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Fouling barnacles: Larval development, settlement behavior and control technology

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Abstract

Macrofouling on ship hulls, cooling conduits and heat exchangers cause serious revenue loss. Though more than 4,000 species have been identified as causative organisms, only a few groups are dominant in a given fouling community. Among them, barnacles are the most troublesome species. They are sedentary, hard-shelled crustaceans, with a worldwide distribution and, therefore, are one of the main targets in the effort to develop antifouling technology. After attaining reproductive maturity, the broods release young larvae known as nauplii, which undergo six stages of metamorphic changes and ultimately develop into what is known as cypris. Cypris is a nonfeeding larva and is remarkably equipped for selection of a suitable site for attachment and subsequent development into an adult barnacle. Biofilms, universally associated with substrata exposed to the marine environment, largely decide the suitability of the substratum for the cypris larva, through molecular cues. Knowledge about barnacle development, settlement cues and mechanism of larval attachment onto the surface is essential not only to understand biofouling process but also to adapt suitable control technology. This paper is an attempt to review information on barnacle larval development, settlement behavior and control technology, especially in the Indian context.

Keywords: Biofouling, barnacle, cypris settlement, biofouling control.

1. Barnacles: a dominant foulant

When a solid substratum is immersed in the sea, marine organisms, particularly sessile benthic forms settle on it, resulting in what is known as marine biofouling. Marine biofouling is a very serious economic problem and constitutes a major impediment to several maritime activities. Fouling affects propulsion of ships, reduces pipeline diameter of cooling-water intake systems, affects the performance of navigational buoys and equipment used for coastal defence, and reduces the heat-transfer efficiency of ocean thermal energy conversion systems (OTEC). Worldwide, the cost of fouling comes to about $1.4 bn a year.[1] According to an Electric Power Research Institute study, macrofouling accounts for about 3–4% loss in the availability of a 600-MW (e) power plant.[2] Barnacle fouling could raise fuel cost of ships by as much as 40%.[1]

Fouling develops on surfaces in a sequential manner. Surface-active macromolecules, particularly protein molecules, coat inert surfaces immersed in sea water[3] to form a conditioning film. Settlement of microorganisms follows the development of this conditioning film. In general, bacteria are the first to settle, followed by microalgae, protozoa and fungi. The last ones to colonize are macroorganisms. Over 4000 species of fouling organisms have so far been listed

among the fouling communities.[4] These include hydroids, serpulids, sponges, barnacles, oysters, bivalves and ascidians. In any given biofouling community, barnacles generally constitute a dominant component.[5] They, in fact, constitute half of the fouling species reported so far from ship hulls.

In recent years, barnacles have come under detailed investigation because of the following reasons: i) they usually dominate a fouling community, irrespective of the geographical region and season, ii) they secure permanent attachment on many kinds of substrata, and iii) their larval stage, the cypris, possesses the remarkable capacity to settle under different environments such as in fast-moving waters, coastal waters, offshore areas, backwaters and polluted ports.[6] In order to develop a successful antifouling system, an accurate knowledge of the organisms involved and the processes that control their settlement are required.

2. Naupliar development and larval rearing

Rearing of sessile invertebrate larvae in the laboratory has gained importance in recent years so as to gain insight into the mechanism of larval settlement[7–9] as well as for laboratory testing of potential antifouling paints and bioactive compounds. Testing of antifouling agents requires continuous supply of test organisms. Barnacle larvae are an excellent test material for screening antifouling compounds.[10–13] A continuous supply of barnacle larvae can only be maintained by their mass rearing in the laboratory. The success of mass culture depends on the type of food supplied to the growing larvae, level of illumination, salinity of the sea water and temperature.[14] A list of diatom and flagellate species used as food for barnacle larvae is given in Table I. The choice of diet for the larval forms of a given species is related to its geographical distribution.[15] It has been shown that the size of algal cells is linked to the intersetular spacing on the antennae of napulii.[16]

