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The interaction of marine fouling organisms with topography of varied scale and geometry: a review

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Abstract

Many studies have examined the effects of surface topography on the settlement behaviour of marine organisms and this article reviews these investigations with more emphasis on the effects of topography scale. It has been observed that macro topographies (1-100 mm) are generally favoured by marine fouling taxa and are unsuitable for antifouling applications. This is because macro topographies are usually large enough to fit fouling organisms and provide refuge from dangers in the marine environment. Micro topographies had only limited success at reducing fouling from a wide range of marine taxa. The antifouling performance of micro topographies (1 to ≤1000 μm) is dependent on the properties of topography features in terms of symmetry, isotropy, width, length, height/depth, separation distance and average roughness. In terms of the antifouling performance of micro topography, topography geometry may only be of secondary importance in comparison to the size of features itself. It is also noted that hydrodynamic stresses also contribute to the settlement trends of foulers on textured surfaces. Future studies on antifouling topographies should be directed to hierarchical topographies because the mixed topography scales might potentially reduce fouling by both micro and macro organisms. Patterned nano-topographies (1- ≤1000 nm) should also be explored because the antifouling mechanisms of these topographies are not yet clear.

Keywords: Marine biofouling; Antifouling; Settlement behaviour; Aspect ratio; Nanostructures; Micro/Macro; Topography; Tortuosity

Review

Introduction

Solid surfaces that spend long periods of time in aquatic environments are susceptible to the accumulation of marine fouling organisms and this phenomenon is known as marine biofouling. This is a natural process which can have significant economic impact on maritime industries. For example, the fouling of ship hulls increases drag and friction which in turn increases fuel consumption by 20.4% or US$2.3 million per ship every year [1]. In the aquaculture industry, approximately US$1.5-3 billion is spent a year on antifoulants and on repair or maintenance fees to manage this phenomenon [2]. Significant effort is focused on the search for an effective, environmentally friendly, antifouling solution and there has yet to be any development that can rival the efficacy of tributyltin (TBT) self-polishing copolymers. Fortunately, these were banned in 2008 because of their detrimental effects on marine life, which included severe deformities in shellfishes and the accumulation of tin in other species [3,4].

Current research to control biofouling is focused on producing non-toxic, effective antifouling systems. Many studies have identified that surface chemistry can affect the settlement trends of marine organisms. These studies resulted in derivations of non-toxic foul-release coatings (FRCs); some of which are commercially available especially for the shipping industry [5-9]. FRCs are made of materials with chemical properties that reduce the adhesion or promote the release of potentially settling organisms [10]. However, these coatings are only effective above a certain level of hydrodynamic shear and still have difficulty preventing the formation of biofilms and the growth

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of microalgae. Several reviews on foul-release coatings were published in recent years and will not be discussed in this review [10-13].

Another non-toxic antifouling approach involves the development of foul-resistant topographies [14-17]. Some of these topography designs were inspired by naturally occurring marine topographies from organisms that are able to prevent fouling on themselves despite spending most of their lives underwater. Examples of these natural topographies include the micro sized riblets on shark's skin and the ridge-pore covered skin of pilot whales [18,19]. Artificially derived geometries of varying scales (from macro to nano) such as meshes, pillars, grooves, bumps and holes were also investigated to determine their effect on the settlement behaviour of marine organisms [20-22]. Studies have shown that micro topography has antifouling properties but topography of this scale alone only reduced the settlement of a few marine species at a time [23,24]. Several studies have mentioned that the mixed scale on hierarchical structures might be able to discourage a wider range of fouling organisms from settling concurrently [23,25,26]. Since not much is known about this topography configuration, future studies are necessary to determine if it would be more effective than current foul-resistant topographies.

Both FRCs and fouling resistant topographies have had a degree of success at reducing or discouraging fouling, but each approach has its limitations. Some studies have suggested that an optimum antifouling system would require manipulating multiple physico-chemical properties of a surface [25,27,28]. It is noted that foul-resistant topographies are made of materials with chemical properties that contribute to the overall antifouling performance of the topography. Several studies explored quantitative models that considered the combined effects of surface topography and chemistry/energy towards the settlement trends of *Ulva* spores [29,30]. A recent study applied a derivation of these models and determined that micro topographies made of cross-linked hydrogels would exhibit better antifouling properties than the same patterns developed with the silicone elastomer, Polydimethylsiloxane (PDMSe) [31]. These are among the many studies that acknowledged the necessity to consider the interaction between multiple physico-chemical properties for an effective antifouling solution. However, for this review, the chemical aspects of foul-resistant topographies are not included. The aims of this review are to focus on certain physical aspects of topography that contribute to its antifouling properties. This article compiles and summarises the influence of topography scale (i.e. width, length, height/depth, space apart or measured roughness) in relation to theories (e.g. attachment point theory) or derived empirical models (Engineered Roughness Index (ERI)) that were developed to better understand the settlement trends of fouling organisms.

