Introduction

Marine microbes exert continuous fouling pressure on any submerged surface, whether dead or living. An average ml of seawater contains up to $10^7$ viruses, $10^6$ bacteria, $10^3$ fungi and $10^3$ microalgae (Cole 1982; Jennings & Fenical 1994). Most of them strive to form biofilms facilitating interaction and proliferation (Grossart 2010). The establishment of a biofilm on a living surface, such as an algal thallus, has multiple and important consequences for the interactions between the host and the environment, eg access to nutrients and light, and modulating the capacity of the alga for further recruitment of microfoulers or macrofoulers (reviewed in Wahl et al. 2012).

Marine macroalgae may bear dense and diverse biofilms (Lachnit et al. 2009). While certain alga–microbe interactions are beneficial (Egan et al. 2001), other associations have been found to be detrimental (Dobretsov et al. 2011). The epibiotic microbial film (Costerton et al. 1995) formed, for instance, on the thallus of a macroalga replaces the original interface between the host organism and its environment by a new epibiotic interface with often strikingly different physical, chemical, mechanical, topographical and biological properties (Wahl 2008). Bacterial colonisers can enhance the colonization of the surface by larvae and spores of macrofoulers (Unabia & Hadfield 1999; Dobretsov et al. 2009, 2011; Mieszkín et al. 2012) along with all the associated consequences, eg hindered transepidermal exchanges, increased weight and friction of the host (Prescott 1990; Dougherty & Russell 2005), or they may hinder colonisation (Nasrolahi et al. 2012). Microbial pathogens may cause extensive tissue damage (Sawabe et al. 1998). Furthermore, the epibiotic biofilm may impede gaseous exchange, reduce the intensity of incoming radiation and thereby reduce the photosynthetic activity of the alga (Wahl 2008). Given the potential consequences of being fouled (de Nys & Steinberg 1999); some control by the host over the type and/or abundance of epibionts is generally expected and should be of selective advantage (Dworjanyn, de Nys et al. 2006).

It has been demonstrated that macroalgae possess chemical defences to control fouling of their surfaces (Lau & Qian 1997; Sneed & Pohnert 2011). Although a number of studies have reported antifouling properties of algal metabolites, until now such roles have only been rigorously demonstrated for 5 species in an ecologically relevant manner (Schmitt et al. 1995; Dworjanyn, de Nys et al. 2006; Paul et al. 2006; Persson et al. 2011; Saha et al. 2011), ie at concentrations that roughly correspond to natural ‘near-surface’ concentrations and against naturally co-occurring fouling species.

Temperate macroalgal species have been reported to exhibit seasonal variation in their antifouling activity in (presumed) phase with fouling pressure and abiotic factors such as light intensity and water temperature (Hellio et al. 2004; Maréchal et al. 2004; Wahl et al. 2010). These studies, however, investigated total rather than surface extracts, and thus, metabolites deployed at the surface and those stored within the thallus could not be distinguished.

Keywords: Fucus; anti-settlement; chemical defence; seasonal variation; antifouling

Seasonal variation in the antifouling defence of the temperate brown alga *Fucus vesiculosus*

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The important role of marine epibiotic biofilms in the interactions of the host with its environment has been acknowledged recently. Previous studies with the temperate brown macroalga *Fucus vesiculosus* have identified polar and non-polar compounds recovered from the algal surface that have the potential to control such biofilms. Furthermore, both the fouling pressure and the composition of the epibiotic bacterial communities on this macroalga varied seasonally. The extent to which this reflects a seasonal fluctuation of the fouling control mechanisms of the host is, however, unexplored in an ecological context. The present study investigated seasonal variation in the anti-settlement activity of surface extracts of *F. vesiculosus* against eight biofilm-forming bacteria isolated from rockweed-dominated habitats, including replication of two populations from two geographically distant sites. The anti-settlement activity at both sites was found to vary temporally, reaching a peak in summer/autumn. Anti-settlement activity also showed a consistent and strong difference between sites throughout the year. This study is the first to report temporal variation of antifouling defence originating from ecologically relevant surface-associated compounds.

Introduction: *Fucus*; anti-settlement; chemical defence; seasonal variation; antifouling

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If defences are costly (Harvell 1998; Dworjanyn, Wright et al. 2006; Jormalainen & Ramsay 2009) and if they can be regulated (which is unproven to date), it would be expected that they would fluctuate seasonally since both light energy (Lehvo et al. 2001) and fouling pressure in temperate latitudes undergo seasonal shifts (Chiavelli et al. 1993; Pinhassi & Hagström 2000; Schauer et al. 2003; Thomsen et al. 2010).