2.1. *Larval diet*

The development time from the first napulius (N1) to cypris in *Balanus amphitrite* and *Balanus cirratus* is approximately 4 days and this is the shortest time interval when compared to other barnacle species (Table I). The duration required for the complete larval development of other barnacle species reared using *Chaetoceros wighamii* is *Balanus reticulatus*–5 days;[17] *M. tintinnabulum*–4 days and *Ch. malayensis* Pilsbry (using *Isochrysis galbana* as food)–9 to 11 days. Rittschof *et al.*[18] obtained cyprid larvae of *B. amphitrite* using *Skeletonema costatum* (Grev.) as food in 4 days and, Karande and Thomas[19] obtained cypris of *C. malayensis* in 9 to 11 days using flagellates as food. A period of 7 to 12 days was reported by Karande[20] for the complete larval development of *B. amphitrite* and *B. cirratus*, which may be attributed to the diet of *Dunaliella primolecta* used by him. Results indicate that warm-water balanids take 4 to 5 days to complete larval development when fed with diatoms, whereas chthamalids require 9 to 11 days, when fed on flagellates.[21] The presence of plumose setae (and the absence of feathery setae, as in chthamalids) on the feeding apparatus (antenna) of balanids facilitates the capture of diatoms more effectively than flagellates and therefore balanids grow better on diatoms, whereas chthamalid larvae grow better on flagellates.[22] The larval development duration of all tropical balanids studied so far is almost similar. Minor variations observed in the laboratory are possibly due to differences in culture conditions.[22] Daniel[23] reported the appearance of *M. tintinnabulum* cypris in laboratory culture within 48 h, whereas our study took 96 h. Daniel

Table I
Earlier studies on larval development in Balanoidea

Species	Food	°C	Time#	References
Balanus kondakovi	DP and diatoms	28	–	Karande 1979
B. amphitrite	Mixed food	20	17	Egan and Anderson 1986
	DP	28	7	Karande 1973[20]
	SC/CW	28	4	Thiyagarajan 1999[27], Rittschof *et al.* 1984[18], Kado 1991
B. reticulatus	CW	28	5	Thiyagarajan *et al.* 1996
B. cirratus	CW	28	5	Thiyagarajan 1999[27]
	DP	28	7	Karande 1974a
B variegatus	Mixed food	20	15	Egan and Anderson 1986
B. albicostatus	NC	25	–	Lee and Kim 1991
B balanus	Mixed food	26	8	Barnes and Costlow 1961
B. glandula	TF	–	–	Branscomb and Vedder 1982
B. improvisus	PT	25	12	Dineen and Hines 1994[34]
B. eburneus	CG/IG	27	6	O'Connor and Richardson 1994[35]
B. subalbidus	PT	25	7	Dineen and Hines 1994[34]
B. crenatus	TF	–	–	Branscomb and Vedder 1982
	No food	20	15	Ovsyannikova and Korn 1984
B. perforatus	–	15	25	Bassindale 1936
B. cariosus	PT	–	–	Branscomb and Vedder 1982
B hameri	Diatom	10	28	Walker 1973
B. trigonus	Flegellates	28	–	Freiberger and Cologer 1966[26]
B balanoides	Diatom	20	7–9	Walker 1973
Megabalanus tintinnabulum	CW	28	5	Thiyagarajan *et al.* 1997[17]
	–	28	2	Daniel 1958[23]
M. rosa	SC	22	6	Kado and Hirano 1994
M. valcano	SC	22	6	Kado and Hirano 1994
M. californicus	–	18	–	Miller and Roughgarden 1994

Time days required for cypris development

DP: *Dunaliella primolecta*; SC: *Skeletonema costatum*; CW: *Chaetoceros wighamii*; NC: *Nitzschia closterium*; TF: *Thalassiosira fluviatils*; PT: *Phaeodactylum tricornutum*; CG: *Chaetoceros gracilis*; IG: *Isochrysis galbana*

however did not describe the culture conditions he maintained and it is possible that sea water he had used might have been contaminated with the advanced naupliar stages of the species.

