



A systematic investigation into the effect of fibrillar microstructures on the settlement and attachment strength of the bay barnacle *Balanus improvisus* under natural conditions

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Abstract

Barnacles are one of the most prominent hardfouling organisms in the marine environment. They are able to adhere efficiently to nearly every surface underwater including artificial ones like ship hulls and maritime installations. This overgrowing can lead to huge economical costs. Previous studies have shown that specific microstructure types including micropillars can reduce the initial settlement of barnacles. However, it is not clear how adult barnacles are influenced by microstructured surfaces and whether microstructures can even decrease the resulting adhesion strength of them under natural conditions. Therefore, the aim of this study was to systematically investigate the influence of height, diameter, aspect ratio and flexibility of fibrillar microstructures made from polydimethylsiloxane (PDMS) on initial settlement of barnacles as well as the permanent attachment of adult ones. Micropillars with three different heights (50 μm , 100 μm , 200 μm) and two different diameters (25 μm , 50 μm) were exposed to the Baltic Sea for 12 weeks. On a weekly basis, all barnacles (*Balanus* [= *Amphibalanus*] *improvisus*) were tracked individually to calculate the release-to-settlement ratio and to capture the average attachment duration prior to detachment. The results have shown that with increasing height, both initial settlement and fouling density development were reduced. An increase of diameter showed a similar relationship but at a much smaller scale. All tested microstructures decreased the detachment rates of barnacles from the surface compared to a flat PDMS control. However, they appear to complicate the development of a strong adhesive joint in the young adult phase. Some grade of flexibility in the microstructures appeared to increase the fouling retention. The results shed light in the interaction between adult barnacle adhesion and microstructures and may help in the development of new antifouling technologies.

Keywords Adhesion · Microstructure · Barnacle · Marine bioadhesives · Biofouling

1 Introduction

Water is an excellent solvent for many inorganic and highly nutritious organic matters. The permanent presence of food in the water column is the reason why many heterotrophic organisms can live sessile by feeding on filtration [50]. Therefore, diverse communities have developed on surfaces

submerged into sea water and contribute to a healthy environment as they supply nutrients for higher trophic stages [33]. This process of overgrowing a surface is called biofouling [12]. Representatives of different kingdoms of organisms including bacteria, animals and plants are involved [48]. To add to the complexity, over 4000 different species are known to settle on man-made structures, but it is assumed that the actual number of relevant fouling organisms is much higher [52].

Suitable living space is scarce in the marine environment, so that sessile marine organisms, such as mussels or barnacles compete with each other for space on the substrate. Normally, sessile organisms use glue-like adhesives to attach to substrates permanently. The overgrowing of other attached organisms, also called epibiosis, occurs frequently in nature and can have negative impacts on the survival of the overgrown organisms (basibionts) [19, 48]. Thus,

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complex defense systems have evolved that are capable to deter a wide range of fouling organisms. They may derive from behavioral (burrowing [35]), chemical (production of toxicants [38]), mechanical (shedding of the outer layer [28], mucus secretion [49]) or physical adaptations (changing the topography of the outer layer [8]) and are often interconnected with each other.

Especially, the physical adaptations of marine organisms have been studied in the scientific community, to find innovative, non-toxic solutions against biofouling on man-made surfaces [45]. For example, the periostracum of some mussels develops homogenous parallel aligned micro-ridges with wavelengths ranging between 0.8 and 2.0 μm which decrease biofouling to some degree [42, 43]. Some sponges (*Pheronema*) produce meshes of tapered mineralized spicules that discourage diatoms and small crustaceans from settling [19]. Egg cases of the dog fish *Scyliorhinus canicula* have a microstructured topography with a mean roughness of 3.7 μm [18] and shall be able to resist macrofouling up to 6 months in field tests.

Inspired by topographic features found in nature, many artificial topographies including bumps, grooves, pyramids, v-shaped structures, meshes, pillars and holes (e.g., honey combs) ranging from the macro- to nano-size have been tested in the last decades (see review by [14, 32]). Some of these microstructured surfaces have been patented (e.g., fibrillar structures: [4, 10, 29, 47] and work efficiently against specific biofouling species. The general idea behind the usage of micro-topographies is that they may discourage fouling organisms from settling on these surfaces. Such microstructuring is believed to negatively impact the formation of a reliable adhesive joint at the larval stage and, thus, significantly lowers the resulting adhesion and settlement rate [2, 9]. However, to guarantee protection against a broad spectrum of biofouling organisms, some authors suggest surfaces with hierarchical structures [20, 24]).

Similar to the results obtained from experiments with natural surfaces, artificial surfaces with specific topographies, so far, have only been able to delay initial settlement of organisms, but could not completely inhibit it [14, 32]. For barnacles in particular, it is unclear how microstructured surface topographies influence the adult permanent attachment after settlement. Lately, Petersen et al. [36] have observed that cylindrical, fibrillar microstructures with a height of 50 μm reduce initial settlement compared to a smooth control of the bay barnacle *Balanus* (= *Amphibalanus*) *improvisus* [37] under natural conditions. However, barnacles detached less frequently from these surfaces compared to the smooth control although a material with good fouling release properties (see [30]) was used.