The rockweed *Fucus vesiculosus* (Phaeophyceae), which has a temperate to arctic distribution is known to up-regulate or down-regulate its anti-herbivory defence in response to variations in grazing pressure (Rohde & Wahl 2008). However, no information is available for ecologically relevant, i.e. surface-deployed, variation in the anti-fouling defence of *F. vesiculosus* or any other macroalga.

Previous studies (Saha et al. 2011, 2012) demonstrated that *F. vesiculosus* possesses surface-associated non-polar (fucoxanthin) and polar (DMSP and proline) metabolites, with anti-settlement activity against a wide range of ecologically relevant fouling bacteria. A first indication for the existence of defence regulation would be if the level of these metabolites was lower when light was limiting (resource-driven regulation) and/or when fouling pressure was low (demand-driven regulation). Since both of these conditions coincide in the winter months, it would be expected that for *F. vesiculosus* in the Western Baltic, the deployment of chemical anti-fouling defences at the thallus surface would be lower in winter than in summer. To test this hypothesis, the anti-microbial activity of surface-bound metabolites was quantified every month over a period of 1 year.

**Materials and methods**

**Sample collection and sample sites**

*F. vesiculosus* was collected monthly between March 2010 and February 2011 from 2 geographically distinct locations separated by ca 300 km of coast line: Gelting, Germany (54°48′ N/9°44′ E) and Poel, Germany (54°01′ N/11°28′ E). Upon collection, the wet algae were individually sealed in zip-lock bags (filled with ambient water) and transported back to the laboratory in a cooler box.

To gain a general overview of the abiotic regime at Gelting and Poel, the temperature and salinity regime between 1 and 3 m depth was extracted from the GEOMAR Kiel Baltic Sea Ice Ocean model with a daily resolution (courtesy Andreas Lehmann, GEOMAR Kiel) with regular ground-truthing by, *inter alia*, using loggers (Star Oddi, Reykyavik, Iceland). Gelting featured a higher salinity, but a similar temperature regime (yearly mean of 15.7 psu (SD 1.1), and 9.4 °C (SD 7.8)) compared to Poel (yearly mean of 13.0 psu (SE 2.1), and 9.9 °C (SD 7.9); Figure S1a, b in the Supplementary information). [Supplementary material is available via a multimedia link on the online article webpage.] The salinity difference was most pronounced from May through to September (Figure S2).

**Surface-specific extraction of algae**

Surface extraction was performed in less than 12 h after collection. Macroscopic epibionts such as filamentous green algae and mussels were gently removed using tweezers. Subsequently, the algal branches were first spin-dried, weighed and then surface-extracted by dipping them for 10 s into a mixture of methanol (MeOH): hexane (1:1 v/v) as described in Saha et al. (2011). This method has been shown previously to be non-destructive for the epithelial cells of *F. vesiculosus* (Saha et al. 2011, Figure S4). Larger thalli had to be cut just prior to spin-drying and extraction. Care was taken to avoid contact between the cut ends and the solvent mixture to avoid contamination by intracellular compounds. The resulting extract was filtered through a GF/A filter (Whatman, Ø = 15 mm) and the solvent was reduced under vacuum at 30 °C using a rotary evaporator. Replication was five-fold for each month and each location, respectively, except for Poel in March and April 2010 and from Gelting in April and November 2010 when only 4 replicates were available.

**Calculating the volume of the extracted surface-associated boundary layer**

Pilot studies had shown that algal growth was more or less isomorphic in the distal thallus parts used for extraction and that 1 g of algal wet weight corresponded to a surface area of 25.57 cm² (both faces added, SD ± 1.88, T. Lachnit (pers. comm.)). Thus, algal surface area was determined by multiplying algal wet weight by 25.57 cm² g⁻¹. Subsequently, the extracted algal surface volume was calculated by multiplying the calculated algal surface area by 30 μm (estimated thickness of the water film adhering to the thallus surface after spin-drying; Wahl et al. 2010).