Methods

This review examined 52 published journal articles from 1988 to 2013 and one PhD thesis. These articles investigated the effects of several physical aspects of surface topography with more focus on topography scale and geometry on the settlement behaviour of marine fouling organisms (Additional file 1: Table S1). Topography features of the same shape or approximate profiles were re-categorised from the original literature and renamed for standardisation purposes. For example, several studies tested a range of macro and micro sized V-shaped trenches or slots [20,32-36]. Although the shape of the tested geometry was the same, a couple of these studies referred to these slots as riblets [20,32]; while the rest named them V-shaped crevices or grooves. Therefore, to compile findings in Additional file 2: Table S2 and Additional file 3: Table S3, the name of these trenches were standardised by naming them V-shaped grooves. Additional file 4: Table S4 provides rough illustrations of the geometries to better elucidate the types of topography and their categorisations with respect to discussions in this article.

The effects of topographies on tested taxa were summarised in Additional file 2: Table S2 and Additional file 3: Table S3 according to taxa, topography scale and topographic geometry. The defined range of macro, micro and nano topography is 1-10 mm, 1 to ≤1000 μm and 1- ≤ 1000 nm respectively. Additionally, studies that investigated the antifouling properties of hierarchical structures are also included. The discussions review topographic effects according to macro algae, micro algae and marine invertebrates. The inhibition or increased fouling by taxa is determined by comparing the settlement densities of textured surfaces with smooth controls where possible. Results of topographies that did not inhibit or encourage fouling are identified by the lack of statistical difference in settlement densities between tested topographies and smooth controls. Inconsistent settlement behaviour within a study or between studies is also noted.

Macro algae fouling

Micro scale *Ulva* spores are quadriflagellated, pyriform microorganisms that are motile and selective about surfaces that are suitable for settlement [37-39]. Upon initial contact with the surface, a spore gauges potential for settlement by spinning in place while being anchored presumably by a temporary adhesive to the same spot. The spinning motion allows the spore to test its adhesion strength to the surface which depends on factors such as the surface's chemical properties. The longer the spinning duration, the more likely the spore will permanently attach to the surface [40,41].

If the spore determines that the surface is suitable for permanent attachment, the spore moulds or conforms to the shape of the substratum and secretes a permanent adhesive to fix itself into position [42,43]. Several studies mentioned that substrate depressions were good locations for spores that looked for refuge from hydrodynamic shear and grazing by predators [17,32,37,44]. These studies also suggested that micro topography influenced the hydrodynamics of the surface by reducing fluid flow where depressions or crevices are present. Reduction of fluid flow decreases hydrodynamic stresses within these textures and increases the likelihood of retaining spore settlements.

Based on the attachment point theory presented in several studies, *Ulva* spores showed enhanced attachment when there were high levels of contact areas with topography features that consisted of depressions (i.e. between grooves or channels) [15,24,45,46]. Substrates with uniform topographies that were 5 μm in width, length, height and distance apart generally encouraged algal spore settlement (Additional file 2: Table S2). *Ulva* spores have diameters of 5 μm and exhibited enhanced settlement densities in substrate depressions that were precisely 5 μm because surface area of contact between the substrate and spores were high [14,15]. The support from surrounding topography features also reduced the amount of adhesive the spores would need to secrete to adhere within the substrate effectively (Figure 1A). 5 μm topographies also provided spores with a refuge from flow shear and did not effectively discourage spore settlements. When exposed to high hydrodynamic stresses from a water jet, only approximately half of the settled spores were removed [26].

Substrates with micro topography that were larger than 5 μm also encouraged *Ulva* spore settlement but were less effective in comparison to features that were precisely 5 μm [26,47]. Under hydrodynamic stresses, the retention of spore settlements in features larger than 5 μm decreased as topography features increased in scale or distance apart. Spores also tended to 'lean' on nearby substrate features to increase its area of contact and reduce the effort required to adhere to the substrate [15]. This behaviour also explains the spores reduced preference to settle on smooth surfaces and the tendency of spores to lean on each other for support if there is no other choice but to settle (Figure 1B) [14]. This is demonstrated in investigations where topographies with average roughness heights (R_z) that ranged from 20-50 μm retained more spore settlements than similar topographies with roughness heights from 50-100 μm [17,20,33]. The larger exposed surface area of topographies with roughness heights from 50-100 μm resulted in a lower net surface area of contact between spores and the substrate. Therefore, the amount of adhesives that spores had to secrete to adhere to the substrate effectively needed to increase.

Substrates with topography features that were less than 5 μm in dimensions were more effective at inhibiting spore attachment because spores were less able to conform into smaller sized depressions. As an effort to increase its contact area with these substrates, spores tended to be 'bridged' among multiple topographical features (Figure 1C). Spores could have landed and temporarily attached in attempts to conduct its initial assessment of the surface (i.e. spinning). However, it would be more likely that the complexity of the topography would have caused most spores to eventually leave and continue exploring [40]. Topography texture at this scale exhibited a level of 'tortuosity' which made the surface less attractive to spores for settlement. Topography tortuosity is a surface roughness parameter that describes the degree of freedom for organism movement on a textured surface (*df*). The more organism movement permitted over the topography, the more difficult it becomes for the organism to decide on an appropriate settlement space and the more 'tortuous' the substrate (Figure 2). It is highly likely that the spore would have rather released temporary adhesion and moved on to more suitable settlement sites. The degree of freedom of movement is one of three parameters associated with the dimensionless ratio derived by Schumacher et al. [16] known as the

