Marine Epibiosis: Concepts, Ecological Consequences and Host Defence

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Abstract The sessile mode of life is widespread in a variety of marine phyla. Sessile life requires a stable substratum. On the benthos, motile life stages and sessile adults compete for rigid surfaces making non-living, i.e. inanimate, hard substratum a limited resource. Epibiosis is a direct consequence of surface limitation and results in spatially close associations between two or more living organisms belonging to the same or different species. These associations can be specifically guided by host chemistry resulting in species-specific symbiotic or pathogenic assemblages. Most colonizers, however, are non-specific substratum generalists. The ecological consequences for the overgrown host (basibiont) and the colonizer (epibiont) can be positive and negative. The predominantly disadvantageous nature of epibiosis by microorganisms for the basibiont has resulted in a variety of defence mechanisms against microcolonizers, including physical and chemical modes of action. Besides antimicrobial effects of secondary metabolites emanating from the host, recent studies increasingly demonstrate that epibiotic bacteria associated with the host deter growth and attachment of co-occurring bacterial species or new epibiotic colonizers competing for the same niche.

1 Introduction

In the marine environment the sessile mode of life is dominant in the majority of phyla. Owing to their low specific weight not only microorganisms (e.g. bacteria, microalgae) but also the small motile life stages of macroorganisms (e.g. larvae and spores) behave like passive propagules in this viscous, hydrodynamically dominated environment. Clearly, for these species an attached filter-feeding mode of life is the energetically advantageous and favourable state, although the lack of locomotion necessitates a variety of new challenging survival strategies, such as reproduction and various defence forms against consumers and overgrowth.

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Sessile life requires a stable substratum. On the benthos, motile life stages and sessile adult forms compete for rigid surfaces making non-living (i.e. inanimate) hard substratum a limited resource. Epibiosis (greek *epi* "on top" and *bios* "life") can be considered as a direct consequence of surface limitation and results in spatially close associations between two or more living organisms belonging to the same or different species. The substrate organism is considered the *basibiont*, while the organism(s) growing attached to the animate surface is referred to as the *epibiont*. Epibionts are further subdivided into *epizoans* (animals) and *epiphytes* (plants, algae). Epibiotic assemblages are rarely species-specific; on the contrary, numerous sessile organisms may live either as basibiont or as epibiont, or both simultaneously (Wahl 1997). Attachment and growth on inanimate surfaces is usually considered as *fouling*, although this term is frequently used synonymously in an epibiotic context in the literature.

Epibiosis is a typical aquatic phenomenon although many examples of terrestrial epibionts are known (e.g. algae, lichens). Depending on seasonality and location the average millilitre of seawater contains 10–100 microscopic larvae and spores, 10^3 fungal cells, 10^6 bacteria and 10^7 viruses. Thus, the colonization pressure exerted by meroplanktonic dispersal stages can be intense on submerged surfaces (Davis et al. 1989) with severe ecological consequences for basibionts and epibionts. The distinctive role of water as a food vector for sessile organisms is the main reason why surface attachment, fouling and hence epibiotic associations predominate in aquatic environments. A large variety of marine phyla have adopted the sessile mode of life for at least one ontogenetic phase. The list includes many bacteria, protozoa, diatoms, molluscs, tube-building polychaetes; most macroalgae, bryozoans, phoronids, cnidarians; some echinoderms, crustaceans; all sponges and tunicates.

2 Ecological Consequences for Epibionts

Marine fouling is an omnipresent phenomenon and the list of foulers is long. The different stages of the fouling process of dispersal stages on solid substrates have been described in successional (Davis et al. 1989) and probalistic models (Clare et al. 1992; Maki and Mitchell 2002) and are presented in detail in this volume. Irrespective of the fouling sequence, for most meroplanktonic larvae settlement is the ultimate prerequisite for successful metamorphosis into sedentary juveniles (Hadfield and Paul 2001). Also, microcolonizers such as bacteria, benthic diatoms and algal spores often proliferate more rapidly or sometimes exclusively when fixed to a solid substratum (Grossart et al. 2003). Once attached, microcolonizers are challenged by other members in the biofilm matrix (Fletcher and Callow 1992; Costerton et al. 1995). To successfully compete in biofilms, many representatives of the bacterial genus *Pseudoalteromonas* release anti-bacterial products that aid the cells in the colonization of host surfaces. Through the production of agarases, toxins, bacteriolytic substances and other enzymes, bacterial cells are assisted in their competition for nutrients and space as well as in their protection against predators grazing on surfaces (Holmström and Kjelleberg 1999).

In densely populated marine environments where competition for space is high, the advantage for colonizers in occupying empty animate surfaces is probably the main reason for epibiosis (Wahl 1989; Todd and Keough 1994) although a variety of other advantages for the colonizer may support specific host–epibiont associations. For instance, settlement on raised or elevated hosts results in a hydrodynamically favourable position of the epibiont (Keough 1986) as flow dynamics increase with distance from the benthos (Butman 1987). Increased flow ensures better supply of planktonic nutrients and more efficient removal of toxic excretory products such as ammonia. An exposed habitat supports phototrophic epibionts, especially in deeper or turbid waters where light penetration is weak (Brouns and Heijs 1986). While filter-feeding epibionts profit from nutrient currents created by the host (Laihonen and Furman 1986) deposit-feeding epibionts benefit from metabolites exuded by the basibiont (Harlin 1973). Regarding the fate of colonizers, epibionts either benefit from the host defence against consumers or other colonizers, a phenomenon termed “associational defence” (Hay 1986), or the fates of epibiont and host are closely interlinked and shared together, a phenomenon termed “shared doom” (Wahl and Hay 1995).