**Bacteria**

Eight common strains of bacteria belonging to the regional pool of microbial foulers were used in the settlement assays: *Cytophaga* sp. was isolated from the brown macroalga *Halidrys siliquosa* and also occurred on the brown macroalga *Saccharina latissima*; *Bacillus aquimaris* (Yoon et al. 2003) was isolated from the brown macroalga *H. siliquosa* and also occurred on the brown macroalga *Desmarestia aculeate* and the red alga *Ahnfeltia plicata*; *Cobetia marina* (Arahal et al. 2002) was isolated from seawater; *Vibrio litoralis* (Nedashkovskaya et al. 2003), *Alpha proteobacterium DG1293* (Submitted to the International Nucleotide Sequence Database Collection (INSDC) by Green et al. in 2006, unpublished data) and *Vibrio* sp. SIGA198 (Hardwick et al. 2003).
were all isolated from the brown macroalga *Fucus serratus; Pseudoalteromonas* BSw20057 (Submitted to the INSDC by Ji et al. in 2008, unpublished data) and *Alteromonadaeae* E1 bacterium (Patel et al. 2003) were isolated from the red alga *Polysiphonia stricta*. Strains were isolated from the named macroalgae by first rinsing the algal surface twice with sterile seawater (to remove non-attached bacteria), then vortexing fragments of the thallus for 20 s in sterile seawater. The resulting bacterial suspension was log-diluted in 6 steps (down to a dilution of $10^{-6}$). From each dilution step, 100 µl were spread onto marine nutrient agar (2.5 g peptone, 0.5 g yeast extract and 15 g agar $1^{-1}$ seawater) and incubated overnight at 28 °C. The next day, single colonies were transferred into liquid culture medium (the same composition as before omitting the agar) and grown over night at 28 °C on a shaker. From these cultures, a dilution spread on nutrient agar was performed triplicate, and grown overnight at 28 °C. Subsequently, a single colony was grown overnight in liquid medium at 28 °C, before being transferred to a Rotistore® cryo tube (Carl Roth GmbH, Karlsruhe, Germany) and stored at $-80^\circ$C. For sequencing, DNA was extracted using an Aqua Pure Genomic DNA isolation kit (Biorad). Bacterial strains were identified by sequencing of their cDNA as described earlier (Lachnit et al. 2010). Strains were isolated and identified by F. Symanowski, GEOMAR.

**Anti-settlement assay**

Anti-settlement activity was used as the most relevant measure of microfouling control because it combines both repellent and/or toxic effects (Wahl et al. 1994). Bacterial strains were grown in nutrient enriched medium (5 g peptone, 3 g yeast $1^{-1}$ filtered seawater) for 18–20 h. Prior to the assays, the optical density (OD) of the bacterial cultures was determined with a Beckman Du® 650 spectrophotometer ($\lambda$ 600 nm). Bacterial cultures with an OD range of 0.5–0.8 were used. Settlement assays were conducted in multi-well plates (96 polystyrene wells, flat bottom, Greiner®). Aliquots (96 µl) of bacterial suspension were added to the wells. Four µl of extract dissolved in DMSO (dimethylsulphoxide) at 25-fold natural concentration were added to the 96 µl of bacterial suspension so that the tested extract in the final mixture were diluted to their natural concentration (ie corresponding to the concentration in the boundary layer covering the algal thallus; Lachnit et al. 2010). Bacteria were not exposed to concentrations of DMSO higher than 5% in order to prevent toxic effects (Wahl et al. 2010). Wells with DMSO and bacterial suspension served as controls ($n=8$). The well plates were incubated for 1 h on a shaking table (100 rpm) at 20 °C (Saha et al. 2011). After that, the bacterial suspension was removed from the wells by overturning the plates and unattached cells were eliminated by gently rinsing ($\times 2$) with 100 µl of sterilised filtered seawater. The attached cells were quantified by staining with the fluorescent DNA-binding dye, Syto 9 (0.005 mM) for 10 min in the dark (Invitrogen, GmbH). A washing step was not necessary since the defence strength of *F. vesiculosus* extracts was calculated by dividing the fluorescence values from the test wells (settled bacteria in the presence of *Fucus* extract in DMSO plus stain) by the fluorescence value in the control well (settled bacteria in the presence of DMSO plus stain). To account for the autofluorescence of the extracts themselves, extract-only samples (extract in DMSO without bacteria) were measured using the same methods. Thus, the fluorescence attributable to the settled bacteria in the test well was obtained by subtracting extract-only fluorescence from the fluorescence values obtained in the test well containing extract, bacteria and stain. Fluorescence, as a proxy for the relative abundance of settled bacteria, was measured at excitation/emission wavelengths of 477–491/540 nm using a plate reader (Hidex Chameleon IV, Turku, Finland). The 8 strains were tested individually in anti-settlement assays. The extract of each of the 5 individuals of *F. vesiculosus* was tested in triplicate to account for variability in bacterial settlement rates. The fluorescence values from the 3 replicates were averaged and represented as 1 true replicate. For certain strains of bacteria replicate testing of individual extracts was not possible due to shortage of material. The defence strength was expressed as the ‘log effect ratio’, ie the logarithm of the fluorescence attributable to settled bacteria of strain Y in the presence of an extract divided by the fluorescence attributable to settled bacteria of strain Y in the absence of that extract (after the appropriate corrections mentioned above). A log effect ratio value of 0 (ie an equal number of bacteria in wells with and without extract) indicates that the tested extract had no effect on settlement, whereas a negative log effect ratio value indicates an inhibitory effect and a positive log effect ratio value indicates an attractive effect. Thus, a log effect ratio of $-1$ represents a 10-fold reduction whereas a value of $+1$ represents a 10-fold enhancement of bacterial settlement caused by the presence of the extract.

