The movement and settlement behaviour of cyprids of *Balanus reticulatus* on the surfaces of the titanium alloys

Ke Chaia,b#, Yaohua Wua,b#, Wei Shia, Dongxia Duan,c, Jinyi Wu a,b and Enhou Hana,b

aInstitute of Corrosion Science and Technology, Guangzhou, China; bSouthern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai, China; cSunrui Marine Environment Engineering Co., Ltd, Qingdao, China

**ABSTRACT**

The motion paths of *Balanus reticulatus* cyprids were similar on all the titanium alloys surfaces. On the parallel grinding surfaces, the temporary attachment duration and the settlement ratio of the cyprids were influenced by the roughness and the composition of the surfaces and correlated positively. The surface roughness could also change the contact area and the numbers of the attachment points of the cyprids in the similar pattern. Consequently, the roughness and the composition of the surfaces regulated the cyprid settlement by the temporary attachment duration. The cross grinding increased the temporary attachment duration but drastically decreased the settlement ratio to 0 compared to the parallel grinding, possibly due to the voids and the drastic decrease of the contact area and the numbers of the attachment points of the cyprids on the cross grinding surface, respectively. The cross grinding therefore significantly reduced the cyprid settlement compared to the parallel grinding.

**ARTICLE HISTORY**

Received 27 August 2021
Accepted 17 October 2022

**KEYWORDS**

*Balanus reticulatus*; cyprids; settlement; titanium alloys

**Introduction**

Marine biofouling refers to the settlement of marine microorganisms like bacteria, diatoms, etc. and marine macroorganisms like barnacles, oysters, etc. on the surfaces of marine facilities such as ships, pipelines, buoys and so on (Lejars et al. 2012; Dobretsov and Rittschof 2020; Uzun et al. 2020). The settlement increases the weight and the maintenance costs of marine facilities, the resistance and the fuel consumption of ships and the corrosion of many metals and decreases the performance and the lifetime of marine facilities (Yebra et al. 2004; de Brito et al. 2007; Schultz et al. 2011). The severe biofouling on the medium-sized navy ship caused the ship a penalty of up to 86% efficiency at the cruising speed (Schultz 2007). Besides, the fouling damages the security of marine facilities and then the ecology.

Barnacles constitute a typical group of marine macrofouling organisms (Berntsson et al. 2000; Clare and Aldred 2009). They can quickly settle and accumulate on the surfaces of materials immersed in seawater (Aldred and Clare 2008). The settlement of barnacles is accompanied by the attachment of other fouling organisms such as diatoms and mussels (Southward et al. 2004; Hadfield 2011). The acids secreted by these fouling organisms around the barnacles accelerate the corrosion of metals in seawater (Kamino 2013). The attached barnacles can bring about crevice corrosion beneath them in many metals, which is derived from the microbial metabolic activities under the barnacles, especially the dead ones, from the inhibition of seawater flow under the base of the barnacles by the barnacle cement and from the oxidation of iron (Fe) by the redox-active, cysteine-rich barnacle cement protein (Eashwar et al. 1992; Koryakova et al. 1995; Neville and Hodgkiess 2000; de Messano et al. 2014; Blackwood et al. 2017; Murugan et al. 2020). Moreover, barnacles live in various sea areas and breed all year round in subtropical and tropical sea areas. To date, barnacles have become one of the most widely used model macrofouling organisms (Clare et al. 1994; Dineen and Hines 1994; Hellio et al. 2004; Clare and Høeg 2008).

In general, the typical barnacle life cycle involves the six nauplius stages, the cyprid stage and the adult stage. The nauplius larvae feed in the last five nauplius stages. Following the metamorphoses of the nauplius larvae into the cyprid larvae, the cyprid larvae...
stop feeding and start to explore the surfaces for settlement. After the settlement, the cyprid larvae metamorphose into the adults (Raman et al. 2013; Guo et al. 2014). Studies have dealt with the settlement of barnacle cyprids on some material surfaces. Petersen et al. (2020) revealed that both the stiffness and the surface free energy of epoxy resin and polyvinylsiloxane played a small role in the settlement of the barnacle Balanus improvisus, while the surface roughness affect the settlement significantly. Aldred and Clare (2008) claimed that the cyprids of Balanus amphitrite would adhere temporarily to the surfaces during the surface exploration before the permanent adhesion. Hills and Thomason (1998) found that the cyprids of the barnacle Semibalanus balanoides were more likely to settle down when the width of the surface texture of polyester casting resin was approximately equal to the cyprid size. However, very little is known about the movement and settlement behaviour of barnacle cyprids on the surface of titanium alloy.

Owing to their high strength, light weight and excellent corrosion resistance (Vanithakumari et al. 2020), titanium alloys have been increasingly involved in marine facilities, such as offshore oil and gas platforms, seawater condensers, etc. (Anandkumar et al. 2019). Nevertheless, the biofouling of titanium alloys especially by barnacles considerably decreases the titanium alloy performances (Burden 2009; Vanithakumari et al. 2017). To clarify the titanium alloy surface characteristics that modulate the settlement of Balanus reticulatus cyprids and the modulation pathways, in this study, a systematic comparison study was carried out to explore the effect of the roughness, the grinding methods and the composition of the titanium alloy surfaces on the movement and settlement behaviour of cyprids of B. reticulatus.

Materials and methods

**B. reticulatus cyprid culture**

The adults of the barnacle, *B. reticulatus*, were collected from intertidal zone of South China Sea (110.39° E, 20.08° N). They were cleaned with fresh seawater and exposed in air in an incubator in the dark for 12 h at 28°C. Then they were immersed in fresh seawater. The larvae released from the brood were induced to gather by light source (Walley 1969; Høeg and Møller 2006). They were confirmed as the nauplii by microscopy and transferred to sterilized seawater in flasks with pipette. The densities of the nauplii were kept to 1 or 2 individuals/mL (O’Connor and Richardson 1994). The nauplii were fed with *Skeletonema costatum* and kept in the dark at 26°C. Meanwhile, they were exposed in natural light twice a day, for 0.5 h every time in the morning and in the afternoon respectively. Under these conditions, *B. reticulatus* nauplii would metamorphose into *B. reticulatus* cyprids within 7 days. *B. reticulatus* cyprids were cultured in sterile seawater filtered by 0.22 μm microporous filter. The day of the metamorphosis of *B. reticulatus* nauplii into *B. reticulatus* cyprids was 0 d. *B. reticulatus* cyprids could be viewed as swimming or floating within the seawater. The floating cyprids were not used in this study. The well-developed and active *B. reticulatus* cyprids were ready for the later experiments (Satuito et al. 1996).

