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BACTERIAL INDUCTION OF SETTLEMENT AND METAMORPHOSIS IN MARINE INVERTEBRATES

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ABSTRACT

In reviewing the complexity and diversity of bacterial mediation of settlement and metamorphosis of marine invertebrate larvae, we give particular attention to settlement and metamorphosis on macroscopic biological substrata, focusing on bacteria-mediated induction of larvae by non-geniculate coralline algae. We consider the evolution of interactions between marine substrata, bacteria and larvae, and offer arguments as to why natural selection may favour these interactions. We speculate that bacterial induction of settlement and metamorphosis in marine invertebrates might be more widespread than is generally recognised.

INTRODUCTION

It has been recognised for at least 60 years, since the experiments of ZoBell and Allen (1935) showed greater recruitment of macroscopic species on slides coated with a bacterial film than on sterile slides, that bacteria have the ability to induce metamorphosis in a taxonomically diverse array of marine invertebrates (Table 1). However, interactions among larvae and bacteria are not always positive and in some species bacteria inhibit settlement and/or metamorphosis (Lewis 1974; Maki et al. 1988,1989,1990,1992; Holmstrom et al. 1992; Avelin et al. 1993), while others show no response to bacteria (Maki et al. 1988,1989; Keough and Raimondi 1995).

The nature of interactions between marine bacteria and invertebrate larvae is highly variable and more complex than simply responding, or not responding, to a non-specific 'primary surface biofilm'. Settlement and metamorphosis of larvae may be induced by soluble products of bacterial metabolism but not by molecules on the surface of cells (e.g. Neumann 1979; Fitt and Hofmann 1985), or by cues on the surface of cells but not by their soluble excreted or secreted products (e.g. Müller et al. 1976; Kirchman et al. 1982), or by cues associated with both the surface and soluble fractions (Spindler and Müller 1972; Weiner et al. 1985; Leitz and Wagner 1993). Larvae of some species respond to cells and/or products of a variety of bacterial genera (e.g. Fitt et al. 1989), while others require particular individual strains or communities of bacteria (e.g. Wilson 1955; Kirchman et al. 1982; see also Johnson and Sutton 1984). Similarly, different bacteria manifest differential ability to inhibit settlement of some barnacles and bryozoans (Maki et al. 1988,1989,1990). Also, the same bacterium may induce settlement in some species but not others (Tritar et al. 1992) or even inhibit settlement in some species but promote settlement in others (cf. Kirchman et al. 1982; Maki et al. 1990,1992).

While there has been little progress in identifying particular bacterially produced chemicals that induce or inhibit settlement/metamorphosis, and despite some contradictory findings in this area (cf. Fitt et al. 1990; Tamburri et al. 1992), available evidence indicates that there is a diverse range of bacterially derived substances which can influence settlement and metamorphosis in invertebrate larvae. For example, several lines of evidence suggest that cholera toxin (ca. 18 kDaltons) from *Vibrio cholerae* (Fitt et al. 1987), a lipid from *Alteromonas espejiana* (Leitz and Wagner 1983), peptide(s) (2.1-9.5 kDaltons) from *Vibrio alginolyticus* (Hofmann and Brand 1987), substances <300 Daltons from *Alteromonas colwelliana* and *V. cholerae* (Fitt et al. 1990), and molecules <5 kDaltons from an unspecified film (Cameron and Hinegardner 1974) induce metamorphosis in different species.

Bacterial films not only affect the chemical properties of a surface but also the physical properties such as wettability, which is known to affect larval settlement patterns (Mihm et al. 1981; Maki et al. 1989; Neal and Yule 1994). This raises the possibility of complex interactions between bacteria and physical and chemical signals (e.g. Neal and Yule 1994), but present evidence suggests that chemical properties of bacterial films override the effects of physical characteristics (Maki et al. 1992; Neal and Yule 1994; see also Mihm et al. 1981).

As a final point to illustrate the complex and variable nature of interactions between invertebrate larvae and bacteria, responses of larvae to bacteria are rarely uniform. Field experiments demonstrate variability in time and space in the responses of larvae to microbial films (Keough and Raimondi 1995), and responses can be dissimilar, or even diametrically opposed, depending on the age of the bacterial film (e.g. Mihm et al. 1981; Neal and Yule 1994; Keough and Raimondi 1995; Maki et al. 1990), the stage of growth of the bacteria (Müller et al. 1976; Fitt et al. 1989), and whether bacteria are in suspension or attached to a surface (Fitt and Hofmann 1985). Perhaps these results should not be surprising given that bacteria are known to produce different exopolymers and exudates depending on their phase of growth and whether they are attached or in the water column (Christensen et al. 1985; Kjelleberg et al. 1987).

We have laboured these points to emphasise that marine invertebrate larvae have evolved a diversity of responses to a variety of cues from different bacteria. These observations raise two important and closely related questions. First, how widespread is bacterial induction of settlement and metamorphosis of marine invertebrates? If the response of larvae to bacteria manifests some degree of specificity, then the absence of responses to particular strains or assemblages of bacteria does not indicate the absence of meaningful physiological and ecological interactions with bacteria. Second, given the large variety of surfaces in the marine environment with particular chemical signatures independent of the presence of bacteria, why might evolution of bacteria-mediated induction of larvae occur at all?