**Fouling regime**

Since most planktonic bacteria prefer to recruit onto solid/liquid interfaces (Grossart 2010), the cell density of bacteria in the plankton may serve as a rough proxy for fouling pressure on macroalgae. Thus, the monthly bacterial density at 1 m depth at Boknis Eck (54°45′N/9°83′E), which is an open-water habitat with a maximum depth of 27 m situated in between the 2 *F. vesiculosus* collection sites and was assessed in a separate project from 2005 to 2008 (data courtesy of H.G. Hoppe & R. Koppe, GEOMAR) was used as an approximate proxy for microfouling pressure.
Statistical analysis

The strength of anti-settlement activity of extracts on bacterial settlement was expressed as the log effect ratio (see above). Mean effect strength values were obtained by averaging the effect of each replicated extract on each of the 8 bacterial strains. A 2-way ANOVA was used to analyse the effect of seasonality and site on the mean antifouling activity of the macroalgae. A Shapiro–Wilks test was used to determine the normality of the distribution while Levene’s test was used to test for homogeneity of variance. Only true replicates (ie extracts of individual algae) were used for the purpose of analysis. Statistica software (Stat Soft, Tulsa, OK, USA) was used to conduct all statistical tests.

Results

Temporal variation in anti-microfouling defence

The anti-settlement defence strength of *F. vesiculosus* varied among seasons, sites and with regard to target bacterial strains (Figures 1–4). The sensitivity of the various bacterial strains differed by a factor of up to 10, with *B. aquimaris* and *Cytophaga* sp. being more sensitive than the other epiphytic bacteria (Figure 4). These relative strain sensitivities were similar at the two sites. Anti-settlement activities were approximately 1.4 times higher in the population of *F. vesiculosus* from Poel than in the population from Gelting (2-way ANOVA, *F* = 18, *p* < 0.001, Figures 1 and 2, Table 1). The pattern of seasonal variability, however, was similar in the 2 populations of *F. vesiculosus*.

The mean anti-settlement activity of surface extracts (averaged over all 8 target strains) showed a clear seasonal pattern (2-way ANOVA, *F* = 5, *p* < 0.001) with stronger repellence in summer and autumn, and weaker repellence in winter and spring. This pattern was consistent between the 2 locations (Figures 1 and 2, Table 1). Anti-settlement activity in summer/autumn was about 1.45 and 1.3 times stronger than in winter/spring for Poel and Gelting, respectively. There was no interaction between the two factors, site and season (2-way ANOVA, *F* = 1.7, *p* > 0.05) illustrating that defence strength in the 2 sites fluctuated synchronously.
Microfouling pressure

The density of bacterial cells in the plankton of Boknis Eck follows a quadratic seasonal curve peaking in summer and autumn (Figure 5) and was about 4 times higher in summer than in winter.