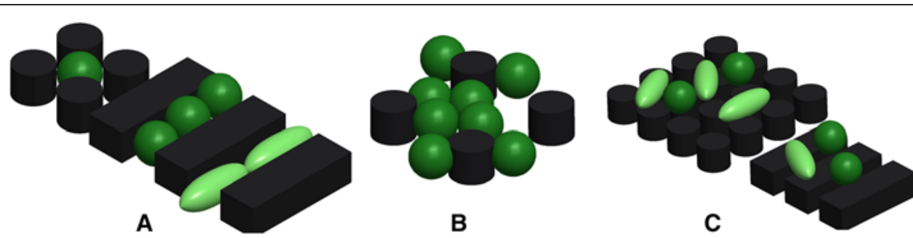

Figure 1 Settlement of organisms on topographically varied structures. (A) Organisms (green and light green) fitting well between adequately spaced topographical features. **(B)** Organisms leaning on each other and on topographies that are spaced too far apart. **(C)** 'Bridging' of organisms on multiple topography features.

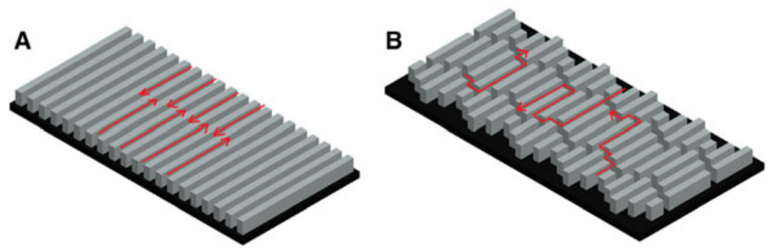

Figure 2 Tortuosity of topography with different complexity. Grooves in **(A)** are less 'tortuous' because organisms perceive the option to move only along one axis (indicated by red arrows); while riblets in **(B)** have higher 'tortuosity' because organisms are allowed the option to explore the surface along several directions.

Engineered Roughness Index (ERI). Other variables that define this ratio include Wenzel's roughness factor (*r*), and the fraction of depression on the surface (f_D).

$$ERI_I = \frac{r \times df}{f_D} \quad (1)$$

Studies showed that spore settlement density decreased as the ratio increased [16,29]. Micro-riblets with periodical lengths (4–12 μm), fixed widths of 2 μm and spaced at 2 μm apart had an ERI ratio of 9.5 and were the most effective at discouraging spore settlements among other topography features when compared with smooth control surfaces with an ERI ratio of 2. The ERI ratio provided a rough quantitative description of topography roughness and complexity but is limited because it is not inclusive of the height limit that is most effective at reducing spore settlement density.

Further investigation by Schumacher et al. [48] on topography of the same micro scale (less than 5 μm) determined that the aspect ratio (feature height/feature width) of topographical features influenced the density of spore settlements [14,48,49]. The same micro riblets tested in Schumacher et al. [16] with the height of 3 μm exhibited the highest antifouling properties when compared to riblets that were 1 or 2 μm high. 3 μm high riblets significantly reduced contact between the spores and the floor of the substrate which reduced effective attachment of the spore to the surface (Figure 3).

A recent investigation of hexagonal pits that gradiently changed to zigzags (1- 10 μm) showed that *Ulva linza* spores preferentially settled at 'kink sites' that provided more contact area for attachment [43]. Settlement density of spores decreased as pit size decreased and when hexagonal pits changed into zigzags (Figure 4A). Apart from being larger than a spore's average size, when the hexagonal pits reached a width of 8.5 μm and changed to zigzags, the number of 'kink sites' reduced from 6 sites to only 2 sites (Figure 4A). 'Kink sites' offered more contact area for spores to mould against and increased their effective attachment (Figure 4B). The reduced number of 'kinks' on zigzags also reduced the number of preferential sites for spores to settle which reduced the overall density of spores settlements. Unlike uniform micro-topographies, the settlement density of spores increased as Wenzel's roughness (*r*) increased. This contradicts the ERI model (equation 1) which demonstrated that the settlement density of spores decreased with increasing Wenzel's roughness. Hence, the ERI model is not suitable to quantify the efficacy of topographies with highly irregular or gradiently changing dimensions.

In contrast to *Ulva* spores, *Centroceras clavulatum* and *Polysiphonia sphaerocarpa* spores exhibited passive settlement behaviours on substrates but were less inclined

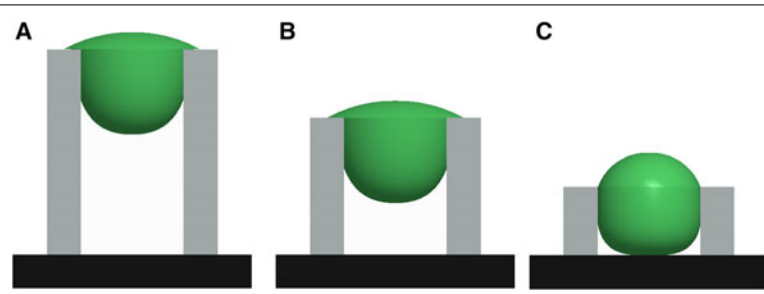

Figure 3 Effect of topography feature height towards effective spore attachment. Spore adhesion is the most effective in **(C)** because there is significant contact between spore and surrounding areas of the topography feature (i.e. walls, floor). Spore settlement in **(A)** and **(B)** is less effective since topography feature height it too high to allow spores more contact with its surroundings, especially the floor of the substrate [15].

