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Biofouling

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713454511>

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First published on: 19 February 2010

To cite this Article Bers, A. V. , Díaz, E. R. , da Gama, B. A. P. , Vieira-Silva, F. , Dobretsov, S. , Valdivia, N. , Thiel, M. , Scardino, A. J. , McQuaid, C. D. , Sudgen, H. E. , Thomason, J. C. and Wahl, M. (2010) 'Relevance of mytilid shell microtopographies for fouling defence - a global comparison', *Biofouling*, 26: 3, 367 – 377, First published on: 19 February 2010 (iFirst)

To link to this Article: DOI: 10.1080/08927011003605888

URL: <http://dx.doi.org/10.1080/08927011003605888>

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Relevance of mytilid shell microtopographies for fouling defence – a global comparison

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(Received 27 October 2009; final version received 6 January 2010)

Prevention of epibiosis is of vital importance for most aquatic organisms, which can have consequences for their ability to invade new areas. Surface microtopography of the shell periostracum has been shown to have antifouling properties for mytilid mussels, and the topography shows regional differences. This article examines whether an optimal shell design exists and evaluates the degree to which shell microstructure is matched with the properties of the local fouling community. Biomimics of four mytilid species from different regional provenances were exposed at eight different sites in both northern and southern hemispheres. Tendencies of the microtopography to both inhibit and facilitate fouling were detected after 3 and 6 weeks of immersion. However, on a global scale, all microtopographies failed to prevent fouling in a consistent manner when exposed to various fouling communities and when decoupled from other shell properties. It is therefore suggested that the recently discovered chemical anti-microfouling properties of the periostracum complement the anti-macrofouling defence offered by shell microtopography.

Keywords: Mytilidae; shell microtopography; antifouling

Introduction

Bivalves of the family Mytilidae feature predominantly among an ever increasing list of invasive aquatic species. Mytilids are widely distributed in inter- and subtidal habitats all over the world. They are considered as key species and important habitat engineers in benthic communities. Mytilidae possess high productivity, high fecundity and wide ecological tolerances that allow them to adapt to various environments (Branch and Steffani 2004), underlining their high invasive potential. Since the nineteenth century, the northern hemisphere mytilids *Mytilus edulis* and *Mytilus galloprovincialis* have spread far

beyond their native ranges, and now populate the east coast of South America, the South Pacific Ocean (Carlton 1999), the northern Pacific coast (McDonald and Koehn 1988; Wonham 2004) and shores of the South Atlantic. In the mid-1970s *M. galloprovincialis* invaded South Africa (Robinson et al. 2005), and now occupies the entire west coast of South Africa and the southern half of Namibia (Branch and Steffani 2004). Other mytilids have been equally invasive: the brown mussel *Perna perna*, a southern hemisphere species, and the green mussel *P. viridis*, an Indo-Pacific species, have recently been found in the Gulf of Mexico (Hicks and Tunnell 1993; Hicks et al. 2001; Ingrao et al. 2001).

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In Brazil, *P. perna* has been reclassified as an old introduction, most likely dating from the sixteenth century (Souza et al. 2004). The Asian mytilid *Musculista senhousia* has been introduced to Australasia, the Pacific coast of North America and the Mediterranean, where it substantially changed habitat complexity (Crooks and Khim 1999). The north-eastern Pacific mytilid *Mytilus trossulus* has not yet populated shores in the southern hemisphere, possibly because it is competitively inferior to the earlier invaders, *M. edulis* and *M. galloprovincialis* (Carlton 1999).

Being released from parasites, predators and epibionts may facilitate invasive success (Colautti et al. 2004). It is suspected that the invasive success of certain mytilid species may be due not only to their physiological adaptability but also to the antifouling (AF) characteristics of their shell surfaces. As for all hard-shelled marine organisms, mytilids offer a potential settlement substratum for larval stages of invertebrates as well as for algal propagules. Being overgrown by epibionts may affect the host organism directly by increasing drag, interfering with filter feeding, or indirectly by modulating ecological interactions (Wahl 1997a, 2008; Lesser et al. 1992; Wahl and Hay 1995). Because the effects of epibiosis are often negative, many invertebrates and algae have evolved AF defence mechanisms (Wahl 1997b).

Mytilid shells often remain remarkably free of epibionts since, like most other organisms, the prevention of epibiosis is vital for their functioning. The basic physical barrier proposed as a putative defence is a distinctive ribbed microtopography on the periostracum which has been shown to repel barnacle cypris larvae (Bers et al. 2006a,b; Wahl et al. 1998; Scardino et al. 2003). Lethal effects on the basibiont can be caused by epibionts, for example the barnacle *Balanus improvisus* impacts upon the functioning of *M. edulis* by: (i) increasing the risk of predation by the crab *Cancer maenas*, (ii) reducing the growth rate of the mussel and (iii) increasing the recruitment of the boring polychaete *Polydora ciliata* (Wahl 2008). Conversely, positive effects of epibiosis for *M. edulis* can be found when the hydroid *Laomedea flexuosa* settles on its shell, where it reduces predation and facilitates the recruitment of more mussels (Wahl et al. 1998; Wahl 2008). Therefore, it is possible that the microtopography of the periostracum of the Mytilidae has evolved as a resistant texture against several harmful epibionts and as a facilitative texture promoting settlement of beneficial epibionts. This can be explained by 'attachment point theory' which explains the mechanisms of epibiosis. This theory assumes that the early stages of life of an epibiont would be more successful in settling on microtextures (eg periostracum