**Materials**

The chemical compositions of the two titanium alloys are given in Tables S1 and S2 (Supporting information). TC4 titanium alloy (Ti-6Al-4V) plates and Ti80 titanium alloy (Ti-6Al-2.8Nb-1.9Zr-1Mo) plates with the dimensions of 30 mm × 30 mm × 2.5 mm were sequentially ground with SiC papers of different grit sizes to produce different surface roughness. For the parallel grinding of the TC4 titanium alloy coupons, the SiC papers of 180 grit, 800 grit and 1500 grit were respectively used for the final grinding. The corresponding ground coupons were named 180pTC4, 800pTC4 and 1500pTC4, which would be used to detect the effect of the surface roughness of the titanium alloy on the movement and settlement behaviour of cyprids of *B. reticulatus*. For the parallel grinding of Ti80 titanium alloy coupons and the cross grinding of TC4 titanium alloy coupons, the SiC paper of 800 grit was used for the final grinding. The corresponding ground coupons were named 800pTi80 and 800cTC4. 800pTC4 and 800cTC4 would be used to explore the effect of the grinding methods of the titanium alloy surfaces on the movement and settlement behaviour of cyprids of *B. reticulatus*. Then, they were degreased with acetone and rinsed with distilled water and ethanol by sonication to remove the grit and dirt, and dried in air aseptically. Sonication is essential for cleaning of structured surfaces to ensure that the patterns formed on the surface do not remain filled with grit or dirt after the cleaning. Subsequently, all the coupons were kept in a desiccator before the measurement.
Measurements of the roughness, the contact angles and the composition of the surfaces

The surface roughness (Ra) of 10 random sites on a coupon of 180pTC4, 800pTC4, 1500pTC4, 800cTC4 and 800pTi80 was measured by a surface roughness meter (MarSurf PS10, Mahr, Germany). The contact angles parallel to the grinding direction and perpendicular to the grinding direction of 10 random sites on a coupon of 180pTC4, 800pTC4, 1500pTC4, 800cTC4 and 800pTi80 were measured by an optical contact angle measurement device (Attension Theta Flex, Biolin, Finland). The surface element composition of 800pTC4 and 800pTi80 were examined in an X-ray photoelectron spectrometer (XPS) (K-Alpha, ThermoFisher, USA). The measurements were carried out in triplicate.

Measurements of the movement and settlement behaviour of the cyprids

The coupon was exposed in 30 mL sterile seawater filtered by 0.22 μm microporous filter. 40 B. reticulatus cyprids were transferred into the seawater. The motion paths, the average step length, the average velocity of B. reticulatus cyprids on the surface of the coupon were measured by a computer-assisted motion analysis system (Thomason et al. 2002). The temporary attachment duration and frequency and the settlement time and ratio of B. reticulatus cyprids on the coupon were also measured. The average step length and the average velocity were respectively the average distance and the average movement velocity between two sequential temporary stop points, while B. reticulatus cyprid walked on the coupon surfaces in a bipedal fashion with the antennules. The temporary attachment was that B. reticulatus cyprid temporarily attached to the surface of the coupon and then left. The temporary attachment duration and frequency were the average duration and the number of times of the temporary attachment in 1 h. The settlement time was the average time from the transfer of B. reticulatus cyprids into the seawater to the metamorphoses of B. reticulatus cyprids on the coupon into the adults. The settlement ratio was calculated by dividing the number of the adults on the coupon by the total number of B. reticulatus cyprids initially added, i.e. 40. The measurements were carried out in triplicate.

Statistical analysis

The quantitative results of the surface roughness and the contact angles of the titanium alloys, the average step lengths, the average velocities, the temporary attachment durations and frequencies and the settlement time and ratios of B. reticulatus cyprids on the titanium alloys were expressed as the mean ± standard error of the mean. One-way ANOVA followed by Tukey post hoc tests was used to make multiple comparisons among these results, respectively. The probability levels < 0.05 were used to indicate significant differences. All statistics were performed using IBM SPSS Statistics 24.0.

Results

As shown in Figure 1, no morphological changes of B. reticulatus cyprids were observed from 0 to 3 d, which suggested that B. reticulatus cyprids did not change morphologically before the settlement.

The roughnesses, the contact angles and the composition of the surfaces

As seen in Table 1, the surface roughness (Ra) significantly increased with the grit size of SiC paper but was not related to the grinding methods and the composition of the titanium alloy surfaces. In Table S3 (Supporting information), the contact angles parallel and perpendicular to the grinding direction of 180pTC4, 800pTC4, 1500pTC4, 800cTC4 and 800pTi80 were at the similar levels. The contact angles of the titanium alloy surfaces therefore did not affect the motion and settlement behaviour of Balanus reticulatus cyprids. The XPS results showed that the surface element composition of 800pTC4 and 800pTi80 was different (Tables S4 and S5) (Supporting information). The 180pTC4, 800pTC4 and 1500pTC4 coupons, the 800pTC4 and 800cTC4 coupons and the 800pTC4 and 800pTi80 coupons could therefore be used to investigated the effect of the surface roughness, the grinding methods and the composition of the titanium alloy surfaces on the motion and settlement behaviour of B. reticulatus cyprids, respectively.

The motion paths, the average step lengths and velocities of the cyprids

Figure 2 shows the motion paths of B. reticulatus cyprids on the titanium alloys. The movement of B. reticulatus cyprids on the titanium alloy coupons was intermittent, which was in agreement with the surface exploration of barnacle cyprids (Eckman et al. 1990; Harder et al. 2001). When B. reticulatus cyprids encountered the titanium alloy coupons, they attached and walked in a bipedal fashion along a straight-line
on a pair of highly specialized antennules. During the straight-line walking, they sometimes actively detached and re-entered into the water column. After walking a short distance, *B. reticulatus* cyprids would stop for a short time. At the stop points, *B. reticulatus* cyprids swung their bodies and turned rapidly. They rarely detached the surfaces and re-entered into the water column in this period, but they could resume the walking. Alternatively, at last, they could attach the surfaces with the both antennules, maintain the positions and settle. Although the single motion path between the two adjacent stop points was straight, the whole motion path of *B. reticulatus* cyprid was not a straight line because almost all the single motion directions of *B. reticulatus* cyprid were different.