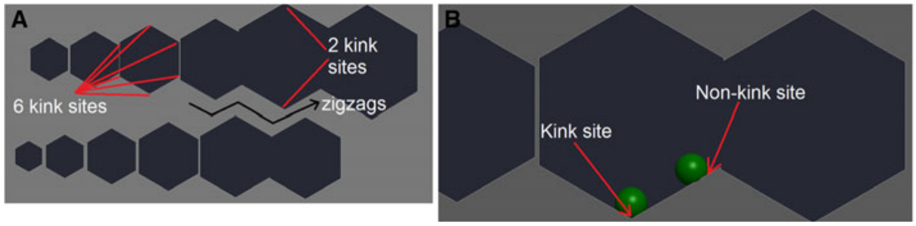

Figure 4 Kink sites for gradient hexagonal pits that change to zigzags. (A) As hexagonal pits change into zigzags, the number of 'kink sites' change from 6 sites to 2 sites when. **(B)** Spores preferred to settle in 'kink sites that provided a higher area of contact from surrounding walls and floors in comparison to 'non-kink sites'.

to settle on textured substrates [24]. When compared to smooth controls, micro-sized sine wave grooves that ranged from 4 – 512 μm showed reduced densities of *C. clavulatum* spores and *Polysiphonia* spores. The lack of difference in *C. clavulatum* spore settlement densities on the same grooves of differing wavelengths suggests that the scale of micro topographical features did not affect the spores' settlement behaviour. Conversely, *P. sphaerocarpa* spores exhibited random trends of settlement density across grooves of differing wavelengths. Even though micro topography reduced the settlement densities of both spores, there is unfortunately insufficient understanding of their settling mechanisms to explain the difference in their settlement trends [50].

Hierarchical topographies Hierarchical structures of micro peaks on irregular micro-cells reduced *Chlorella* (9-10 μm) and *Nannochloropsis maritima* (1-2 μm) settlements [51]. The micro peaks were 0.3 μm wide with an average height of 2 μm and were densely packed on cells that had an average width of 30 μm. This topography exhibited antifouling properties because gaps between peaks were too small (2-3 μm) to accommodate high settlement densities of both types of spores. 'Bridging' of spores between peaks also indicated that this topography was high in tortuosity which made it difficult for spores to establish contact with the substrate to adhere effectively. The difficulty to attach would have made the substrate less 'attractive' to the spores for settlement. Hence, results indicated that only sufficiently large gaps between micro cells that were not covered with micro peaks retained more spore settlements.

In contrast, hierarchical structures of periodical micro- and nano-sized sine-wave wrinkles that ranged from 5 nm to 500 μm enhanced *Ulva rigida* spore settlements [23]. Spores settled abundantly especially in depressions that were larger than the spore's body size and a majority of these features retained spore settlements even after being rinsed with water. Hierarchical structures of micro riblets with the same dimensions in the study by Schumacher et al. [16] layered on top of micro rectangular grooves (20 μm or 200 μm) also enhanced the same spores to settle [48]. Closer analysis of the laboratory assays under a Scanning Electron Microscope (SEM) determined that spaces between riblets and adjacent walls of grooves or areas of grooves that were not patterned with riblets were the areas that permitted spores to settle.

Microalgae fouling

Macro topographies Field studies on microalgae communities of *Licmophora* sp. and other unidentified species of diatoms settled successfully on macro sized bumps that ranged from 1-5 mm (Additional file 2: Table S2). Settlement densities were particularly abundant on the highest elevations of these bumps [52]. Diatoms exhibit passive settlement onto a substrate and subsequent motility only when physical contact is made with the substrate to select preferred attachment sites [46]. Therefore, diatoms could have settled passively onto random areas on the substrate and migrated to elevated areas of bumps which would be the most strategic position for diatoms to reach sunlight and other nutrient supplies. Based on a study by Verran et al. [53], passive retention of micro-sized diatoms should be minimal on significantly larger macro-sized bumps. However, the presence of a biofilm layer could have conditioned the textured surface and provided a complex matrix that protected the diatoms from turbulent shear forces at the peaks of the bumps.

Micro topographies The implications of micro topographies were evident when topographical feature sizes were proximate to the width of the diatoms' bodies [54]. Since tests on diatoms were limited to observing passive settlement on topographied surfaces with laboratory assays, hydrodynamic stresses would have not been consequential to the results. Although the ERI ratio has yet to be tested on diatoms, this ratio could be applicable to these organisms since both diatoms and spores are rather similar in size.

Based on the attachment point theory, *Nitzschia* cf. *paleacea* (2 μm) and *Fallacia carpentariae* (1 μm) settled on sine-wave grooves that were 4 and 2 μm in wavelength because multiple contact points were formed between the diatoms' bodies and the topographies. Cylindrically shaped diatoms were able to fit securely in the recessed areas of the grooves especially when the diatoms' bodies were aligned longitudinally to the grooves (Figure 1A) [46].

In contrast, the densities of larger diatoms *Amphora* sp. (7 μm) and *Navicula jeffreyi* (4 μm) were reduced on the same grooves because only an estimated maximum of six contact points were formed between these diatoms and the grooves. Both diatoms showed the tendency to be 'bridged' across several grooves and were not slim enough to fit securely in between the peaks of grooves.