Although *S. costatum*, a chain-forming diatom, has been successfully used as food for barnacle larvae[18], it has some disadvantages such as rapid sinking rate that reduces its availability to swimming nauplii larvae.[24] Thiyagarajan *et al.*[21] have reported that the use of *Chaetoeceros wighamii* supports higher survival rate than *S. costatum*. It supports faster growth, not only of *B. reticulatus* but also of other tropical balanids (Table I). *Chaetoeceros wighamii* is usually in single-cell state and therefore sinks slower, as compared to the sticky *S. costatum* cells. This particular characteristic of *C. wighamii* is possibly an important reason for its good dietary value to barnacle larvae than *S. costatum*.[21] Kado[24] has successfully used another species of this genus, namely, *C. calcitrans* as food for *B. amphitrite* larvae. Algal density used for naupliar development was found to affect both survival and metamorphic success.[25] Larvae of *Balanus eburneus*, *B. amphitrite*, *B. trigonus* and *B. improvisus* have been successfully reared with *Cyclotella nana* at 2×10^5 cells/ml.[26] Similarly, larvae of four tropical species have been

successfully reared using *C. wighamii* at a density of 2×10^5 cells/ml.[27] However, Rittschof *et al.*[18] reported that *B. amphitrite* larvae required a diatom (*S. costatum*) density of 2×10^6 cells /ml for optimum growth.

2.2. *Illumination*

Light is an important factor that affects barnacle larval development. When larvae were grown under dark condition, the growth of diatom as well as larvae was retarded.[14] However, when sufficient food is available, the growth of diatom is found to be similar to the larvae growing in constant light condition.[28] For successful development of *B. amphitrite* larvae, 15 h of light is necessary.[10]

2.3. *Temperature*

As reported by Tighe-Ford *et al.*[14] in the case of *B. amphitrite*, *B. reticulatus* too grows faster at elevated temperatures, possibly due to faster feeding rate at higher temperatures.[28] Apart from the effect on inter-molt period between successive larval stages, temperature also affects the survival of the larvae in the culture.[25] It was found that temperature above 23°C and 28°C reduces the rate of survival of *Elminius modestus* and *B. amphitrite*, respectively. Studies also show that sea water of 30–35 ppt salinity supports good growth of *B. reticulatus* larvae under constant illumination and at a temperature of 28–30°C.[27]

3. Behavior of cyprid larvae during settlement

In recent years, barnacle larval settlement behavior and mechanism have been subjected to intensive investigations for understanding the factors which determine the temporal and spatial distribution of these organisms as well as for the control of their settlement on man-made surfaces.[8, 29–31] Laboratory-reared cyprids have been extensively used to understand the mechanism controlling metamorphosis and to investigate the possibilities of using nontoxic natural bioactive products in antifouling systems.[25] Cyprid larvae of *Balanus amphitrite*,[1, 4, 10, 31, 32] *B. balanoides*,[33] *B. eburneus*, *B. improvisus*, *B. subalbidus*,[34, 35] *Elminius modestus*[36] and *Megabalanus rosa*[37] have been successfully reared in the laboratory and used for studying settlement behavior (Tables I, III and IV). *B. amphitrite* larvae are widely used as a model test organism[8, 18, 38] for testing antifouling coatings[39] and for screening natural bioactive compounds.[10, 12, 37] Studies by O'Connor and Richardson[36] on the settlement behavior of *B. amphitrite*, *B. improvisus* and *B. eburneus* cyprids at varying salinity, cyprid age and using different types of substrata showed that the settlement behavior of *B. amphitrite* cyprid was not generalizable and that it could be different from that of other balanomorphs. Moreover, assay procedure used for *B. amphitrite* larvae may not be suitable for other balanomorphs, because of species-specific settlement behavior.[18, 35] Compared to *B. amphitrite*, whose settlement behavior has been extensively studied (Table IV), much information is not available on the larval settlement behavior of other common fouling barnacles.