Figure 3 shows the average step lengths of *B. reticulatus* cyprids on the titanium alloy coupons. The average step length of *B. reticulatus* cyprids significantly increased from Ra 0.512 \(\mu m\) to Ra 0.198 \(\mu m\) and Ra 0.157 \(\mu m\), slightly decreased from Ra 0.198 \(\mu m\) to Ra 0.157 \(\mu m\) (Figure 3A). In addition, it significantly decreased from the parallel grinding to the cross grinding and from the TC4 surface composition to the Ti 80 surface composition (Figure 3B and C).

The temporary attachment durations and frequencies of the cyprids

As seen in Figure 5A, when the titanium alloy surface roughness was Ra 0.512 \(\mu m\), the temporary attachment duration of *B. reticulatus* cyprids was at the lowest level. It significantly increased at Ra 0.198 \(\mu m\), and then significantly decreased at Ra 0.157 \(\mu m\). It significantly increased from the parallel grinding to the cross grinding and from the TC4 surface composition to the Ti 80 surface composition (Figure 5B and C).

The temporary attachment frequency of *B. reticulatus* cyprids reached the highest level, when the titanium alloy surface roughness was Ra 0.512 \(\mu m\) (Figure 5D). It significantly decreased at Ra 0.198 \(\mu m\) and continuously significantly decreased at Ra 0.157 \(\mu m\) (Figure 5D). The temporary attachment frequencies of *B. reticulatus* cyprids corresponding to the cross grinding and the Ti 80 surface composition were equal to and significantly

---

**Table 1. Roughness of the titanium alloy surfaces.**

| Titanium alloy coupons | Ra (mean ± standard error of mean)/\(\mu m\) |
|------------------------|---------------------------------------------|
| 180pTC4                | 0.512 ± 0.033 \(^a\)                         |
| 800pTC4                | 0.198 ± 0.011 \(^b\)                         |
| 1500pTC4               | 0.157 ± 0.009 \(^c\)                         |
| 800cTC4                | 0.203 ± 0.014 \(^b\)                         |
| 800pTi80               | 0.211 ± 0.019 \(^b\)                         |

The results were presented as the mean ± standard error of the mean. Different letters indicated significant differences (\(p < 0.05\)) among the values. The measurements were carried out in triplicate.
lower than that of the parallel grinding and that of the TC4 surface composition, respectively (Figure 5E and F).

**The settlement time and ratios of the cyprids**

Figure 6 shows the settlement time. It significantly increased from Ra 0.512 μm to 0.198 μm, significantly decreased from 0.198 μm to Ra 0.157 μm (Figure 6A). No settlement was observed on the cross grinding surface in 240 h (Figure 6B). The settlement time was significantly higher than that of the parallel grinding (Figure 6B). For the Ti 80 surface composition, the settlement time significantly lower than that of the TC4 surface composition (Figure 6C).

The settlement ratios of *B. reticulatus* cyprids are shown in Figure 7. The settlement ratio significantly increased from Ra 0.512 μm to 0.198 μm but significantly decreased from 0.198 μm to Ra 0.157 μm (Figure 7A). The settlement ratio for the cross grinding was 0 (Figure 7B). The settlement ratio significantly decreased from the parallel grinding to the cross grinding and significantly increased from the TC4 surface composition to Ti 80 surface composition (Figure 7B and C). The temporary attachment duration of *B. reticulatus* cyprids was positively related to the settlement ratio on the titanium alloy surfaces except the cross grinding surface (Figure 7D).

**Discussion**

The settlement of organisms leads to the fouling of the material surfaces, in which the material surface characteristics and components certainly influence the movement and settlement behaviour of the fouling...
Figure 4. Effect of (A) the roughness (Ra), (B) the grinding methods and (C) the composition of the titanium alloy surfaces on the average velocities of *B. reticulatus* cyprids. The results were presented as the mean ± standard error of the mean. Different letters indicated significant differences (*p* < 0.05) among or between the values. The measurements were carried out in triplicate.

Figure 5. Effect of (A) the roughness (Ra), (B) the grinding methods and (C) the composition of the titanium alloy surfaces on the temporary attachment durations of *B. reticulatus* cyprids and effect of (D) the roughness (Ra), (E) the grinding methods and (F) the composition of the titanium alloy surfaces on the temporary attachment frequencies of *B. reticulatus* cyprids. The results were presented as the mean ± standard error of the mean. Different letters indicated significant differences (*p* < 0.05) among or between the values. The measurements were carried out in triplicate.

Figure 6. Effect of (A) the roughness (Ra), (B) the grinding methods and (C) the composition of the titanium alloy surfaces on the settlement time of *B. reticulatus* cyprids. The results were presented as the mean ± standard error of the mean. Different letters indicated significant differences (*p* < 0.05) among or between the values. The measurements were carried out in triplicate.
organisms on the surfaces. In this work, the contact angles parallel to the grinding direction and perpendicular to the grinding direction of the titanium alloys did not affect the movement and settlement behaviour of the cyprids of *B. reticulatus* on the surfaces of the titanium alloys due to their insignificant differences. Therefore, the present effort focused on the effect of the roughness, the grinding methods and the composition of the titanium alloy surfaces on the movement and settlement behaviour of *B. reticulatus* cyprids.

**Effect of the roughness, the grinding methods and the composition of the surfaces on the motion paths of the cyprids**

Although the surface roughness changed among 180pTC4, 800pTC4 and 1500pTC4 coupons and the grinding methods and the surface composition were different between 800pTC4 and 800cTC4 and between 800pTC4 and 800pTi80 respectively, all the motion paths of *B. reticulatus* cyprids on the coupons were similar arcs which were made up of the straight short motion paths of different directions (Figure 2). The analogous paths were found in the cyprids of *Balanus Amphitrite* and *Semibalanus balanoides* on other surfaces (Prendergast et al. 2008; Aldred et al. 2018). These results revealed that the roughness, the grinding methods and the composition of the titanium alloy surfaces did not affect the motion paths of the cyprids of *B. reticulatus* on the surfaces of the titanium alloys. The pair of antennules is the most important apparatus of barnacle cyprid for the motion and the sensing, with lots of chemo- and mechano-receptive setae (Crisp 1976; Maruzzo et al. 2011). For the settlement sites, the cyprids of *B. reticulatus* could search the wide ranges of the surfaces of the titanium alloys using the antennules in the straight-line walking motion. They might find the acceptable areas at the stop points and continue to detect these smaller areas around them with the body swinging and turning. If the areas were more acceptable, they could proceed to examine the local area and settle with the secretion of cement. This process was consistent with the classic model of barnacle cyprid exploration, in which cyprid explores a large area of surface by the antennules in the straight-line and bipedal walking, that is wide search; then it explores the positive feedback area immediately proximate to the attachment point, namely close search; finally, it inspects the more positive feedback area before the settlement with the cementation, i.e. inspection (Knight-Jones and Crisp 1953; Crisp 1961; 1976). These cyprid exploration
behaviours had been proved by many researches (Lagersson and Høeg 2002; Amsler et al. 2006; Faimali et al. 2006; Maleschlijski et al. 2015; Aldred et al. 2018). In Balanus amphitrite, cyprids could returned to the water column in exploration (Maleschlijski et al. 2015; Aldred et al. 2018). The similar behaviour was also observed in B. reticulatus cyprids in the present study. Generally, young barnacle cyprids repeated wide search, close search and even swimming in water column many times before inspection and settlement (Maleschlijski et al. 2015; Aldred et al. 2018).