In addressing these questions we use as an example the 3-way interaction between non-geniculate coralline algae (NCA), bacteria and larvae of the crown-of-thorns starfish to (1) suggest why there may be strong selection for mechanisms of induction involving bacteria, and (2) raise the possibility that other examples of induction of metamorphosis by NCA, and other macroscopic biological surfaces, may be mediated by bacteria. As one of the best studied examples of NCA/larva interactions, we give some attention to induction of abalone larvae by NCA in posing the question whether this interaction might be mediated by surface bacteria; we suggest that present evidence is inconclusive but does not exclude this possibility.

In addressing the evolution of a bacterial role in induction of settlement and/or metamorphosis, we focus on benthic invertebrates. In considering the critical consequences of larval choice of settlement site, we suggest that inclusion of a bacterial link in the processes of settlement and/or metamorphosis allows for evolution of systems that are able to provide similar cues to larvae across a diversity of substrata while at the same time facilitating recognition of particular substrata.

We speculate that bacterial mediation of settlement and/or metamorphosis may be more complex and widespread than is recognised currently, but emphasise that research has been insufficient to enable reasonable assessment of the extent of the phenomenon, or provide understanding of how marine invertebrate larvae interact with bacteria and bacterially derived molecules in the sea. We have scant understanding of the chemicals and mechanisms involved, of spatial and temporal variability in interactions, and of the ecological and commercial implications of bacteria-mediated settlement and metamorphosis.

BACTERIA AND METAMORPHOSIS OF LARVAE ON BIOLOGICAL SURFACES

Larvae of many invertebrate species settle on the surface of other macroscopic organisms, and their selection of these substrata may be non-specific, moderately specific or highly specific. A well known example of specificity in settlement is that of species of the polychaete *Spirorbis*; *S. borealis*, *S. corallinae*, *S. rupestris* and *S. tridentatus* settle with high fidelity on the brown alga *Fucus serratus*, geniculate coralline alga *Corallina officinalis*, the NCA *Lithothamnion polymorphum*, and on rocks with a microbial film respectively (De Silva 1962;

Table 1: Marine invertebrate species in which bacteria or bacterial products induce metamorphosis and/or settlement. Examples are selected to indicate the range of taxa in which metamorphosis is influenced by bacteria, but the list does not represent an exhaustive review. The nature of the evidence is of variable specificity and quality.

Species	Nature of Evidence	Reference(s)
COELENTERATA		
Hydractinia echinata	Metamorphosis induced by washed bacterial cells coated onto filters and medium with added bacteria, but not by sterile filtered medium.	Spindler and Müller 1972
	Metamorphosis induced by bacteria (*Alteromonas espejiana*) on filter paper and by a lipophilic extract of the bacterium, but not in controls. Other bacteria (species of *Alteromonas* and *Oceanospirillum*) also induce metamorphosis.	Leitz and Wagner 1993
Cassiopea andromeda	Metamorphosis induced by untreated sea water and soluble products of several species of marine *Vibrio* bacteria, but not by sterilised or antibiotic-treated sea water.	Hofmann et al. 1978; Neumann 1979; Fitt and Hofmann 1985; Fitt et al. 1987; Hofmann and Brand 1987
Heteroxenia fuscescens	Metamorphosis induced by coral skeleton fouled with bacteria/microalgae, but not by bleached and autoclaved skeleton.	Henning et al. 1991
Pocillopora damicornis	Settlement and metamorphosis occurs at higher rates on filmed than unfilmed surfaces.	Harrigan 1972
ANNELIDA		
Janua brasiliensis	Settlement and metamorphosis induced by unspecified multi-species bacterial film, *Pseudomonas marina* and to a lesser extent by 4 other separate bacterial strains; settlement on 7 other strains was low or zero.	Kirchman et al. 1982
Ophelia bicornis	Semi-quantitative experiments rank rates of metamorphosis: untreated sediment > acid-washed sediment reinoculated with microbes from seawater and sediment > acid-washed, or formalin- or alcohol-treated sediment. Some evidence that different suites of microbes induce metamorphosis/ settlement at different rates.	Wilson 1955
Spirorbis tridentatus	Settles on stones with, but not without, microbial films.	De Silva 1962
MOLLUSCA		
Crassostrea gigas	Metamorphosis induced by a film of *Shewanella colwellina* at higher rates than controls.	Tritar et al. 1992
	Soluble products of several bacteria (*Alteromonas (Shewanella) colwellina*, *Vibrio cholerae* and *Escherichia coli*) induce metamorphosis.	Fitt et al. 1989, 1990
Crassostrea virginica	Metamorphosis induced at higher rates by a film of sea water bacteria than in controls, and by a monospecific film, and soluble product, of the bacterium 'LST' (later identified as *Shewanella colwellina*; Coyne et al. 1989).	Weiner et al. 1985
Ostrea edulis	Metamorphosis induced by film of *Shewanella colwellina* at higher rates than controls.	Tritar et al. 1992
Rangia cuneata	Larvae settle on untreated sediment at greater rates than on similar sediment autoclaved or treated with antibiotics or H_2O_2, but bacterial cue non-obligatory.	Sundberg and Kennedy 1993
BRYOZOA		
Bugula simplex *B. stolonifera* *B.turrita*	Settlement higher on surfaces with than without multi-species microbial film in experiments offering a choice of substrata; without choice, settlement was low on unfilmed surfaces except for *B. stolonifera*.	Brancato and Woollacott 1982
ECHINODERMATA		
Acanthaster planci	Rates of settlement and metamorphosis significantly lower on coralline algae treated with antibiotic than on untreated algae.	Johnson et al. 1991b
	Settlement and metamorphosis significantly greater on antibiotic-treated shards of coralline algae re-inoculated with coralline surface bacteria than on shards treated with antibiotics but not re-inoculated.	Johnson and Sutton 1994
Coscinasterias calamaria	Settles on a variety of surfaces with 'primary' film of microorganisms, and where tested, at greater rates than on surfaces without a primary film.	Barker 1977
Strongylocentrotus droebachiensis	Settles at greater rates on substrata with greater development of microbial film, but on a large variety of other substrata as well.	Pearce and Scheibling 1991
Lytechinus pictus	Induction of metamorphosis in laboratory experiments requires bacterial film or soluble compound of bacterial origin.	Cameron and Hinegardner 1974