The predominantly advantageous associations of epibionts with host organisms indicate that the mere presence of a surface is often not the only criterion for successful colonization. Numerous colonizers are reported to be guided by specific “cues” mediating the suitability of the settlement site (Rodriguez et al. 1993; Wieczorek and Todd 1998; Steinberg et al. 2002). The recognition of appropriate cues activates the genetically scheduled sequence of behavioural and physiological processes during settlement (Morse 1990) and many larvae delay or even avoid settlement in the absence of appropriate settlement cues (Coon et al. 1990; Qian and Pechenik 1998).

3 Settlement Cues

There is clear experimental evidence for physical settlement cues, such as surface roughness (Berntsson et al. 2000) and wettability (Qian et al. 2000); environmental conditions in direct proximity to the surface, such as irradiation (Maida et al. 1994) and microhydrodynamics (Mullineaux and Butman 1991); and biogenic chemical signals emanating from the basibiont or other epibionts (e.g. bacteria) already present on the host surface (Johnson et al. 1991a,b; Krug and Manzi 1999). Several authors have presented experimental evidence for selective settlement of both generalist and specialist epibionts in response to invertebrate or plant host cues. In most of these studies, the host served as the obligate prey source for larvae or adults. This raises the question of how planktonically dispersed larvae locate their patchily distributed hosts. Given the large spatial scales that need to be screened by potential colonizers, one would expect either strong or very distinct cues that govern such host–epibiont associations. To address this question, a number of studies have focused on the selective response of sea slugs to host corals. For example, water-soluble cues from corals induce settlement and metamorphosis in larvae of the opisthobranchs *Phestilla sibogae* (Hadfield and Scheuer 1985), *Adalaria proxima* (Lambert and Todd 1997) and *Alderia modesta* (Krug and Manzi 1999). Other well-investigated host plants are coralline algae that govern larval settlement of taxonomically distinct invertebrates such as the sea urchin *Holopneustes purpurascens* (Williamson et al. 2000), the starfish *Acanthaster planci* (Johnson et al. 1991b; Johnson and Sutton 1994) and the mollusk *Haliotis* (Morse and Morse 1984; Hahn 1989). Other well-studied systems comprise obligate associations that seemingly benefit from close proximity of conspecifics to enhance reproductive output, such as in oysters (Tamburri et al. 1992; Turner et al. 1994) and barnacles (Clare and Matsumura 2000). However, the settlement cue(s) involved in the establishment of these systems were rarely identified at the molecular level.

In contrast to the numerous partially characterized inducers, only few settlement cues isolated from natural sources were in fact chemically identified, e.g. delta-tocopherols from *Sargassum tortile* that induce settlement of the hydroid *Coryne uchidai* (Kato et al. 1975); jacarone isolated from the red alga *Delesseria sanguinea* that induces settlement of the scallop *Pecten maximus* (Yvin et al. 1985); narains and anthosamines A and B isolated from marine sponges and lumichrome isolated from conspecifics that induce settlement of ascidian larvae (Tsukamoto et al. 1994, 1995, 1999); *N*-acylhomoserine lactone quorum sensing signal molecules that aid zoospores of the green macroalgae *Ulva* to exploit a bacterial sensory system and select permanent attachment sites by responding to bacteria already present on the surface (Joint et al. 2002). In most cases, the ecological relevance of these compounds in situ is not clear, either because the source of the settlement cue is not necessarily related to the recruitment patterns of the organism (Yvin et al. 1985; Tsukamoto et al. 1994, 1995), or because the availability of the cue to settling larvae has not been demonstrated unequivocally (Tsukamoto et al. 1999).

Interestingly, there is a high similarity in host recognition by pathogens in marine and terrestrial plants (Kolattukudy et al. 1995). For example, the pathogenic filamentous green alga *Acrochaete operculata* recognized its host, the red alga *Chondrus crispus,* by cell wall polysaccharides. *C. crispus* has an isomorphic life history, in which the gametophytic and sporophytic generations differ only in minor traits, such as sulfate-ester group distribution of their matrix polysaccharides, known as κ- and λ-carrageenans. Remarkably, the sporophytic generation is highly susceptible to infection whereas the gametophytic phase is naturally resistant. The virulence of the green algal endophyte is modulated by the presence of λ-carrageenan, which stimulates protein synthesis and elicits the production of specific polypeptides in the pathogen (Bouarab et al. 2001).

