone (overlaying roughnesses) with a tenacity similar to a manufactured surface with a grain size of 35–1000 µm.

The size of the fish clearly affected the maximum surface roughness *G. maeandricus* was able to cling to (Table 1, Fig. 6). The smallest fish (length <60 mm) failed on all roughnesses over 1000 µm grain size, while the largest fish (>100 mm) were able to cling to even the roughest test surfaces (2000–4000 µm).

Pull-off force varied with fish size on different surface roughness (Fig. 6). The regression lines (see also supplementary material Table S1) show that the intersection with the x-axis increases with increasing surface roughness while the slope is similar for substrates of 35–2000 µm grain size. This implies that for any roughness there is a size threshold below which no fish will be able to cling to. For example, fish must be at least 53 mm in length to cling to substrates of 500–1000 µm grain size and 74 mm in length to cling to substrates of 1000–2000 µm grain size. For substrates of 2000–4000 µm grain size, only fish larger than 97 mm would cling at all. A significantly different relationship between pull-off force and fish length was found between smooth substrates and those with a grain size of 35 µm (ANCOVA, $F_{1,41}$=45.7, P<0.001), grain sizes of 269 and 500–1000 µm (ANCOVA, $F_{1,41}$=9.47, P<0.005), grain sizes of 500–1000 and 1000–2000 µm (ANCOVA, $F_{1,41}$=34.28, P<0.001) as well as grain sizes of 1000–2000 and 2000–4000 µm (ANCOVA, $F_{1,41}$=17.93, P<0.001). In contrast, the substrates of grain sizes 35 and 78 µm (ANCOVA, $F_{1,41}$=0.01, P>0.05) as well as of grain sizes 78 and 269 µm (ANCOVA, $F_{1,41}$=3.71, P>0.05) showed no differences in the relationship between pull-off force and fish length.

DISCUSSION

While walking on the rocks of the intertidal, it is evident that the environment is slippery. The northern clingfish seems to share the human experience to some extent. Once surfaces are fouled, the tenacities on rough surfaces decrease to match values for smooth surfaces, around 14–15 N and approximately 150 times the body weight of the fish. On rough, unfouled surfaces, clingfish can use their elastic disc margin covered with hierarchical papillae made of microvilli to adapt to surface irregularities. These structures probably increase the friction when pulled in a horizontal direction and thereby delay inward slipping and failure of the disc to cling (Wainwright et al., 2013). In contrast, a fish attached to a fouled substrate makes no direct contact with the primary substrate, but instead contacts the fouling organisms.

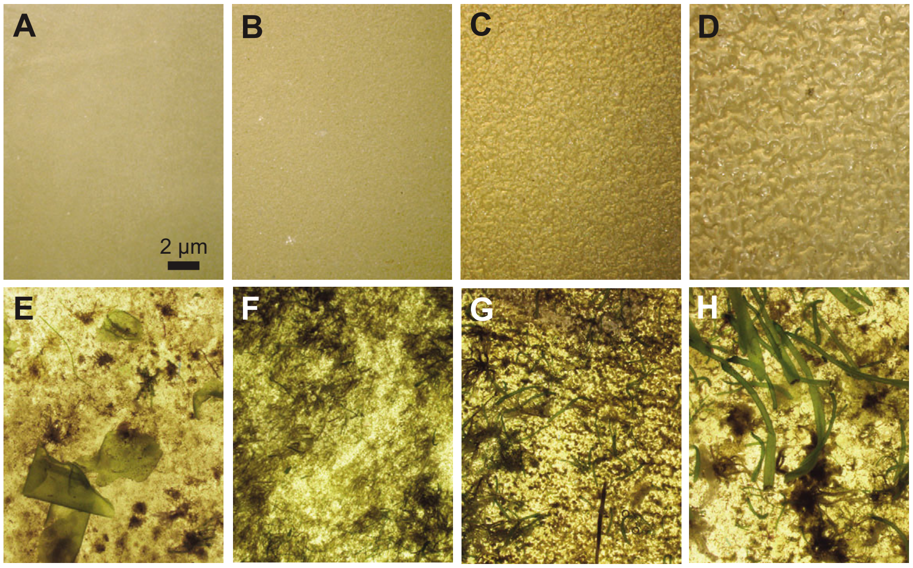

Fig. 3. Substrates of four different types of surface roughness. (A,E) Smooth or grain size 0 µm; (B,F) grain size 35 µm; (C,G) grain size 78 µm; (D,H) grain size 269 µm. The first row (A–D) shows the primary substrates while the second row (E–H) shows the same substrate types after exposure, covered with periphyton (secondary surface structure). The scale is the same for all images.