Effect of the roughness, the grinding methods and the composition of the surfaces on the average step lengths and velocities of the cyprids

Barnacle cyprid attaches temporarily to the surface by the antennules while it explores the surface in search of the appropriate settlement sites. It has been found that the surface topographies and composition influenced the exploration and the settlement of barnacle cyprids (Schumacher et al. 2007a; Prendergast et al. 2008; Aldred et al. 2010a, 2010b). In B. reticulatus cyprids, decreasing the surface roughness of TC4 titanium alloy significantly increased the average step length and the cross grinding and the surface composition of Ti80 significantly decreased the average step length compared to the parallel grinding and the surface composition of TC4, respectively (Figure 3), which indicated that the surface roughness, the grinding methods and the surface composition of the titanium alloys significantly affected the average step length of B. reticulatus cyprids. The step length changes were also observed in Balanus amphitrite cyprids. The close-range microscopy revealed that the mean step length of Balanus amphitrite cyprids was larger on the hydrophilic bare and NH2-treated glasses than on the hydrophobic CH3-treated glasses (Chaw and Birch 2009). It was worth noting that the 30 μm-high and 5 μm-diameter pillars in polycarbonate decreased the mean step length of Balanus amphitrite cyprids compared to the 5 μm-high and 5 μm diameter pillars and the smooth surface of polycarbonate (Chaw et al. 2011), which was in agreement with the average step length changes of B. reticulatus cyprids with the surface roughness of TC4 titanium alloy in the present study. In addition, the increased settlement was observed on the 30 μm-high and 5 μm diameter pillars compared with the 5 μm-high and 5 μm diameter pillars and the smooth surface, which was contrary to the mean step length changes (Chaw et al. 2011). The increased settlement possibly derived from the cyprid cement secreted into the voids between micropillars, resulting in the stronger attachment in the settlement (Chaw et al. 2011). Nevertheless, the average step lengths of B. reticulatus cyprids were not related to the settlement ratios and the other movement and settlement behaviour (Figures 3–7), which could be attributed to the much smaller scales of the microtextures, the species specific differences in larval responses to surface texture and surface chemistry differences (Berntsson et al. 2000; Prendergast et al. 2008).

The average velocities of B. reticulatus cyprids were between 0.0964 and 0.80 mm/s on the titanium alloys (Figure 4). Similarly, in Balanus amphitrite cyprids, the walking velocity values between 0 and 1 mm/s represented 95% of all velocities on the self-assembled monolayers (Maleschlijski et al. 2015) and the average walking velocity was about 0.1 mm/s on the glass (Lagersson and Høeg 2002). The average velocity results of B. reticulatus cyprids showed that decreasing the surface roughness of TC4 titanium alloy significantly increased the average velocity, though further decreasing the surface roughness would slightly decrease the average velocity (Figure 4A). For Semibalanus balanoides cyprids, the mean velocity reached the maximum on the smooth texture and the minimum on the coarse texture of the resin tile (Prendergast et al. 2008). Yet the most settlement always occurred on the coarse and the least on the smooth texture (Prendergast 2007). Many studies have found that the attachment of barnacle cyprids was increased on the textures above the larval size (Scardino et al. 2008; Crisp and Barnes 1954), which applied to the case of Semibalanus balanoides cyprids (Prendergast et al. 2008). Several researches indicated that the barnacle cyprids moved faster on the surfaces unfavourable to the attachment (Hills et al. 2000; Berntsson et al. 2004; Maleschlijski et al. 2014). Perhaps the smooth texture induced Semibalanus balanoides cyprids to travel faster to abandon the exploration (Prendergast et al. 2008). In the current case, the average velocities of B. reticulatus cyprids did not correlate with the settlement ratios of B. reticulatus cyprids and the other movement and settlement behaviour (Figures 3–7). Comparably, the mean velocities of Balanus amphitrite cyprids were about 0.7 mm/s on glass and poly(carboxybetaine methacrylate) (polyCBMA) but the settlement ratios were 48% and 0, respectively (Aldred et al. 2010a). Though the mean velocities of Balanus amphitrite cyprids were 20% higher on poly CBMA than on poly(sulfobetaine methacrylate) (polySBMA), both of the settlement ratios were 0 (Aldred et al. 2010a). Many researches have proved the microtextures below the size of
barnacle cyprid inhibited the settlement of the cyprids compared to smooth surfaces (Scardino et al. 2008). The experiments showed that the 30–45 μm height topographic surface textures decreased the settlement and recruitment of Balanus improvisus cyprids by 92% as compared to the smooth surfaces (Berntsson et al. 2000). Balanus amphitrite cyprid settlement was reduced by 97% by the barnacle-specific topography (40 μm feature height, aspect ratio of 2) (Schumacher et al. 2007a). Balanus amphitrite cyprids seldom settled on the sinusoidal linear textures of 64–256 μm in polycarbonate and polydimethylsiloxane and did not settle on the texture of 256 μm in a polydimethylsiloxane (Aldred et al. 2010b). The above microtextures were between 30 and 256 μm. When the scales of the microtextures in polycarbonate and polydimethylsiloxane were 0–32 μm, Balanus amphitrite cyprids settled preferentially, nonetheless, the settlement ratios fluctuated (Aldred et al. 2010b). At present, the roughness scales of the titanium alloys were constrained to a smaller range (Ra 0.157 μm–Ra 0.512 μm) (Table 1). The settlement ratios and the average velocities of B. reticulatus cyprids fluctuated more severely and did not match (Figures 4 and 7), which suggested that the average velocities of B. reticulatus cyprids could not serve as an indicator for settlement preferences in this case. The cross grinding and the surface composition of Ti80 significantly decreased the average velocity of B. reticulatus cyprids compared to the parallel grinding and the surface composition of TC4, respectively (Figure 4), suggesting that the grinding methods and the surface composition of the titanium alloys had significant effect on the average velocities of B. reticulatus cyprids.