Only recent years have witnessed some complete characterizations of marine invertebrate larval settlement cues. In a series of investigations Matsumura et al. (1998) identified the key molecule responsible for gregarious settlement in the fouling barnacle *Balanus amphitrite* as a settlement-inducing protein complex (SIPC). This protein complex has now been fully elucidated as a α_2-macroglobulin-like glycoprotein (Dreanno et al. 2006). Although the SIPC is regarded as an adult cue that is recognized by the cyprid at settlement, it is also expressed in juveniles and in larvae, where it may function in larva–larva settlement interactions. In another series of investigations the structure and the different sources of coralline algae-derived settlement cues for two larval species of sea urchins, *Holopneustes purpurascens* and *Heliocidaris erythrogramma,* have been fully elucidated. The biogenic amine histamine was isolated from the red alga *Delisea pulchra* by bioassay-guided fractionation and identified as the inducer of settlement of *H. purpurascens* (Swanson et al. 2004). The alga still evoked larval settlement after antibiotic treatments, which effectively removed epiphytic bacteria on the algal surface, demonstrating that histamine was indeed an alga-derived cue. In contrast, the coralline alga *Amphiroa anceps,* which also stimulates larval settlement of *H. purpurascens,* lacked detectable amounts of histamine. Interestingly, antibacterial treatment of *A. anceps* removed the settlement cue, suggesting a bacterial origin of the cue from this alga; indeed bacterial films of two isolates from the surface of *A. anceps* induced settlement of *H. purpurascens* in laboratory assays (Swanson et al. 2006). The role of algae-associated bacteria as producers of settlement cues has been examined in more detail for the sea urchin *H. erythrogramma* (Huggett et al. 2006). The hypothesis of a bacterially derived settlement signal was supported by the fact that a variety of bacterial isolates from the surface of coralline algae triggered larval settlement at levels comparable to those of the positive control of coralline algae. One bacterial isolate from *A. anceps, Thallasomonas viridans*, is a known histamine producer. Given that larvae of both urchin species settle in response to histamine, these findings demonstrate a common settlement cue in coralline algae produced by the host alga and/or by associated bacteria.

4 Ecological Consequences for Basibionts

Any potential basibiont, i.e. the majority of sessile, relatively long-lived organisms, must either tolerate epibiosis or employ some sort of defence against this phenomenon. While epibiosis entails both benefits and disadvantages for epi- and basibionts the investment into defence depends on a finely tuned and often variable energy budget of the basibiont (Wahl 1989). Epibiosis causes a variety of beneficial effects to the basibiont, such as the induction of morphogenesis in macroalgae by symbiotic bacteria (Tatewaki et al. 1983; Nakanishi 1999), the interaction between macroalgae and nitrogen-fixing bacteria (Thevanathan et al. 2000), and the protection of seaweed surfaces from bacterial colonizers by associated bacteria (Lemos et al. 1985). A well-investigated example of a symbiotic association between host and epibiotic bacteria is the embryo of the American lobster, *Homarus americanus*, which is resistant to the fungus *Lagenidium callinectes*, a pathogen of many crustaceans. The surfaces of healthy lobster embryos are covered almost exclusively by a single, Gram-negative bacterium, which produces the antifungal substance 4-hydroxyphenethyl (Gil-Turness and Fenical 1992). Testing the effects of epibiosis on herbivory and predation, research by Wahl and colleagues suggested that epibionts on the blue mussel *Mytilus edulis* affected its susceptibility to predation by the shore crab *Carcinus maenas* (Wahl et al. 1997). Similarly, epibiosis by a variety of plants and animals altered the host susceptibility of the omnivorous sea urchin *Arbacia punctulata* (Wahl and Hay 1995). Furthermore, Wahl and Mark (1999) investigated the hypothesis that if the effects for epibiont and basibiont were predominantly beneficial then co-evolution would be expected to lead to some sort of associational specificity. However, by analyzing over 2000 patterns of epibiotic associations the authors concluded that many colonizers are non-specific substratum generalists and that epibiosis is predominantly facultative (Wahl and Mark 1999).

The adverse effects of epibiosis on the basibiont often outweigh the beneficial ones (Table 1). For instance, soft-bodied marine invertebrates and algae are susceptible to diseases and tissue necrosis induced by bacteria, fungi and microalgae (Mitchell and Chet 1975; Bouarab et al. 2001; Cooney et al. 2002). The sometimes drastic changes of pH and redox conditions created by microepibionts may attack chemically sensitive surfaces of the basibiont (Terry and Edyvean 1981). Importantly, the adverse effects of microbial epibiosis may reach beyond pathogenicity and virulence. Since microbial films are important sources of chemical cues for larval settlement in many benthic marine invertebrates (Lau et al. 2002; Harder et al. 2002), microbial epibiosis may promote subsequent colonization by rigid crustose epibiotic macroorganisms, which in turn significantly impair the basibiont's ability to exchange gases and nutrients (Jagels 1973), damage the tissue by increased weight, rigidity and drag (Dixon et al. 1981), and decrease the growth rate of photosynthetic basibionts by cutting surface irradiance levels (Sand-Jensen 1977; Silberstein et al. 1986). From a nutritional perspective it is evident that if the host and the epibiont share the same trophic requirements then planktonic nutrients reaching the basibiont may already be partially depleted after their passage through the epibiotic barrier. As epibionts may fall victim to predators of their hosts, so may basibionts suffer from

Table 1 Ecological consequences for epibiont and basibiont as a result of epibiotic associations (summarized from Wahl 1989)

	Advantages	Disadvantages
Epibiont	Colonization of new substrate	Unstable, non-durable substrate
	New surface due to growth of basibiont	Biologically variable substrate
	Nutrient flow from basibiont	Exposure to detrimental host defence
	Favourable hydrodynamic conditions	Shared doom
	Favourable exposure to light	
	Associational resistance	
Basibiont	Camouflage	Increased weight and drag
	Insulation against desiccation	Decreased elasticity
	Nutrient flow from epibiont	Increased surface roughness
	Associational resistance	Increased deposition of particulate material
		Insulation against exchange of gas and waste products
		Increased mechanical damage
		Increased chemical damage
		Decreased nutrient flow through epibiotic filter

"shared doom", i.e. damage due to grazers preying on epibionts (Dixon et al. 1981). Table 1 summarizes the advantages and disadvantages of epibiosis for epi- and basibionts.