Whether decisions made by juvenile barnacle larvae during the settlement stage influence the attachment strength in the sessile adult stage is unknown especially

for artificial substrates. Aldred et al. [2] have shown under laboratory conditions that cyprid larval settlement positively correlates with their attachment strength on surfaces with varying roughness. However, it is still difficult to make assumptions regarding the permanent adhesion of adult barnacles. Overall, there is little research of the influence of microstructures on adult barnacle adhesion. Therefore, the main aim of this work is to systematically investigate the influence of fibrillar microstructures with different height and diameter on the initial settlement of the bay barnacle *Balanus improvisus* and to put these results into perspective with the permanent attachment in the adult stage under natural conditions. Hexagonally distributed fibrillar microstructures with three different heights (50 μm , 100 μm , 200 μm) and two diameters (25 μm , 50 μm) made from PDMS, a material known to have good fouling release properties [30], were tested in a field experiment in the Baltic Sea for 12 weeks starting from May 2018. The overall fouling dynamics and attachment strength of adult barnacles were evaluated systematically on a weekly basis for each sample (see [36]).

2 Materials and methods

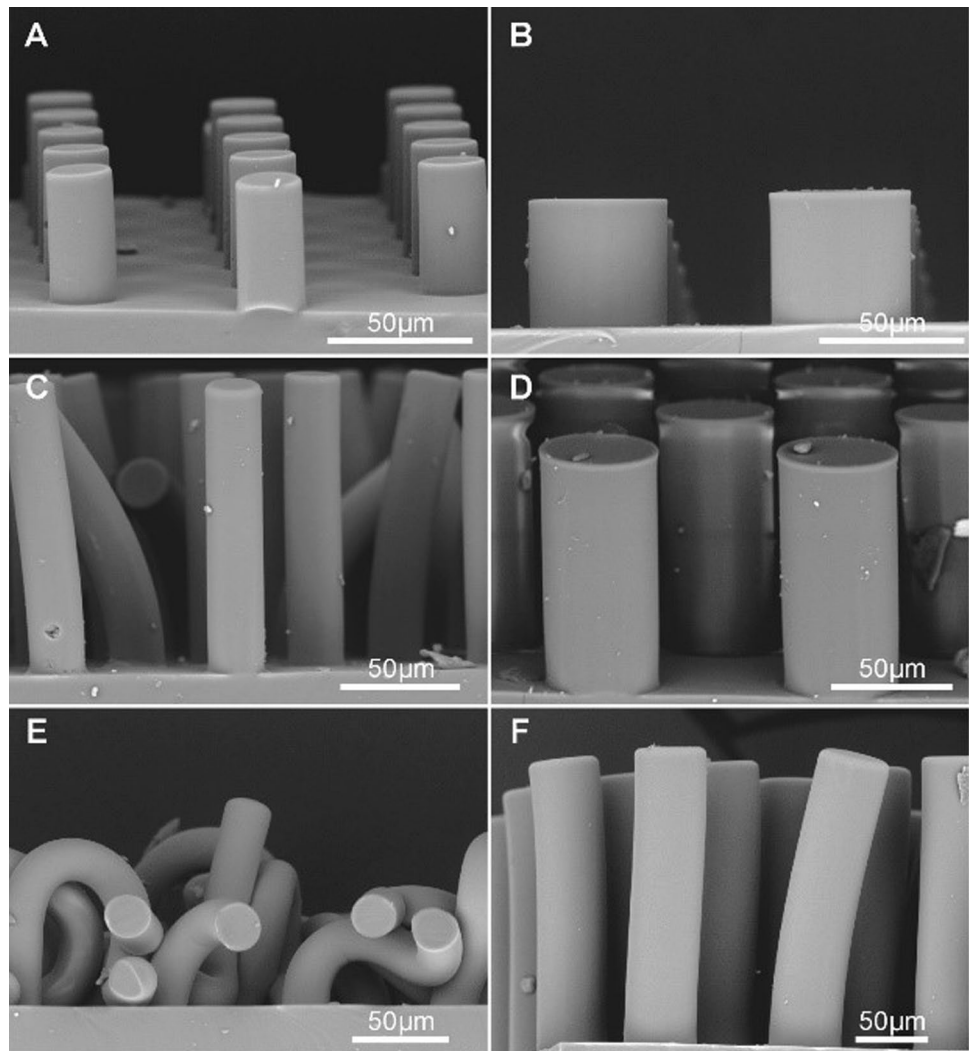
2.1 Sample types and sample preparation

Crystalline silica wafers (Micromotive GmbH, Mainz, Germany) were used as master templates with a pattern-forming photoresist coating, to mould the fibrillar microstructures used in the study. The wafers had holes with two different diameters (25 μm and 50 μm) and three different depths (50 μm , 100 μm , 200 μm). All holes were hexagonally distributed with a distance of 50 μm to each other. The wafer surfaces were silanized with dichlorodimethylsilane in a Büchi V-100 desiccator (BÜCHI Labortechnik AG, Flawil, Switzerland) before polydimethylsiloxane Sylgard 184 (PDMS) (DOWSIL, Midland, MI, USA) was poured. In total, five replicates per treatment were moulded with a size of 8 cm \times 4 cm.

2.2 Sample characterization

Scanning electron microscopy analysis was performed with a Hitachi S-4800 (Hitachi High-Technologies Corp., Tokyo, Japan) to check the integrity of the silicone moulds (Fig. 1). Fibrillar microstructures with a height of 100 μm or 200 μm and a diameter of 25 μm (Fig. 1c, e), as well as fibrillar microstructures with a height of 200 μm and a diameter of 50 μm (Fig. 1f) began to collapse due to their high aspect ratio (see [15]).

