Reference: *Biol. Bull.* **189**: 272–279. (December, 1995)

Behavioral Responses of *Concholepas concholepas* (Bruguière, 1789) Larvae to Natural and Artificial Settlement Cues and Microbial Films

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Abstract. The behavioral responses of veliger larvae of the gastropod *Concholepas concholepas* were studied in the presence of different natural and artificial settlement cues and microbial films. Early pre-competent larvae stopped swimming, sank (due to ciliary arrests, retraction of the velum into the shell, or both), and remained inactive on the substratum when exposed to conspecific mucus and hemolymph. In both cases the effect was time-dependent and the number of larvae showing these behaviors decreased over time. Larvae exposed to NH_4Cl (ammonium ion) showed a similar time- and dose-dependent response. A positive and time-dependent response was also observed when larvae were exposed to different extracellular matrix (ECM) components (*i.e.*, collagen, gelatin, and fibronectin) and sulfated polysaccharides (*i.e.*, carrageenan, heparin, and chondroitin sulfate). In this case the larvae remained attached to the substratum. However, the effect of sulfated polysaccharides on *C. concholepas* larval behavior was faster than that observed with other ECM molecules. We also studied the responses of pre-metamorphic *C. concholepas* larvae exposed to different microbial films. In chemotaxis experiments with different films, with glass as the substratum, larvae showed a significant preference for multispecific and diatoms films. When shells of *C. concholepas* were used as the substratum, the preference for multispecific films was clear and significant. Likewise, larvae showed velar contractions in the presence of all the films tested. Larvae exposed to multispecific films and to the microalga *Prasinocladus marinus* showed an increased ciliar movement. The finding that mucus and hemolymph of conspecific adults and ECM molecules (mainly sulfated polysaccharides) induce the cessation of swimming of *C. concholepas* larvae suggests a possible role for cell-surface receptors in mediating the larval response of marine organisms. Likewise, the positive chemotaxis responses of *C. concholepas* larvae to different microbial films suggest that microorganisms may have a role in bringing larvae close to settlement inducers on the marine benthos.

Received 11 November 1994; accepted 28 July 1995.

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Introduction

Settlement and metamorphosis are key steps during the life cycle of benthic marine invertebrates. A number of artificial and natural settlement-inducing substances have been described (Pawlik, 1992; Rodríguez *et al.*, 1993). Most of the artificial inducers are neuroactive molecules such as neurotransmitters, neurotransmitter precursors, and ions (Morse *et al.*, 1979; Hirata and Hadfield, 1986; Yool *et al.*, 1986; Bonar *et al.*, 1990). Natural inducers are associated with three main sources: conspecific individuals (*e.g.*, Pawlik, 1986), microbial films (Maki *et al.*, 1989), and prey species (Hadfield and Pennington, 1990). Concerning the first source, conspecific mucus is known to induce larval settlement in the gastropod *Haliotis rufescens* (Slattery, 1992). Moreover, it has been proposed that growth factors associated with the mucus could trigger the settlement response in at least some molluscan species (Cantillana and Inestrosa, 1993; Rodríguez *et al.*, 1993). Bacterial films have been reported to induce larval settlement in a number of marine invertebrates (*e.g.*,

Kirchman *et al.*, 1982). In some cases, exopolymers are the stimulus. These are produced by bacteria, possibly as adhesive factors during attachment to the substratum (Maki *et al.*, 1989). In other cases, the active inductive factor from bacterial supernatants could be ammonium ion (NH_4^+) (Bonar *et al.*, 1990). For example, oyster larvae exposed to solutions of NH_4Cl exhibit stereotypical settlement behavior similar to that which normally precedes metamorphosis (Coon *et al.*, 1990).