The settlement behavior of barnacles has been reviewed by Mary and Sarojini.[25] Contact with substratum by cypris in dynamic waters is governed by both physical and behavioral mechanisms.[5] Cyprid larvae recognize their adults for gregarious settlement through adult-borne protein called arthropodin.[40] Factors such as food density used during naupliar develop-

Table II
Earlier studies on *Balanus amphitrite* larval settlement behavior

Cyprid behavior during settlement	References
After exploration, cypris rejects a surface more frequently in fast flow than in slow flow conditions	Mullineaux and Butman 1991
Cyprids tend to attach in higher number on glass than on polystyrene	O'Connor and Richardson 1994[35]
Salinity has little effect on settlement	O'Connor and Richardson 1994[35]
Cyprid foot print, arthropodins, soluble barnacle odor act as settlement pheromone	Crisp and Meadows 1962, 1963 Rittschof 1985
Larva–larva interaction affects the percentage settlement	Rittschof *et al.* 1998[8]
Settlement is sensitive to light and color of the substratum	Clare *et al.* 1994[38]
Settles in a wide range of salinity, temperature and hydrostatic pressure	Kon-ya and Miki 1994[41]
Gregarious behavior is conspecific	Crisp 1990
Bacteria elicit different settlement responses on different substrata	Maki *et al.* 1990[58]
Settles at higher rate on surface with higher wettability	Rittschof and Costlow 1989
Surface chemistry of a surface plays a significant role in determining distribution and abundance of barnacles	Rittschof and Costlow 1989
Cyprids caught in plankton respond similar to young laboratory-reared cyprids	Branscomb and Rittschof 1984[52]
Low-frequency (25 to 35 Hz) sound waves reduce settlement	Branscomb and Rittschof 1984[52]
Settlement factor has no effect on older cyprids	Rittschof *et al.* 1984[18]
Alteration of ionic concentrations does not affect settlement	Rittschof *et al.* 1984[18]
Rate of settlement is positively correlated with wettability of a surface. Cyprids are stimulated to investigate by high-surface energy surfaces	Gerhardt *et al.* 1992
Storing cyprids at low temperature result in loss of substratum discrimination	Kitamura and Yasuo 1996,[15] Rittschof *et al.* 1984[18]
Cyclic AMP may be involved in larval signal transduction	Clare *et al.* 1995

ment,[28] light,[41] substratum type,[18, 35] salinity,[34] cyprid age,[4, 39, 42, 43] cyprid density, cyprid foot prints[39] and water flow[6] influence settlement of barnacle larvae. A study of the nature of influence of these factors on the settlement of barnacles is desirable because their response to these specific cues determines the final settlement and metamorphosis in the preferred habitat.[29] The study will also help to design a suitable laboratory bioassay protocol.[41]

Quality and quantity of food used for naupliar development influence larval survival and rate of metamorphosis.[27, 44] Food concentration used for naupliar development has a notable influence on the settlement ability of the larvae. The quantity/quality of food determines the

Table III
Earlier studies on cyprid settlement behavior (other than *Balanus amphitrite*)

Species	Cyprid behavior during settlement	References
Balanus balanoides	Cyprid with a propensity to swim exhibit low temporary adhesion force than cyprid with a propensity to explore the surface	Neal and Yule 1992
	Strength of adhesion is more on arthropodin-treated slates than control	Yule and Walker 1987
	Not all protein coatings cause an increase in the magnitude of temporary adhesion	Yule and Walker 1984
	Cypris does not settle on glass even in the presence of settlement factor nor on other surfaces unless they are previously soaked in settlement factor	Crisp and Meadows 1963
B. perforatus	Attach less strongly to low shear biofilm	Neal and Yule 1994
Elminius modestus	Attach strongly to low shear biofilm in contrast to *B. perforatus*	Neal and Yule 1994
	Bacteria–substratum adhesion plays a significant role in cyprid settlement	Neal and Yule 1994
B. improvisus	Attach more on polystyrene than on glass	O'Connor and Richardson[35]
	Cyprid age has little influence on settlement	O'Connor and Richardson[35]
	Salinity affects settlement	O'Connor and Richardson[35]
B. eburneus	Larval behavior at settlement could play a substantial role in determining adult distribution	Dineen and Hines[34]
B. subalbidus	Oligohaline distribution of adult is not determined by larval behavior at settlement in contrast to *B. eburneus*	Dineen and Hiens[34]
B. reticulatus	Physical (light, temperature, substratum), chemical (larval foot prints, organic coatings) and biological (biofilms, feeding history) factors significantly influence larval settlement	Thiyagarajan[27]