5 Defence

Many marine invertebrates and plants have evolved a variety of physical and chemical defence mechanisms to suppress epibiosis and/or remove epibionts. Epibiont removal can be physically achieved by continuous or periodic surface renewal or by means of mucus secretion (e.g. in cnidaria, algae, molluscs, echinoderms and tunicates) and periodical shedding of the cuticula or epidermis (Sieburth and Tootle 1981; Littler and Littler 1999; Nylund and Pavia 2005) (see Fig. 1).

To create unfavourable or toxic conditions at or immediately above the living surface is a wide-spread adaptation of host organisms to cope with epibionts.

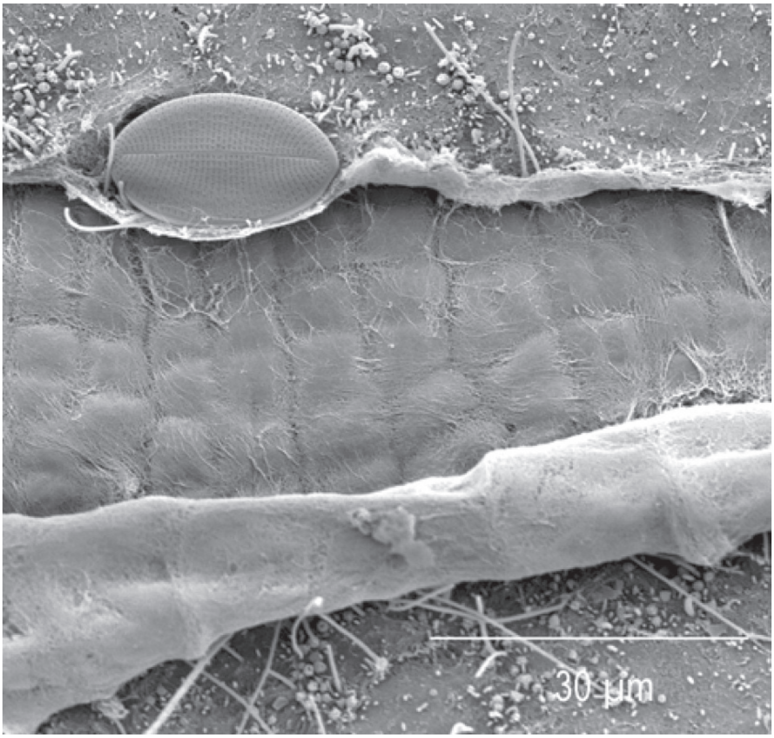

Fig. 1 Surface of the macroalga *Laminaria digitata* showing sloughing of the cuticle-containing bacteria and diatoms to reveal an uncolonized algal surface. *Scale bar*: 30 µm

The brown alga *Laminaria digitata* and the red alga *Gracilaria conferta* react with an oxidative burst to the presence of either alginate oligosaccharides or agar oligosaccharides, both of which are degradation products of their own cell walls (Küpper et al. 2001), resulting in the efficient elimination of bacterial epiflora (Weinberger et al. 2000). Moreover, sessile marine organisms feature a variety of chemical defence metabolites effective against different phyla of potential epibionts (reviewed by Clare 1996; Faulkner 2000). There are numerous studies on the inhibition of micro- and macroorganisms by extracts from diverse marine eukaryotes, such as corals, sponges, tunicates, ascidians and macrophytes (e.g. Michalek and Bowden 1997; Jensen et al. 1996; Wilsanand 1999; Slattery et al. 1995; Hellio et al. 2000; Dobretsov et al. 2006). Mostly, these investigations were descriptive and did not result in the purification and elucidation of inhibitory compounds. It remains unclear whether these extracts deter epibiosis at or near surfaces in situ and, if so, at what concentrations these effects are elicited. In this context, one of the better-studied models for algal secondary metabolism is the Australian red alga *Delisea pulchra*, which produces a range of structurally similar halogenated furanones (Steinberg et al. 2001). These metabolites are encapsulated in vesicles in the gland cells of *D. pulchra*, which provide a delivery mechanism to the surface of the alga at concentrations that deter a wide range of prokaryote and eukaryote epibionts (Maximilien et al. 1998). Being structurally related to acylated homoserine lactones (AHLs), halogenated furanones inhibit bacterial colonization through direct antagonism of bacterial cell-to-cell signalling. The AHL-mediated gene expression of bacteria is inhibited when halogenated furanones occupy the AHL-binding site of LuxR-like proteins, which represent the transcriptional activators in AHL regulatory systems (Manefield et al. 1999).

Information on the localization, identity and surface concentration of defence secondary metabolites is rapidly advancing (e.g. Salomon et al. 2001; Kubanek et al. 2002; Nylund et al. 2005; Paul et al. 2006) and the relevance of defence metabolites is increasingly discussed in a chemical ecological context. Moreover, recent studies on the deterrence of microbial colonization highlight that chemical antifouling defences cannot be generalized as broadly bacteriostatic or bactericidal, instead the effects are quite selective and targeted against particular microbial species (Maximilien et al. 1998; Egan et al. 2000; Kubanek et al. 2003).