The settlement densities of *Amphora* sp. were reduced on micro sized sine wave grooves when compared to smooth surfaces. This study also observed that sine wave grooves larger than diatoms showed slightly more diatom densities than grooves that were smaller than the diatoms' size, but these increases were considered statistically insignificant.

Apart from supporting the attachment point theory, it is possible that this observation also concurs with a derivation of the ERI ratio (equation 1) where the parameter that accounts for surface tortuosity is replaced with *n,* which is defined as the number of distinct features that is visible on the textured surface [29].

$$\mathrm{ERI}_{II} = \frac{r \times n}{f_D} \tag{2}$$

From equation (2) substrates with constant surface areas would fit more grooves with smaller widths in comparison to larger width grooves. Similar to equation (1), the antifouling efficacy of the textured surface is proportional to the ERI value. Therefore, the slightly better antifouling performance of smaller width sine-wave grooves at resisting Amphora sp. settlement is warranted.

Invertebrate fouling

Macro topographies Most macro invertebrates reviewed in Additional file 3: Table S3 settled effectively on macro-sized topographies that consisted of bumps, holes and V-shaped grooves [22,36,55-57]. These topographies were generally larger in scale relative to the size of invertebrates and were likely considered to be suitable by these organisms for settlement and adhesion. Turbulent flows over macro sized topographies creates disturbances in the viscous sublayer of the boundary layer that is directly adjacent to the surface [44]. Disturbance in the viscous sublayer increases hydrodynamic drag or friction on the surface of the substrate which is a condition that must be avoided particularly in the shipping industry. Hence, it could be argued that topographies at this scale are not suitable for marine antifouling applications. However, the differences in macro-invertebrate settlement patterns observed on macro-scale topographies highlight essential factors that are of interest to antifouling research.

The preference of *Crambe crambe* and *Scopalina lophyropoda* sponge larvae (average size of 1 mm^2) to settle in 5 mm wide V-shaped grooves large enough to shade them suggests that these larvae were affected by light. This is an example of an instance where negative phototaxis cues affected the distribution of sponge larvae [58]. It is also likely that barnacle cyprids, ascidians and bryozoan larvae tend to settle at the base of bumps or in large holes to take refuge from hydrodynamic shear (rheotaxis) and protection from desiccation, being grazed over or eaten by predators [35,59]. Another probable reason for this observation is that hydrodynamic forces from turbulent water flow could have also caused these larvae to passively settle within these holes. However, holes with heights of 2 mm lacked *B. neritina* settlement because the average height of this bryozoan (2.8 mm) exceeded the height of these holes and significantly increased its exposure to predation.

Unlike *B. neritina*, the hydroid *Tubularia crocea* exhibited preference for the high areas of macro topographies that were most exposed to hydrodynamic shear stress and turbulence [60]. The reasons for this behaviour were uncertain but it is possible that the turbulent areas of macro topographies act as sediment traps that supply nutrients to the hydroid [52]. If the flow over the substrate is an ambient current, there is also the possibility that the water flow could have carried *T.crocea* larvae passively to the highest elevations of the topography instead of dragging larvae into protected crevices [61]. Macro holes seemed to be effective at preventing *T. crocea* settlements but the reasons for this finding were not substantiated. Among the possibilities for this observation includes rejection of holes by the hydroid larvae or that macro holes might have been too small to fit adult hydroids. It is also likely that the formation of eddies in the holes from turbulent fluid flow could have prevented larvae from settling successfully.

The bryozoan *Membranipora membranacea* has a flexible body that is flat which often puts it at risk of being encrusted over by competing organisms that settle in the same vicinity [36]. This encourages the bryozoan to respond to spatial refuge cues and seek the highest elevations of topographical features [36]. This explained their preference to settle on the top of macro bumps and on the outer rims of macro holes where the probability of getting encrusted over is lower.

Some species showed gregarious settlement behaviour responding to conspecific cues [34]. Barnacle cyprids and pediveligers of mussels respond positively to conspecific cues left by preceding or current colonies of their respective species. These larvae have 'feet' that explore substrates and settle when they detect familiar chemical compounds, the physical presence of living or deceased predecessors and in certain cases, even chemical secretions from their prey.

Many studies have shown that biofilms provide an important settlement cue for macro invertebrates. Biofilms layers consist of polymeric substances, microorganisms, and miscellaneous matter that can attract many invertebrates to settle. For example, larvae of *Hydroides elegans*, and *Mytilus galloprovincialis* exhibit positive settlement on substrates that were coated with layers of biofilms that contain chemical substances that are familiar to them [62-64]. Biofilm layers that are formed under different hydrodynamic conditions also affected the attachment strength of cyprids. Biofilms formed under high shear stresses are more densely linked than biofilms formed under low shear stresses. The denser matrix of biofilms that grew in high shear conditions allowed cyprids to attach more firmly in comparison to substrates with biofilms that grew under low shear stresses [65,66].

Micro topographies Like *Ulva* spores, there are studies that explained the settlement mechanisms of barnacles [40,67]. A barnacle nauplius matures into cyprids that swim about in the water column looking for a place to settle. When it comes into contact with a substrate, it tracks or walks about leaving 'footprints' to assess the suitability of the surface for settlement. While it has been mentioned that cyprids respond to conspecific cues, surface topography also contributes to the settlement trends of these organisms [22]. Macro topographies are favourable for cyprids to settle but comparisons between studies have shown that significantly smaller micro topographies were effective at deterring cyprids from settling [22,68]. Many studies have tested the effects of micro topographies on the settlement behaviour of barnacle cyprids and these studies consolidate the importance of topography scale in relation to the size of the cyprids [48,69-71].