Discussion

Anti-microfouling activity of *F. vesiculosus* varied among target bacterial strains, sites and seasons. *Cytophaga* sp. and *B. aquimaris* were the most sensitive, while *C. marina* along with strains isolated from the brown alga *F. serratus* and the red alga *P. stricta* were relatively less sensitive to the total surface extract (Figure 4). Specific knowledge is insufficient to explain the causes of the differences in sensitivity among strains. However, the fact that the bacterial strains react differently to the surface metabolites of *F. vesiculosus* may contribute to the commonly observed non-random distribution of epibiotic bacteria. Indeed, *F. vesiculosus* is known to possess a biofilm that differs conspicuously from the biofilm on the surfaces of other neighbouring macroalgal species, non-living substrata and from the coloniser pool in the surrounding water column (Lachnit et al. 2009). Such an observation, along with the strain-specific sensitivities observed in the present study, is indicative of selective recruitment of bacterial foulers on the algal surface. This is explainable by either strain-specific preferences for certain traits of the algal surface (e.g. wettability, surface free energy, exudates suitable as an energy source; Wahl et al. 2010) or by the strain specific stimulation or antifouling activities of compounds on the surface of the alga (Saha et al. 2012). Epibacteria associated with basibionts are also known to modulate further microfouling and macrofouling (Holmström et al. 1992; Dobretsov & Qian 2004). Bacterial strains isolated from *F. serratus* inhibited fouling by diatoms (S. Alpert, GEO-MAR, pers. comm.) as well as by cypris larvae of the barnacle *Amphibalanus improvises* (Nasrolahi et al. 2012). Similarly, monospecific bacterial films of *Sheawella baltica* and *Pseudoalteromonas arctica*, isolated from the red alga *P. stricta*, reduced the attachment of barnacle cypris larvae (Nasrolahi et al. 2012). Biofilm communities which differed in their composition and were from different environments (Stratil et al. 2013) also differed in their potential to modulate fouling, while all biofilms isolated from the surfaces of *Fucus* spp. repelled barnacle larvae. The latter effect was stronger by 39 and 23% for biofilms from algae reared at 5 and 15 °C, respectively, cf. to the repellency observed for biofilms from algae reared at 20° (Nasrolahi et al. 2012).

| Month   | Poel     | Gelting   |
|---------|----------|-----------|
| April   | Spring   | 0.51 (0.28) | 0.43 (0.21) |
| May     | Summer   | 0.36 (0.29) | 0.40 (0.20) |
| June    | Autumn   | 0.56 (0.28) | 0.53 (0.20) |
| July    | Winter   | 0.78 (0.37) | 0.50 (0.28) |
| August  |          | 0.61 (0.30) | 0.60 (0.28) |
| September |        | 0.65 (0.38) | 0.51 (0.26) |
| October |          | 0.65 (0.39) | 0.50 (0.25) |
| November |        | 0.74 (0.40) | 0.48 (0.30) |
| December |         | 0.66 (0.39) | 0.54 (0.28) |
| January |          | 0.64 (0.36) | 0.23 (0.29) |
| February |         | 0.68 (0.37) | 0.50 (0.30) |
| March   |          | 0.49 (0.27) | 0.38 (0.19) |
While the set of strains used in this study is not identical to that in previous monostrain or multistrain assays, it is apparent that the selective activity of secondary metabolites against microfouling may play an important role in moderating the epibiotic biofilm of the alga (Lachnit et al. 2010; Wahl et al. 2012).

The observation that defences in the Poel population were stronger than those in the Gelting population may be due to (1) genetic differences between the two populations, (2) small-scale differences in fouling pressure between the 2 sites (if fouling pressure drives defence strength, which is not yet proven) and/or (3) site differences in salinity (Figure S2, Supplementary information) or (4) possible differences between the sites regarding grazing, nutrients or hydrodynamic conditions, which have been shown to have the potential to affect chemical defence production in brown seaweeds (Henmi et al. 2004; Macaya et al. 2005; Yun et al. 2007). In addition, fluctuations in water level at the Poel site often result in the algae being temporarily exposed to air (K. Maczassek and S. Stratil, GESMAR, pers. comm.). Transient exposure to air facilitates the accumulation of antifouling products at the surface of the thallus (Brock et al. 2007). However, despite this geographical heterogeneity, a similar seasonal pattern of defence strength was found in the 2 populations.