Gee and Knight-Jones 1962; Gee 1965). Another polychaete, *Janua brasiliensis*, demonstrates only moderate substratum specificity in settling on a diversity of surfaces including the green alga *Ulva lobata* and the seagrass *Zostera marina* (Nelson 1979; Kirchman et al. 1982). There are many examples of larvae that are induced to metamorphose and settle by contact with non-geniculate coralline algae (NCA), although the specificity of the interaction with NCA varies among species (the topic has been reviewed extensively by CR Johnson, manuscript submitted to American Naturalist). Examples include larvae of the crown-of-thorns starfish which are induced to metamorphose at high rates on one species of NCA but not others (Johnson et al. 1991b; Johnson and Sutton 1994), the coral *Agaricia agaricties humilis* which is induced by several NCA but not others (Morse et al. 1988), larvae of the abalone *Haliotis rufescens* which are induced by any of several species of NCA in its adult habitat (Morse and Morse 1984; A. Morse pers. comm.) and by microbial films (Hahn 1989), and the starfish *Coscinasterias calamaria* in which metamorphosis is induced by NCA and several other substrata (Barker 1977).

In these and other cases in which larvae settle and metamorphose on other species, and particularly when they do so preferentially, the question arises as to how larvae recognise the substrata on which they settle. If the cue is chemical, as it often is (Pawlik 1992), might it be bacteria that are the source of such cues? If bacteria do mediate metamorphosis and settlement, then a critical requirement to account for the specificity of larval settlement responses is that bacterial assemblages or their metabolic characteristics must be dissimilar on different surfaces. Johnson et al. (1991a) showed this to be the case for the surfaces of different species of NCA. They compared bacterial communities on the surface of two species of NCA with those developed on (initially) sterile glass slides incubated *in situ* for 11 days alongside the NCA, and in samples of seawater collected on separate occasions from above the NCA. Bacterial communities in the seawater samples and on the glass slides were similar, but clearly different to those on the surfaces of the NCA which were also distinctive from each other. Thus, their results were consistent with the idea that bacteria may be the source of inductive molecules on surfaces or macroscopic organisms. Lewis et al. (1985) also showed that bacteria associated with the surfaces of NCA are distinctive.

It appears that bacteria do provide the cue for metamorphosis of *J. brasiliensis* on *Ulva lobata*. Some bacteria isolated from *U. lobata* were inductive as mono-specific films, but there was virtually no metamorphosis on clean surfaces or on a non-specific (but bacteria free) organic film (Kirchman et al. 1982). However, a multi-species film developed by incubating slides in seawater induced metamorphosis at higher rates than films of individual strains isolated from *Ulva*.

INTERACTIONS BETWEEN CORALLINE ALGAE, BACTERIA AND LARVAE

Induction of crown-of-thorns starfish, *Acanthaster planci*

In induction of metamorphosis by NCA, the possibility of a bacteria-mediated mechanism has been examined only for larvae of the crown-of-thorns starfish (COT) which are induced to settle and metamorphose at high rates by a particular species of NCA, although they will settle at low rates on a variety of other substrata (Johnson et al. 1991b). This work indicated that bacteria growing on the plant surface induce metamorphosis of COT larvae, and that the morphogenic cues do not derive from the plant itself (Johnson and Sutton 1994). This finding explained initial observations that larvae metamorphose on shards of NCA with high, but not low, densities of bacteria (Johnson et al. 1991b).

Although induction of COT larvae appears to be mediated by bacteria on the surface of the plant, an important result is that the coralline also plays an integral role in the interaction. Several lines of evidence indicate that the bacteria produce the morphogenic agent from a substrate derived from the plant; strains of bacteria isolated from inductive NCA and filmed onto several surfaces singly or in multispecies assemblages are not inductive, fractions of cell debris containing intact and damaged bacterial cells but not soluble compounds from the alga are not inductive, and bacteria proliferate only on damaged sections of the NCA surface where plant-derived material is available (Johnson et al. 1991a,b; Johnson and Sutton 1994).

In this example there is likely to be strong selection for a 3-way larva-bacteria-NCA interaction since all three gain from the interaction; the NCA is the preferred food of the juvenile starfish and provides protection from predators, the NCA is likely to benefit from the grazing activities of the starfish reducing fouling of its surface, and the bacteria proliferate when surface cells of the plant are damaged by grazers.