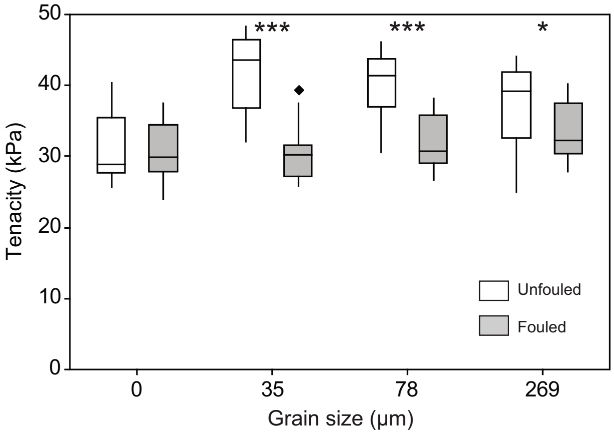

Fig. 4. Tenacity of *G. maeandricus* on four substrates of different surface roughness: unfouled and fouled surface. The impact of fouling on tenacity depended on the surface roughness of the primary substrate. Boxplots show median, upper and lower quartiles, interquartile range and outliers (black diamond). Asterisks indicate level of significance: ***$P<0.001$; *$P<0.05$.

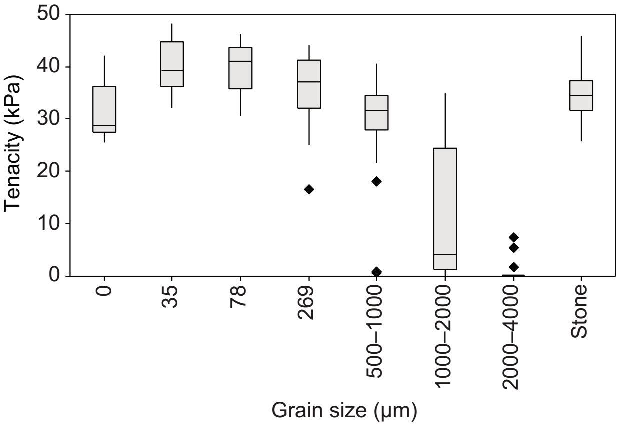

Fig. 5. Tenacity of *G. maeandricus* on seven substrates of increasing surface roughness (smooth to very coarse roughness). Tenacity increased on rough compared with smooth substrates. At a grain size over 500–1000 µm, the tenacity decreased. On substrates with 2000–4000 µm grain size, most specimens failed to cling. Boxplots show median, upper and lower quartile, interquartile range and outliers (black diamonds).

How do the biomechanical properties of the biofilm affect suction adhesion?

In our experiments, these fouled surfaces include both biofilm and periphyton. The resistance of these materials to deformation, characterized by Young's modulus of elasticity (E), has been measured as 100–500 kPa for algae, 50–2000 kPa for bacteria, <600 kPa for soft parts of a biofilm developed in drinking water (>0.5 µm height), and approximately 200 kPa for a freshwater biofilm (Abe et al., 2011; Ditsche et al., 2014; Francius et al., 2008). This range is encompassed by various preparations of agar and is indicative of the low stress needed to stretch or compress the soft biofilm (Nayar et al., 2012). However, aggregates inside the biofilm can cause high variation of E (200–9000 kPa) for the same biofilm, so different components of biofilm will resist different levels of shearing stress (Abe et al., 2011). Nevertheless, EPS secreted by organisms in biofilm have a gel-like structure, which is viscoelastic, making the rate of applied strain a determinant in stiffness. In the context of our experiments, the low stiffness of biofilm combined with the viscoelastic nature of EPS causes the film to act as a lubricant, explaining the decrease in tenacity from unfouled to fouled rough substrates. It is likely that the properties of the biofilm as height and composition can influence the effect of the biofilm on clingfish attachment.