microtopography) slightly wider than their own size. This is because the maximisation of the number of contact points between the epibiont and the settlement substratum will provide maximum adhesion and hence resistance against hydrodynamic drag forces (Scardino et al. 2006, 2008, 2009a,b). However, as a competing hypothesis, it is also possible that larvae and spores of species that are not obligate epibionts have co-evolved to avoid basibionts, because they can represent a weak substratum for settlement increasing the risk of mortality, eg mussels which can be easily detached. This notion is convergent with the fact that larvae and spores have to be able to recognise basibionts, perhaps using chemical compounds or the microtexture of the basibiont.

The Mytilidae are notorious as ship hull epibionts and are able to reach new areas as a consequence of increasingly intensive maritime traffic (Champ 2000; Minchin and Gollasch 2003). It is hypothesised that this global success is linked to the existence of a defensive mechanism related to the microtexture of the periostracum on which larvae and spores of epibionts have difficulty settling, and/or avoid. It is possible that this periostracal microtopography could vary regionally due to adaptation to local fouling species, assuming selection pressure was strong enough. On the other hand a broadly efficacious AF defence system would favour a strong ability to invade new areas. In general biogeographical differences in the AF defence systems of marine invertebrates have not been extensively studied (Becerro et al. 2003).

The aim of this study was to investigate the AF performance of biomimics of the shell microtopographies of various mytilid species originating from different regional provenances when exposed to autochthonous and allochthonous epibiotic challenges.

Material and methods

Study organisms

Four mytilid species from eight different regional provenances were used in this study: (1) *M. edulis* from Kiel Fiord in the Western Baltic, Germany (54°22'N, 10°9'E), from Matryonin Island in the Kandalakshsky Gulf of the White Sea, Russia (66°18'N, 33°40'E), from Toothacker Cove on Swan's Island, Maine, USA (44°10'N, 68°25'W) and from Hartlepool Marina in the North East of England (54°41'N, 1°11'W); (2) *P. perna* individuals from Itaipu Beach, Rio de Janeiro State, Brazil (22°56'S, 43°03'W) and from Bushman's, South Africa (33°42'S, 26°40'E); (3) *P. viridis* from Long Harbour, South China Sea, Hong Kong (22°27'N, 114°21'E); (4) *M. galloprovincialis planulatus* from Eden, New South Wales, Australia (37°5'S, 149°57'E).

Replication of mussel shells

To investigate the influence of mytilid surface topography on epibiosis separately from other possible anti-settlement properties such as chemical defences, high resolution resin (Devcon 2-TON) replicas of the specimen from the eight provenances were made (see Marrs et al. 1995; Bers and Wahl 2004; Bers et al. 2006b for details). Only specimens with intact periostracum were used for replication. There is little literature on periostracum structure and condition in many of the regions examined. However, strong correlations have been found between the degree of periostracum abrasion and epibiosis and/or endolithic organisms. This relationship provides indirect information about the health of the periostracum in natural habitats where the mussels were collected (Wahl et al. 1998; Kaehler 1999). It is well documented that abrasion of the periostracum is caused by wave action (Wahl et al. 1998; Kaehler 1999; Zardi et al. 2009). For example, the percentage of abrasion of the periostracum of *P. perna* in South Africa can be higher on headlands (96%) than in sheltered bays (5–20%) (Kaehler 1999; Zardi et al. 2009). In this investigation, it was found that the proportion of mussels exhibiting epibiosis and periostracum damage varied between 0% and 10% among the regions studied. Smooth control surfaces were made by sealing a second resin replica of the same individual shell with an additional layer of the epoxy resin. Rough control surfaces were made by sanding a third resin replica of the same individual with a Dremel[®] rotary tool and sanding bands (no. 408/Grit 60) to provide standardised roughness. Thus, the surfaces of all replicas of each shell were identical in every respect (size, shape, colour, chemistry) except surface microtopography. All resin replicas were checked for the presence of micro-bubbles under a dissecting microscope, and poor replicas were discarded. Only perfectly replicated biomimics were used in this study. The casts are robust, non-toxic and highly accurate copies of natural mussel surfaces and therefore suitable for use in replicated experimental designs (Bers and Wahl 2004; Bers et al. 2006b).

The mytilid periostracum is believed to have evolved from a primitive flat form *via* a rough anisotropic form to the present ribbed isotropic texture (Bers et al. 2006b). Thus the two controls (rough and smooth) represent the isotropic and anisotropic forms. Therefore no difference in effect between a specific periostracum microtexture and the smooth control or rough control would indicate that epibiotic organisms cannot distinguish between these two surfaces, thus denoting similarity in their functions at that scale. In contrast, if a specific mussel microtexture exhibited differences to either of the

control surfaces, this would imply that the microtexture has evolved specifically to either deter or attract certain epibionts.