The findings of bacterial induction of metamorphosis in *Janua* and COT, and particularly the complex and specific nature of the interaction between COT larvae, surface bacteria and the host coralline, should encourage a more extensive analysis of the extent and nature of bacterial-mediated induction of larvae that settle and metamorphose on macroscopic organisms. It cannot be assumed that macroscopic hosts are the source of inductive principles.

Induction of abalone, *Haliotis* spp.

Induction of metamorphosis of the Pacific red abalone (*Haliotis rufescens*) by NCA has been studied extensively. Red abalone larvae are induced by crude soluble extracts of NCA and by contact with NCA, and there is evidence that one inductive agent associated with the surface of the plant is a peptide associated with phycobiliproteins (Morse et al. 1984; Morse and Morse 1984). However, it is possible that there is more than one type of inductive molecule associated with surfaces of NCA. If the morphogenic peptide is ubiquitous on all NCA species as implied, then it is unlikely to be involved in induction of *Haliotis asinina*, since all but one species of NCA tested so far are unable to induce metamorphosis in this species (R. Counihan and B. Degnan, unpub. data). Although it is likely that COTs and abalone have different signal-receptor systems (Johnson and Sutton 1994), it is instructive to examine more closely the possibility of a bacterial-link in induction of abalone.

There are several lines of evidence consistent with bacterial mediation of induction of larvae of at least some *Haliotis* species. Plates covered with a bacterial biofilm (which may also include diatoms) is the most widespread method of inducing settlement and metamorphosis of abalone (including *H. rufescens*) in commercial hatcheries (Hahn 1989). In New Zealand, rates of metamorphosis of *H. iris* and *H. virginea* on plastic tags increased with development of a surface biofilm (0–36 days) in the absence of NCA (R. Roberts, unpub. data). Also, inductive ability of NCA in trials with *H. iris* was reduced significantly (from ca. 90% to <60% metamorphosis) by scrubbing the algal surface (R. Roberts, unpub. data), indicating a possible role for surface microbes. Other evidence that the morphogen is attached to the NCA surface but can be physically dislodged, which is consistent with the possibility that the cue is associated with bacteria, is that abalone normally require contact with the NCA for induction, but will metamorphose after exposure to water in which NCA surfaces have been brushed (Morse and Morse 1984). These observations do not demonstrate a role for bacteria, but neither do they discount it.

Bacterial production of γ-aminobutyric acid (GABA)

In an important early paper, Morse et al. (1979) showed that metamorphosis of *H. rufescens* is induced by GABA, although it is generally thought that GABA does not play an inductive role in nature. However, since marine bacteria are able to produce GABA from a variety of substrates including glutamate (its precursor amino acid) and the amines putrescine and spermidine (Mountfort and Pybus 1992a,b), the possibility of GABA production by bacteria on the surface of NCA warrants critical attention. These substrates are available in various red algae and presumably in NCA; glutamate occurs as an intermediary in the GOGAT pathway for nitrogen metabolism in red algae (G. Stewart, unpub. data) and is one of the most abundant amino acids in red algae (Bird et al. 1982; Horrocks et al. 1995), and putrescine, which is a precursor of spermidine and is formed from the amino acids ornithine or arginine, is a biogenic polyamine present in all eukaryotic cells. However, many marine microbes are also able to degrade GABA (Kaspar et al. 1991; Kaspar and Mountfort 1995). Kaspar and Mountfort (1995) examined the balance of GABA production and degradation by heterotrophic bacteria on the surface of NCA. They found that NCA surface bacteria did not produce GABA when glutamate or putrescine were added to seawater in which corallines were incubated, but that they readily 'degraded' GABA added to the seawater. However, we suggest that their conclusion, that GABA is unlikely to induce metamorphosis of abalone larvae on NCA, extrapolates beyond the indications of their results.

Given that abalone larvae are induced to metamorphose at high rates by GABA at low concentrations (10^{-6}M), and that the morphogenic cue associated with NCA is not

released into seawater but is attached to the surface of the plant (Morse and Morse 1994), ecological interpretation of Kaspar and Mountfort's (1995) results, which report on release into seawater of bacterially produced GABA and microbial utilisation of free GABA in seawater, is equivocal. This work does not examine the possibilities that surface bacteria might produce but not exude GABA, or that exuded GABA is taken up by other microbes in the biofilm, or that GABA may not be degraded in the process of uptake. In a pilot study, we examined whether bacteria from the surface of NCA produce GABA but do not exude it, and whether GABA that is taken up by NCA bacteria ('degraded' sensu Kaspar and Mountfort) is metabolised immediately or is available within the cell as free GABA, as outlined below.

Methods

Bacteria were isolated from surfaces of subtidal NCA collected in Tasmania. Intact NCA were rinsed with sterile seawater (SSW), swabbed with sterile cotton swabs, the swabs were inoculated onto Zobell's Marine Agar supplemented with vitamins (MAV, Lewis et al. 1985), and incubated at 20°C for seven days. Colonies of four common morphotypes were streaked to purification and stored on MAV slopes at 2°C.