The magnitude, and likely the mechanism, of decrease in tenacity is similar to attaching to a nanoscale smooth surface, with the patterned edges of the disc failing to interlock with surface structure. The reduced friction at the edges of the suction disc will reduce the force needed to create inward slipping of the disc edges, which results in failure at lower stresses. Though we measured a decrease in tenacity with biofilm accretion, it is worth emphasizing that the actual adhesive tenacity was still very high (~150 times body weight).

Does the biofilm have adhesive properties?

Gels can have adhesive properties and are used by many invertebrates for adhesion. However, animal mucus shows a variety of properties, ranging from non-adhesive to highly adhesive (Smith, 2006), and it is difficult to say anything definite about the adhesive properties of the biofilm alone from our experiments. However, adhesion is inherently a two-surface problem and when the surface adhering to the biofilm is a clingfish disc, we found no evidence of increased adhesion for any biofilm on any surface roughness. This was surprising, especially on the smoothest surfaces, because adhesion in these fishes is probably driven in part by viscous resistance to flow between the adhering surfaces. As biofilms have a higher viscosity than seawater, we supposed that under some conditions this would lead to increased total adhesion of the fish. Our results suggest that the reduced friction of the biofilm is more important than the increased viscosity.

Fouling and surface roughness

Two competing processes affect surface roughness as a biofilm develops. The deposition of EPS smooths rough surfaces by filling in the 'valleys', but the growth of macroalgae and the settlement of invertebrates increase surface roughness (Ditsche et al., 2014). Compared with the surface irregularities of the primary substrates, the surface irregularities caused by macroalgae can be considerably larger (Fig. 3). The clingfish's flexible suction disc and hierarchical microstructures can adapt to these surfaces if a few flexible macroalgae are present. However, if too many higher macroalgae were growing on the surface, the fish was unable to cling onto the surface (P.D., personal observation). Our observation is in accordance with the field observations for the sister species *Gobiesox barbatulus*, which prefers habitats with little or no periphyton and macroalgae (Pires and Gibran, 2011).

Furthermore, the development of biofilm is influenced by the properties of the substratum (Donlan, 2002). For example, biofilm has been found to grow faster on rougher surfaces (Characklis et al., 1990). In our study, faster biofilm growth may have induced faster growth of macroalgae on rougher surfaces. This impact of surface roughness on fouling growth can affect the comparability between different surfaces. Nevertheless, this effect is unavoidable if we want to test the substrates under the same conditions. Moreover, we generally have to be aware of the large heterogeneity of species composition and density on fouled surfaces in nature. Natural substrates are certainly variable in fouling growth, which may influence properties such as surface structure, elasticity and hardness. Despite this, a layer of EPS will be present in substrates covered with biofilm and algae. Therefore, we expect our perceived

Table 1. Percentage of northern clingfish adhering to surfaces of different surface roughness for different size classes

	Grain size of substrate (µm)						
Length of fish (mm)	0	35	78	269	500–1000	1000–2000	2000–4000
<60 (*N*=2)	100	100	100	100	50	0	0
60–80 (*N*=7)	100	100	100	100	100	20	0
80–100 (*N*=11)	100	100	100	100	100	100	9
>100 (*N*=3)	100	100	100	100	100	100	100

trend of decreased tenacity on fouled rough surfaces compared with unfouled surfaces to hold true. On substrates heavily fouled by macroalgae or invertebrates, the effect on suction adhesion may differ.

The effect of surface roughness on suction adhesion

We found an adhesive threshold for surface roughness, which is dependent on fish size. Adaptation of the adhesive disc to the surface irregularities can happen on different hierarchical layers: (1) adaptation of the elastic disc margin to large surface irregularities; (2) adaptation of the papillae on the disc margin to surface irregularities in the range of a few 100 µm; (3) adaptation of the rods to the surface irregularities in the range of a few micrometers; and (4) adaptation of the filaments on the tips of the rods to surface irregularities in the range of a few 100 nm. The adaptation to surface irregularities at different size scales allows good sealing of the disc