Regardless of geometry, micro topographies with geometry widths that ranged from 1-5 μm and approximately 64-256 μm reduced cyprid settlements (Additional file 3: Table S3). Cyprids track or walk about with 'feet' before settling permanently and the tips of their 'feet' consist of ovoid antennular discs that are approximately 20-30 μm [70]. It could be argued that topographies ranging from 1-5 μm were rejected by cyprids because there were insufficient contact points to support settlement and attachment. Likewise, topographies that ranged from 64-256 μm would have been too small to accommodate metamorphosed juvenile barnacles with body widths of approximately 500 μm.

Micro topographies that ranged from 8-64 μm and 500-1000 μm enhanced cyprid settlements. Sine wave grooves that were 16 μm in wavelength were very supportive of *B. amphitrite* attachment which suggests that this size could be a critical dimension for this particular cyprid species. However, it is difficult to ascertain the reasons for this finding and further studies are warranted to determine this. It is probable that similar grooves which were 32-64 μm provided strong anchor points for attachment because cyprid antennular discs (30 μm) were compatible in size to these grooves. Topographies that were 500-1000 μm were expected to encourage cyprids to settle because cyprids were close in size and would be able to attach easily onto topographies of this range [70].

A study that compared between V-shaped grooves and peaks of the same structure height (69 μm) and base size (97 μm) with smooth controls found that both micro topographies reduced *Balanus improvisus* cyprid settlement. However, based on the overall settlement density of cyprids, grooves were more effective than peaks at resisting cyprid settlements [32]. A separate study found that micro-sized sine wave grooves with peak wavelengths of 1.5-2 μm were better than roughened grooves of similar geometry and magnitude at deterring *Semibalanus balanoides* cyprids from settling [68]. The reduction in settlement density of cyprids in both studies can be attributed to topography size being too small to accommodate the larger bodies of cyprids (500-1000 μm). However, the higher antifouling performance of V-shaped grooves and unroughened sine-wave grooves in both studies respectively also suggests that cyprids are likely discouraged to settle on micro textures that have a degree of symmetry and isotropicity (Figure 5). These results also explain the enhanced antifouling efficacy of isotropic micro mesh patterns at preventing cyprids from settling in a number of investigations [33,71,72].

Although there are fewer studies of micro topographies on other marine invertebrates, these studies also indicated that micro topographies have varying effects on other larvae settlements. Larvae of *Bugula neritina* have diameters that are approximately 300-400 μm which explains the reduced settlements on sine wave grooves that were only 256 μm wide. As expected, the largest sine wave grooves (512 μm) were able to fit the zooids of this bryozoa. The increased settlement densities of *B. neritina* larvae on smaller grooves that ranged from 8–64 μm were unexpected and difficult to explain. Grooves of this range have an overall exposed surface area that is greater than larger sine wave grooves. Therefore, it is possible that the amount of adhesives adsorbed on grooves of this roughness range would be high and could have aided in retaining more bryozoan larvae [44].

Laboratory assays found that rectangular grooves ranging from 100-1000 μm enhanced the passive settlement of *Mytilus galloprovincialis* pediveligers when compared to smooth controls [5]. The settlement trend

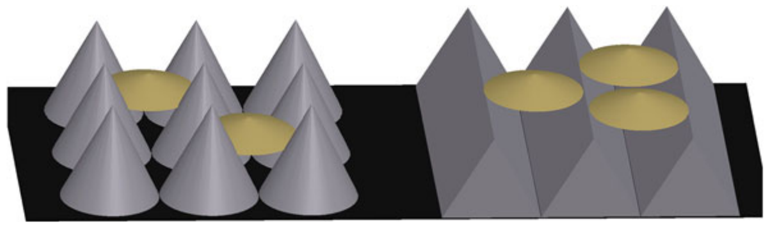

Figure 5 Influence of isotropy on similarly scaled but geometrically different topography. The illustration shows that peaks are better than the more isotropically inclined V-shaped grooves of approximate size at providing secure settlement points for the attachment of cyprid base plates (beige).

of pediveligers indicated sensitivity to texture size of micro topographies. The average body size of *Mytilus galloprovincialis* pediveligers are approximately 190 μm and 260 μm in width and length respectively [73]. Unlike *Ulva* spores, pediveligers are fixed in shape and would find it difficult to conform or fit into grooves that are near to its size. This substantiates the reduction in settlement on grooves that ranged from 100-250 μm.

The single flexible 'foot' of pediveligers is approximately 40 μm in diameter which is small enough to fit square grooves that were 40-80 μm in width and height [25,73]. These studies also determined that pediveligers showed poor retention on topographies of this range when subjected to hydrodynamic shear tests. One possibility for this observation is that settled pediveligers could have metamorphosed to sizes that could no longer fit these grooves within a matter of hours after initial settlement and be removed easily when rinsed with water. Several separate studies also observed that topographies with heights that ranged from 40-100 μm showed a reduction in retained settlements of barnacle cyprids, *Mytilus edulis* larvae and *Ulva* spores after being subjected to hydrodynamic shear [17,25,32,48]. It can be observed that topography with roughness heights within this range are effective at reducing invertebrate larvae and algae spore settlements if the textured surface is subjected to hydrodynamic shear. It is likely that the resultant drag and lift subjected to these topographies reduced the adhesion strength of these fouling organisms. From these studies, it is possible to postulate that the maximum cutoff height that would be most effective at discouraging fouling by the aforementioned larvae could be approximately 100 μm.