Growth of bacteria, and their utilisation and/or production of GABA and glutamate, was examined after incubation in broth cultures with different carbon sources. To basic autoclaved media (artificial sea salts 3% w/v; NH4Cl 0.2% w/v; tris buffer 0.1% w/v; mineral salts solution 0.2% v/v) were added sterile filtered phosphate buffer solution (0.2% v/v) and one of four carbon sources, viz., (1) glucose + sodium acetate; (2) glucose + sodium acetate + GABA; (3) NCA aqueous extract; and (4) sodium glutamate. NCA medium was aqueous extract of NCA to the equivalent of 0.2% NCA wet weight w/v, and all other nutrients were added at 0.2% w/v each.

Following incubation (7d at 20°C), cells were harvested by centrifuging and washed several times in SSW before extraction in HPLC-grade methanol in glass vials for 7 days at 4°C. Presence of glutamate and GABA was examined by gas chromatographic (GC) and mass spectrometric analyses. For derivatisation of amino acids from the cells, methanol extracts were evaporated to dryness under nitrogen before adding 500 µL MilliQ water. N(O,S) ethoxycarbonyl ethyl esters of amino acids (ECEEs) were prepared as outlined in Wang et al. (1994). ECEEs were extracted twice after cessation of gas evolution; first with 10 mL and then 5 mL of chloroform. The combined extracts were evaporated under nitrogen and concentrated for GC analysis. In the GC analysis components were identified by relative retention time compared to standards, and identification was confirmed by mass spectrometric analysis (we used a Hewlett-Packard 5890 GC equipped with a 59970 mass selective detector).

Results and Conclusions

All four strains of NCA bacteria grew on all media (Table 2). However, while bacteria grew well on both glutamate and soluble nutrients extracted from NCA as sole carbon sources, and despite that most strains produced glutamate from most carbon sources, there was no evidence that they produced GABA from any of them. However, cells grown with GABA as a carbon source contained GABA, indicating that GABA is not necessarily metabolised as it is taken up. The inability of some NCA surface bacteria to produce GABA from potentially suitable substrates (i.e. glutamate and NCA extract) parallels the findings of Kaspar and Mountfort (1995). However, the finding that free GABA occurs in cells that take up GABA is important. While the few strains we isolated from NCA did not produce GABA, other marine bacteria are able to produce GABA and release it into the water column (Mountfort and Pybus 1992a,b). Since bacteria on NCA can take up ('degrade') free GABA from the water column at low concentrations (Kaspar et al. 1991; Kaspar and Mountfort 1995), providing it is not metabolised in the process of uptake, then larvae contacting live bacteria may be exposed to GABA and therefore the inductive stimulus. A key question is whether GABA can be taken up and made available on the surface of the bacterial film.

Our overall conclusion is that while a bacterial role has not been demonstrated in the induction of settlement and metamorphosis of abalone larvae by NCA, the available evidence does not rule out this possibility. There is insufficient evidence to dismiss the possibility that GABA associated with bacteria on the surface of NCA is one possible mechanism of induction of abalone larvae, and the idea warrants further attention.

Table 2: Growth and production and utilisation of glutamate and GABA in four strains of bacteria isolated from the surface of subtidal NCA in Tasmania.

Carbon source and bacteria strain	Growth	Glutamate in cell extracts	GABA in cell extracts
NCA Aqueous Extract			
strain A	+	-	-
strain B	+	+	-
strain C	+	+	-
strain D	+	-	-
Glutamate			
strain A	+	+	-
strain B	+	+	-
strain C	+	+	-
strain D	+	+	-
Acetate + Glucose			
strain A	+	+	-
strain B	+	+	-
strain C	+	+	-
strain D	+	+	-
Acetate + Glucose + GABA			
strain A	+	+	+
strain B	+	+	+
strain C	+	+	+
strain D	+	+	+

EVOLUTION OF BACTERIA-MEDIATED INDUCTION OF METAMORPHOSIS OF BENTHIC MARINE INVERTEBERATES

The question arises as to why systems in which bacteria mediate the induction of settlement and metamorphosis might evolve at all. In particular, why would 3-way substratum-bacteria-larva, or 2-way bacteria-larva, interactions develop instead of a 2-way substratum-larva interaction?

The decision to settle and metamorphose is crucial for sessile and sedentary benthic animals since selection of settlement site to a large degree defines the likelihood of post settlement survival (e.g. see Keough and Downes 1982). Like most other aspects of life history, there is a spectrum of 'strategies' among marine invertebrates between the extremes of generalists, which will settle readily on a variety of substrata, and specialists, which manifest strong preferences for settlement on particular substrata. For settling larvae, particularly those demonstrating higher levels of substratum specificity, there are two requirements: first, competent larvae must be are able to identify a consistent cue that is characteristic of microhabitats in which their likelihood of post settlement survival is greatest, and second, in the absence of preferred substrata, larvae should be induced by cues on suboptimal, but not deleterious, substrata since some rate of settlement in suboptimal microhabitats realises greater fitness than complete failure to settle. In this respect we point out that, in most cases, species with clear preferences for specific substrata, e.g. crown-of-thorns starfish (Johnson et al. 1991b), abalone (Hahn 1989), any of several species of *Spirorbis* (Gee and Knight-Jones 1962; De Silva 1962; Gee 1965), the Atlantic rangia (Sundberg and Kennedy 1993), and the polychaete *Janua brasiliensis* (Nelson 1979; Kirchman et al. 1982), will also settle at low rates on a diversity of substrata other than the preferred type.