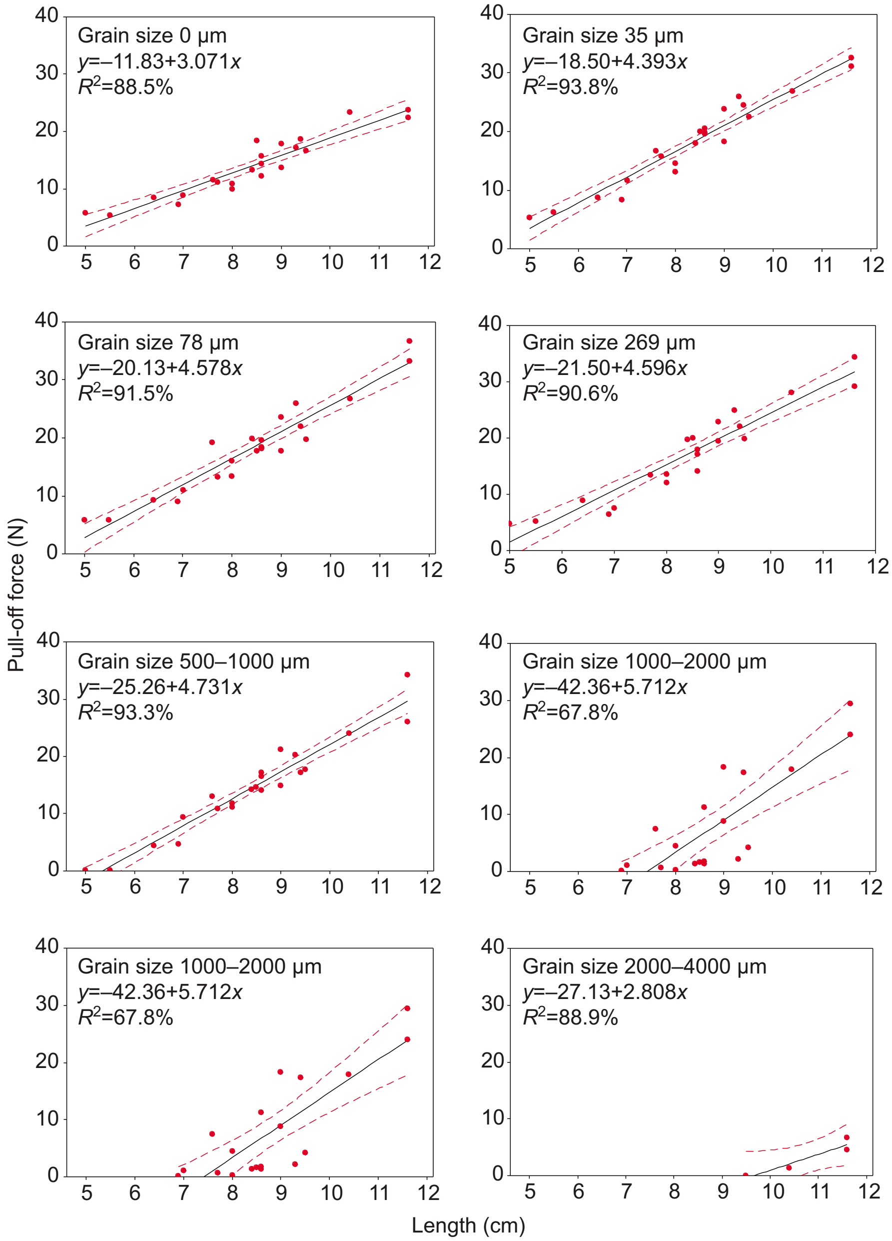

Fig. 6. Pull-off force of *G. maeandricus* versus fish length on seven substrates of increasing surface roughness. Regression lines are shown with 95% confidence intervals.The pull-off force shows a strong dependency on fish length. The intersection of the regression line with the *x*-axis increases with the surface roughness. This value shows the minimum length needed by the fish specimen to cling to a substrate of a certain surface roughness.

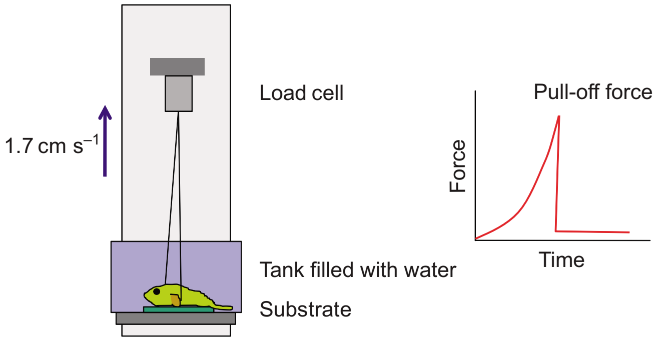

Fig. 7. Experimental design for force measurements. The diagram (left) shows a test specimen connected to a material testing machine. The graph (right) shows an example force–time curve.

margin. However, it also causes an increased area of real surface contact, which increases friction with the support. It is likely that the adaptation in the first hierarchical layer is the one that fails at a certain grain size. The gaps between the single grains become too deep to be overcome by the adaptation of the elastic disc margin.