number of oil droplets (food reserve) in the cyprids. Cyprids with higher number of oil droplets are likely to have better chances of settlement than those with less number. Qiu and Qian,[45] and Anil and Kurian[28] have shown that food concentration used during naupliar growth has clear influence on the duration of larval development. Cypris of *B. reticulatus* prefers darkness to light or dark/light cycle for settlement and this behavior of the larva is important in designing an assay protocol.[27] In contrast to *B. reticulatus*, Kon-ya and Miki[41] have reported that cyprid larvae of *B. amphitrite* prefer light to darkness. However, Thiyagarajan[27] has reported that cypris of *B. amphitrite* like *B. reticulatus* appears to settle more in dark than in light. Since candidate bioactive compounds used for bioassay are likely to be denatured by light,[41] it will be better if the assay is done in complete darkness. In this respect, *B. reticulatus* is preferable to *B. amphitrite* as a test organism.

Planktonic naupliar stages of barnacles prevailing in coastal waters may enter in to estuaries. They are thus subjected to severe salinity stress. Cyprid larvae of both *B. reticulatus* and *B.*

Table IV
Earlier studies on biofilm effect on barnacle larval settlement

Species		Effect of biofilm	Surface	Composition of biofilm	References
Balanus amphitrite	L	No effect/inhibition	Polystyrene	Single-species bacterial strains	Maki *et al.* 1988
B amphitrite	L	Inhibition	Polystyrene	Single-species bacterial strains	Mitchell and Maki 1988
B. amphitrite	L	Inhibition	Polystyrene	*Deleya marina*	Rittschof and Costlow 1989
B. amphitrite	L	Facilitation/ inhibition	Polystyrene	Natural biofilm	Maki *et al.* 1990
B. amphitrite	L	Inhibition	Polystyrene	2 strains of *D. marina*	Maki *et al.* 1992
B amphitrite	L	Inhibition	Polystyrene	Bacterial strain isolated from biofilm	Holmstrom *et al* 1992
B amphitrite	L	No effect/inhibition	Polystyrene	Single species bacterial strains	Mary *et al.* 1993
B. amphitrite	L	Facilitation/ inhibition	Polystyrene	Natural biofilm	Wieczorek *et al* 1995
B amphitrite	L	Facilitation	Glass	Natural biofilm	Tsurumi and Fusetani 1998
B. improvisust	L	Facilitation	Glass	Single-species bacterial strains	O'Connor and Richardson 1996
B improvisus	L	Inhibition	Polystyrene	Single-species bacterial strains	O'Connor and Richardson 1996
B cariosus	F	Facilitation/ inhibition	Slate	Natural biofilm	Strathmann and Branscomb 1979
B crenatus	F	Inhibition	Plastic	Natural biofilm	Hudon *et al.* 1983
B glandula	F	Facilitation/ inhibition	Slate	Natural biofilm	Strathmann and Branscomb 1979
B perforatus	L	No effect/inhibition	Glass	Natural biofilm	Neal and Yule 1994
Elminius modestus	L	Facilitation/ inhibition	–	*D. marina*	Neal and Yule 1994
E. modestus	L	Facilitation/ inhibition	Glass	Natural biofilm	Neal and Yule 1994
Notomegabalanus algicola	F	Facilitation	PVC	Natural biofilm	Cook and Henschel 1984
Semibalanus balanoides	F	Inhibition	Wood	Natural biofilm	LeTourneux and Bourget 1988
Barnacles	F	Facilitation	Glass	Natural biofilm	Meenakumari and Nair 1994
B. reticulatus	L	Inhibition	Polystyrene	Natural biofilm	Thiyagarajan 1999
B. reticulatus	L	Inhibition/no effect/facilitation	Polystyrene	Bacterial film	Thiyagarajan 1999
B. reticulatus	L	Inhibition	Polystyrene	Diatom film	Thiyagarajan 1999