Morse and Morse (1991) reported that the morphogenetic molecule for a scleractinian coral larvae is a sulfated glycosaminoglycan. In spite of this finding, the possibility that extracellular matrix (ECM) macromolecules play a role in the settlement of marine invertebrate larvae has not been widely explored. Several studies have examined the behavioral response of gastropod pre-competent larvae to different settlement-inducing cues. Results indicate that pre-competent larvae are able to show some of the typical settlement behaviors observed during the metamorphosis of competent ones, such as ciliary arrests and contractions of the velar lobes, when exposed to settlement-inducing substances (Arkett *et al.*, 1987; Barlow, 1990). The pre-competent larvae sometimes retract the velum into the shell, probably due to overstimulation (Barlow, 1990). As a consequence of the settlement behaviors described above, larvae sink and remain transiently inactive on the substratum.

The prosobranch mollusc *Concholepas concholepas* ("loco"), an economically important benthic marine resource along the Chilean coast, is in danger of extinction resulting from overexploitation (Castilla, 1988). We have been studying this species to generate basic information that will eventually allow us to culture it (Urrea *et al.*, 1992; Cantillana and Inestrosa, 1993; Inestrosa *et al.*, 1993a,b; Campos *et al.*, 1994). We previously showed that an excess of K^+ induces metamorphosis in planktonic as well as in laboratory-reared larvae of *C. concholepas* (Inestrosa *et al.*, 1993a; Campos *et al.*, 1994).

Here we report the effect of conspecific mucus and hemolymph, ECM macromolecules, sulfated polysaccharides, and ammonium ion on the behavior of early pre-competent *C. concholepas* larvae. Likewise, we report on the behavioral response of pre-metamorphic larvae to different microbial films isolated from a native area of recruitment of *C. concholepas*.

Materials and Methods

Experimental animals

Adult specimens and egg capsules of *Concholepas concholepas* were collected from the subtidal zone off the central Chilean coast (Las Cruces; 33° 30′ S, 71° 30′ W) and immediately transported to our laboratory in fresh seawater. Capsules were maintained in aerated, membrane-filtered (0.45 μm) seawater at 20–22°C until hatching. For the experiments with natural and artificial cues, early pre-competent veliger larvae just hatched from capsules were acclimated 1–2 days before being used. For experiments with microbial films, pre-metamorphic larvae were obtained from a culture of 75 days as described by Riquelme and Chavez (1995). In brief, veliger larvae were obtained from mature capsules and maintained in 1-l bottles containing membrane-filtered (0.22 μm) seawater at 20°C and with a 14:10 LD photoperiod (60 larvae per liter). The seawater was changed every 2 days. The microalga *Isochrysis galbana* was used as food at a density of around 10^3 cells per liter of larval culture. Larvae of about 1650 μm were maintained at a density of 10 larvae per liter and acclimated for 2 days before using in experiments. These larvae showed all the characteristics of the pre-metamorphic stage of *C. concholepas* described by DiSalvo (1988).

Obtaining mucus and hemolymph

Conspecific mucus was obtained by smoothly scraping the muscular foot of living adult *C. concholepas* with a spatula. After that, the individuals were broken into pieces and placed inside a funnel; the drained hemolymph was collected. Both procedures were carried out in a cold room (4°C). The mucus and the hemolymph were used in experiments immediately after collection.

Obtaining microorganisms

The microorganisms used to create microbial films were isolated from the surface of rocks obtained in the natural area of recruitment of *C. concholepas* on the north coast of Chile (Antofagasta Bay; 23° 39′ S, 71° 30′ W). Four types of microbial films were used for larval behavior experiments: (1) multispecific bacteria–microalgal films (MBM), scraped directly from rocks; (2) monospecific bacterial films, constituted by a periphytic bacterium able to develop a strong film on glass and polystyrene plates; (3) *Prasinocladus marinus* films, produced by a periphytic dominant microalga present on the rocks; and (4) multispecific diatom films. To isolate bacteria, different rocks were scraped. The resulting material was inoculated in agar St 10 for marine bacteria (Ishida *et al.*, 1986) and incubated at 20°C for a week. Different bacterial strains growing in St 10 medium were recognized on the basis of some morphological characters (size, shape, color, and height) of their colonies. These bacterial strains were isolated and tested for their ability to develop a strong film on polystyrene plates. Monospecific bacterial suspensions were placed on petri dishes and rinsed with sterile seawater after 24 h. The strain that was able to remain attached to plates after rinsing was considered a strong periphytic bacterium.