Fig. 1 Scanning electron micrographs of the fibrillar microstructures made from PDMS. Left column: 25- μm -diameter pillars. Right column: 50- μm -diameter pillars. **a, b** 50- μm height. **c, d** 100- μm height. **e, f** 200- μm height



2.3 Field experiment

In total, 30 rectangular samples of 8 cm \times 4 cm and five control surfaces (flat polydimethylsiloxane—PDMS) were tested. The samples were fixated within polymethylmethacrylate (PMMA) sample holders by a thin layer of PDMS underneath. 35 PMMA frames with silicone surfaces on top and five frames without silicone were mounted on pipes of acrylic glass (Fig. 2b). The pipes were fixated with zip ties inside a perforated polyethylene (PE) barrel. The barrel was immersed in the Kiel Fjord (54° 22' 16.5" N, 10° 09' 23.9" E) in May 2018 (Fig. 2a). During the field experiment, water temperature ranged between 18 and 21 °C (<https://www.wassertemperatur.org> accessed on 20.10.2018). The protective barrel was used to reduce the influence of water currents and to minimize the impact of predation.

2.4 Evaluation of barnacle settlement and fouling dynamics

Biofouling of the bay barnacle *Balanus* (= *Amphibalanus*) *improvisus* [37] was documented weekly for 12 weeks beginning from May 2018. For this purpose, the samples were shortly removed from the sea water and then photographed individually once a week. During image acquisition, all samples were kept in sea water. Afterwards the samples were re-exposed to the sea. All samples were carefully inspected for the presence of predators that might have led to detachments of barnacles. Individual barnacles were counted, consecutively numbered and tracked throughout the entire time of the experiment. Once a barnacle had been detached from a surface, the time between initial settlement and detachment was monitored (Fig. 3). Several parameters were defined to further describe the fouling dynamics on the tested surfaces.

Fig. 2 Experimental setup. **a** The tested samples were fixated within the blue plastic barrel to minimize predation and the influence of water current. **b** The entire field experiment took 12 weeks. Fouling development was evaluated on a weekly basis

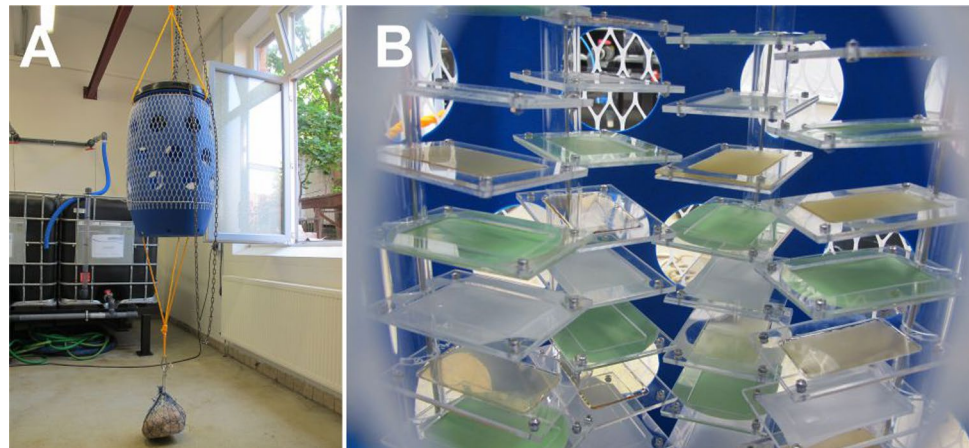
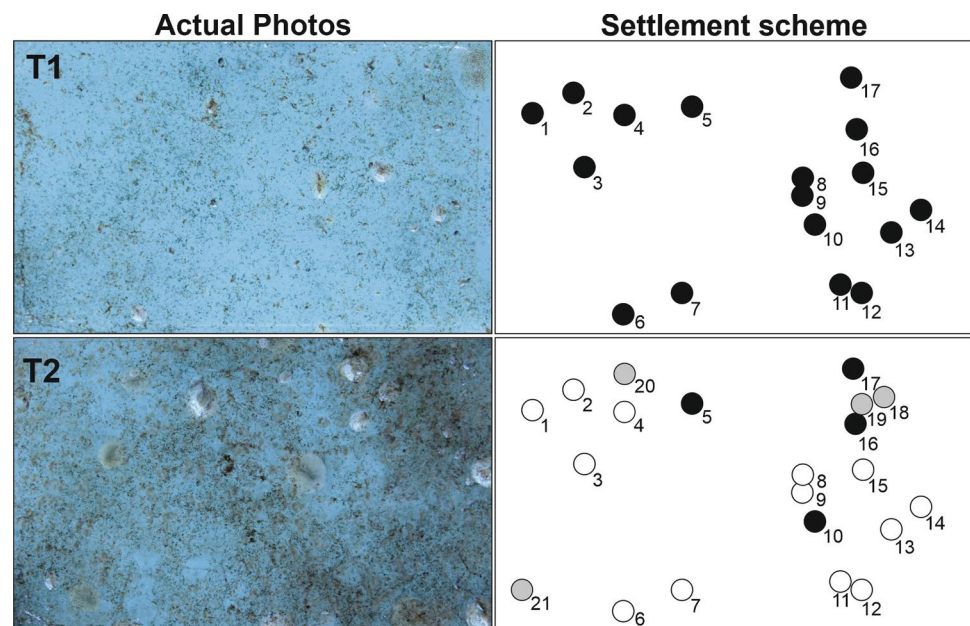