It was postulated in an earlier study (Wahl et al. 2010), that if the production of antifouling defence compounds competes with other metabolic budgets for limited resources, e.g., growth rate or reproductive output (Dworjanyn, Wright et al. 2006), defence might be jeopardised when energy input in winter is low (Lehvo 2001) and/or when growth (mainly in early summer) and reproduction (mainly in spring) consume stored or newly produced energy. This investigation showed a seasonal defence pattern with a maximum in late winter/early spring. The apparent contradiction is explainable by the different extraction techniques used. The earlier investigation (Wahl et al. 2010) used whole tissue extracts, a method that has been state-of-the-art for decades. The present investigation used a surface-extraction technique that has been optimised in recent years. One interpretation of the contrasting temporal pattern of defence could be that the whole extract mainly contained metabolites responsible for ‘internal’ defence against pathogens and which was different in structure and seasonal dynamics from the surface-bound metabolites. However, it is considered more likely that the distribution of defence metabolites on the thallus, vs inside the thallus, differs among seasons. The results presented here are more relevant ecologically than those published earlier, since they quantify the effect of the metabolites that potential foulers encounter physically on the surface of the macroalga. However, the earlier results are also interesting, and can be interpreted as indicating the potential for defence, ie the amount of defensive metabolites produced and stored within the alga ‘waiting’ to be deployed when the need arises at the onset of spring and summer; this scenario assumes that antifouling defence is regulated. If defence metabolites that are produced exude through the thallus epithelium in an uncontrolled manner, the seasonal patterns detected by the 2 extraction procedures should coincide. The fact that they do not may be interpreted as indicating a decoupling between production and deployment of anti-microfouling defences, ie their regulation. This interpretation of the apparently conflicting seasonal defence patterns within and on the surface of algae requires further research in order to address a number of key questions. (1) Whether the chemical antifouling defences in F. vesiculosus and other macroalgae are costly to an extent that they compete for resources with other metabolic processes. (2) Can antifouling defence be regulated as suggested by the seasonal pattern described in the present study and if so what is the mechanistic basis for such regulation? (3) Are the drivers for such regulation the availability of free energy and resources (ie that not appropriated for growth or reproduction), and/or the number and nature of fouling organisms contacting the algal surface? If the latter driver is of most consequence how does the alga perceive fouling on the surface of the thallus? The robustness of the seasonal pattern in defence strength at the thallus surface suggests that the fluctuation is not random. The drivers remain to be identified.

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References

Arahal DR, Castillo AM, Ludwig W, Schleifer KH, Ventosa A. 2002. Proposal of Cobetia marina gen. nov., comb. nov., within the family Halomonadaceae, to include the species Halomonas marina. Syst Appl Microbiol. 25:207–211.

Brock E, Nylund GM, Pavia H. 2007. Chemical inhibition of barnacle larval settlement by the brown alga Fucus vesiculosus. Mar Ecol Prog Ser. 337:165–174.

Chiavelli DA, Mills EL, Threlkeld ST. 1993. Host preference, seasonality, and community interactions of zooplankton epi-bionts. Limnol Oceanogr. 38:574–583.

Cole JJ.1982. Interactions between bacteria and algae in aquatic ecosystems. Annu Rev Ecol Syst. 13:291–314.

Costerton JW, Lewandowski Z, Caldwell D, Korber D, Lappin-Scott HM. 1995. Microbial biofilms. Annu Rev Microbiol. 49:711–745.
de Nys R, Steinberg PD. 1999. Role of secondary metabolites from algae and seagrasses in biofouling control. In: Fingerman M, Nagabhushanam R, Thompson MF, editors. Recent advances in marine biotechnology, Vol. 3. Enfield (NH): Science; p. 223–244.

Dobretsov S, Qian PY. 2004. The role of epibotic bacteria from the surface of the soft coral Dendronephthya sp. in the inhibition of larval settlement. J Exp Mar Biol Ecol. 299:35–50.

Dobretsov S, Teplitski M, Bayer M, Gunasekra S, Proksch P, Paul VJ. 2011. Inhibition of marine biofouling by bacterial quorum sensing inhibitors. Biofouling. 27:893–905.

Dobretsov S, Teplitski M, Paul V. 2009. Mini-review: quorum sensing in the marine environment and its relationship to biofouling. Biofouling. 25:413–427.

Dougherty JR, Russell MP. 2005. The association between the coquina clam Donax fijor Say and its epibiotic hydroid Lovenella gracilis Clarke. J Shellfish Res. 24:35–46.

Dworjanyn SA, Wright JT, Paul NA, de Nys R, Steinberg PD. 2006. Cost of chemical defence in the red alga Delisea pulchra. Mar Ecol Prog Ser. 318:153–163.