In the case of the microalgal isolation, the scraped material was diluted, inoculated in agar (Provasoli *et al.*, 1957), and incubated at 20°C for 2 weeks with a 14:10 LD photoperiod. The dominant microalgal species, *Prasinocladus marinus*, was isolated by hand under a microscope and also inoculated in Provasoli medium. This species and the diatoms were identified by Professor Gerald Boalch (Citadelhill Plymouth Laboratories, U.K.).

Preparing microbial films

Pieces of *C. concholepas* shell and glass coverslips were offered as substrata to microorganisms. Before being used, substrata were washed with acid and rinsed with abundant seawater to remove all tissue residue. After that, they were deposited in bottles containing 150 ml of seawater. This material was autoclaved before being inoculated with the different strains. The substrata were incubated with the microorganisms in suspension until they developed film. The substrata were washed with sterile seawater and immediately used in experiments. Preliminary experiences showed that 48 h of incubation was sufficient to create a film able to adhere after washing.

Behavioral response bioassays

Larval response to different natural and artificial cues. Conspecific mucus was spread over 24-well culture plates in a homogeneous film. Twenty to thirty early pre-competent veliger larvae were assayed per well in a final volume of 1 ml of filtered seawater. The number of *C. concholepas* larvae that sank as a result of a cessation of swimming (due to ciliary arrest, retraction of the velum into the shell, or both) and remained inactive on the bottom of the wells during a 2-h incubation was recorded using a Wild dissecting microscope. The behavior of 20 to 30 control larvae maintained in wells containing nothing but 1 ml of normal filtered seawater was followed simultaneously with each treatment. Each treatment and each control were performed in triplicate. Hemolymph was loaded into 24-well culture plates (100 μl/plate) and dried overnight. The same procedure was followed with 100 μl of solutions containing 2 μg of fibronectin, carrageenan, chondroitin sulfate, or heparin; or 12 μg of collagen; or 200 μg of gelatin. Higher concentrations of collagen and gelatin were used because no larval response was observed at lower concentrations. The wells were filled with filtered seawater (1 ml) before 20 to 30 larvae were placed in each well. The experiments were followed for 30 min in the case of the hemolymph and 24 h for the ECM components and sulfated polysaccharides. The number of larvae that sank and remained attached to the substratum was recorded as described above. A similar experiment was carried out with hemolymph boiled for 3 min. Together with each treatment, the behavior of 20 to 30 control larvae maintained in wells containing nothing but 1 ml of normal filtered seawater was followed. Each treatment and each control were performed in triplicate.

Larvae were exposed to a range of concentrations of NH_4Cl (*i.e.*, 2, 5, 8, and 10 m*M*). A stock solution of 100 m*M* NH_4Cl was made in seawater and adjusted to pH 8.0 with 1 N NaOH. At the beginning of each bioassay, enough stock solution was added to a volume of seawater (pH 8.0) to generate 1 ml of the desired NH_4Cl final concentration in 24-well culture plates containing 20 to 30 larvae per well. The experiments were followed for 30 min and the number of larvae that sank and remained inactive on the bottom of wells was recorded as previously described. Treatments and controls were performed in triplicate, as described for the other bioassays. The ECM molecules and sulfated polysaccharides were obtained from Sigma Chemical Co. (St. Louis, MO).

The concentration of larvae used in all the above experiments (*i.e.*, 20 to 30 larvae/ml) was similar to the concentration at which larvae were acclimated after hatching and before the assays. In many species, repeated encounters with others causes larvae stop swimming and settle to the bottom of the culture vessel; thus the high density used in our experiments could have affected the results. However, the control larvae in our assays were never observed to stop swimming or settle as a result of encounters among them. The time courses followed in the above experiments were different because the assays were carried out until a clear response was observed.