Effect of the roughness, the grinding methods and the composition of the surfaces on the temporary attachment durations and frequencies and the settlement time and ratios of the cyprids

Besides the average velocities and the average step lengths, the surface topographies and the surface composition also affected some other exploration behaviour of barnacle cyprids. In the present study, for B. reticulatus cyprids, the surface roughness and the surface composition of the titanium alloys significantly influenced the temporary attachment duration and frequency but the grinding methods of the titanium alloys only significantly affected the temporary attachment duration and had no effect on the temporary attachment frequency (Figure 5), suggesting that some exploration behaviour of barnacle cyprids might not be affected by some surface topographies or the surface composition. Similarly, the frequencies of touchdowns by the antennules of Semibalanus balanoides cyprids were not significantly different for the surfaces of four types of self-assembled monolayers (SAMs) and an ultrathin hydrogel coating (Aldred et al. 2011). Interestingly, the cross grinding not only significantly affected the settlement time and the settlement ratio of B. reticulatus cyprids compared to the parallel grinding but also led to no settlement (Figures 6 and 7), suggesting that the cross grinding might override the other surface topographies and the surface composition of the titanium alloys for the effect on the settlement time and the settlement ratio. It therefore might be based on the parallel grinding that the surface roughness and the surface composition of the titanium alloys significantly affected the settlement time and the settlement ratio. Additionally, the temporary attachment frequency and the settlement time of B. reticulatus cyprids did not correlate to the settlement ratio and the other movement and settlement behaviour (Figures 3–7). Comparably, the number of the wild Semibalanus balanoides cyprids arriving on the textured surfaces during the first minute of immersion attained the maximum, the medium and the minimum value on the fine, the smooth and the coarse texture, respectively, which bore no relation to the settlement pattern (Prendergast et al. 2008).

In Semibalanus balanoides cyprids, the frequency and the thickness of footprint deposition of the antennule adhesive similarly varied with the surface chemistry, which was consistent with the barnacle settlement-inducing protein complex (SIPC) binding change except the amine-terminated surface (Aldred et al. 2011). The frequency and the thickness of footprint deposition could be therefore indicatives of the settlement on the different surfaces tested except the amine-terminated surface. Though the average velocity, the average step length and the frequency and the thickness of footprint deposition of barnacle cyprids showed the relations to the settlement in some researches, more data revealed that the time spent exploring, i.e. the temporary attachment duration of barnacle cyprids correlated to the settlement and it might be the easily measured indicator of the settlement of barnacle cyprids. Now, the temporary attachment duration of B. reticulatus cyprids correlated positively to the settlement ratio on the titanium alloy surfaces except the cross grinding surface (Figures 5A–C and 7), which was supported by the following data. In the field-based studies, the wild Semibalanus
balanoides cyprids achieved the maximum value of the time spent exploring on the coarse texture and the minimum value on the smooth texture, which concurred with the settlement pattern (Prendergast et al. 2008). The surface textures with profile heights within a topographic range of 20–100 μm reduced the settlement of Balanus improvisus cyprids as compared to the smooth surface, which could be best explained by the behavioural responses of the cyprids to surface topographies that the cyprids spend more time exploring the smooth surface than the textured surfaces (Berntsson et al. 2000). For Balanus amphitrite cyprids, the time spent on the surface exploring relevant behaviour in the lower swimming regions (LSR, extending from the surface into ≈1.5 mm solution with a high probability of finding cyprids) over the 1-dodecanethiol (DDT) surface and the N,N-trimethyl-(11-mercaptoundecyl) ammonium chloride (TMA) surface was significantly higher than that over the 11-mercapto-1-undecanol (HUDT) surface and the TMA + 12-mercaptoundecanoic acid (MUDA) surface, which correlated positively to the significant higher settlement values on the DDT surface and the TMA surface (Maleschlijski et al. 2014).

The temporary attachment duration and the settlement ratio of B. reticulatus fluctuated consistently with the titanium alloy surface roughness in the range much smaller than the barnacle cyprid antennular discs from Ra 0.157 μm to Ra 0.512 μm (Figures 5A and 7A). The similar results have been reported in some researches. The proportions of settled and metamorphosed Hydroides elegans larvae, Bugula neritina larvae and Balanus amphitrite cyprids fluctuated with the sinusoidal shape microtexture wavelength in the ranges smaller than the three larvae from 0 to 128 μm, from 0 to 256 μm and from 0 to 32 μm, respectively (Scardino et al. 2008; Aldred et al. 2010b). During the surface exploration, B. reticulatus cyprid antennule discs covered with numerous micro-scale cuticular villi temporarily attach to the grinded titanium alloy surfaces with numerous micro-scale irregular valleys by the temporary adhesive secreted onto the disc surfaces. The scales of the cuticular villi and the valleys could match. The antennule discs highly deformed to adapt to the valley profiles (Santos et al. 2005). The cuticular villi therefore filled the valleys and contacted the surfaces of the valleys beneath the discs. According to the concept of the aspect ratio of the surface features proposed by Schumacher et al. (2007a), the contact area beneath the B. reticulatus cyprid antennule discs would increase with the aspect ratio of the valleys. For the parallel grinding TC4 titanium alloy surfaces, the aspect ratio of the valleys might increase from Ra 0.157 μm to Ra 0.198 μm and decrease from Ra 0.198 μm to Ra 0.512 μm. The contact area beneath the B. reticulatus cyprid antennule discs therefore reached the maximum at Ra 0.198 μm and the similar values at Ra 0.157 μm and Ra 0.512 μm, which was consistent with the changes of the numbers of the attachment points beneath the B. reticulatus cyprid antennule discs based on the attachment point theory illustrated by Scardino et al. (2006; 2008). This mechanism could well explain the temporary attachment duration changes of B. reticulatus cyprids with the titanium alloy surface roughness, which led to the changes of the settlement ratio. For the cross grinding TC4 titanium alloy surface of Ra 0.203 μm, the valleys were broken and the voids formed at the crossing sites. The contact area and the numbers of the attachment points beneath the B. reticulatus cyprid antennule discs decreased drastically compared to the parallel grinding TC4 titanium alloy surface of Ra 0.198 μm. Accordingly, the settlement ratio decreased to 0 (Figure 7B). Comparably, the ridges and the pillars had the same widths, depths and intervals on the polydimethylsiloxane elastomer (PDMS) surfaces but the significantly higher numbers of Enteromorpha zoospores settled were found on the surfaces with the valleys between the ridges compared to the surfaces with the voids between the pillars (Callow et al. 2002). Under the same distance between the adjacent protruding micro-squares with the same height on the PDMS surfaces, the surface coverage of E. coli RP437/pRSH103 biofilms was significantly higher on the surface with the valley length of 20 μm than on the surfaces with the valley lengths of 5, 10 or 15 μm, respectively (Hou et al. 2011). Though all the topographies had the feature spacing of 2 μm and the height of 3 μm, the higher mean Ulva spore density was measured on the topography of 2 μm wide ridges with the channels continuous in length compared to the topographies of 2 μm wide ribs of lengths 4, 8, 12, and 16 μm combined, 2 μm diameter circular pillars and 10 μm equilateral triangles combined with 2 mm diameter circular pillars (Schumacher et al. 2007b). However, with the similar surface roughness of the two TC4 titanium alloy surfaces, B. reticulatus cyprids might spend much temporary attachment duration to identify the voids on the cross grinding TC4 titanium alloy surface and rejected the surface. For the effect of the composition of the titanium alloy surfaces, the composition of Ti60 titanium alloy surface was significantly more attractive to the temporary attachment.
and the settlement by *B. reticulatus* cyprids than that of TC4 titanium alloy surface. In brief, the roughness and the composition of the titanium alloy surfaces dominated the settlement of *B. reticulatus* cyprids through the temporary attachment duration and the cross grinding significantly decreased the settlement of *B. reticulatus* cyprids compared to the parallel grinding.