The encrusting tube worms *Hydroides elegans* and *Polydora* sp. have bodies that are approximately 100-250 μm wide and also exhibited settlement behaviours that concurred with the attachment point theory [24,45]. Scardino et al. [24] observed increased *H. elegans* larvae settlement on sine wave grooves with wavelengths of 125 μm and above while Bers and Wahl [25] found that sine wave grooves less than 5 μm reduced settlements of the same larvae. Bers and Wahl [25] also observed reduced densities of *Polydora* sp. larvae on rectangular grooves with widths that ranged from 30 – 50 μm.

Several field studies found inconsistent settlement behaviours of *Polydora* sp. larvae on micro bumps that range from 10-500 μm [25,52]. Reasons for this behaviour were not clear but environmental factors such as the presence of biofilms, conspecific cues and hydrodynamic stresses on the textured surface could have contributed to this inconsistency. This result also indicates that environmental cues in natural settings might have more influence than micro topographies towards polychaete larvae settlement behaviour.

Both these studies were the only studies that observed the effects of topography towards ciliates which makes it difficult to ascertain the reasons for their settlement behaviour. Nonetheless, with the exception of *Vorticella* sp., it is likely that other ciliates were less inclined to settle on textured or roughened substrates across the macro, and micro scales (Additional file 3: Table S3).

Hierarchical topographies Hierarchical structures that consisted of periodical sine wave wrinkles (5 nm-500 μm in wavelength) effectively deterred cyprids while micro bumps (200 μm in diameter) overlapped with smaller micro peaks (1-5 μm in width and height) reduced cyprid and mussel settlement [23,25]. In these cases, the topographies were often too small to fit both types of larvae. Since both investigations were field studies, it is also likely that hydrodynamic stresses on this hierarchical surface would have contributed to the antifouling efficacy of these topographies.

Another possibility for the enhanced antifouling performance of hierarchical topographies influenced the surface exploration time of cyprids and pediveligers which discouraged these larvae to settle and attach. Several studies have shown that reducing the exploration time of cyprids and mussel larvae decreased attachment and also increased the rejection rate of the substrate for attachment and metamorphosis [20,33,72].

Nano topography

Antifouling research applying nano-sized topographies is recent and technically more complicated. The AMBIO collaboration was an effort that investigated the potential of nanostructures for antifouling applications [74]. These studies involved the development of nanostructures with novel, non-toxic materials to develop better antifouling solutions. Apart from concepts of superhydrophobicity, surface energy and surface wettability, there are yet to be established theories that can confirm the direct correlation of the antifouling properties of nano-topographies with the settlement trends of marine fouling organisms [74-76]. Nonetheless, with the exception of marine bacteria, settlement densities of other taxa were reduced on substrates with topographies of this scale (Additional file 2: Table S2, Additional file 3: Table S3).

There are not many studies that discussed the effect of topography on the settlement behaviour of marine bacteria to determine a distinct trend that could explain their behaviour during settlement and attachment [77-79]. Several studies that analysed the settlement behaviour of marine bacteria on substrates did not indicate that topography had a significant effect on their settlement behaviour. Nonetheless, marine bacteria density increased on substrates with increased nano-scale surface roughness particularly where the measured root mean square roughness (R_q) equals to 0.01 μm [78,80]. This is likely because nano-rough surfaces have increased surface area for adhesion of extracellular polymeric substances (EPS) which improved the attachment of marine bacteria [45].

The greater reduction of diatoms and spores on substrates of nano peaks compared to nano bumps suggests that sharper nano peaks might have provided less support than broad nano bumps for attachment [81]. Similar to observed settlement trends of marine bacteria, several studies proposed that nanostructured substrates influenced surface wettability which influences organism adhesion. For example, the hydrophobic nature of adhesives secreted by diatoms makes them less inclined to adhere to hydrophilic nanostructures [6,82]. In contrast, *Ulva* spores attached weakly on hydrophobic nanostructures due to the hydrophilic nature of its adhesive [42].

Another proposed antifouling mechanism involving nano-topographies involved the surface reconstruction of nanostructures after immersion in water [6,83-86]. This shift in surface morphology might contribute to varying surface wettability of the substrate and influence organism adhesion. There has yet to be sufficient evidence or studies to support or deny these theories. The exact antifouling mechanism of nano-topographies remains unfounded and still lacks adequate understanding.

Several studies have argued that nano-topographies created trapped ‘air pockets’ between the solid–liquid interface that impedes larvae or microorganisms from having direct contact with the substrate [38,80,87-89]. The nature of these substrates was described as superhydrophobic and commonly characterised by measured contact angles that are at least 135° [90]. Due to their significantly larger bodies, it is unlikely that macro invertebrate larvae would be able to sense undulating irregularities at the nano-scale. Topography with features that are this small would not be large enough to induce significant drag or skin friction beyond the viscous sublayer of the water column in direct contact with the substrate. It is more probable that the ‘air pockets’ reduced macro invertebrate larvae density by preventing them from having physical contact with the substrate.