Fig. 3 Actual photos taken during the field experiment and observed settlement scheme. At given T1, all barnacles were counted and indexed (black circles). At a later stage (T2), all detached barnacles were tracked (white circles) and newly barnacles (gray circles) were indexed consecutively



“Actual barnacle density” is defined as the true number of barnacles observed during image acquisitions for each week. “Cumulative barnacle density” is defined as the total number of barnacles observed without subtracting detached barnacles. “Attachment duration” is defined as the time that has passed between initial attachment and possible detachment of barnacles on a surface. “Release-to-settlement ratio” (r/s) is defined as the total number of released barnacles divided by the total number of settled ones. Both attachment duration and r/s are used to quantify the fouling release abilities of the tested surfaces against barnacles. A counting error of 0.1 barnacles per 10 cm² was assumed.

2.5 Statistical analysis

Chi-squared tests were performed, to evaluate an influence of topography on initial and accumulated fouling densities.

The total count of all attached barnacles across all sample types was used to calculate expected barnacle densities, if no difference in fouling accumulation was assumed. A one-way ANOVA on ranks test was performed, to evaluate the influence of topography on average attachment duration prior to release. If significant differences were found, a Holm–Sidak post hoc test was additionally performed. All statistical analyses were executed using SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA). If not stated otherwise, the data were normally distributed and showed homoscedasticity.

3 Results

3.1 General fouling dynamics

The temporal course of settlement and fouling development by *B. improvisus* was monitored for a duration of 12 weeks in the Baltic Sea near Kiel. PDMS samples with fibrillar microstructures having different heights and diameters are abbreviated as h_d , where h is the height of fibers in μm and d is the fiber diameter in μm (i.e., fibrillar microstructures with a height of 50 μm and a diameter of 25 μm are abbreviated by 50_25).

Figure 4a represents the actual barnacle densities recorded (ABD, barnacles per 10 cm^2) for all micropillar samples and the smooth PDMS control. No settlement was observed up to week two. PDMS control, 100_25 and 100_50 were the first samples started to overgrow in week three. From week three on, 50_25, 50_50 and 200_25 also started to overgrow. Apart from 200_25, all samples showed comparable ABDs (4.0–5.0 barnacles per 10 cm^2) with 50_25 having the highest one (5.2 ± 0.1

barnacles per 10 cm^2) in the week five. 200_50 was the last sample type being overgrown after 4 weeks of exposure and also showed the lowest total ABD after 12 weeks. After 5 weeks of exposure, the ABD remained stable at around 1.1 ± 0.1 barnacles per 10 cm^2 until the end of the experiment. 200_25 showed the highest ABD at week five (2.2 ± 0.1 barnacles per 10 cm^2). No big changes were observed until the end of the experiment for 200_25. After 5 weeks of exposure, barnacles had begun to detach in irregular patterns from 50_25 and 100_25. However, the highest ABD on 50_25 was observed in week seven with 6.1 ± 0.1 barnacles per 10 cm^2 . ABDs decreased after 7–8 weeks of exposure 50_50 and 100_50. The PDMS control showed the highest ABD of all sample types with 8.0 ± 0.1 barnacles per 10 cm^2 in week seven. From week seven on, ABDs drastically decreased on the PDMS control and had reached its minimum in week 12 with 2.3 ± 0.1 barnacles per 10 cm^2 . The ABDs observed after 12 weeks of exposure are statistically different between the different sample types (Chi Square Test, $p = 0.001$, $df = 6$, $F = 21.902$).

Figure 4b displays the temporal course of cumulative barnacle densities (CBD, barnacles per 10 cm^2) for each sample type over the duration of 12 weeks. Cumulative barnacle density in this case means that all barnacles that have settled on the samples are considered during evaluation regardless of their detachment time at a later stage. The CBDs during the first weeks were in line with the actual barnacle densities observed in Fig. 4a. From week 8 on, three clustered groups of samples were observed with respect to their CBDs. The first group showed the highest CBDs and consists of the PDMS flat control, 50_25 and 50_50 samples. The development pattern of the CBDs was similar across all three sample types and becomes more flattened towards later weeks. 50_50 showed the highest CBD (12.5 ± 0.1 barnacles per 10 cm^2) followed by the PDMS control (11.0 ± 0.1 barnacles per 10 cm^2) and 50_25 (10.2 ± 0.1 barnacles per 10 cm^2) samples. The second group consists of 100_50 and 100_25. The CBDs were higher compared to the first group with 8.0 ± 0.1 barnacles per 10 cm^2 on 100_25 and 7.5 ± 0.1 barnacles per 10 cm^2 on 100_50. The last group consists of 200_50 and 200_25 samples and showed the lowest CBDs after 12 weeks of exposure. The lowest CBD was observed on 200_50 sample with 3.0 ± 0.1 barnacles per 10 cm^2 after 12 weeks of the experiment. The CBDs observed after 12 weeks of exposure are statistically different between the different sample types (Chi-Square Test, $p = 0.001$, $df = 6$, $F = 23.763$).

Empty sample holders (PMMA) were used as controls for a material with no fouling release abilities. The ABD and CBD values can be found in the supplementary material. No barnacles detached from these surfaces during the field experiment.