Experimental design and field experiments

The experiment followed a randomised block design with within-block replication. Level one: block or region from which mussels originated. The biomimics were permanently hung on eight ropes submerged at 1 m water depth to exclude benthic predators. Level two (treatments): each block comprised three treatments or biomimic types: (1) natural, (2) smooth and (3) rough microtopography. The blocks were randomly distributed along each rope. The total replication was 8 sites \times 8 (ropes per site) \times 8 (blocks) \times 3 (treatments) = 1536 individual biomimics. All biomimics were exposed to natural fouling or epibiotic communities in the following regions: USA, Australia, Brazil, Germany, Chile, Hong Kong, South Africa and the UK, either hanging from piers or on floating buoys in sheltered conditions such as bays/marinas, and therefore protected from major wave action. Thus the immersion depth of the biomimics was constant. Most of the mussel species from which biomimics were made can be found naturally in low wave action conditions so that the experiments were carried out in natural environments, exposing the biomimics to natural epibiotic communities.

Identical experiments were conducted at eight regions (or locations) for 6 weeks in the summer season of each country during 2004/2005 (see Table 1 for details). *M. edulis* from the USA were only tested at sites in the Northern Hemisphere, and at the US site they were only replicated four times due to limitations on space.

Abundances of common (> 5% cover) fouling organisms were checked weekly by estimating the percentage cover under a dissecting microscope (visual estimation) and by analysing digital photographs of the resin mussels using Image Tool 3.0. Agreement between visual and digitised estimates was previously confirmed based on a large number of local datasets in which both methods were employed (data not shown). Only a central area (1 cm²) of each cast was used for the estimates to avoid possible edge effects. This was considered to be a sufficiently large sampling area since the ratio larvae to microsites is below 1 cm² (Scardino et al. 2003; 2006; 2008; 2009a,b; Bers and Wahl 2004; Bers et al. 2006b). All replicates were immediately re-deployed after counting. The abundances of fouling organisms on the microstructured surfaces were compared to those on the corresponding control surfaces. Due to technical problems, data from the German experimental site are missing for week 3, and

Table 1. Details of the experimental sites.

Country	Australia	Brazil	Chile	Germany	Hong Kong	South Africa	UK	USA
Longitude	146°50'E	43°08'W	71°22'W	10°9'E	114°21'E	26°40'E	1°11'W	68°25'W
Latitude	19°15'S	22°52'S	29°58'S	54°22'N	22°27'N	33°42'S	54°41'N	44°10'N
Start of experiment	29.09.2004	29.10.2004	12.10.2004	23.05.2005	14.05.2005	11.10.2004	06.05.2005	16.06.2005
End of experiment	10.11.2004	11.12.2004	26.11.2004	04.07.2005	25.06.2005	22.11.2004	19.06.2005	31.07.2005
Sea surface temperature (°C)	24–28	24–27	19–21	12–16	23–27	14–20	15–20	n/a
Tidal amplitude (m)	3.27	1.2	1.3–1.5	0.2	0.5–2.3	2	0	n/a
Species richness (with > 1% cover)	6	8	5	8	4	4	4	4
Salinity (psu)	35.5–36	24–34	34	12–17	20–35	35	35–38	n/a

from the experimental sites in South Africa and the UK for week 6.

Surface characterisation

The natural surface topography of mussel shells from each species was visualised by scanning electron microscopy (SEM). The shells were sputter coated with a 20 nm thick gold – palladium alloy using a Balzer Union SCD 004 instrument and viewed with a Zeiss DSM 940 Digital Scanning Microscope (Figure 1). Casting accuracy was assessed by the evaluation of SEM pictures (Bers and Wahl 2004). Additionally, the amplitude of the ripples and their aspect ratio of each natural biomimic were characterised by measuring them from the SEM photographs using Image Tool 3.0. Five measurements were taken for each regional biomimic.

Statistical analysis

Three types of analysis were employed: (1) In order to be able to compare results from different fouling communities affected by different climatic and environmental conditions in different parts of the world all within a single experimental design, a meta-analysis approach (Gurevitch and Hedges 2001) was used. Data for settlement after 3 and 6 weeks were analysed to determine the influence of time within the duration of the experiment. In order to compare settlement effects between experimental sites with highly different fouling communities and between sampling dates, a meta-analysis based on the natural log (ln) effect ratio was used to assess the relative and overall impact of surface microtopography on fouling (Gurevitch and Hedges 2001):

$$\text{In effect ratio} = \ln \left[\frac{\text{Average \% cover of recruits (natural surface structure)}}{\text{Average \% cover of recruits (rough control)}} \right]$$

$$\text{In effect ratio} = \ln \left[\frac{\text{Average \% cover of recruits (natural surface structure)}}{\text{Average \% cover of recruits (smooth control)}} \right],$$

respectively

First, The effect ratios were calculated for each biogeographic block by dividing the average of epibiont cover in the natural biomimic ($n = 8$) treatment by the rough ($n = 8$) and smooth control ($n = 8$) treatments, respectively. Confidence intervals were calculated using Student's t -distribution: upper and lower limit = mean (effect ratio per bio-geographical region) $\pm t_{0.05(\infty)}$ SD (where SD means standard deviation). Non-overlap of confidence intervals with zero indicated significant differences or effects (level of significance $\alpha = 0.05$), which can be positive, indicating epibiotic attraction by the microtopography in comparison to a specific control, or negative,