(F: field study, L: laboratory study)

amphitrite are capable of settling in moderately wide range of salinities.[46] However, post-settlement osmotic/ionic stress could potentially be a factor influencing their distribution.[34] Ionic stimulation has been shown to enhance metamorphosis in some invertebrates, but not in barnacle cypris.[47] Cyprid larvae of *B. reticulatus*[27] and *B. improvisus*[36] attach very well on polystyrene. *B. reticulatus* larvae do not settle well on glass surfaces. In contrast, cyprid larvae

of *B. amphitrite* prefer glass.[18, 27, 35] Wettability of substratum has been reported to influence larval adhesion[30, 48] and this could be a reason for the preference of *B. reticulatus* larvae to polystyrene (a hydrophobic substratum), compared to glass (hydrophilic).

Cyprid–cyprid interaction in *B. amphitrite* has been found to be an important factor in the design of a settlement assay because cyprid settlement could be density-dependent.[27,38] However, this is not evident in the case of *B. reticulatus* larvae because varying the number of larvae in the range 5–200 larvae/5 ml does not seem to affect settlement.[27] Larval foot prints, which are proteinaceous secretions left on a surface by exploring cyprids,[38, 49, 50] enhance conspecific settlement.[38] Cyprid foot prints induce conspecific settlement in *B. reticulatus* as reported in the case of *B. amphitrite*[27, 38] and *B. balanoides*.[49]

A simple and reliable method of storing cyprid larvae at low temperature provides opportunities to use them for extended period.[18] It has been shown that cyprid larvae aged in this manner attach in higher numbers, when compared to young cyprids.[18, 27, 43, 51] This is due to reduced discriminating ability to select the right surface, with advancing age.[4, 18, 43, 51, 52] Contrary to the above observations, Willemsen *et al.*[39] have reported that per cent settlement of *B. amphitrite* cypris on polystyrene was similar (70%) for all age groups (3, 6, 10 and 17 day). Satuito *et al.*[53] found that cyprid larvae having sufficient amount of cyprid major protein (CMP) are capable of settling at higher rate, while those aged more than three days at low temperature have depleted CMP and reduced settlement success. Depleted stock of CMP cannot support the production of adult structures following settlement.[53] Ability of a cyprid to undergo aging varies with the species and depends on the length of time it can postpone metamorphosis, which, in turn, depends on lipid energy reserves.[52] It has been suggested that postponing metamorphosis of *B. reticulatus* larvae beyond 5 days decreases settlement rate and increases mortality.[27] This may be due to the depletion of CMP, as suggested by Satuito *et al.*[53]

4. Biofilm cues for larval settlement

An important function of cypris is to respond to physical and chemical cues and to achieve successful settlement and metamorphosis in a location that provides good environment for survival and successful reproduction.[42] Barnacle larvae are known to receive both negative and positive cues from the environment.[29] Biofilms act as a source of cues for barnacle larvae to recognize between favorable and unfavorable surfaces for settlement and metamorphosis.[54, 31] The presence of biofilm, though, is not a prerequisite for cyprid settlement.[32] On the other hand, it has been suggested that the pattern of fouling community in a habitat is decided by biofilm.[55] Therefore, knowledge about the cues provided by biofilms to settling larvae would be useful to develop suitable control strategies against biofouling as well as to understand spatial variations in larval settlement.[56]

Marine biofilms are generally dominated by bacteria, sessile diatoms and large amount of polymeric substances that bind cells and other organic and inorganic materials together and to the substratum.[29] Biofilms act as a potential source of settlement and metamorphic cues for a wide range of marine invertebrate larvae (see reviews[29, 31]). Effect of biofilm on larval settlement depends on its composition,[57] age,[33, 58] film volume,[59] as well as the barnacle species (Table IV). Earlier studies show that larval response to biofilm cues are very subtle and specific, and that small-scale differences in successional composition, physiological conditions

and growth phase of the biofilm community may affect larval behavior at the time of settlement.[60, 61]