Besides antimicrobial effects of secondary metabolites emanating from the host, recent studies have increasingly demonstrated that epibiotic bacteria associated with the host deter growth and attachment of co-occurring bacterial species or new epibiotic colonizers competing for the same niche (Armstrong et al. 2001; Harder et al. 2004a). A well-investigated bacterium in this context is *Pseudoalteromonas tunicata*, which has been isolated from a tunicate and a green macroalga. *P. tunicata* has been found to produce at least five extracellular compounds that inhibit other organisms from establishing themselves in a epibiotic community by inhibiting settlement of invertebrate larvae and algal spores, growth of bacteria and fungi, and surface colonization by diatoms (Holmström and Kjelleberg 1999; Holmström et al. 1996). Thus, in terms of the chemical ecology of host–epibiont associations, it seems evident that there is a significant protective role of symbiotic microbial epibionts, which in turn release antifouling compounds. However, after more than 20 years of research there is no experimental evidence demonstrating if and how host organisms selectively attract such epibionts.

With the advancement of molecular biological tools to analyse the diversity and abundance of bacteria in biofilms (Dahllöf 2002), several studies have demonstrated that quantitative and qualitative bacterial occurrence on host organisms differs significantly from inanimate reference surfaces (Harder et al. 2003, 2004b; Lee and Qian 2004; Dobretsov et al. 2006; Rao et al. 2005). These findings firstly suggest strong host defence mechanisms against non-culturable epibiotic bacteria but also support the notion of potent effects of non-culturable epibiotic bacteria against subsequent colonizers of host organisms. It will be interesting to see follow-up studies that utilize advanced molecular biological tools, such as cloning techniques, to directly test the metabolites of non-culturable symbiotic prokaryotes on epibiotic eukaryotes.

References

Armstrong E, Yan L, Boyd KG, Wright PC, Burgess JG (2001) The symbiotic role of marine microbes on living surfaces. *Hydrobiologia* 461:37–40

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

Bouarab K, Potin P, Weinberger F, Correa J, Kloareg B (2001) The *Chondrus crispus Acrochaete operculata* host-pathogen association, a novel model in glycobiology and applied phycopathology. *J Appl Phycol* 13:185–193

Brouns JJWM, Heijs FML (1986) Production and biomass of the seagrass *Anhalus acovoides* (Lf) Royle and its epiphytes. *Aquat Bot* 25:21–45

Butman CA (1987) Larval settlement of soft-sediment invertebrates: the spatial scales of pattern explained by active habitat selection and the emerging role of hydrodynamical processes. *Oceanogr Mar Biol A Rev* 25:113–165

Clare AS (1996) Marine natural product antifoulants. *Biofouling* 9:211–229

Clare AS, Matsumura K (2000) Nature and perception of barnacle settlement pheromones. *Biofouling* 15:57–71

Clare AS, Rittschof D, Gerhart DJ, Maki JS (1992) Molecular approaches to non-toxic antifouling. *Invert Reprod Dev* 22:67–76

Coon SL, Fitt WK, Bonar DB (1990) Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas*. *Mar Biol* 106:379–387

Cooney RP, Pantos O, Le Tissier MDA, Barer MR, O'Donnell AG, Bythell JC (2002) Characterization of the bacterial consortium associated with black band disease in coral using molecular microbiological techniques. *Environ Microbiol* 4:401–413

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. *Annu Rev Microbiol* 49:711–745

Dahllöf I (2002) Molecular community analysis of microbial diversity. *Curr Opin Biotechnol* 13:213–217

Davis AR, Targett NM, McConnell OJ, Young CM (1989) Epibiosis of marine algae and benthic invertebrates: natural products chemistry and other mechanisms inhibiting settlement and overgrowth. In: ScheuerPJ (ed.) Bioorganic marine chemistry, vol 3. Springer, Heidelberg Berlin New York, pp. 85–114

Dixon J, Schroeter SC, Kastendick J (1981) Effects of encrusting bryozoan, *Membranipora membranacea* on the loss of blades and fronds by the giant kelp, *Macrocystis pyrifera* (Laminariales). *J Phycol* 17:341–345

Dobretsov S, Dahms HU, Harder T, Qian PY (2006) Allelochemical defense of the macroalga *Caulerpa racemosa* against epibiosis: evidence of field and laboratory assays. *Mar Ecol Prog Ser* 318:165–175

Dreanno C, Matsumura K, Dohmae N, Takio K, Hirota H, Kirby R, Clare AS (2006) An α-2-macroglobulin-like protein is the cue to gregarious settlement of the barnacle *Balanus amphitrite*. *Proc Natl Acad Sci U S A* 103:14396–14401

Egan S, Thomas T, Holmström C, Kjelleberg S (2000) Phylogenetic relationship and antifouling activity of bacterial epiphytes from the marine alga *Ulva lactuca*. *Environ Microbiol* 2:343–347

Faulkner DJ (2000) Marine pharmacology. *Antonie Van Leeuwenhoek* 77:135–145

Fletcher RL, Callow ME (1992) The settlement, attachment and establishment of marine algal spores. *Br Phycol J* 27:303–329

Gil-Turness MS, Fenical W (1992) Embryos of *Homarus americanus* are protected by epibiotic bacteria. *Biol Bull* 182:105–108