**Conclusions**

In this work, the effect of the roughness, the grinding methods and the composition of the titanium alloys surfaces on the movement and the settlement behaviour of the cyprids of *B. reticulatus* was investigated. All the motion paths of *B. reticulatus* cyprids on the titanium alloys consisted of the straight short motion paths in various directions, implying that *B. reticulatus* cyprids explored the surfaces of the titanium alloys for settlement in the movement. The roughness and the composition of the titanium alloy surfaces significantly influenced the average step length, the average velocity, the temporary attachment duration and frequency and the settlement time and ratio of *B. reticulatus* cyprids. The grinding methods also significantly affected the other five types of behaviour except the temporary attachment frequency. Meanwhile, the temporary attachment duration of *B. reticulatus* cyprids correlated positively to the settlement ratio on the titanium alloy surfaces except the cross grinding titanium alloy surface, which could be attributed to the changes of the contact area and the numbers of the attachment points beneath the *B. reticulatus* cyprid antennule discs caused by the surface roughness changes of the parallel grinding TC4 titanium alloy and the more attraction of the composition of Ti80 titanium alloy surface to the temporary attachment and the settlement by *B. reticulatus* cyprids compared to that of TC4 titanium alloy surface. The lowest settlement ratio of 0 was detected on the cross grinding titanium alloy surface, which might be due to the great decrease of the contact area and the numbers of the attachment points by the cross grinding compared to the parallel grinding. Taken together, the roughness and the composition of the titanium alloy surfaces regulated the settlement of *B. reticulatus* cyprids by the temporary attachment duration and the cross grinding significantly decreased the settlement of *B. reticulatus* cyprids compared to the parallel grinding.

**Acknowledgments**

We would like to thank Mr. Wenhao Cao for his kind assistance and intensive discussions in the culture of *B. reticulatus* cyprids.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by Innovation Group Project of Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai) (No. 311020012) and Guangzhou Basic and Applied Basic Research Foundation (No. 202102080638).

**ORCID**

Yaohua Wu

[http://orcid.org/0000-0002-1603-792X](http://orcid.org/0000-0002-1603-792X)

Wei Shi

[http://orcid.org/0000-0001-5859-2115](http://orcid.org/0000-0001-5859-2115)

**References**

Aldred N, Alsaab A, Clare AS. 2018. Quantitative analysis of the complete larval settlement process confirms Crisp’s model of surface selectivity by barnacles. Proc R Soc B. 285:20171957. doi:10.1098/rspb.2017.1957

Aldred N, Clare AS. 2008. The adhesive strategies of cyprids and development of barnacle resistant marine coatings. Biofouling. 24:351–363. doi: 10.1080/08927010802256117

Aldred N, Ekblad T, Andersson O, Liedberg B, Clare AS. 2011. Real-time quantification of microscale bioadhesion events in situ using imaging surface plasmon resonance (iSPR). ACS Appl Mater Interfaces. 3:2085–2091. doi: 10.1021/am2003075

Aldred N, Li GZ, Gao Y, Clare AS, Jiang SY. 2010a. Modulation of barnacle (*Balanus amphitrite* Darwin) cyprid settlement behavior by sulfobetaine and carboxybetaine methacrylate polymer coatings. Biofouling. 26:673–683. doi:10.1080/08927014.2010.506677

Aldred N, Scardino A, Cavaco A, de Nys R, Clare AS. 2010b. Attachment strength is a key factor in the selection of surfaces by barnacle cyprids (*Balanus amphitrite*) during settlement. Biofouling. 26:287–299. doi:10.1080/08927014.2010.506672

Amsler MO, Amsler CD, Rittschof D, Becerro MA, Mclintock JB. 2006. The use of computer-assisted motion analysis for quantitative studies of the behaviour of barnacle (*Balanus amphitrite*) larvae. Mar Freshw Behav Physiol. 39:259–268. doi:10.1080/10236240600980640

Anandkumar B, George RP, Rao CJ, Philip J. 2019. In situ application of alternate potentials with chlorination synergistically enhanced biofouling control of titanium condenser materials. Int Biodeter Biodegr. 144:104746. doi: 10.1016/j.ibiod.2019.104746
Berntsson KM, Jonsson PR, Larsson AI, Holdt S. 2004. Rejection of unsuitable substrata as a potential driver of aggregated settlement in the barnacle *Balanus improvisus*. Mar Ecol Prog Ser. 275:199–210. doi:10.3354/meps275199

Berntsson KM, Jonsson PR, Lejhall M, Gatenholm P. 2000. Analysis of behavioural rejection of micro-textured surfaces and implications for recruitment by the barnacle *Balanus improvisus*. J Exp Mar Biol Ecol. 251:59–83. doi:10.1016/S0022-0981(00)00210-0

Blackwood DJ, Lim CS, Teo SL, Hu X, Pang J. 2017. Macrofouling induced localized corrosion of stainless steel in Singapore seawater. Corros Sci. 129:608–612. doi:10.1016/j.corsci.2017.10.008

Burden JF. 2009. Marine antifouling laboratory bioassays: an overview of their diversity. Biofouling. 25:297–311.