Regardless of whether a species is a specialist or generalist in the substrata on which it settles, all species must demonstrate some degree of substratum selectivity, and all require an appropriate cue for settlement and/or metamorphosis. Larva alighting a surface in the marine environment will soon encounter marine bacteria, and we suggest that bacteria provide appropriate cues for both 'generalist' and 'specialist' settlers. For generalist species, some portion of the bacterial assemblage is likely to provide a uniform cue across a diversity of substrata given the ability of attached bacteria to utilise similar substrates in the water column independent of the substratum to which they are attached, and their ability to synthesise the same compound from different substrates (e.g. the amino sugar *N*-acetylglucosamine, which is a component of bacterial cell walls and the antigen specific lipopolysaccharide layer of the outer membrane, can be synthesised from several different hexose phosphates; see Gottschalk 1986). For 'specialist' species, we argue that because of

rapid generation times and the diversity of metabolic pathways expressed by bacteria, they are likely to be a source of rapid evolution of novel settlement cues and increase the diversity of cues associated with any one substratum, which facilitates evolution of specificity in larva-substratum interactions. Generation of unique cues is facilitated by microbes in biofilms living in close proximity to one another, thus fostering complex chemical interactions with one another as well as with the substratum. Further, genetic exchange, and therefore rapid evolution of chemical phenotypes, is likely to occur in circumstances where bacterial cells are attached in close proximity. Plasmid transfer has been observed in epilithic populations and rates of DNA transformation increase when bacteria are associated with a substratum (see Fletcher 1991).

A diversity of chemical signals generated by bacteria will best facilitate evolution of substratum-bacteria-larva interactions if larvae have the ability to discern among and differentially respond to diverse chemical cues. While many marine invertebrate larvae can detect specific settlement environments, evidence suggests larvae can respond to a wide range of chemicals. Although larvae are structurally simple, chemosensation can be complex and discerning. Based on gross morphological and behavioural comparisons, the level of chemosensation in the highly characterised nematode *Caenorhabditis elegans* is probably similar to that of many marine invertebrate larvae. In this animal, a wide range of chemosensory responses has been defined, with 32 chemosensory neurons sensing diverse chemical signals (White et al. 1986). Recently over 40 divergent G protein-coupled receptors have been identified in *C. elegans* (Troemel et al. 1995). Importantly, G protein-mediated second messangers have been strongly implicated in abalone chemoreception and settlement (reviewed in Morse, 1992). By direct comparison, it is likely that many marine invertebrate larvae have the potential to sense and respond to a wide range of soluble and insoluble chemicals.

It is likely therefore that larvae are able to sense many chemical signals, and that a variety of chemical signals is able to trigger settlement or metamorphosis (see also Pawlik 1992). Our suggestion is that the bacterial assemblage on particular substrata will comprise both microbes that generate a characteristic chemical signature, and a component that will be common to many substrata. For sessile invertebrate species with specific habitat requirements, or who interact with substrata that convey particular benefits (e.g. in supply of food and/or shelter), bacteria readily provide a characteristic and consistent cue to enable distinguishing among substrata (see Johnson et al. 1991a). Recognition of these cues on suitable substrata will ensure selection for and maintenance of substratum-bacteria-larva interactions by post settlement processes. Chemical signals other than those characteristic of optimal substrata may also induce metamorphosis, but will also be subject to post settlement selection and lead to avoidance of deleterious substrata but some level of settlement or metamorphosis on sub-optimal substrata. Strong selection for marked preferences in settlement mediated by bacteria can be expected when, as in the case of COT larvae (Johnson and Sutton 1994), the bacteria and/or substrata also benefit from the interaction. We propose that bacteria can also facilitate substratum selection by invertebrate species that are advantaged by settlement on a wide range of substrata by (1) providing consistent cues through growth on soluble nutrients independent of the substratum, and (2) their ability to synthesise particular molecules from different chemical substrates.

We expect evolution of interactions between marine invertebrate larvae, bacteria and marine substrata to be widespread because bacteria are likely to be predictably encountered, provide for rapid evolution of chemosensory response to unique chemical signals, and generate a greater diversity of substratum-specific chemical signals (but also provide a greater similarity of cues across a diversity of substrata) than other sources of cues. Our general conclusion is that relatively little is known of the extent and nature of these interactions, and that the topic warrants greater attention by researchers.

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REFERENCES

Avelin M, Vitalina M, Rittschof D, Nagabhushanam R (1993) Bacterial-barnacle interaction: potential of using juncellins and antibiotics to alter structure of bacterial communities. J Chem Ecol 19:2155-2167

Barker MF (1977) Observations on the settlement of the brachiolaria larvae of *Stichaster australis* (verrill) and *Coscinasterias calamaria* (Gray)(Echinodermata: Asteroidea) in the laboratory and on the shore. J Exp Mar Biol Ecol 30:95-108

Bird KT, Habig C, DeBusk T (1982) Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). J Phycol 18:344-48

Brancato, MS Woollacott, RM (1982) Effect of microbial films on settlement of bryozoan larvae (*Bugula simplex*, *B. stolonifera* and *B. turrita*). Mar Biol 71:51-56

Cameron RA, Hinegardner RT (1974) Initiation of metamorphosis in laboratory cultured sea urchins. Biol Bull 146:335-342