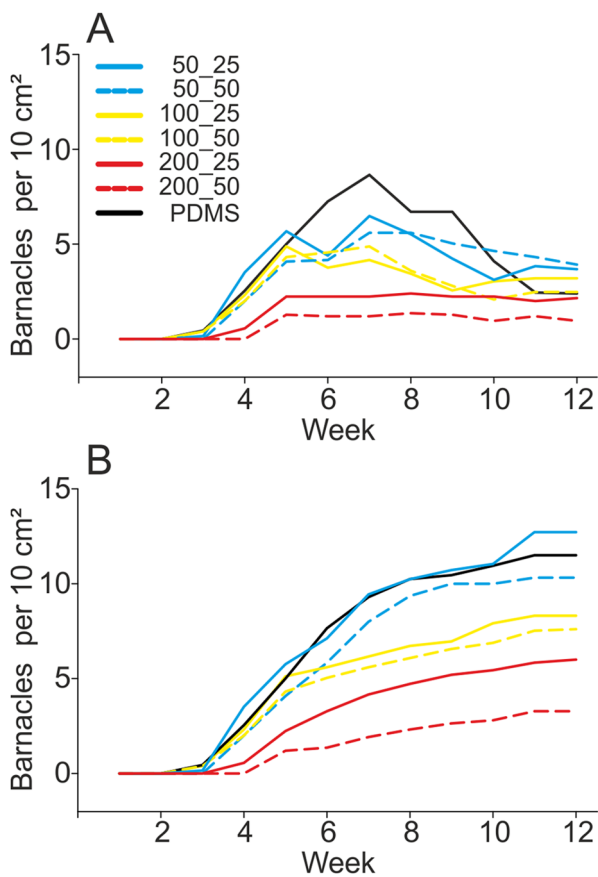


Fig. 4 Settlement dynamics of barnacles per 10 cm^2 . **a** Actual monitored fouling densities. **b** Accumulated fouling densities (CBD) neglecting the detachment time of barnacles

3.2 Release-to-settlement ratio and attachment duration

Release-to-settlement ratios (r/s) were calculated according to Petersen et al. [36]. In brief, the r/s ratio is defined as the total number of released organisms divided by the total number of settled organisms and is used as a quantification tool for the fouling release ability of the surface.

Figure 5 shows the r/s ratio for each sample type. The highest r/s ratio was observed for the PDMS control ($r/s=0.82$). All other surfaces covered with fibrillar microstructures showed a decrease in fouling release ability, ranging from $r/s=0.60$ – 0.72 , if compared to the control. Among structured samples, the highest r/s ratio was observed for 50_25 and 200_50 samples with $r/s=0.72$, the lowest ones for 50_50 and 100_25 samples with $r/s=0.61$. Fibrillar microstructures with fiber heights of 100 μm and 200 μm displayed higher r/s ratios with increasing fiber diameter.

Figure 6 represents boxplots of the attachment duration prior to release for all tested surfaces. Barnacles that had been attached for 1 week only were excluded from the data processing. No distinct differences with respect to the average attachment duration and variance were observed between different sample types except for 200_50. The average attachment duration prior to release ranged between 3 and 4 weeks except for 200_50. The sample 200_50 showed the lowest average attachment duration among all sample types with an average of 2 weeks and an upper 1.5 interquartile range of only 4 weeks.

Figure 7 displays the frequency histograms of the barnacle number in relation to their attachment duration prior to release for different samples. In general, all sample types tended to have a left-skewed distribution. The PDMS control showed a peak at attachment durations of 3–4 weeks with 65 and 55 barnacles, respectively. The

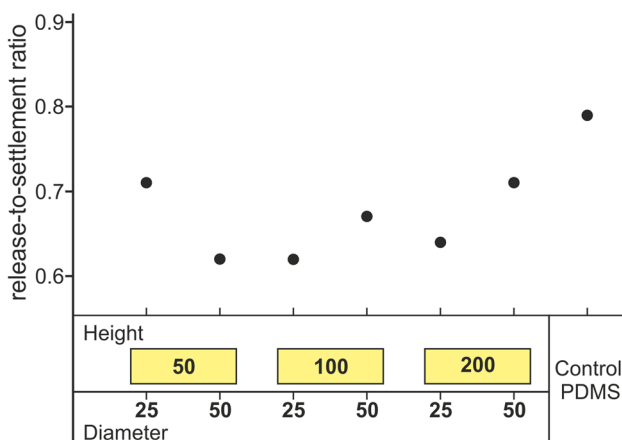


Fig. 5 Release-to-settlement ratios (r/s) for each sample type. The r/s ratio is used as a measure for the attachment strength of adult barnacles

number of barnacles then decreased continuously with an increasing attachment duration up to 9 weeks. The sample 50_25 showed a strong peak at 2 weeks with 65 barnacles followed by a more uniform distribution up to 6 weeks (~ 15 – 20 barnacles per week). Only very few barnacles detached with longer attachment durations. The sample 50_50 did not show such a characteristic peak at 2 weeks, but the overall distribution was comparable to the one observed for 50_25 sample. Both sample types with fibrillar microstructures having a height of 100 μm showed a left-skewed distribution of the same dimension with the highest number of detached barnacles after 3 weeks of exposure (28 barnacles in both cases). It is important to note that more barnacles detached after a longer period of time on the sample 100_25 compared to 100_50. The longest fibrillar microstructures tested (200_25, 200_50) displayed a left-skewed distribution comparable to the other sample types, but at smaller total numbers of individuals. The highest frequency of detached barnacles was observed after 2 weeks with 22 barnacles on the sample 200_25 and 19 barnacles on the sample 200_50. The sample 200_50 was also the only the sample type, on which barnacles have not been detached later than 6 weeks after exposure. Empty sample holder was used as controls with no fouling release abilities during the field experiment (Fig. 7, PMMA). The histogram shows a right skewed distribution with its highest peak at 9 weeks.