Callow ME, Jennings AR, Brennan AB, Seegert CE, Gibson A, Wilson L, Feinberg A, Baney R, Callow JA. 2002. Microtopographic cues for settlement of zoospores of the green fouling alga *Enteromorpha*. Biofouling. 18:229–236. doi:10.1080/08927010290014908

Chaw KC, Birch WR. 2009. Quantifying the exploratory behaviour of *Amphibalanus amphitrite* cyprids. Biofouling. 25:611–619. doi:10.1080/08927010903363261

Chaw KC, Dickinson GH, Ang KY, Deng J, Birch WR. 2011. Surface exploration of *Amphibalanus amphitrite* cyprids on microtextured surfaces. Biofouling. 27:413–422. doi:10.1080/08927014.2011.577210

Clare AS, Aldred N. 2009. Surface colonisation by marine organisms and its impact on antifouling research. In Hello C, Yebra D, editors. Advances in marine antifouling coatings and technologies. Cambridge: Woodhead Publishing; p. 46–79.

Clare AS, Freet RK, McClary M. 1994. On the antennular secretion of the cyprid of *Balanus amphitrite*, and its role as a settlement pheromone. J Mar Biol Ass. 74:243–250. doi:10.1017/S0025315400035803

Clare AS, Høeg JT. 2008. *Balanus amphitrite* or *Amphibalanus amphitrite*? A note on barnacle nomenclature. Biofouling. 24:55–57. doi:10.1080/08927010701830194

Crisp DJ. 1961. Territorial behaviour in barnacle settlement. J Exp Biol. 38:429–446. doi:10.1242/jeb.38.2.429

Crisp DJ. 1976. Adaptation to environment: Essays on the physiology of marine animals: Two settlement responses in marine organisms. London: Butterworths; p. 83–124.

Crisp DJ, Barnes H. 1954. The orientation and distribution of barnacles at settlement with particular reference to surface contour. J Anim Ecol. 23:142–162. doi:10.2307/1664

de Brito LVR, Coutinho R, Cavalcanti EHS, Benchimol M. 2007. The influence of macrofouling on the corrosion behaviour of API 5L X65 carbon steel. Biofouling. 23:193–201. doi:10.1080/08927010701258966

de Messano LVR, Reznik LY, Sathler L, Coutinho R. 2014. Evaluation of biofoucrosion on stainless steels using laboratory-reared barnacle *amphibalanus amphitrite*. Anti-Corros Method M. 61:402–408. doi:10.1108/ACMM-07-2013-1278

Dineen JF, Hines AH. 1994. Effects of salinity and adult extracts on settlement of the oligohaline barnacle *Balanus subalbidus*. Marine Biology. 119:423–430. doi:10.1007/BF00347539

Dobretsov S, Ritschof D. 2020. Love at first taste: introduction of larval settlement by marine microbes. IJMS. 21:731. doi:10.3390/ijms21030731

Eashwar M, Subramanian G, Chandrasekarapan, Balakrishnan K. 1992. Mechanism for barnacle-induced crevice corrosion in stainless steel. Corrosion. 48:608–612. doi:10.1006/jcor.1991.0062

Eckman JE, Savidge WB, Gross TF. 1990. Relationship between duration of cyprid attachment and drag forces associated with detachment of *Balanus amphitrite* cyprids. Mar Biol. 107:111–118. doi:10.1007/BF01313248

Faimali M, Garaventa F, Piazza V, Greco G, Corrà C, Magillo F, Pittore M, Giacco E, Gallus L, Falugi C, et al. 2006. Swimming speed alteration of larvae of *Balanus amphitrite* as a behavioural end-point for laboratory toxicological bioassays. Mar Biol. 149:87–96. doi:10.1007/s00227-005-0209-9

Guo S, Puniredd SR, Janiczewski D, Lee SSC, Teo SLM, He T, Zhu X, Vancso GJ. 2014. Barnacle larvae exploring surfaces with variable hydrophilicity: influence of morphology and adhesion of "footprint" proteins by AFM. ACS Appl Mater Interfaces. 6:13667–13676. doi:10.1021/am503147m

Hadfield MG. 2011. Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. Ann Rev Mar Sci. 3:453–470. doi:10.1146/annurev-marine-120709-142753

Harder TN, Thiagarajan V, Qian PY. 2001. Effect of cyprid age on the settlement of *Balanus amphitrite* Darwin in response to natural biofilms. Biofouling. 17:211–219. doi:10.1080/08927010109378480

Hello C, Simon-Colin C, Clare A, Deslandes E. 2004. Isethionic acid and floridoside isolated from the red alga, *Grateloupia turuturu* in response to natural biofilms. Biofouling. 17:211–219. doi:10.1080/08927010109378480

Hills JM, Thomason JC. 1998. The effect of scales of surface roughness on the settlement of barnacle (*Semibalanus balanoides*) cyprids. Biofouling. 12:57–69. doi:10.1080/08927010412331279605

Hills JM, Thomason JC, Davis H, Kohler J, Millett E. 2000. Exploratory behaviour of barnacle larvae in field conditions. Biofouling. 16:171–179. doi:10.1080/08927010009378442

Høeg JT, Møller OS. 2006. When similar beginnings lead to different ends: constraints and diversity in cirripede larval development. Invertebr Reprod Dev. 49:125–142. doi:10.1080/08927010412331279605

Hoern P, Smith C, Ren DC. 2011. Microtopographic cues for settlement by marine microbes. IJMS. 21:470. doi:10.1007/s10783-010-0200-z

Hou SY, Gu H, Smith C, Ren DC. 2011. Microtopographic patterns affect *Escherichia coli* biofilm formation on poly(dimethylsiloxane) surfaces. Langmuir. 27:2686–2691. doi:10.1021/la1046194

Kamino K. 2013. Mini-review: barnacle adhesives and adhesion. Biofouling. 29:735–749. doi:10.1080/08927014.2013.80863

Knight-Jones EW, Crisp DJ. 1953. Gregarioussness in barnacles in relation to the fouling of ships and to antifouling research. Nature. 171:1109–1110. doi:10.1038/1711109a0
Koryakova MD, Filonenko NY, Kaplin YM. 1995. Barnacle-induced corrosion of high-alloyed steels. Prot Met. 31: 219–221.

Lagersson NC, Høeg JT. 2002. Settlement behavior and antennulary biomechanics in cypris larvae of Balanus amphitrite (Crustacea: Thecostraca: Cirripedia). Mar Biol. 141:513–526.