indicating deterrence of fouling by the natural microtopography with respect to the control. Finally, confidence intervals that overlap with zero imply the absence of significant attraction or deterrence. (2) A total of 112 individual tests were carried out to

determine the significance of effect ratios in the meta-analysis at the end of week 3, while 96 tests were carried out at the end of week 6. Positive, negative and null effects were pooled for week 3 and week 6, and the results were tested with a χ^2 -goodness of fit test to check if the frequencies were likely to have been due to chance. (3) Two one-way ANOVAs were used to compare differences between peak to peak amplitude and aspect ratio (height/width) of micro-ripples that represented the microtopography of each regional mussel and/or species; data were natural log ($\ln + 1$) transformed to achieve normality and variance homogeneity. The sample size was $n = 5$ for each bioregional mussel. Student-Newman Keuls (SNK) *post hoc* tests were used to locate sources of variation causing any significant differences.

Results

Characterisation of the microtopography of shells from different regions of the world

All of the Mytilidae used in this study possessed a distinct microtopography of the periostracum (ANOVA $F_{7,32} = 33.5, p < 0.0001$). The surface structure of the genus *Mytilus* consisted of microripples with an amplitude of 0.4–4 μm (Figure 2), running more or less at right angles to the growth rings. *P. viridis* from Hong Kong exhibited the largest peak to peak ripple amplitude of $3.5 \pm 1.05 \mu\text{m}$. The four regional forms of *M. edulis* exhibited significant differences in the

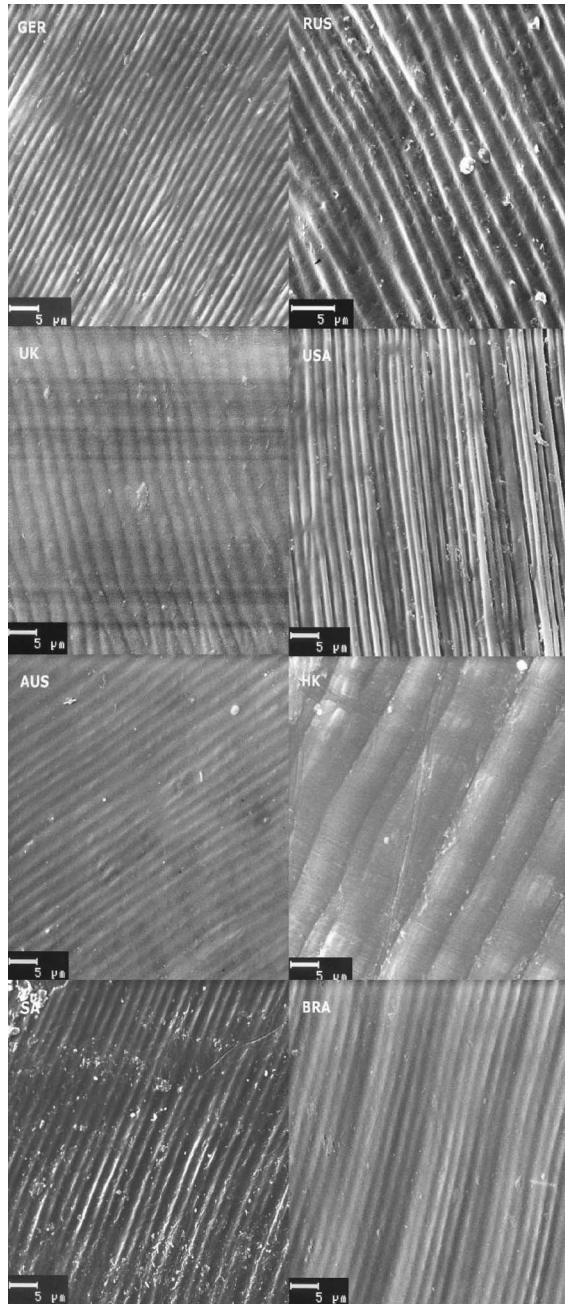


Figure 1. Scanning electron micrographs of the natural shell microtopographies of all Mytilidae investigated: GER, *M. edulis* from Germany; RUS, *M. edulis* from Russia; UK, *M. edulis* from the UK; USA, *M. edulis* from the US; AUS, *M. galloprovincialis planulatus* from Australia, HK, *P. viridis* from Hong Kong; SA, *P. perna* from South Africa; and BRA = *P. perna* from Brazil.

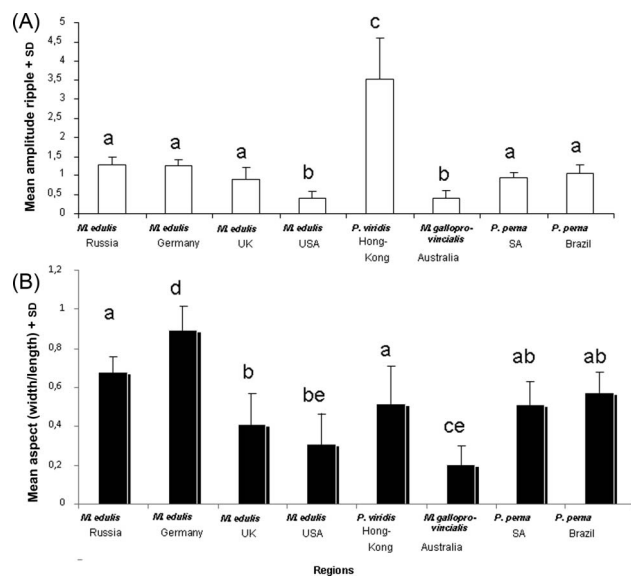


Figure 2. (A) Differences in amplitude between species of mussels. (B) Differences in aspect ratio between mussel species. Results were extracted from an SNK *post hoc* test after significant ANOVA. Different letters indicate differences between species.