Dworjanyn SA, Wright JT, Paul NA, de Nys R, Steinberg PD. 2006. Cost of chemical defence in the red alga Delisea pulchra. Oikos. 113:13–22.

Egan S, James S, Holmström C, Kjelleberg S. 2001. Inhibition of algal spore germination by the marine bacterium Pseudoalteromonas tunicata. FEMS Microbiol Ecol. 35:67–77.

Grossart HP. 2010. Ecological consequences of bacterioplankton lifestyles: changes in concepts are needed. Environ Microbiol Rep. 2:706–714.

Hardwick EO, Ye W, Moran MA, Hodson RE. 2003. Temporal dynamics of three culturable gamma-Proteobacteria taxa in salt marsh sediments. Aquat Ecol. 37:55–64.

Harvell CD. 1998. Genetic variation and polymorphism in the inducible spines of a marine bryozoan. Evolution. 52:80–86.

Hellio C, Maréchal JP, Veron B, Bremer G, Clares AS, Le Gal Y. 2004. Seasonal variation of antifouling activities of marine algae from the Brittany Coast, France. Mar Biotechnol. 6:67–82.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.

Holmström C, Rittschof D, Kjelleberg S. 1992. Inhibition of settlement by larvae of Balanus amphitrite and the marine bacteria Cobetia marina and Pseudoalteromonas haloplanktis. J Exp Mar Biol Ecol. 313:47–62.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.

Hemmi A, Honkanen T, Jormalainen V. 2004. Inducible resistance to herbivory in Fucus vesiculosus — duration, spreading and variation with nutrient availability. Mar Ecol Prog Ser. 273:109–120.
Schauer M, Balague V, Pedros-Alio C, Massana R. 2003. Seasonal changes in the taxonomic composition of bacterioplankton in a coastal oligotrophic system. Aquat Microb Ecol. 31:163–174.

Schmitt TM, Hay ME, Lindquist N. 1995. Constraints on chemically mediated coevolution: multiple functions for seaweed secondary metabolites. Ecology. 76:107–123.

Sneed JM, Pohnert G. 2011. The green alga Dictyosphaeria ocellata and its organic extracts alter natural bacterial biofilms. Biofouling. 27:347–356.

Stratil SB, Neulinger SC, Knecht 2013, Friedrichs AK, Wahl M. 2013. Temperature-driven shifts in the epibiotic bacterial community composition of the brown macroalga Fucus vesiculosus. Microbiol Open. Available from: http://doi.wiley.com/10.1002/mbo3.79

Thomsen J, Gutowska MA, Saphörster J, Heinemann A, Fietzke J, Hiebenthal C, Eisenhauer A, Körtzinger A, Wahl M, Melzner F. 2010. Calcifying invertebrates succeed in a naturally CO2-rich coastal habitat but are threatened by high levels of future acidification. Biogeosciences. 7:3879–3891.

Unabia CRC, Hadfield MG. 1999. Role of bacteria in larval settlement and metamorphosis of the polychaete Hydroides elegans. Mar Biol. 133:55–64.

Wahl M. 2008. Ecological lever and interface ecology: epibiosis modulates the interactions between host and environment. Biofouling. 24:427–438.

Wahl M, Goecke F, Labes A, Dobretsov S, Weinberger F. 2012. The second skin: ecological role of epibiotic biofilms on marine organisms. Front Microbiol. 3:1–21.

Wahl M, Jensen PR, Fenical W. 1994. Chemical control of bacterial epibiosis on ascidians. Mar Ecol Prog Ser. 110:55–57.

Wahl M, Shahnaz L, Dobretsov S, Saha M, Symanowski F, David K, Lachnit T, Vasel M, Weinberger F. 2010. Ecology of antifouling resistance in the bladder wrack Fucus vesiculosus: patterns of microfouling and antimicrobial protection. Mar Ecol Prog Ser. 411:33–48.

Yoon JH, Kim IG, Kang KH, Oh TK, Park YH. 2003. Bacillus mariisflavi sp. nov. and Bacillus aquimaritis sp. nov., isolated from sea water of a tidal flat of the Yellow Sea in Korea. Int J Syst Evol Microbiol. 53:1297–1303.

Yun H, Cruz J, Treitschke M, Wahl M, Molis M. 2007. Testing for the induction of anti-herbivory defences in four Portuguese macroalgae by direct and waterborne cues of grazing amphipods. Helgol Mar Res. 61:203–209.