Grossart HP, Kiørboe T, Tang K, Ploug H (2003) Bacterial colonization of particles: growth and interactions. *Appl Environ Microbiol* 69:3500–3509

Hadfield MG, Scheuer D (1985) Evidence for a soluble metamorphic inducer in *Phestilla sibogae:* ecological, chemical and biological data. *Bull Mar Sci* 37:556–566

Hadfield MG, Paul VJ (2001). Natural chemical cues for settlement and metamorphosis of marine-invertebrate larvae: 431–461. In: McClintock JB, Baker BJ (eds.) Marine chemical ecology. CRC, Boca Raton, pp. 1–610

Hahn KO (1989) Induction of settlement in competent abalone larvae. In HahnKO (ed.) Handbook of culture of abalone and other marine gastropods. CRC, Boca Raton, pp. 101–112

Harder T, Lam C, Qian PY (2002) Induction of larval settlement of the polychaete *Hydroides elegans* (Haswell) by marine biofilms: an investigation of monospecific diatom films as settlement cues. *Mar Ecol Prog Ser* 229:105–112

Harder T, Lau SCK, Dobretsov S, Fang TK, Qian PY (2003) A distinctive epibiotic bacterial community on the soft coral *Dendronephthya* sp. and antibacterial activity of coral tissue extracts suggest a chemical mechanism against bacterial epibiosis. *FEMS Microbiol Ecol* 43:337–347

Harder T, Dobretsov S, Qian PY (2004a) Waterborne, polar macromolecules act as algal antifoulants in the seeweed *Ulva reticulata*. *Mar Ecol Prog Ser* 274:131–141

Harder T, Lau SCK, Tam WY, Qian PY (2004b) A bacterial culture-independent method to investigate chemically mediated control of bacterial epibiosis in marine invertebrates by using TRFLP analysis and natural bacterial populations. *FEMS Microbiol Ecol* 47:93–99

Harlin MM (1973) Transfer of products between epiphytic marine algae and host plants. *J Phycol* 9:243–248

Hay ME (1986) Associational plant defenses and the maintenance of species diversity: turning competitors into accomplices. *Am Nat* 128:617–641

Hellio C, Bremer G, Pons AM, Le Gal Y (2000) Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany, France. *Appl Microbiol Biotechnol* 54:543–549

Holmström C, Kjelleberg S (1999) Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. *FEMS Microbiol Ecol* 30:285–293

Holmström C, James S, Egan S, Kjelleberg S (1996) Inhibition of common fouling organisms by pigmented marine bacterial isolates. *Biofouling* 10:251–259

Huggett MJ, Williamson JE, De Nys R, Kjelleberg S, Steinberg PD (2006) Larval settlement of the common Australian sea urchin *Heliocidaris erythrogramma* in response to bacteria from the surface of coralline algae. *Oecologia* 149:604–619

Jagels R (1973) Studies of a marine grass, *Thalassia testudinum* I. Ultrastructure of the osmoregulatory leaf cells. *Am J Bot* 60:1003–1009

Jensen PR, Harvell CD, Wirtz K, Fenical W (1996) Antimicrobial activity of extracts of Caribbean gorgonian corals. *Mar Biol* 125:411–419

Johnson CR, Sutton DC (1994) Bacteria on the surface of crustose coralline algae induce metamorphosis of the crown-of-thorns starfish *Acanthaster planci*. *Mar Biol* 120:305–310

Johnson CR, Muir DG, Reysenbach AL (1991a) Characteristic bacteria associated with surfaces of coralline algae: a hypothesis for bacterial induction of marine invertebrate larvae. *Mar Ecol Prog Ser* 74:281–294

Johnson CR, Sutton DC, Olson RR, Giddins R (1991b) Settlement of crown-of-thorns starfish: role of bacteria on surfaces of coralline algae and a hypothesis for deepwater recruitment. *Mar Ecol Prog Ser* 71:143–162

Joint I, Tait K, Callow ME, Callow JE, Milton D, Williams P (2002) Cell-to-cell communication across the procaryote – eucaryote boundary. *Science* 298:1207

Kato T, Kumanireng AS, Ichinose I, Kitahara Y, Kakinuma Y, Nishihira M, Kato M (1975) Active components of *Sargassum tortile* effecting the settlement of swimming larvae of *Coryne uchidai*. *Experientia* 31:433–434

Keough MJ (1986) The distribution of a bryozoan on seagrass blades: settlement, growth and mortality. *Ecology* 67:846–857

Kolattukudy PE, Rogers LM, Li D, Hwang CS, Flaishman MA (1995) Surface signaling in pathogenesis. *Proc Natl Acad Sci U S A* 92:4080–4087

Krug PJ, Manzi AE (1999) Waterborne and surface-associated carbohydrates as settlement cues for larvae of the specialist marine herbivore *Alderia modesta*. *Biol Bull* 197:94–103

Kubanek J, Whalen KE, Engel S, Kelly SR, Henkel TP, Fenical W, Pawlik JR (2002) Multiple defensive roles for triterpene glycosides from two Caribbean sponges. *Oecologia* 131:125–136

Kubanek J, Jensen PR, Keifer PA, Sullards MC, Collins DO, Fenical W (2003) Seaweed resistance to microbial attack: a targeted chemical defense against marine fungi. *Proc Natl Acad Sci U S A* 100:6916–6921