amplitude of the periostracal ripples, for example those from the USA and Australia exhibited the smallest ripple amplitude ranging from 0.4 to 0.6 μm , while those from the UK, Russia, South Africa and Brazil at 0.96–1.3 μm were intermediate in magnitude (Figure 2A). *P. perna* from Brazil and South Africa did not exhibit differences between the amplitude of their ripples, nor did most of the *Mytilus* species from these sites. The general trend in the magnitude of

ripple amplitude from the smallest to the largest was according to the SNK test: USA < Russia = Germany < Australia < Brazil = South Africa = UK < Hong Kong (Figure 2A). The microtopography of *M. galloprovincialis planulatus* was similar to that of its congeners. *P. perna* also exhibited microripples, but on a less flat general surface formed of larger sets of hills and valleys composed of smaller ripples; *P. viridis* (Hong Kong), however, had a surface

Table 2. Fouling species and their abundance at the different sites.

Fouling species	AUS	BR	CHL	GER	HK	UK	USA	SA
Chlorophyta								
Cell size range (5–7 μm)								
<i>Ulva linza</i>	○	++	+++	+	++			○
<i>Ulva sp.</i>	○	+	○		+		+	
<i>Cladophora vagabunda</i>	○	+	○					
<i>Spongomorpha sp.</i>								
Rhodophyta								
Cell size range (30–40 μm)								
<i>Polysiphonia sp.</i>			+++					
Phaeophyta								
Cell size range (about 5 μm)								
<i>Desmarestia viridis</i>							+++	
<i>Ectocarpus siliculosus</i>						++		
Ciliata								
Cell size range (about 100 μm)								
<i>Folliculina sp.</i>			+					
Crustacea								
Size larvae (about 500 μm)								
<i>Amphibalanus amphitrite</i>	+							
<i>B. improvisus</i>				++				
<i>Austromegabalanus pssittacus</i>			++					
Ascidacea								
Size larvae (about 200 μm)								
<i>Botrylloides nigrum</i>		+						
<i>Botrylloides leachi</i>	+						○	
<i>Styela plicata</i>	++							
<i>Ascidia sp.</i>	○							
<i>Diplosoma listeranium</i>	++							
Bryozoa								
Size larvae (about 300 μm)								
<i>Watersipora subtorquata</i>	+							
<i>Schizoporella sp.</i>						+		
<i>Bugula neritina</i>		+++						+
Polychaeta								
Size larvae (about 200 μm)								
<i>Hydroides elegans</i>	++					++		
Serpulidae		+						
Spionidae		+++						
<i>Spirorbis spirorbis</i>						+		
Hydrozoa								
Size larvae (10 width \times 500 length μm)								
<i>Obelia dichotoma</i>		+++						
<i>Obelia sp.</i>			++				○	
Bivalvia								
Size larvae (300 μm)								
<i>M. edulis</i>				++				
Anthozoa								
Size larvae (200 μm)								
<i>Metridium senile</i>							○	

+++ , very abundant; ++ , abundant; + , present; ○ , occasionally. Field site codes: AUS, Townsville, Australia; BRA, Mocangú Island, Guanabara, Brazil; GER, Kiel, Germany; HK, Hong Kong; UK, Hartlepool, England; USA, Swan's Island, USA; SA, Bushman's, South Africa; CHL, Bahía de Herradura, Chile.

where sets of hills and valleys were accentuated and ripples were attenuated.

The aspect ratios of the periostraca also were variable between regions (ANOVA $F_{7,32} = 12.1$, $p < 0.0001$), but did not follow the trend in size as found for amplitude. The range of aspect ratio varied between 0.2 and 0.9. The largest was observed for Germany, while the smallest aspect ratio was observed for *M. edulis* from the USA and *M. galloprovincialis* from Australia (Figure 2B).

Effects of microtopographies in different bio-geographical regions

Even though the mussels and the regions studied represent only a small fraction of the biogeographic zones in the world, epibiotic settlement was highly variable with regard to macrofoulers (Table 2). Nevertheless, in all instances the resin casts were rapidly covered by biofilms, recognised visually by a change of coloration of the casts. The total percentage of cover only refers to macrofouling species and both biofilm and benthic diatoms were excluded from the analysis. For all mytilid species tested at all experimental sites, the general picture was similar: the microtopography did not have a persistent significant effect, either repellent or attractive, in comparison to the rough and smooth controls. In the few cases where a significant effect was found, the natural microtopography almost exclusively reduced fouling. Overall there were significantly more neutral relationships, meaning attributable to chance, during the first ($\chi^2 = 168.76$, $df = 2$, $p < 0.0001$) and sixth week ($\chi^2 = 147.25$, $df = 2$, $p < 0.0001$).