Lejars M, Margaillan A, Bressy C. 2012. Fouling release coatings: a non-toxic alternative to biocidal antifouling coatings. Chem Rev. 112:4347–4390. doi:10.1021/cr200350v

Maleschlijski S, Bauer S, Aldred N, Clare AS, Rosenhahn A. 2015. Classification of the pre-settlement behaviour of barnacle cyprids. J R Soc Interface. 12:20141104. doi:10.1098/rsif.2014.1104

Maleschlijski S, Bauer S, Di Fino A, Sendra GH, Clare AS, Rosenhahn A. 2014. Barnacle cyprid motility and distribution in the water column as an indicator of the settlement-inhibiting potential of nontoxic antifouling chemistries. Biofouling. 30:1055–1065. doi:10.1080/08927014.2014.966097

Maruzzo D, Conlan S, Aldred N, Clare AS, Høeg JT. 2011. Video observation of surface exploration in cyprids of Balanus amphitrite: the movements of antennular sensory setae. Biofouling. 27:225–239. doi:10.1080/08927014.2011.555534

Murugan VK, Mohanram H, Budanovic M, Latchou A, Webster RD, Miserez A, Seita M. 2020. Accelerated corrosion of marine-grade steel by a redox-active, cysteine-rich barnacle cement protein. NPJ Mater Degrad. 4:20. doi:10.1038/s41529-020-0124-z

Neville A, Hodgkiess T. 2000. Localised effects of macrofouling species on electrochemical corrosion of corrosion resistant alloys. Brit Corros J. 35:54–59. doi:10.1179/0007059000101501083

O’Connor NJ, Richardson DL. 1994. Comparative attachment of barnacle cyprids (Balanus amphitrite Darwin, 1854; B. improvisus Darwin, 1854; & B. ehurneus Gould, 1841) to polystyrene and glass substrate. J Exp Mar Biol Ecol. 183:213–225. doi:10.1016/0022-0981(94)90088-4

Petersen DS, Gorb SN, Heepe L. 2020. The influence of material and roughness on the settlement and the adhesive strength of the barnacle Balanus improvisus in the Baltic Sea. Front Mar Sci. 7:664. doi:10.3389/fmars.2020.00664

Prendergast GS. 2007. Settlement and succession of benthic marine organisms: interactions between multiple physical and biological factors [dissertation]. UK: University of Newcastle.

Prendergast GS, Zurn CM, Bers AV, Head RM, Hansson LJ, Thomason JC. 2008. Field-based video observations of wild barnacle cyprid behaviour in response to textural and chemical settlement cues. Biofouling. 24:449–459. doi:10.1080/08927010802340135

Raman S, Karunamoorthy L, Doble M, Kumar R, Venkatesan R. 2013. Barnacle adhesion on natural and synthetic substrates: adhesive structure and composition. Int J Adhes Adhes. 41:140–143. doi:10.1016/j.ijadhadh.2012.11.003

Santos R, Gorb S, Jamar V, Flamman P. 2005. Adhesion of echinoderm tube feet to rough surfaces. J Exp Biol. 208:2555–2567. doi:10.1242/jeb.01683

Satuito CG, Shimizu K, Natoyama K, Yamazaki M, Fusetani N. 1996. Age-related settlement success by cyprids of the barnacle Balanus amphitrite, with special reference to consumption of cyprid storage protein. Mar Biol. 127:125–130. doi:10.1007/BF00993652

Scardino AJ, Guenthner J, de Nys R. 2008. Attachment point theory revisited: the fouling response to a microtextured matrix. Biofouling. 24:45–53. doi:10.1080/0892701071784391

Scardino AJ, Harvey E, de Nys R. 2006. Testing attachment point theory: diatom attachment on microtextured polylime biominims. Biofouling. 22:55–60. doi:10.1080/08927010500506094

Schultz MP. 2007. Effects of coating roughness and biofouling on ship resistance and powering. Biofouling. 23:331–341. doi:10.1080/08927010701461974

Schultz MP, Bendick JA, Holm ER, Hertel WM. 2011. Economic impact of biofouling on a naval surface ship. Biofouling. 27:87–98. doi:10.1080/08927014.2010.542809

Schumacher JF, Aldred N, Callow ME, Finlay JA, Callow JA, Clare AS, Brennan AB. 2007a. Species-specific engineered antifouling topographies: correlations between the settlement of algal zoospores and barnacle cyprids. Biofouling. 23:307–317. doi:10.1080/08927010701393276

Schumacher JF, Carman ML, Estes TG, Feinberg AW, Wilson LH, Callow JA, Finlay JA, Brennan AB. 2007b. Engineered antifouling microtopographies-effect of feature size, geometry, and roughness on settlement of zoospores of the green alga Ulva. Biofouling. 23:55–62.

Southward AJ, Hiscock K, Kerckhof F, Moyse J, Elijmov AS. 2004. Habitat and distribution of the warm-water barnacle Solidobalanus fallax (Crustacea: Cirripedia). J Mar Biol Ass. 84:1169–1177. doi:10.1017/S0025315404010616h

Thomson JC, Hills JM, Thomason PO. 2002. Field-based behavioural bioassays for testing the efficacy of antifouling coatings. Biofouling. 18:285–292. doi:10.1080/0892701021000034391

Uzun D, Ozyurt R, Demirel YK, Turan O. 2020. Does the barnacle settlement pattern affect ship resistance and powering? Appl Ocean Res. 95:102020. doi:10.1016/j.aporo.2019.102020

Vanithakumari SC, George RP, Mudali UK. 2017. Environmental stability and long-term durability of superhydrophobic coatings on titanium. J Mater Eng Perform. 26:2640–2648. doi:10.1007/s11665-017-2708-5

Vanithakumari SC, Jena G, Sofia S, Thinaharan C, George RP, Philip J. 2020. Fabrication of superhydrophobic titanium surfaces with superior antibacterial properties using graphene oxide and silanized silica nanoparticles. Surf Coat Tech. 400:126074. doi:10.1016/j.surfcoat.2020.126074

Walley LJ. 1969. Studies on the larval structure and metamorphosis of Balanus balanoides (L.). Phil. Trans. R Soc Lond B Biol Sci. 256:237–280.

Yebu DM, Kil S, Dam-Johansen K. 2004. Antifouling technology-past, present and future steps towards efficient and environmentally friendly antifouling coatings. Prog Org Coat. 50:75–104. doi:10.1016/j.porgcoat.2003.06.001