Chemotaxis to microbial films. Individual assays of chemotactic response to microbial films were carried out in sterile petri dishes containing 15 ml of sterile seawater. Pre-metamorphic larvae and substrata containing microorganisms were placed on opposite sides of the dishes. We considered a response to be positive when larvae moved directly to the substrata and remained close to them, and negative when larvae moved to the edge of the dishes or remained close to the starting point (random movement). Ten pre-metamorphic larvae were simultaneously placed for each type of substratum and continuously observed for 1 h with a Wild dissecting microscope. The controls were carried out using sterile substrata. Each treatment was performed in triplicate. A *G* test (Sokal and Rohlf, 1981) was used for statistical analysis.

Larval activity in response to microbial films. The activity of *C. concholepas* larvae exposed to microbial films was observed. The films were prepared on glass coverslips and assayed in petri dishes as described above. An increased ciliar movement and the presence of contractions of the velum were used as criteria for larval activity. Larvae swimming with the velum extended and retracting it briefly but repeatedly were categorized as presenting velar contractions. Likewise, larvae moving their cilia faster

than the rate observed during a normal swim were considered to be showing an increased ciliary beating. Larvae were directly placed on the films and observed with a Wild dissecting microscope; the number showing an activity response was recorded at intervals of 0–2, 2–4, 4–6, 6–8, and 8–10 min (hereafter 2, 4, 6, 8, and 10 min, respectively). A total of 30 individual bioassays were carried out per film. A G test was used for statistical analysis.

Results

Larval response to different natural and artificial cues

Mucus and hemolymph. To learn about the effect of conspecific natural substances on the behavioral response of *C. concholepas*, early pre-competent larvae of this gastropod were exposed to mucus and hemolymph. In the presence of conspecific mucus, the veliger larvae stopped swimming and sank. The effect of mucus was time-dependent and reached a plateau after 5 min of continuous exposure to the film (Fig. 1). At this time, about 50% of the larvae were inactive on the bottom of the wells and remained in this state for 30 min of incubation (Fig. 1). Thereafter, the number of larvae swimming normally increased, leaving only 10% of the total larvae sunk after 2 h (Fig. 1). Larvae exposed to hemolymph showed a pronounced and quick response. After just 2 min of exposure, the sinking rate was 94% (Fig. 2). Thereafter, the number of larvae showing a cessation of swimming slowly decreased, resulting in a 75% sinking rate after 30 min (Fig. 2). Only a few larvae remained inactive on the bottom (16%) after 2 h of incubation (data not shown). Larvae exposed to boiled hemolymph showed a response similar to that observed with normal hemolymph during the first 2 min. However, the effect was transient because only 50% of the larvae remained sunk after 5 min of incubation (Fig. 2). The number of larvae induced to sink with boiled hemolymph was less than that observed with normal hemolymph throughout the experiment (Fig. 2).