Deterrent effects after three weeks exposure (Figure 3 and Table 3)

At the US site, the natural surface structure of the UK *M. edulis* significantly reduced fouling when compared to the rough control. Similarly, at the Hong Kong site, the natural microtopography of the autochthonous *P. viridis* significantly reduced fouling when compared to both rough and smooth controls, and at the South African site, the natural surface structure of the German *M. edulis* reduced fouling in comparison to smooth and rough controls, while at the Hong Kong site the microstructured surface was less fouled than the smooth control. In the UK, natural microtopography of *M. edulis* from Russia deterred foulers in comparison to the rough control. Finally, the natural microtopography of *M. galloprovincialis planulatus* of Australia performed best in Hong Kong, where it was significantly less fouled than both smooth and rough controls.

Deterrent effects after 6 weeks exposure (Figure 4 and Table 3)

At the US site, the better performance of the UK *M. edulis* natural microtopography in comparison to the rough control persisted after 6 weeks, while all other effects detected after 3 weeks vanished. However, at the German field site, fouling on the natural surface structure of the South African *P. perna* was significantly lower than on the smooth and rough control surfaces. Similarly, *P. viridis* from Hong Kong diminished the settlement of foulers on the natural surface compared to the smooth and rough controls at the Australian site. In Chile, casts of natural *M. edulis*

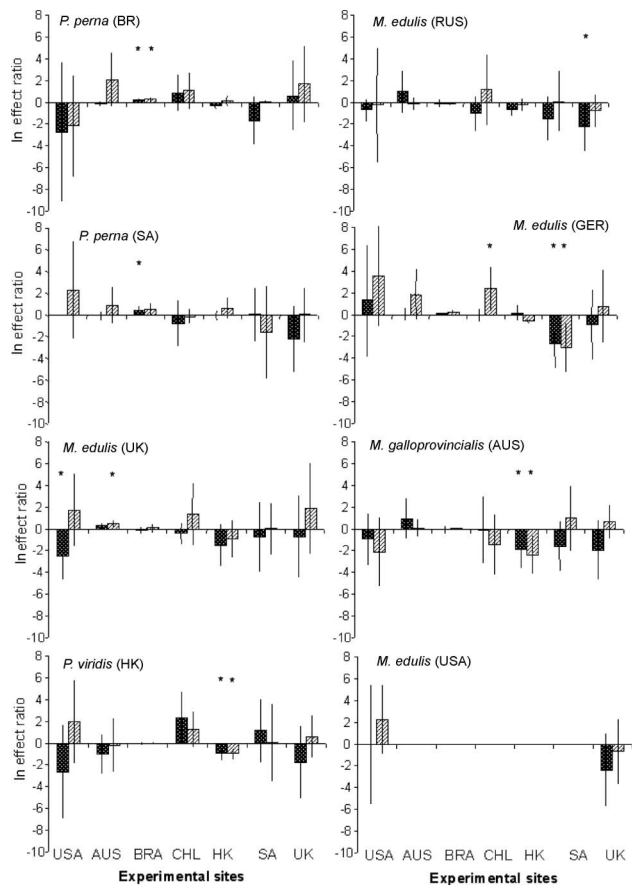


Figure 3. Fouling after 3 weeks exposure on natural shell microtopographies vs rough and smooth controls, using a ln effect ratio. Error bars indicate 95% confidence intervals. ■ = $\ln(\text{total \% cover on natural microtopography} / \text{total \% cover on rough control})$; ▨ = $\ln(\text{total \% cover on natural microtopography} / \text{total \% cover on smooth control})$. Experimental sites: USA, Swan's Island, USA; AUS, Townsville, Australia; BRA, Mocanguê Island, Brazil; CHL, Bahía de Herradura, Chile; HK, Hong Kong; SA, Bushman's, South Africa; UK, Hartlepool, UK; *, significant differences from ln effect ratio = 0.

Table 3. Synthesis of results from meta-analysis.

Mussel spp. and region	Autochthonous effects?	Allochthonous effects?
After 3 weeks		
<i>P. perna</i> , Brazil	Attraction of fouling	No effect
<i>P. perna</i> , South Africa	No effect	Attraction in Brazil
<i>M. edulis</i> , UK	No effect	Deterrence in USA, attraction in Australia
<i>P. viridis</i> , Hong Kong	Deterrent effects	No effect
<i>M. edulis</i> , Russia	Not tested	Deterrence in UK
<i>M. edulis</i> , Germany	No effect	Attraction in Brazil
<i>Mytilus galloprovincialis planulatus</i> , Australia	No effect	Deterrence in Hong Kong
<i>M. edulis</i> , USA	No effect	No effect
After 6 weeks		
<i>P. perna</i> , Brazil	No effect	No effect
<i>P. perna</i> , South Africa	No effect	Deterrence in Germany
<i>M. edulis</i> , UK	Not tested	Deterrence in USA
<i>P. viridis</i> , Hong Kong	No effect	Deterrence in Australia
<i>M. edulis</i> , Russia	Not tested	Attraction in Australia, deterrence in Chile
<i>M. edulis</i> , Germany	No effect	Attraction in Brazil
<i>Mytilus galloprovincialis planulatus</i> , Australia	No effect	No effect
<i>M. edulis</i> , USA	No effect	No effect

The table describes the effects of the microtopography of different species and regions on their own autochthonous epibiotic community and when it was exposed to an allochthonous community. Attraction means the microtopography stimulated epibiosis, deterrence means a repulsive effect on epibionts.

shells from Russia had fewer epibionts than the rough controls.