4 Discussion

4.1 Initial settlement and fouling dynamics on microstructured samples

The effects of differently sized fibrillar microstructures on the surface exploration patterns have been previously examined under laboratory conditions for barnacle cyprid larvae [16]. It has been shown that the height of fibrillar microstructures was the most important factor leading to changes in the surface exploration pattern and to some degree in their settlement rates. The aim of our study was to take a step further in our understanding of the fibrillar microstructure effects on barnacle settlement. In this study, attachment strength was evaluated indirectly by monitoring the average attachment duration and by calculating the release-to-settlement ratios [36]. Our comparison provides a detailed evaluation of fibrillar microstructure-related effects on hardfouling and their assessment for further use as environmentally friendly antifouling solutions.

Several studies have already shown that microtopography even at a small feature size discourages cyprid larvae from settling (see [14, 32]). In this study, no differences in

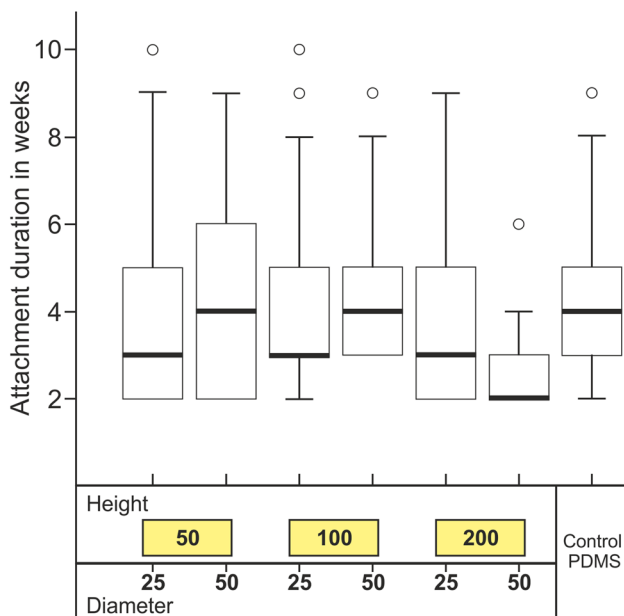


Fig. 6 Boxplot showing the attachment durations for each sample type. Height and diameter are shown in μm

initial settlement between surfaces having fibrillar microstructures and the flat PDMS control in the first weeks were documented with an exception of the longest fibrillar microstructures (200 μm) (Fig. 4). Later, samples, having fibrillar microstructures with a height of 100 μm , showed a decrease in fouling accumulation. Both 50_25 and 50_50 samples did not differ in total cumulative barnacle densities from the flat PDMS control after 12 weeks of exposure. These results deviate from our own data, obtained in the previous field experiment in 2014 with microstructures of similar size, and highlight strong variability of results during field tests [36]. External factors, such as larval density, predation pressure, biofilm formation and presence/directionality of water current are important factors that can shape the fouling density development significantly [27]. Therefore, all conclusions made regarding initial settlement under natural conditions should be always treated with caution.

If the histograms of the barnacles' attachment durations prior to release (Fig. 7) are taken into account, the first 2–3 weeks are the most important time windows with respect to detachment on all microstructured surfaces. By increasing the height of the fibrillar microstructures at a given diameter, they become more flexible, similar to seagrass under action of water currents. This “seagrass effect” could be the reason, why surfaces with longer and slender microstructures become less overgrown over time in this study. Cyprid larvae and recently metamorphosed barnacles might be hindered in developing a strong and reliable joint, because of the dynamically moving nature of such a