Küpper FC, Kloareg B, Guern J, Potin P (2001) Oligoguluronates elicit an oxidative burst in brown algal kelp, *Laminaria digitata*. *Plant Physiol* 12:278–291

Laihonen P, Furman ER (1986) The site of settlement indicates commensalism between blue mussel and its epibiont. *Oecologia* 71:38–40

Lambert WJ, Todd CD, Hardege JD (1997) Partial characterization and biological activity of a metamorphic inducer of the dorid nudibranch *Adalaria proxima* (Gastropoda: Nudibranchia). *Invertebr Biol* 116:71–81

Lau SCK, Mak KKW, Chen F, Qian PY (2002) Bioactivity of bacterial strains from marine biofilms in Hong Kong waters for the induction of larval settlement in the marine polychaete *Hydroides elegans*. *Mar Ecol Prog Ser* 226:301–310

Lee O, Qian PY (2004) Potential control of bacterial epibiosis on the surface of the sponge *Mycale adhaerens*. *Aquat Microb Ecol* 34:11–21

Lemos ML, Toranzo AE, Barja JL (1985) Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. *Microbiol Ecol* 11:149–163

Littler MM, Littler DS (1999) Blade abandonment/proliferation: a novel mechanisms for rapid epiphyte control in marine macrophytes. *Ecology* 80:1736–1746

Maida M, Coll JC, Samarco PW (1994) Shedding new light on scleractinian coral recruitment. *J Exp Mar Biol Ecol* 180:189–202

Maki JS, Mitchell R (2002) Biofouling in the marine environment. In: Bitton G (ed.) Encyclopedia of environmental microbiology. Wiley, New York, pp. 610–619

Manefield M, de Nys R, Kumar N, Read R, Givskov M, Steinberg PD, Kjelleberg S (1999) Inhibition of LuxR-based AHL regulation by halogenated furanones from *Delisea pulchra*. *Microbiology* 145:283–291

Matsumura K, Nagano M, Fusetani N (1998) Purification of a larval settlement-inducing protein complex (SIPC) of the barnacle, *Balanus amphitrite*. *J Exp Zool* 281:12–20

Maximilien R, de Nys R, Holmström C, Gram L, Givskov M, Crass K, Kjelleberg S, Steinberg PD (1998) Chemical mediation of bacterial surface colonization by secondary metabolites from the red alga *Delisea pulchra*. *Aquat Microb Ecol* 15:233–246

Michalek K, Bowden BF (1997) A natural algacide from soft coral *Sinularia flexibilis* (Coelenterata, Octocorallia, Alcyonacea). *J Chem Ecol* 23:259–273

Mitchell R, and Chet I (1975) Bacterial attack of corals in polluted seawater. *Microb Ecol* 2:227–233

Morse DE (1990) Recent progress in larval settlement and metamorphosis: closing the gaps molecular biology and ecology. *Bull Mar Sci* 46:465–483

Morse ANC, Morse DE (1984) Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surface of crustose red alga. *J Exp Mar Biol Ecol* 75:191–215

Mullineaux LS, Butman CA (1991) Initial contact, exploration and attachment of barnacle (*Balanus amphitrite*) cyprids settling in flow. *Mar Biol* 110:93–103

Nakanishi K, Nishijima M, Nomoto AM, Yamazali A, Saga N (1999) Requisite morphologic interaction for attachment between *Ulva pertusa* (Chlorophyta) and symbiotic bacteria. *Mar Biotech* 1:107–111

Nylund G, Cervin G, Hermansson, Pavia H (2005) Chemical inhibition of bacterial colonization by the red alga *Bonnemaisonia hamifera*. *Mar Ecol Prog Ser* 302:27–36

Nylund G, Pavia H (2005) Chemical versus mechanical inhibition of fouling in the red alga *Dilsea carnosa*. *Mar Ecol Prog Ser* 299:111–121

Paul NA, de Nys R, Steinberg PD (2006) Chemical defence against bacteria in the red alga *Asparagopsis armata*: linking structure with function. *Mar Ecol Prog Ser* 306:87–101

Qian PY, Pechenik JA (1998) Effects of larval starvation and delayed metamorphosis on juvenile survival and growth of the tube-dwelling polychaete *Hydroides elegans* (Haswell). *J Exp Mar Biol Ecol* 227:169–185

Qian PY, Rittschof D, Sreedhar B (2000) Macrofouling in unidirectional flow: miniature pipes as experimental models for studying the interaction of flow and surface characteristics on the attachment of barnacle, bryozoan and polychaete larvae. *Mar Ecol Prog Ser* 207:109–121

Rao D, Webb S, Kjelleberg S (2005) competitive interactions in mixed-species biofilms containing the marine bacterium *Pseudoalteromonas tunicata*. *Appl Environ Microbiol* 71:1729–1736

Rodriguez SR, Ojeda FP, Inestrosa NC (1993) Settlement of benthic marine invertebrates. *Mar Ecol Prog Ser* 97:193–207

Salomon CE, Deerinck T, Elissman MH, Faulkner DJ (2001) The cellular localization of dercitamide in the Palauan sponge *Oceanapia sagittaria*. *Mar Biol* 139:313–319

Sand-Jensen K (1977) Effect of epiphytes on eelgrass photosynthesis. *Aquat Bot* 3:55–63

Sieburth JM, Tootle JL (1981) Seasonality of microbial fouling on *Ascophyllum nodosum* (L.) Lejol., *Fucus vesiculosus* L., *Polysiphonia lanosa* (L.) Tandy and *Chondrus crispus* Stackh. *J Phycol* 17:57–64