Attraction effects after 3 weeks (Figure 3, and Table 3)

The biomimics of the natural topography of *P. perna* from Brazil exhibited an enhanced cover of autochthonous epibionts in comparison to smooth and rough controls. The same enhancement was observed for *P. perna* from South Africa in comparison to the rough control in Brazil. The natural topography of *M. edulis* from the UK enhanced the settlement of epibionts at the Australian site. Finally, in Chile, *M. edulis* from Germany with natural topography suffered enhanced settlement in comparison to the smooth treatment.

Attraction effects after 6 weeks (Figure 4, and Table 3)

M. edulis from Russia enhanced the settlement of macrofouler in Australia. Similarly, *M. edulis* from Germany enhanced settlement of foulers in Brazil.

Discussion

Living mussels with intact periostracum are well known to maintain a fouling-free surface over months to years. Hence, one or more mechanisms must be responsible for maintaining a clean shell over a long time periods. Investigation of the surface chemistry of *M. edulis* has revealed that the periostracum contains several compounds that not only inhibit attachment of

bacteria and diatoms but also slow down diatom growth and prevent bacterial growth (Bers et al. 2006a). At the same time *Mytilus* is able to maintain, at least for a short period, a relatively epibiont-free surface using only its shell microtexture, therefore both mechanisms may complement one another (Bers et al. 2006a,b; Scardino et al. 2008).

This study explored for the first time the performance of the shell microtexture of different mytilid species cross-exposed to fouling communities in different regions of the world. This was done to test the contribution of the purely textural AF property of the shell. Since all mytilids tested in this study are known as very good invaders it was hypothesised that their shell microtopography could serve as a multi-regional AF mechanism in a wide range of native and non-native regions. All the epoxy resin biomimics at all field sites (regardless of surface structure: roughened, smooth or naturally textured) were rapidly covered with a biofilm and then by macrofoulers (multicellular epibionts) consistent with the normal fouling succession (Dobretsov et al. 2006). Neither persistent nor conspicuous AF mechanisms associated with periostracum microtexture were observed.

According to the 'contact point theory', fouling organisms that match the size of the texture can increase the number of contact points, facilitating their settlement (Scardino et al. 2008). This theory suggests therefore that deterrent effects would occur when epibiont size is larger than basibiont microtexture as there would not be enough contact points between them for substantial adherence. A cursory examination

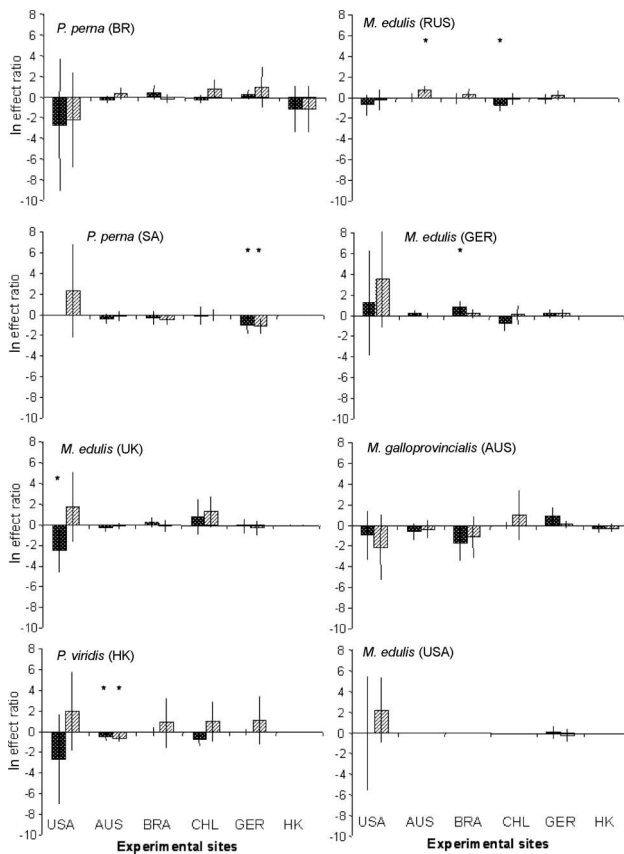


Figure 4. Fouling on natural microtopographies vs. rough and smooth control, using a \ln effect ratio, after 6 weeks exposure. Error bars indicate 95% confidence interval. \blacksquare = \ln (total % cover on natural microtopography/total % cover on rough control); \square = \ln (total % cover on natural microtopography/total % cover on smooth control). Experimental sites: USA, Swan's Island, USA; AUS, Townsville, Australia; BRA, Mocanguê Island, Brazil; CHL, Bahía de Herradura, Chile; GER, Kiel fiord, Germany; HK, Hong Kong; *, significant differences from \ln effect ratio = 0.