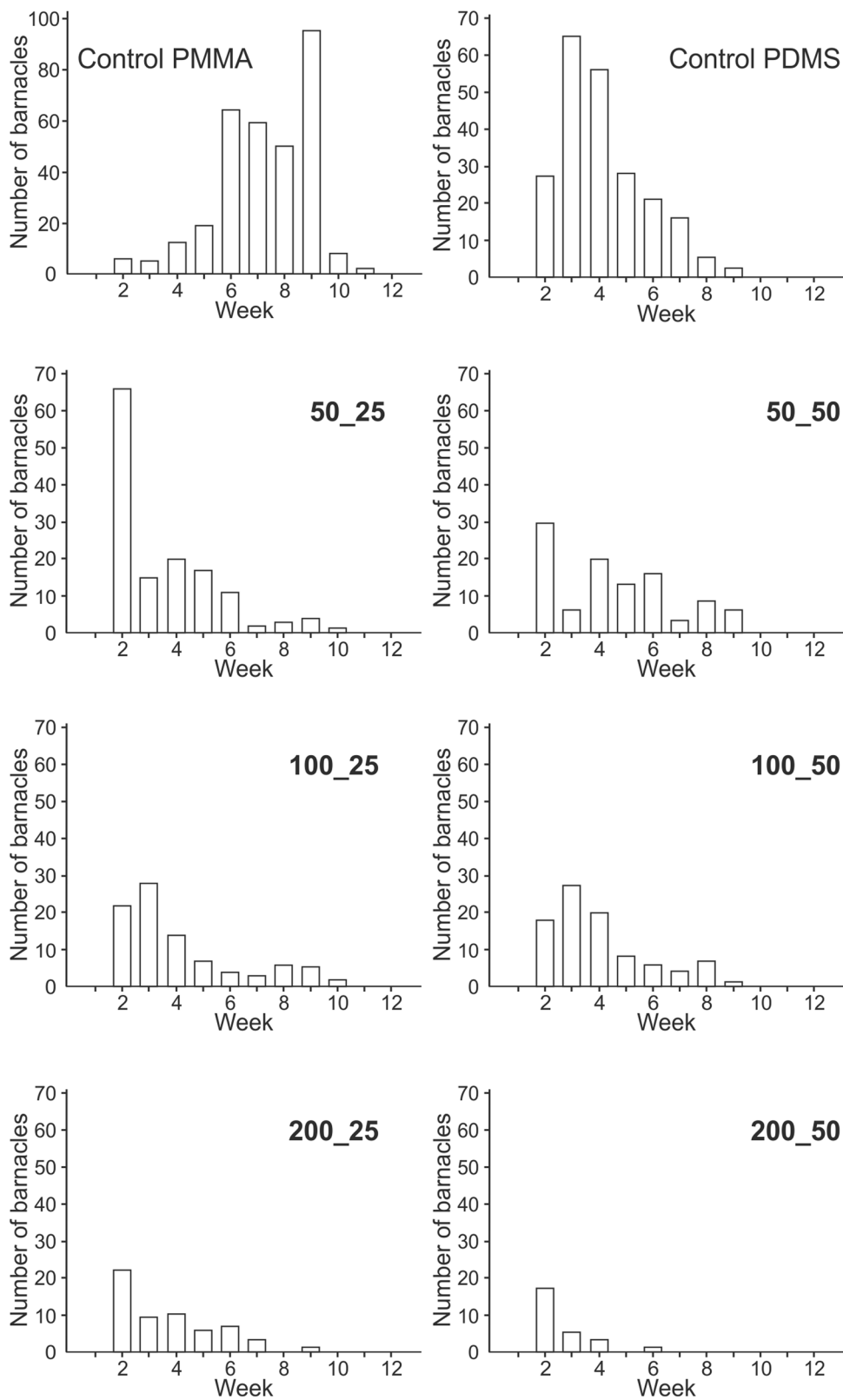
“seagrass effect” surface, due to (1) the potentially incomplete embedding of the surface structures into the barnacle glue and (2) the remaining water at the interface of the adhesive bond that changes the strength of the barnacle adhesive bond. Barnacles are known to produce a white, rubbery adhesive on silicone substrates, [6, 51]. Holm et al. [25, 26] have shown that the development of this white, rubbery adhesive may be linked to the genetics of the organism and also depends on the type of silicone they grow on. However, if the substrate surface microstructures are far too flexible, the negative effect of height on the settlement seems to be reduced. When the microstructures become too flexible, they can be completely bent even at very low water currents. Such a resulting mesh-like configuration of the collapsed fibrillar microstructures provides better surface topography for barnacles to adhere to, thereby increasing settlement and attachment strength.

As earlier stated by Briand [11], results from field experiments performed under natural conditions sometimes do not always support results gained from laboratory experiments. In our study, there was neither information available about the initial cyprid larval density, nor the exploration behavior. Thus, it is rather difficult to interpret the actual initial fouling retention. Nonetheless, it is very clear that the height of the surface microstructure played the most important role in reducing the settlement rate. Additionally, it also strongly influenced the overall fouling dynamics under natural conditions (Fig. 7). If the cumulative barnacle densities (Fig. 4b) are considered, the total settlement correlates inversely with height of the surface features, as indicated by the stacked curves.

The factor diameter shows some effect on the general fouling dynamics, but at a much smaller scale than the height. Only small differences in initial settlement between both diameters can be observed for each tested height (Fig. 4). The larger diameter (50 μm) showed a slight decrease in cumulative barnacle densities, if compared to the smaller diameter (25 μm) for all pillar lengths. However, the reasons for the reduced settlement on the larger diameter remain unclear. According to the literature [13, 44, 46], lower settlement ratios would have been expected on the smaller diameter due to a more difficult contact formation during the cyprid larval phase, because the attachment discs have diameters ranging from 20 to 25 μm [7].

Important to note is that silicone may leach compounds that can alter the curing behavior of barnacle adhesives [40]. This leaching may have also affected the results presented in this study.

Fig. 7 Histograms of attachment duration for each sample type. In addition, PMMA (empty sample holders) is shown as a control surface with no fouling release abilities



4.2 Attachment strength in dependence of height and diameter

The relationship between height, diameter and the r/s ratio, an indicator for the attachment strength, is not trivial. Each tested type of the microstructure increased the attachment strength, as indicated by the lower r/s ratios of microstructured samples in comparison to the flat PDMS control (Fig. 5). From a contact mechanics point of view, this makes perfect sense, because the resulting contact area is increased and mechanical interlocking between the microstructures and the cured barnacle adhesive can additionally take place [36]. The samples 50_25 and 200_50 still have shown rather good fouling release abilities with an $r/s = 0.72$ that is only 0.1 lower than that on the flat PDMS control.