This finding is consistent with a separate field study which found that superhydrophobic nano-substrates prevented macro and microorganisms from settling for a period of 6 months [91]. In many cases, there has yet to be a solution which allows these substrates to maintain their impervious nature for prolonged periods of time. Subsequent fouling occurred because ‘air pockets’ eventually dissolved in water and also because the substrate was damaged by grazing from marine organisms.

Future directions

The critical range for microorganisms and macro organisms to settle differ significantly. This increases the difficulty to identify a range that would be effective at preventing both micro and macro organisms from settling. Micro topographies that usually consist of one uniform arrangement of geometry and scale have shown limited success at stopping settlement and did not have the same level of efficacy at inhibiting a wider range of taxa.

Hierarchical topographies are relatively new and have yet to be extensively explored for marine antifouling applications. Nonetheless, there were promising results which indicated that these topographies might have the potential to reduce fouling by both micro and macro organisms. Therefore, future studies on antifouling topographies should consider mixed scales of hierarchical structures that might have the potential to prevent a wider array of taxa from settling.

Unlike macro and micro topographies, nanostructures that were tested for marine antifouling purposes have been limited to irregular self-assembled geometries of mixed nano sizes. There are studies that have studied the effects of regular arrays of nano topographies at deterring biofouling but these have been limited to other applications [92,93]. Hence, future investigations should develop regular, nano-sized topographies to be analysed for marine antifouling purposes.

Surface topography also affects hydrodynamic stresses exerted on their ambient surroundings which also influence the settlement of fouling organisms. It would be beneficial to conduct more field assays taking this and other environmental factors into consideration especially on less explored nano or hierarchical topographies. Laboratory assays and field trials often yield results that differ from each other. This indicates the influence of environmental variables such as biofilm growth and conspecific cues on organism settlement trends [25,94].

A recent study by Vucko et al. [94] determined that the settlement trends of a range of marine organisms differed when comparing results from laboratory assays and field trials. It is speculated that biofilm growth could have contributed to the differences in observations. Biofilm formation on submerged substrates could have rendered the antifouling properties of topographies irrelevant. The next best course of action would be to combine antifouling topography with other antifouling mechanisms such as surface energy to devise better solutions and reduce biofilm formation [95]. It is probable that only combinations of several mechanisms could result in more robust topographies with better antifouling abilities.

This might now be possible with improvements made to the ERI_{II} ratio (equation 2) to quantify the combined effects of substrate material (i.e. surface energy) and topography to optimise future designs of antifouling solutions [29-31]. Decker et al. [96] further improved these results to predict not just the inhibitory but also the enhancing aspects of foul resistant topographies that influence organism settlement trends. These quantitative models are necessary and useful to evaluate the performance of foul-resistant topographies and also to validate results from laboratory and field trials. Future studies on these models could consider a wider range of environmental variables such as salinity and temperature to make the results more comparable to experimental trials.

Conclusions

Macro topographies encouraged most organisms to settle and are not suitable to be applied as antifouling topographies. In general, sub-macro topographies exhibited antifouling properties and there were encouraging results but there has yet to be a topography configuration capable of reducing the settlement of a wide range of marine taxa. Closer inspection of micro topographies determined that the topography scale in terms of height/depth, width, symmetry, distance apart from each other and average roughness were important parameters that can have polarising effects on different taxa.

As it stands, it is difficult and possibly not practical to develop optimum topographies with scales that can deter the settlement of all types of taxa. Nonetheless, uniformly patterned nano-topographies and hierarchical topographies have yet to be widely explored and might have the potential to deter fouling from a wider range of marine taxa and should be investigated further. Both scales of topography also warrant further investigations to clarify their exact antifouling mechanisms to increase the antifouling efficacy of both types of topography.

A solution is also required to increase the durability of antifouling topographies in marine environments to be considered a viable option for commercial antifouling applications. Moreover, there has yet to be any solution to prevent the formation of biofilms that would eventually hide and overcome the antifouling properties of topographies. This suggests that environmental factors (e.g. hydrodynamic shear, biofilms, conspecific cues) are influential towards antifouling topographies and current topographies are still not well equipped to be in marine environments for long periods of time. Hence, combinations of several strategies are likely the best option to maintain the durability and performance of antifouling topographies. More field trials are necessary to corroborate observations from laboratory tests and calculations from quantitative models for optimum designs of foul-resistant topographies.

Additional files

Additional file 1: Table S1. List of reviewed studies. List of studies that have been considered for the purpose of this review paper.

Additional file 2: Table S2. Microorganism settlement on topography of various scales and geometries. This table compiles the known effects of topography that were varied in terms of scale or geometry towards encouraging or inhibiting the settlement of microorganisms.

Additional file 3: Table S3. Macroorganism settlement on topography of various scales and geometries. This table compiles the known effects of topography that were varied in terms of scale or geometry towards encouraging or inhibiting the settlement of macroorganisms.

Additional file 4: Table S4. Rough illustrations of reviewed topography geometries. This table aims to clarify the classification of topography geometries which lead to the compilation of Additional file 2: Table S2 and Additional file 3: Table S3.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
FWYM drafted the manuscript, figures and tables under the frequent advisement of OP and JW. Both OP and JW proof read the manuscript and all authors agree with the final version of the manuscript.

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