We showed that northern clingfish with a disc diameter of 13 mm could attach to surfaces up to a grain size of 269 µm, but not rougher. Larger fishes, with a disc width of 34 mm, can attach to surfaces with a grain size between 2000 and 4000 µm. Considering the attachment limit in terms of the ratio of grain size to disc width, we found that fish can attach with significant tenacity to surfaces with a grain size of 2–9% of the disc diameter. We can use this to explore attachment in a comparative context as well as make predictions about attachment surfaces for manufactured suction devices based on the clingfish morphology. The largest species of clingfish, found in South America and South Africa, reach lengths of 34 cm. On the basis of a disc diameter of 66 mm for a 22-cm-long specimen of *Sicyases sanguineus* (K. W. Conway, personal communication), we predict that these fish could stick to fully wetted surfaces with a grain size on the order of 6 mm or more. This corresponds to the roughest sandstones. Furthermore, a manufactured disc just 15 cm in diameter could stick to surfaces with a grain size of 1.3 cm. This corresponds to the roughest surfaces on the exterior of buildings and even natural structural surfaces such as tree bark.

MATERIALS AND METHODS

Animals

We collected specimens of *Gobiesox maeandricus* in the intertidal region of San Juan Island, Washington, USA, and transported the live fish immediately to sea tables in a flow-through system. Directly before the adhesion measurement, each specimen was euthanized with MS-222 and length and mass were determined. The procedures used in this study were approved by an Institutional Animal Care and Use Committee protocol at the University of Washington.

Substrates

We created four substrates of different surface roughnesses using a molding technique. In contrast to natural substrates, they provide not only a homogeneous surface roughness, but also identical material properties because all surfaces were created with the same material. We used a mold made from a smooth glass surface and three different types of sandpaper (P320, P180 and P60; Buehler® Carbimet, Lake Bluff, IL, USA, matching grain sizes of 35, 78 and 269 µm) using dental wax (President Light Body, Coltene Whaledent, Lagenau, Germany), and then cast surfaces in epoxy resin (Low Viscosity Spurr Kit, SPI Supplies®, West Chester, PA, USA) in accordance with Koch (Koch et al., 2008). To let fouling organisms grow on the substrates, we exposed 10 surfaces of each roughness type (primary surface roughness) for a time span of 6 weeks (June–July) in the Salish Sea at Friday Harbor Laboratories, WA, USA. For adhesion experiments, we compared unfouled and fouled substrates. Unfouled substrates were created using the same method, without the exposure to the ocean.

Additionally, we produced three coarser sandpapers to investigate the upper threshold of surface roughness the fish can adhere to. Because our roughest sandpaper was not rough enough, we glued sand and little stones of grain sizes 500–1000 µm, 1000–2000 µm and 2000–4000 µm to cardboard and made molds of these surfaces using the steps outlined above. For comparison of these artificial substrates with a natural surface structure, we collected a single large rock that a clingfish had been found sticking to and molded the surface in the manner described previously. Here, different orders of roughness overlap each other, so that it cannot be assigned to a particular grain size.

Adhesive force

Adhesive forces were measured with a MTS Synergie 100 materials testing system (Cary, NC, USA) using a 500 N load cell. We built a tank that allowed surfaces to be easily interchanged on the bottom; this tank was mounted under the crosshead of the MTS materials testing machine and filled with seawater during testing. Fish were harnessed by threading suturing thread through the opercular gill openings and through the body of the fish above the suction disc, thereby creating a loop on either side of the specimen. The loops were hung on the moving crosshead attached to the MTS machine (Fig. 7; supplementary material Movie 1). Before starting the test, we pressed the fish gently onto the substrate to ensure adhesion (preliminary experiments had shown that higher push-on forces did not lead to higher attachment forces). Tests were run with the crosshead moving at a constant speed of 1 m min^{-1} and force was continually recorded at 500 Hz. Under natural conditions, speed might vary in a large range and both lower and higher speeds might occur, but for data comparison we had to define a specific speed. Adhesive force was measured in the plane perpendicular to the surfaces. The maximal adhesive force of the suction disc is the pull-off force, also called force at failure, which we used for further calculations. The disc always had some amount of mucus that was removed during a failure event. The first trials usually showed a bit higher adhesive forces compared with the following ones. The higher viscosity of the mucus probably causes a better sealing of the disc margin and, therefore, a delayed detachment and higher adhesive forces. To reduce the effect of mucus and achieve a better comparability of our data, we discarded the first three trials for each fish. For each specimen, all of the surfaces were tested in random order. This was done three times, so there were three maximal adhesion events for every specimen–surface pair. The highest pull-off force of the three performed trials for each surface was chosen for analysis because we are interested in maximal and not average performance. We assume the maximum tenacities measured for euthanized fish can be used as a conservative minimum of what is possible in a live fish, and there are data to suggest that live fish can perform no more than 30% better than dead ones (Arita, 1962). Fifteen specimens were measured to investigate the impact of fouling, and 22 specimens were measured to investigate the size effect.