In the present study, samples having fibrillar microstructures with a height of 50 μm showed a lower r/s ratio with an increasing diameter of fibers. As mentioned above, this effect can be explained by the higher resulting contact area and mechanical interlocking. It has been previously shown that barnacles are able to completely embed microstructures with a height of 50 μm [36]. The microstructures with a height of 50 μm do not bend strongly due to their small aspect ratio, which may increase the mechanical interlocking between the glue and substrate.

However, on samples having fibrillar microstructures with heights of 100 μm and 200 μm , samples with the bigger diameter (50 μm) showed better fouling release abilities. Several reasons might have led to these results. It could be possible that barnacles are not able to completely embed the longer microstructures, so that the still uncured glue was in permanent contact with water. Under this circumstance, the resulting adhesive strength might be weakened, due to the water uptake by the glue [1, 39]. Furthermore, the barnacle could not effectively seal the outer edge of the basal plate from the marine environment. If the overbending microstructures are not completely embedded into the barnacle glue, small water inclusions can be developed within the adhesive joint. These water inclusions may generate capillary forces that can lead to detachment of the barnacles during growth.

These hypotheses do not explain why fibrillar microstructures with a bigger diameter show higher r/s values at microstructure heights of 100 μm and 200 μm . It seems that height alone does not necessarily induce an increase or decrease in attachment strength. The resulting r/s ratio may be also dependent on the flexibility of the microstructures. A complete bending even at low external forces (e.g., through the water current or through the normal load exerted by adult barnacles at an early stage of settlement) could help in generating a stronger adhesive joint, because the glue can better embed the fibrillar microstructures and water can be displaced more effectively.

The histograms of the average attachment durations prior to detachment display a general difference between the tested microstructured surfaces and the flat PDMS control (Fig. 7). On the microstructured surfaces, a majority of barnacles has already been detached after only 2 weeks of exposure. On the flat PDMS, most barnacle detachments took place after 3–4 weeks of exposure. This result indicates that the most crucial phase during attachment for barnacles on fibrillar microstructured surfaces is the young adult stage. During this life phase, the fibrillar microstructures seem to hinder the organism to generate reliable adhesive joints probably because the barnacle dewetting ability is reduced on these surfaces [21]. Another possible reason might be that the organism is not capable to produce sufficient amount of glue, to completely embed the microstructures at this life stage. On PDMS controls, these effects do not occur and the detachment of barnacles after 3–4 weeks is attributed to the weak adhesion between surface and barnacle adhesive [30].

4.3 The relationship between cyprid larvae settlement and attachment strength of adult barnacles

Most research investigating the influence of surface topography/microstructure on the attachment of barnacles has been restricted to the settlement of cyprid larvae [14, 30, 32]. Cyprid larvae are equipped with chemoreceptors and mechanoreceptors on their antennules [34] and are able to sense chemical, physical and biological cues during surface exploration prior to settlement [3, 31]. This so-called “search and settlement” stage is one of the most crucial phases in the life of barnacles, because later for the rest of their life as adults, they remain sessile [5, 17, 41].

Several studies have shown that cyprid larvae are discouraged from settling on surfaces with microstructures in laboratory experiments (for detailed review, see [14, 32]). It is believed that cyprid larvae actively reject surfaces [41] and that attachment strength prior to metamorphosis may be the key factor in the decision-making [2]. Although no actual cyprid larvae rejection rates were monitored in our study, the histograms of the attachment durations prior to detachment (Fig. 7) suggest that especially young adult barnacles have issues staying attached in the first 2 weeks on fibrillar microstructures. Limitations in the contact formation, difficulties with biological dewetting of the surface [21, 22] and the shortage of adhesive for the complete embedment of the fibrillar microstructures may be the reasons. However, after surpassing this critical phase, adult barnacles attach much stronger to surfaces covered by fibrillar microstructures than to smooth surfaces, as indicated in our study by the lower r/s ratios, possibly

due to an increased mechanical interlocking between adhesive bond and substrate [23]. Materials with strong fouling release ability, such as silicones [30], may not only increase [36], but also lose their properties against biofouling in combination with a microstructure. Also based on the results of this study, one may conclude that behavioral rejection of fibrillar microstructures by cyprid larvae is not directly linked to the resulting attachment strength of adult barnacles at later stages of their life.

5 Conclusions

The presence of fibrillar microstructures on surfaces can greatly affect the initial settlement of barnacles and their fouling density development. The height of microstructures was found to reduce both initial settlement and fouling density development. An increase of the diameter also has shown a reduction in initial settlement but at a much smaller scale. Overall, fibrillar microstructures decrease the fouling release ability of a surface. However, they seem to affect the attachment strength of barnacles differently during their lifespan. Shortly after metamorphosis, fibrillar microstructures appear to complicate the development of a good adhesive joint. Thus, attachment ability of adult barnacles on microstructured surfaces is decreased at this stage. After this critical phase, the attachment strength increases most likely due to the increased contact area and the strong mechanical interlocking between the fibrillar microstructures and the cured adhesive. The bending ability of microstructures seems to be an important parameter influencing the attachment strength. A certain grade of flexibility in the microstructures seems to be beneficial and may reduce settlement and attachment strength. The results shed light on the interaction between initial settlement rates and attachment strengths of barnacles on fibrillar microstructures. A decrease in initial settlement does not necessarily mean that barnacles show weak attachment strengths once they have settled.

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Author contributions Conceptualization: DSP, LH, SNG; Methodology: DSP, LH, Project administration: LH; Investigation: DSP, MS; Visualization: DSP, MS, Original Draft: DSP, MS, Review and editing: LH, SNG.

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