Silberstein K, Chiffings AW, McComb AJ (1986) The loss of seagrass in Cockburn Sound, Western Australia. 3. The effect of epiphytes on productivity of *Posidonia australis. Hook F Aquat Bot* 24:355–371

Slattery M, McClintock JB, Heine JN (1995) Chemical defenses in Antarctic soft corals: evidence for antifouling compounds. *J Exp Mar Biol Ecol* 190:61–77

Steinberg PD, De Nys R, Kjelleberg S (2001) Chemical mediation of surface colonization, In: McClintock JB, Baker JB (eds.) Marine chemical ecology. CRC, Boca Raton, pp. 355–387

Steinberg PD, de Nys R, Kjelleberg S (2002) Chemical cues for surface colonization. *J Chem Ecol* 28:1935–1951

Swanson RL, Williamson JE, De Nys R, Kumar N, Bucknall MP, Steinberg PD (2004) Induction of settlement of larvae of the sea urchin *Holopneustes purpurascens* by histamine from a host alga. *Biol Bull* 206:161–172

Swanson RL, de Nys R, Huggett MJ, Green JK, Steinberg PD (2006) In situ quantification of a natural settlement cue and recruitment of the Australian sea urchin *Holopneustes purpurascens*. *Mar Ecol Prog Ser* 314:1–14

Tamburri MN, Zimmer-Faust RK, Tamplin ML (1992) Natural sources and properties of chemical inducers mediating settlement of oyster larvae: a re-examination. *Biol Bull* 183:327–338

Tatewaki M, Provasoli L, Pintner IJ (1983) Morphogenesis of *Monostroma oxyspermum* (Kuetz.) Doty (Chlorophyceae) in axenic culture, especially in bialgal culture. *J Phycol* 19:409–416

Terry LA, Edyvean RGJ (1981) Microalgae and corrosion. *Bot Mar* 24:177–183

Thevanathan R, Nirmala M, Manoharan A, Gangadharan A, Rajarajan R, Dhamotharan R, Selvaraj S (2000) On the occurrence of nitrogen fixing bacteria as epibacterial flora of some marine green algae. *Seaweed Res Utiln* 22:189–197

Todd CD, Keough MJ (1994) Larval settlement in hard substratum epifaunal assemblages: a manipulative field study of the effects of substratum filming and the presence of incumbents. *J Exp Mar Biol Ecol* 181:159–187

Tsukamoto S, Kato H, Hirota H, Fusetani N (1994) Narains: *N,N*-dimethylguanidinium styryl sulfates, metamorphosis inducers of ascidian larvae from a marine sponge *Jaspis* sp. *Tetrahedron Lett* 35:5873–5874

Tsukamoto S, Kato H, Hirota H, Fusetani N (1995) Pipecolate derivatives, anthosamines A and B, inducers of larval metamorphosis in ascidians, from a marine sponge *Anthosigmella aff. raromicrosclera*. *Tetrahedron* 51:6687–6694

Tsukamoto S, Kato H, Hirota H, Fusetani N (1999) Lumichrome – a larval metamorphosis-inducing substance in the ascidian *Halocynthia roretzi*. *Eur J Biochem* 264:785–789

Turner EJ, Zimmer-Faust RK, Palmer MA, Luckenback (1994) Settlement of oyster *Crassostrea virginica* larvae: effects of water flow and a water-soluble chemical cue. *Limnol Oceanogr* 39:1579–1593

Wahl M (1989) Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Mar Ecol Prog Ser* 58:175–189

Wahl M (1997) Living attached: aufwuchs, fouling, epibiosis. In: Nagabushanam R, Thompson MF (eds.) Fouling organisms of the Indian Ocean: biology and control technology. Oxford and IBH, New Delhi, pp. 31–83

Wahl M, Hay ME (1995) Associational resistance and shared doom: effects of epibiosis on herbivory. *Oecologia* 102:329–340

Wahl M, Mark O (1999) The predominantly facultative nature of epibiosis: experimental and observational evidence. *Mar Ecol Prog Ser* 187:59–66

Wahl M, Hay ME, Enderlein P (1997) Effects of epibiosis on consumer-prey interactions. *Hydrobiologia* 355:49–59

Weinberger F, Friedlander M (2000) Response of *Gracilaria conferta* (Rhodophyta) to oligoagars results in defense against agar-degrading epiphytes. *J Phycol* 36:1079–1086

Wieczorek SK, Todd CD (1998) Inhibition and facilitation of the settlement of epifaunal marine invertebrate larvae by microbial biofilm cues. *Biofouling* 12:81–93

Williamson JE, De Nys R, Kumar N, Carson DG, Steinberg PD (2000) Induction of metamorphosis in the sea urchin *Holopneustes purpurascens* by a metabolite complex from the algal host *Delisea pulchra*. *Biol Bull* 198332–345

Wilsanand V, Wagh AB, Bapuji M (1999) Effect of alcohol extracts of demospongiae on growth of periphytic diatoms. *Ind J Mar Sci* 28:274–279

Yvin JC, Chevolet L, Chevolet-Maguer AM, Cochard JC (1985) First isolation of jacarone from an alga, *Delesseria sanguinea:* a metamorphosis inducer of *Pecten* larvae. *J Nat Prod* 48:814–816