Imaging

Before adhesion measurements, we took photographs of the ventral side of each fish. We then determined the area of the adhesive disc in ImageJ (National Institutes of Health, http://rsbweb.nih.gov/ij/). Images of the substrates were taken with a Zeiss Discovery V.20 Stereo microscope in combination with AxioCam HRc and the Software AxioVision (both Zeiss, Jena, Germany).

Data processing

In suction adhesion, the pull-off force depends on the size of the adhesive disc (Smith, 1991). Thus, we calculated tensile stress, or tenacity (P_{ad}), from pull-off force (F_{ad}) and the surface area of the adhesive disc (A):

$$P_{ad} = \frac{F_{ad}}{A} . \qquad (1)$$

Statistical analyses were performed using the software MINITAB 14. The distribution of the data was normal and homoscedastic, so *t*-tests were used to compare the means of unfouled and fouled surface pairs for each roughness type. To compare tenacity on different kinds of surface roughness, we used an ANOVA. We calculated least squared regression lines to investigate the dependency of the pull-off force on the size of the adhesive disc and fish length. ANCOVA was used to compare the relationship between tenacity and fish length on different levels of surface roughness.

Acknowledgements

We thank Dr Thomas F. Mumford (University of Washington) for help with the identification of the algae on the fouled substrates. Kevin Conway (Texas A&M University) and Maha Mahadevan (Harvard University) were excellent sounding boards for the concepts we are tackling.

Competing interests

The authors declare no competing financial interests.

Author contributions

Conception: P.D. and A.S. Design: P.D., A.S. and D.K.W. Execution: P.D. and D.K.W. Interpretation of the findings: P.D. and A.S. Drafting and revising the article: P.D. and A.S.

Funding

The German Academic Exchange Service (DAAD, D/12/40258) and the National Science Foundation (IOS-1256602) financially supported this study. The Seaver Institute also provided funding.

Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.100149/-/DC1

References

Abe, Y., Polyakov, P., Skali-Lami, S. and Francius, G. (2011). Elasticity and physico-chemical properties during drinking water biofilm formation. *Biofouling* **27**, 739-750.

Arita, G. S. (1962). *A Comparative Study of the Structure and Function of the Adhesive Apparatus of the Cyclopteridae and Gobiesocidae*. MSc thesis, University of British Columbia, Vancouver, BC, Canada.

Budney, L. A. and Hall, B. K. (2010). Comparative morphology and osteology of pelvic fin-derived midline suckers in lumpfishes, snailfishes and gobies. *J. Appl. Ichthyol.* **26**, 167-175.

Characklis, W. G., McFeeters, G. A. and Marshall, K. C. (1990). Physiological ecology in biofilm systems. In *Biofilms* (ed. K. C. M. William and G. Characklis), pp. 341-394. New York, NY: Wiley and Sons.

Denny, M. and Gaines, S. (2007). *Encyclopedia of Tidepools and Rocky Shores*. Oakland, CA: University of California Press.

Ditsche, P., Michels, J., Kovalev, A., Koop, J. and Gorb, S. (2014). More than just slippery: the impact of biofilm on the attachment of non-sessile freshwater mayfly larvae. *J. R. Soc. Interface* **11**, 20130989.

Ditsche-Kuru, P., Koop, J. H. E. and Gorb, S. N. (2010). Underwater attachment in current: the role of setose attachment structures on the gills of the mayfly larvae *Epeorus assimilis* (Ephemeroptera, Heptageniidae). *J. Exp. Biol.* **213**, 1950-1959.