A majority of the studies carried out so far have examined cyprid settlement response to biofilm either in the field[57, 62, 61] or to individual films of bacteria and their exopolymers in the laboratory.[27, 32, 58, 63–65] Biofilms have been observed to facilitate settlement in the laboratory in *B. amphitrite* until the film volume was in the range 0.1–1 $\mu m^3/\mu m^{-2}$; the response decreased thereafter.[59] Biofilms developed in laboratory aquaria inhibit barnacle settlement,[33] while they enhance the settlement in field conditions.[66] Settlement of *B. variegatus* and *E. modestus* cypris was found to be more on unfilmed or less-filmed surfaces, when compared to filmed surface in the field conditions.[62] Biofilms developed on polystyrene dishes constitute a surface which is not preferred by barnacles.[27] Earlier studies give conflicting views on the effect of biofilm on barnacle settlement and indicate lack of any uniform pattern (Table IV). Any meaningful interpretation of settlement dynamics needs knowledge of the response to biofilm across all or most of the fouling species.[62] In a number of studies on the effect of biofilm on barnacle settlement, single-species biofilms have been used.[32, 58, 63–65] This may not help in generalization of larval responses to biofilm under natural conditions. At the same time, it is only by utilizing single-species film in the laboratory that progress can be made in identifying the specific components of a biofilm that act as important cue for larval settlement in the field.[31]

Inhibition or induction of larval settlement by biofilms is influenced by many factors such as surface-free energy of a substratum and production of negative cue by its components such as bacteria and diatoms.[32, 58, 12] Substratum properties (e.g. wettability) influence the adsorption of organic molecules and continue to exert control over bacterial adsorption, even after a conditioning film is established.[67] Adsorbed molecules alter properties of substratum making all surfaces wettable.[68] The presence of biofilms, bacteria, diatoms and organic polymers renders the surfaces more wettable.[48] Since *B. reticulatus* larvae do not prefer wettable surface, the presence of biofilms on hydrophobic (polystyrene) surface might have reduced the cyprid settlement when compared to clean, untreated hydrophobic surfaces, due to an increase in surface wettability.[27] O'Connor and Richardson[65] have reported that settlement of *B. improvisus* was decreased by bacterial cells, when present on hydrophobic (polystyrene) surface, whereas it was facilitated when they were coated on hydrophilic (glass) surface. Type of surface (surface energy of a substratum) and cues (positive/negative) associated with biofilms determine the strength (tenacity) of adhesion of barnacle larvae.[36] Maki *et al.*[58] suggest that the species composition of a biofilm may play an important role in the settlement of cypris larvae.

Marine bacteria have been isolated from biofilms and their influence on *Balanus amphitrite, B. reticulatus* and *B. improvisus* settlement has been studied in the laboratory.[12, 27, 32, 58, 65, 66] In general, bacterial films inhibit settlement, when exposed to the larvae individually. Among the bacterial species, *Deleya marina* was found to have a potent biomolecule causing the inhibition of *B. amphitrite* settlement.[12] Similarly, diatoms have been examined for their influence on *B. reticulatus* settlement. Diatoms too inhibited the larval settlement in a density-dependent manner.[27] Bacteria can change the physico-chemical nature of the substratum by altering the surface wettability or by exposing different cell-surface domains.[32, 58, 63] Bacteria can also produce surface-bound and soluble chemical cues that either stimulate or inhibit larval settlement.[68, 60] Thus bacteria present in the biofilm play a significant role in influencing larval settlement. Exopolymeric film formed on the surface due to bacterial action may act as an ad-

Table V
Control of cypris settlement through various physical and chemical methods