Christensen BE, Kjosbakken J, Smidsrod O (1985) Partial chemical and physical characterisation of two extracellular polysaccharides produced by marine, periphytic *Pseudomonas* sp. Strain NCMB2021. Appl Environ Microbiol 50:837-845

Coyne VE, Pillidge CJ, Sledjeski DD, Hori H, Ortiz-Conde BA, Muir DG, Weiner RM, Colwell RR (1989) Reclassification of *Alteromonas colwelliana* to the genus *Shewanella* by DNA-DNA hybridization, serology and 5S ribosomal RNA sequence data. System Appl Microbiol 12:275-279

De Silva PHDH (1962) Experiments on choice of substrate by *Spirorbis* larvae (Serpulidae). J Exp Biol 39:483-490

Fitt WK, Hofmann DK (1985) Chemical induction of settlement and metamorphosis of planulae and buds of the reef-dwelling coelenterate *Cassiopea andromeda*. Proc 5th Int Coral Reef Symp 5:239-244

Fitt WK, Hofmann DK, Wolk M, Rahat M (1987) Requirement of exogenous inducers for metamorphosis of axenic larvae and buds of *Cassiopea andromeda* (Cnidaria: Scyphozoa). Mar Biol 94:415-422

Fitt WK, Labare MP, Fuqua WC, Walch M, Coon SL, Bonar DB, Colwell RR, Weiner RM (1989) Factors influencing bacterial production of inducers of settlement behaviour of larvae of the oyster *Crassostrea gigas*. Microb Ecol 17:287-298

Fitt WK, Coon SL, Walch M, Weiner RM, Colwell RR, Bonar DB (1990) Settlement behaviour and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. Mar Biol 106:389-394

Fletcher M (1991) The physiological activity of bacteria attached to solid surfaces Adv Microb Physiol 32:53-85

Gee JM (1965) Chemical stimulation of settlement in larvae of *Spirorbis rupestris* (Serpulidae). Anim Behav 13:181-186

Gee JM, Knight-Jones EW (1962) The morphology and larval behaviour of a new species of *Spirorbis* (Serpulidae). J Mar Biol Assoc UK 42:641-654

Gottschalk G (1986) Bacterial metabolism (2nd ed) Springer-Verlag, New York

Hahn KO (1979) Induction of settlement in competent abalone larvae. In: Hahn KO (ed) Handbook of culture of abalone and other marine gastropods. CRC Press, 101-112

Harrigan JF (1972) Behavior of the planula larva of the scleractinian coral *Pocillopora damicornis* (L.). Am Zool 12:723

Henning G, Benayahu Y, Hofmann DK (1991). Natural substrates, marine bacteria and a phorbol ester induce metamorphosis in the soft coral *Heteroxenia fuscescens* (Anthozoa: Octocorallia). Verh Dtsch Zool Ges 84:486

Hofmann DK, Brand U (1987) Induction of metamorphosis in the symbiotic scyphozoan *Cassiopea andromeda*: Role of marine bacteria and of biochemicals. Symbiosis 4:99-116

Hofmann DK, Neumann R, Henne, K (1978) Strobilation, buding and initiation of scyphistoma morphogenesis in the rhizostome *Cassiopea andromeda* (Cnidaria: Scyphozoa). Mar Biol 47:161-176

Holmstrom C, Rittschof D, Kjelleberg S (1992) Inhibition of settlement by larvae of *Balanus amphitrite* and *Ciona intestinalis* by a surface-colonizing marine bacterium. Appl Exp Microb 58:2111-2115

Horrocks JL, Stewart GR, Dennison WC (1995) Tissue nutrient content of *Gracilaria* spp. (Rhodophyta) and water quality along an estuarine gradient. Mar Freshwat Res 46:975-983

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 crustose coralline algae and a hypothesis for deepwater recruitment. Mar Ecol Prog Ser 71:143-162

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

Kaspar HF, Mountfort DO, Pybus V (1991) Degradation of gamma aminobutyric acid (GABA) by marine microorganisms. FEMS Microbiol Ecol 85:313-318

Kaspar HF, Mountfort DO (1995) Microbial production and degradation of γ-aminobutyric acid (GABA) in the abalone larval settlement habitat. FEMS Microbiol Ecol 17:205-212

Keough MJ, Downes BJ (1982) Recruitment of marine invertebrates: the role of active larval choices and early mortality. Oecologia 54:348-352

Keough MJ, Raimondi PT (1995) Responses of settling invertebrate larvae to bioorganic films: effects of different types of films. J Exp Mar Biol Ecol 185:235-253

Kirchman D, Graham S, Reish D, Mitchell R (1982) Bacteria induce settlement and metamorphosis of *Janua (Dexiospira) brasiliensis* Grube (Polychaeta: Spiorbidae). J Exp Mar Biol Ecol 56:153-163

Kjelleberg S, Hermansson M, Marden P (1987) The transient phase between growth and nongrowth of heterotrophic bacteria, with emphasis on the marine environment. Ann Rev Microbiol 41:25-49

Leitz T, Wagner T (1993) The marine bacterium *Alteromonas espejiana* induces metamorphosis of the hydroid *Hydractinia echinata*. Mar Biol 115:173-178

Lewis JB (1974) The settlement behaviour of planulae larvae of the hermatypic coral *Favia fragum* (Esper). J Exp Mar Biol Ecol 15:165-172