Dobretsov, S. (2002). Inhibition and introduction of marine biofoulng by biofilms. In *Marine and Industrial Biofouling* (ed. H.-C. Flemming, P. S. Murthy, R. Venkatesan and K. E. Cooksey), pp. 293-314. Berlin: Springer.

Donlan, R. M. (2002). Biofilms: microbial life on surfaces. *Emerg. Infect. Dis.* **8**, 881-890.

Francius, G., Tesson, B., Dague, E., Martin-Jézéquel, V. and Dufrêne, Y. F. (2008). Nanostructure and nanomechanics of live *Phaeodactylum tricornutum* morphotypes. *Environ. Microbiol.* **10**, 1344-1356.

Fulcher, B. A. and Motta, P. J. (2006). Suction disk performance of echeneid fishes. *Can. J. Zool.* **84**, 42-50.

Gerstner, C. L. (2007). Effect of oral suction and other friction-enhancing behaviors on the station-holding performance of suckermouth catfish (*Hypostomus* spp.). *Can. J. Zool.* **85**, 133-140.

Green, D. M. and Barber, D. L. (1988). The ventral adhesive disc of the clingfish *Gobiesox maeandricus*: integumental structure and adhesive mechnisms. *Can. J. Zool.* **66**, 1610-1619.

Hadfield, M. G. (2011). Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Ann. Rev. Mar. Sci.* **3**, 453-470.

Koch, K., Schulte, A. J., Fischer, A., Gorb, S. N. and Barthlott, W. (2008). A fast, precise and low-cost replication technique for nano- and high-aspect-ratio structures of biological and artificial surfaces. *Bioinspir. Biomim.* **3**, 046002.

Maie, T., Schoenfuss, H. L. and Blob, R. W. (2012). Performance and scaling of a novel locomotor structure: adhesive capacity of climbing gobiid fishes. *J. Exp. Biol.* **215**, 3925-3936.

Maki, J. S. and Mitchell, R. (2003). Biofouling in the marine environment. In *Encyclopedia of Environmental Microbiology* (ed. G. Bitton), pp. 610-619. New York, NY: Wiley and Sons.

Nayar, V. T., Weiland, J. D., Nelson, C. S. and Hodge, A. M. (2012). Elastic and viscoelastic characterization of agar. *J. Mech. Behav. Biomed. Mater.* **7**, 60-68.

Pennisi, E. (2012). Clingfish stick like geckos. *Science* **335**, 277.

Pires, T. H. S. and Gibran, F. Z. (2011). Intertidal life: field observations on the clingfish *Gobiesox barbatulus* in southeastern Brazil. *Neotrop. Ichthyol.* **9**, 233-240.

Railkin, A. (2004). *Marine Biofouling, Colonization Processes and Defenses*, 376 pp. Boca Raton, FL: CRC Press.

Reinhardt, U. G., Eidietis, L., Friedl, S. E. and Moser, M. L. (2008). Pacific lamprey climbing behavior. *Can. J. Zool.* **86**, 1264-1272.

Roberts, T. R. (1998). Systematic revision of the balitorid loach genus *Sewellia* of Vietnam and Laos, with diagnoses of four new species. *Raffles Bull. Zool.* **46**, 271-288.

Scherge, M. and Gorb, S. N. (2001). *Biological Micro- and Nanotribulogy*. Berlin: Springer.

Schoenfuss, H. L. and Blob, R. W. (2003). Kinematics of waterfall climbing in Hawaiian freshwater fishes (Gobiidae): vertical propulsion at the aquatic–terrestrial interface. *J. Zool.* **261**, 191-205.

Smith, A. M. (1991). Negative pressure generated by octopus suckers: a study of the tensile strength of water in nature. *J. Exp. Biol.* **157**, 257-271.

Smith, A. M. (2006). The biochemistry and mechanics of gastropod adhesive gels. In *Biological Adhesives* (ed. A. M. Smith and J. A. Callow), pp. 167-180. Berlin: Springer.

Wainwright, D. K., Kleinteich, T., Kleinteich, A., Gorb, S. N. and Summers, A. P. (2013). Stick tight: suction adhesion on irregular surfaces in the northern clingfish. *Biol. Lett.* **9**, 20130234.