Name	Concentration/Treatment	Reference
Toxic metals		
Cuprous oxide	16 Cu μg/cm^2/day	De la Court 1987
Organotin compounds	2 Sn μg/cm^2/day	De la Court 1987
Oxidizing biocides		
Chlorine	0.2 to 1*	Nair *et al.* 1997
Chlorine-activated sodium bromide	0.05 to 0.1*	Ekis and Trulear 1992
Bioactive compounds		
Diterpenoid, Pukalide, Epoxypukalide	–	Rittschof *et al* 1985, Mizobuchi *et al.* 1994
Renillafoulin		Rittschof *et al.* 1986
Zosteric acids	–	Clare 1995
Tribromogramine	–	Miki 1994
Bromine-containing furanones	–	De Nys *et al.* 1995
Juncellin	–	Mary *et al.* 1993
Furan-2-carboxylic acid	–	Mizobuchi *et al.* 1994
Eudistomin G and H	–	Davis and Wright 1990
Other methods		
Heat treatment	100% mortality in 17 min at 40°C	Sasikumar 1991
Mechanical methods	–	Little and DePalma 1988
Water flow	3.3 m/s	Whitehouse *et al.* 1985

* total residual concentration

hesive layer or may provide unfavorable surface for barnacle larvae, depending on the chemical composition.[32, 58, 63] Some strains do not affect larval settlement, while others induce or inhibit larval settlement (Table IV).[12, 32] The age, density and growth phase of the bacterial strains have also been shown to influence the larval settlement of barnacles.[32, 58, 63, 12, 27] However, the mechanism by which bacteria and their exopolymers act on barnacle larvae is not clear so far.[7]

5. Control of larval settlement

Various antifouling methods that are currently in use are shown in Table V. Presently the principal antifouling technology for the control of barnacle larval settlement is based either on toxic metals or oxidizing biocides. On surfaces such as ship hulls, antifouling paints or coatings with toxic ingredients are used to prevent cypris settlement. Such paints, in practice, keep the surface free of fouling for one to five years. The toxic metals are held in soluble, insoluble or self-polishing paint matrix. Upon contact with water, the toxicant is slowly released into sea water. Metal toxins prevent fouling either by repelling or by killing the larvae. A major breakthrough in the development of antifouling paints was the introduction of self-polishing paints. These paints keep the surface smooth due to the hydrolysis and dissolution of the matrix with simultaneous release of the toxin. They thus prevent the settlement of barnacle larvae as well as provide a smooth hull surface. Antifouling compounds based on organotin are banned in several countries as a result of environmental pollution.[69] There is a necessity for effective, nontoxic and nonpolluting fouling control methods. In recent years, researchers have investi-

gated environmentally friendly alternatives to traditional antifouling systems.[70, 71, 72] These alternatives include antifouling systems such as low-surface energy coatings, naturally produced toxins, enzymes and nonleaching biocides.[13] Earlier studies[75] have reported that the low-surface-free energy approach could be an attractive alternative. Silicon-based polymers provide a surface with low-surface-free energy (non-wettable). Such surfaces do not favour strong attachment of marine organisms. However, more investigations on their mode of action are necessary for the efficient manipulation of paint composition.[72]

Another attractive alternative to toxic metals is naturally available compounds which, though not toxic, interfere with the larval settlement process, thereby preventing fouling.[1] A list of such bioactive compounds is given in Table V. These compounds have been shown to prevent the settlement of barnacle larvae at least in the laboratory. It is still not clear how the bioactive compounds repel/prevent the settlement of barnacle larvae without being toxic to them.[1] Moreover, it is generally felt that before such compounds can be exploited for commercial purpose, they will have to be engineered to provide a coating that stays active for a few years.[1]

6. Future research

Even though researchers are actively working for the past five decades on the ability of cypris for surface discrimination, a thorough understanding of larval site selection and settlement is still elusive. To date, only a limited number of field experiments on larval responses to biofilms have been carried out. Only a few of these have assessed the effects on more than one barnacle species or have examined temporal variations in response. Thus, though it is well established in many laboratory experiments that biofilm cues can affect larval behavior during settlement, little is known about the ecological significance of these cues under natural conditions. No effort has been made to study the influence of natural biological cues in association with physical factors, which affect the settlement of major fouling organisms. This could be a rewarding approach in larval settlement research.

Further progress in the control of barnacle larval settlement through enzymatic digestion of larval cement, interference of larval signal transduction pathways, use of neuropharmacological agents, application of endocrine manipulations during larval metamorphosis and use of natural antifoulants/analogs will be made easier, once we know how barnacle larvae sense environmental cues and settle in response to such cues.

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