Lewis TE, Garland CD, McMeekin TA (1985) The bacterial biota on crustose (nonarticulated) coralline algae from Tasmanian waters. Microb Ecol 11:221-230

Maki JS, Rittschof D, Costlow JD, Mitchell R (1988) Inhibition of attachment of larval barnacles, *Balanus amphitrite*, by bacterial surface films. Mar Biol 97:199-206

Maki JS, Rittschof D, Mitchell R (1992) Inhibition of larval barnacle attachment to bacterial films: An investigation of physical properties. Microb Ecol 23:97-106

Maki JS, Rittschof D, Samuelsson MO, Szewzyk U, Yule AB, Kjelleberg S, Costlow JD, Mitchell R (1990) Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. Bull Mar Sci 46:499-511

Maki JS, Rittschof D, Schmidt AR, Snyder AG, Mitchell R (1989) Factors controlling attachment of bryozoan larvae: A comparison of bacterial films and unfilmed surfaces. Biol Bull 177:295-302

Mihm JW, Banta WC, Loeb GI (1981) Effects of adsorbed organic and primary fouling films on bryozoan settlement. J Exp Mar Biol Ecol 54:167-179

Morse DE (1992) Molecular mechanisms controlling metamorphosis and recruitment in abalone larvae. In: Shepherd SA, Tegner MJ, Guzman del Proo (eds) Abalone of the world: ecology, fisheries and culture. Blackwell, Oxford, 107-119

Morse ANC, Froyd CA, Morse DE (1984) Molecules from cyanobacteria and red algae that induce larval settlement and metamorphosis in the mollusc *Haliotis rufescens*. Mar Biol 81:293-298

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

Morse DE, Hooker N, Duncan H, Jensen, L (1979). γ-aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. Science 204:407-410

Morse DE, Hooker N, Morse ANC, Jensen RA (1988). Control of larval metamorphosis and recruitment in sympatric agariciid corals. J Exp Mar Biol Ecol 116:193-217

Mountfort DO, Pybus V (1992a) Regulatory influences on the production of gamma-aminobutyric acid by a marine pseudomonad. Appl Environ Microbiol 58:237-242

Mountfort DO, Pybus V (1992b) Effect of pH, temperature and salinity on the production of gamma aminobutyric acid (GABA) from amines by marine bacteria. FEMS Microbiol Ecol 101:237-244

Müller WA, Wieker F, Eiben R (1976) Larval adhesion, releasing stimuli and metamorphosis. In: Mackie, GO (ed) Coelenterate Ecology and Behaviour, Plenum Press NY, 339-346

Neal AL, Yule AB (1994) The interaction between *Elminius modestus* Darwin cyprids and biofilms of *Deleya marina* NCMB1877. J Exp Mar Biol Ecol 176:127-139

Neumann R (1979) Bacterial induction of settlement and metamorphosis in the planula larvae of *Cassiopea andromeda* (Cnidaria: Scyphozoa, Rhizostomeae). Mar Ecol Prog Ser 1:21-28

Nelson WG (1979) Observations on the settlement patterns of *Janua (Dexiospira) brasiliensis* (Polychaeta: Serpulidae). Estuaries 2:213-217

Pawlik JR (1992) Chemical ecology of the settlement of benthic marine invertebrates. Oceanogr Mar Biol Annu Rev 30:273-335

Pearce CM, Scheibling RE (1991) Effect of macroalgae, microbial films, and conspecifics on the induction of metamorphosis of the green sea urchin *Strongylocentrotus droebachiensis* (Müller). J Exp Mar Biol Ecol 147:147-162

Spindler K, Müller WA (1972) Induction of metamorphosis by bacteria and by a lithium-pulse in the larvae of *Hydractinia echinata* (Hydrozoa). Wilhelm Roux Arch dev Biol 169:271-280

Sundberg K, Kennedy VS (1993) Larval settlement of the Atlantic rangia, *Rangia cuneata* (Bivalvia: Mactridae). Estuaries 16:223-228

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

Tritar S, Prieur D, Weiner R (1992) Effects of bacterial films on the settlement of the oysters, *Crassostrea gigas* (Thunberg, 1793) and *Ostrea edulis*, Linnaeus, 1750 and the scallop *Pecten maximus* (Linnaeus, 1758). J Shellfish Res 11:325-330

Troemel ER, Chou JH, Dwyer ND, Colbert HA, Bargmann CI (1995) Divergent seven transmembrane receptors are candidate chemosensory receptors in C. Elegans. Cell 83:207-218

Wang J, Huang ZH, Gage DA, Watson JT (1994). Analysis of amino acids by gas chromatography-flame ionisation detection and gas chromatogrphy-mass spectrometry: Simultaneous derivatisation of functional groups by an aqueous-phase chloroformate-mediated reaction. J Chromatography 663:71-78

Weiner RM, Segall AM, Colwell RR (1985) Characterization of a marine bacterium associated with *Crassostrea virginica* (the eastern oyster). Appl Environ Microbiol 49:83-90

White JG, Southgate E, Thompson JN, Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. Phil Trans Roy Soc (Lond) B 314:1-340

Wilson DP (1955) The role of micro-organisms in the settlement of *Ophelia bicornis* Savigny. J Mar Biol Assoc UK 34:531-543

ZoBell CE, Allen EC (1935) The significance of marine bacteria in the fouling of submerged surfaces. J Bacteriol 29:239-251