of the relationship between settler size of the abundant and very abundant epibionts (see Table 2) and ripple size (Figure 2) provides no evidence for this theory, *viz* globally the amplitude of the periostracal ripples ranged from 0.4 to 4 μm whilst the size of settlers ranged between 5 and 500 μm . Therefore, these findings challenge the 'contact point theory' (at least with regards to the natural microtextures from the Mytilidae studied), because the performance of a given microtexture is not dependent on the relation between its surface (scale of complexity) and the size and specificity of potential fouling organisms. Other factors can be important in keeping mytilid shells free, such as chemical defences and the aspect ratio of the microtexture (Bers et al. 2006a,b; Schumacher et al. 2007). Microtopographies with a larger aspect ratio may have

a stronger AF property, for example Schumacher et al. (2007) showed that the density of spores of *Ulva* was reduced by 42% with each unit of increase in aspect ratio, but only if the microtopography was at the same scale of the spores. In this study there was no obvious relationship between AF efficacy and aspect ratio. Indeed, the species that settled had larvae or spores larger than the microtexture, thus aspect ratio becomes somewhat irrelevant to these organisms.

The reduction of epibiosis on mussel shells can also be related to the complexity generated by the behaviour and the physiology of epibionts, for example, the contrast in settlement between motile and non-motile spores/larvae (eg Schumacher et al. 2007; Scardino et al. 2008). All the investigated microtopographies failed to prevent fouling in a persistent manner when decoupled from other factors, even more so when exposed to their natural fouling community. The few repulsive effects observed were restricted to a relatively early period of colonisation and diminished afterwards; by weeks 3 and 6, there were few significant effects of surface microtopography. This is in agreement with the findings of Scardino et al. (2003), Bers and Wahl (2004) and Scardino and de Nys (2004), who also described the short-term nature of the AF effects of natural microtopographies. It is possible that the microtopography interferes with the attachment strength of specific epibiont organisms and then a chemical property of it interacts that induces the release of the epibionts.

Furthermore, it is possible that microorganisms such as bacteria and diatoms gradually fill up the mytilid microtopographies, thus masking them for settling macrofouling organisms and creating an attractive substratum for colonisation. Interestingly, many of the biomimics exhibited different effects on allochthonous epibiotic communities and these effects were either positive or negative. The only positive effect on allochthonous fouling communities that was consistent in time (ie detected during both week 3 and 6) was the case of *M. edulis* from Germany, which experienced enhanced biofouling in Brazil. Multi-allochthonous effects (+ve and -ve) were observed in *M. edulis* from Russia, which were deterrents in Chile but attractive in Australia. These positive effects can be explained as a mechanism for protection against predation, where a basibiont attracts an epibiont that contains some noxious chemical compound (Wahl et al. 1998), such as in the case of the brown alga *Desmarestia* spp. (Molis et al. 2008). Similarly, the presence of chemical defences in the Ulvales has been reported in some places (Van Alstyne et al. 2001).

It was clear that shell microtopography alone does not contribute substantially to the successful

establishment of invasive Mytilidae in new habitats through the deterrence of epibionts. It is possible that the isotropic texture of mytilids has evolved in response to other selective pressures such as erosion. For example, more textured shells can delay abrasion of the periostracum, and delay infestation by endolithic bacteria (Zardi et al. 2009).

The ecologically complex interactions of basi- and epibionts and/or predators may require a multi-level defence system with chemical, physical and mechanical mechanisms (Krug 2006). These natural defence systems with two or more synergistic mechanisms have been studied before. In the gorgonians *Leptogorgia* and *Pterogorgia* toxic secondary compounds slow down surface fouling and are combined with a periodic ablation of the outer surface (Targett et al. 1983). The ascidian *Polysyncraton lacazei* possesses one mechanical, one associational and four chemical mechanisms targeting various types of fouling organisms (Wahl and Banaigs 1991), and similarly in four Caribbean sponges (*Agelas clathrodes*, *Cinachyrella alloclada*, *Clathria virgultosa* and *Xestospongia muta*) a chemical defence acts synergistically with glass spicules against generalist fish predators (Jones et al. 2005). Bacteria associated with marine macro-invertebrates furthermore appear to be a major source of AF compounds and their highly specific symbiosis may complement natural AF systems (Dobretsov et al. 2006, 2009). Finally, the hypothesis of the existence of a microtopography that is effective in repelling fouling organisms in multiple regions of the world is rejected. If mussels adapt the microtopography of their periostracum to repel local coloniser species efficiently, then it would be expected to reduce defence performance when these mussels are faced with a different coloniser pool. Thus, allochthonous mussels should foul more intensively than autochthonous mussels. Regional adaptations of mussels to local fouling organisms were not detected in the present study; there were no obvious differences in how autochthonous and allochthonous species performed. The question of regional optimisation of an invasive species should therefore be re-examined by taking into account more complete natural AF systems that combine both chemistry and microtopography in this kind of global comparison.

Acknowledgements

The authors are grateful to Peter Petraitis (University of Pennsylvania) for providing the experimental data for the US site, Ms Erika Carlson Rhile and Tiago F. Porto for assistance in the field, and Allan Henderson for access to Hartlepool Marina. JCT was supported by the Royal Society. BAPdG thanks CNPq and FAPERJ for his Research Productivity and Young Scientist of the State fellowships. The work in South Africa is based upon research supported by the South African Research Chairs

initiative of the Department of Science and Research foundation.

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