

Chapter 6
Summary

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Recruitment of the larvae of fouling organisms and their metamorphosis is the most important step in the fouling process. Cypris larvae do a substratum search before undergoing metamorphosis. The third antennular segment with its attachment disc is the most obvious point of contact between the cyprid and the substratum during the search. Flicking of the fourth antennular segment is also evident while a cyprid explores a substratum (Clare et al. 1994). While exploring some surfaces, cyprids leave behind 'footprints' of temporary adhesive, which are believed to be secreted by the glands of the antennular disc. The exploratory behavior and subsequent metamorphosis response of cypris when subjected simultaneously to sugars and adult extract (AE) is reported through this study. Evaluation of sugar-treated cyprids was carried out with AE coated and non-coated multiwells containing filtered seawater (FSW). This was done in order to observe how a cyprid would behave when the polar groups associated with CTA are blocked by cues like sugars and under such conditions how AE influences cyprid's search behavior and metamorphosis response.

The detection of AE even after blockage of polar groups of CTA on the third antennular segment with its attachment disc, suggests the availability of alternate sites for pheromone reception. It is possible that the settlement proteins of AE are detected by the receptors on the fourth antennular segment via olfaction. The absence of AE rendered these sites non-functional thus the cyprids responded to sugars in either promotion or inhibition of metamorphosis without further search.

This aspect needs attention and will be helpful in identifying the role of alternate pheromone reception sites.

Gregarious settlement in barnacles has been related to the settlement inducing compounds from adult conspecifics, microbes and their interaction. Efforts have been made to elucidate these settlement cues from these sources. Bacteria, *P. aeruginosa*, *B. pumilus* and *C. freundii* isolated from the shell surface of *B. amphitrite* and a thraustochytrid protist identified as a component of marine microbial films were used as candidate organisms. The facilitation of metamorphosis by *P. aeruginosa*, *B. pumilus* or *C. freundii* depended on the salinity or temperature. The variations in larval behavior in response to salinity and temperature can be attributed to alteration in metabolic activities and expression of different cell surface organic molecules. These surface associated specific biochemicals may not only function in the role of stimulating or inhibiting larvae but may change the surface chemistry in a more general fashion. By doing so they either mask important signals or block the receptors responsible for eliciting the larval responses (Maki 1999).

The leachants of *P. aeruginosa* culture supernatant fraction CS1 proved to be most inhibitory, whereas the cyprids did not settle in presence of leachants of the fraction, CS3 of *B. pumilus* and *C. freundii* at the end of day 1. The interaction of sugars with water is highly specific and depends strongly on the stereoisomerism of the hydroxyl group (Maggio et al. 1985; Kutteneich et al. 1988). Culture supernatant fractions obtained from the bacteria by growing them

in BSS consisted mainly of carbohydrates. The differences in metamorphosis induction by them with respect to salinity may be the result of positional effect due to isomerism.

The culture supernatant of *P. aeruginosa* obtained by semi-solid culture was proteinaceous and showed the presence of terpenoids and steroids. The degree of inducement by this was greater than the supernatant obtained from bacteria using any other nutrient media and was protein concentration dependent. The culture supernatant isolated from cells growing in a semi-solid environment also represents a closer approximation to the natural environment existing between a bacterium and its substratum (Abu et al. 1991). According to Boyle and Reade (1983), such conditions may be similar to the effects of exposure to intertidal zones. However, in case of *C. freundii*, cyprids metamorphosed in higher percentages when exposed to culture supernatant obtained by using BSS and was carbohydrate concentration dependent. In the case of culture supernatants of *B. pumilus*, such an inducement was not evident.

In the present investigation the bacteria were assessed separately, whereas, in nature they occur in conjunction. The contradictory signals from each bacterium would give different set of signals to the settling larvae. Spatial and chemical heterogeneities of surfaces in marine environment have been illustrated by lectin probes and its role in possible microscale cues for biofouling is suggested (Michael and Smith 1995). The understanding of the synchronization of all the

contradictory signaling molecules in presence of each other needs further validation.

It has been reported earlier that in *B. amphitrite* cyprids, the degree of inducement of metamorphosis varied with various combinations of exopolymers of different bacterial strains with or without AE (Anil et al. 1997; Anil and Khandeparker 1998). Some bacteria in biofilms are capable of genetic exchange (Fry and Day 1990). The cell-cell communication between their own species and perhaps others through N-acyl-L-homoserine lactones (AHL) cannot be ruled out as AHL activity has been demonstrated in natural biofilms (McLean et al. 1997). The ability of larval receptors to distinguish between the EPS that are intimately associated with the cell surface and those that are released in surrounding medium would be a key for production of probes for such saccharides and the genes that produce them can then be explored. The response of the cyprids to bacteria and its products thus seems to be regulated by both contact chemoreception and/or olfaction, which depend on the properties of the settlement inducing compounds.

The involvement of lectins in the settlement and metamorphosis of invertebrates has been hypothesized for many years. Lectins belong to a class of naturally occurring proteins or glycoproteins and can recognize and bind carbohydrates specifically and noncovalently. In the present investigation the bacterial films were probed with lectins in order to identify the specific carbohydrate molecules responsible in signaling the cyprids. *P. aeruginosa* when tagged with lectins

specific to glucose and its derivatives, mannose and fructofuranose negated the promotory effect. Tagging of galactose derivatives translated the inhibitory effect of *B. pumilus* and *C. freundii* into a promotory one. This shows that the lectins can transform the signals in either direction. Galactose-binding lectins have been identified in the haemolymph of barnacles, which could find their way through the excretory system to the surface. It is possible that the presence of lectins could provide this organism with an ability in altering the signals or cues. Microscale patchiness of bacteria is also evidenced in marine communities. In view of this, and the conflicting cues available, such patchiness can help piloting the larvae to the destination by the basibionts. Understanding these controlling mechanisms and interfering with the pathways that are involved in lectin synthesis would be a step ahead in antifouling technology.

Thraustochytrid protists that have been detected in microbial films recently facilitated metamorphosis of cyprids to an even greater extent than adult extract (AE). It is likely that in nature, thraustochytrids on marine surfaces play an important role in the settlement of larvae belonging to macrofouling invertebrates. The need to characterize and distinguish the receptors, which act via different signaling systems on a particular settlement cue, will advance our understanding of the complexities of invertebrate larval recruitment.

Cypris prolong their larval duration until a conducive substratum is available for settlement and subsequent metamorphosis. Cyprids, like other invertebrate larvae derive their energy from stored lipids. In the absence of stimuli, these

competent larvae delay metamorphosis. In the present study the aged cyprids showed higher rates of metamorphosis. A possible explanation could be that young cyprids are more discriminating than the old ones but become less discriminating with age during settlement (Rittschof et al. 1984; Crisp 1988) presumably due to decline in their energy reserves and thus the physiological quality (Anil et al. 2001). The evidence gathered from the present experiments indicate that the naupliar experience clearly determines the capability of the cyprids to metamorphose as well. The RNA content of larvae raised at 20° C, which had longer total naupliar duration, was considerably less compared to those raised at 30° C. This difference in RNA content was reflected in the capability of the cyprids to survive ageing at 5° C. The larvae raised at 20° C could only successfully metamorphose until 2 to 4 days, whereas, those raised at 30° C could do so for 8 to 16 days. Clare et al. (1994) observed that the cyprid settlement of *B. amphitrite* is uncharacteristically low when reared with *D. tertiolecta* and that, although the cyprids look normal, they may be deficient in some respect. It was also observed earlier that, by day 3, the cyprids lose their power to discriminate the substrata and are less useful for assaying metamorphosing inducers (Rittschof et al. 1984; Crisp 1988; Clare et al. 1994). Maki et al. (1988) showed from their experimental results on inhibition of attachment of *B. amphitrite* to bacterial films, 4 day old cyprids showed an increase in larval attachment as compared to 2 day old ones. It was observed with cyprids of *Balanus* (= *Semibalanus*) *balanoides* after 4-5 weeks at 10° C a final threshold level was reached wherein they possessed insufficient energy to

accomplish metamorphosis into a feeding juvenile barnacle (Lucas et al.1979). Høeg and Ritchie (1987) observed with *Lernaeodiscus porcellanae* (Cirripedia: Rhizocephala) larvae, which have lecithotrophic nauplii and small sized cyprids, the energy threshold, where metamorphosis was no longer possible, was seemingly reached in less than 15 days and attributed this to the rearing temperature.

The results of the present investigation show that the variations in the larval response to different cues and the influence of aging of cyprids on larval metamorphosis can thus be related to the larval quality and / or nutritional stress experienced by the larvae which is governed by naupliar rearing conditions. This will be of critical importance to recruitment and early post-settlement mortality.

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Publications

1. Lidita Khandeparker, A C Anil and S Raghukumar

Barnacle larval destination: piloting possibilities by bacteria and lectin interaction.

***Journal of Experimental Marine Biology and Ecology*, Vol.289: 1-13, April, 2003.**

2. Lidita Khandeparker, A C Anil and S Raghukumar

Factors regulating the production of different inducers in *Pseudomonas aeruginosa* with reference to larval metamorphosis in *Balanus amphitrite*.

***Aquatic Microbial Ecology*, Vol. 28: 37-54, May, 2002.**

3. Lidita Khandeparker, A C Anil and S Raghukumar

Exploration and metamorphosis in *Balanus amphitrite* Darwin (Cirripedia; Thoracica) cyprids: significance of sugars and adult extract.

***Journal of Experimental Marine Biology and Ecology*, Vol. 281: 77-88, December, 2002.**

4. A C Anil, Dattesh Desai and **Lidita Khandeparker**

Larval development and metamorphosis in *Balanus amphitrite* Darwin (Cirripedia:Thoracica) significance of food concentration, temperature and nucleic acids.

***Journal of Experimental Marine Biology and Ecology*, Vol. 263(2):125-141, August, 2001.**

5. S. Raghukumar, A C Anil, **Lidita Khandeparker** and J S Patil

Thraustochytrid protists as component of marine microbial film.

***Marine biology*, Vol. 136 (4):603-609, May, 2000.**