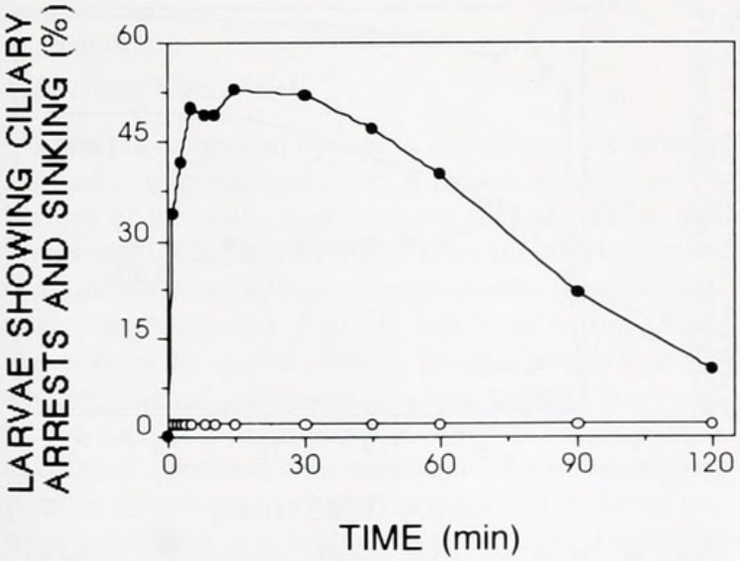

Figure 1. Mean percentage of early pre-competent larvae of *Concholepas concholepas* induced to sink by conspecific mucus after 2 h of incubation. ● = Mucus, and ○ = control.

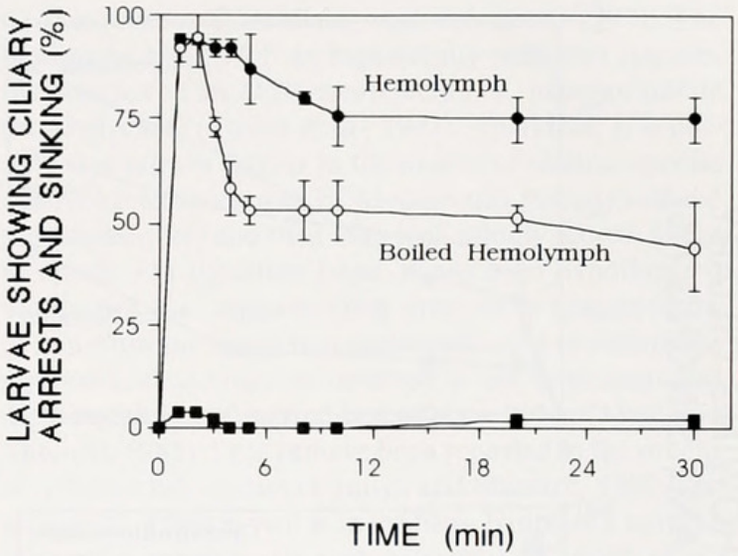

Figure 2. Mean percentage of early pre-competent larvae of *Concholepas concholepas* induced to sink by conspecific hemolymph. ● = Hemolymph, ○ = boiled hemolymph, and ■ = control.

ECM components, sulfated polysaccharides, and NH_4Cl. To study the effect of some artificial cues on the behavioral response of *C. concholepas*, early pre-competent larvae of this mollusc were exposed to different ECM molecules and sulfated polysaccharides. A positive and time-dependent response was observed for all the assayed molecules. After 24 h of incubation, collagen, gelatin, and fibronectin induced sinking rates of 44%, 67%, and 89%, respectively (Fig. 3a). At the same time, rates of 70%, 78%, and 87% were observed when larvae were exposed to carrageenan, heparin, and chondroitin sulfate, respectively (Fig. 3b). The larvae responded to the sulfated polysaccharides more quickly than to the ECM molecules, reaching more than the 50% of the final response after just 2 h of incubation (*i.e.*, sinking rates of 39%, 73%, and 68% with carrageenan, heparin, and chondroitin sulfate, respectively) (see Fig. 3a, b). To test the effect of NH_4Cl on the response of *C. concholepas*, early pre-competent larvae of this gastropod were exposed to different concentrations of ammonium ion. The behavioral response observed was time- and dose-dependent (Fig. 4): it increased rapidly during the first 2 min, reaching sinking rates of 4%, 45%, 48%, and 84% at NH_4Cl final concentrations of 2, 5, 8, and 10 mM, respectively (Fig. 4). Thereafter, the number of larvae that stopped swimming and remained inactive stayed relatively constant, resulting in respective rates of 1%, 39%, 74%, and 98% after 30 min of incubation (Fig. 4). At the end of the experiment, larvae exposed to

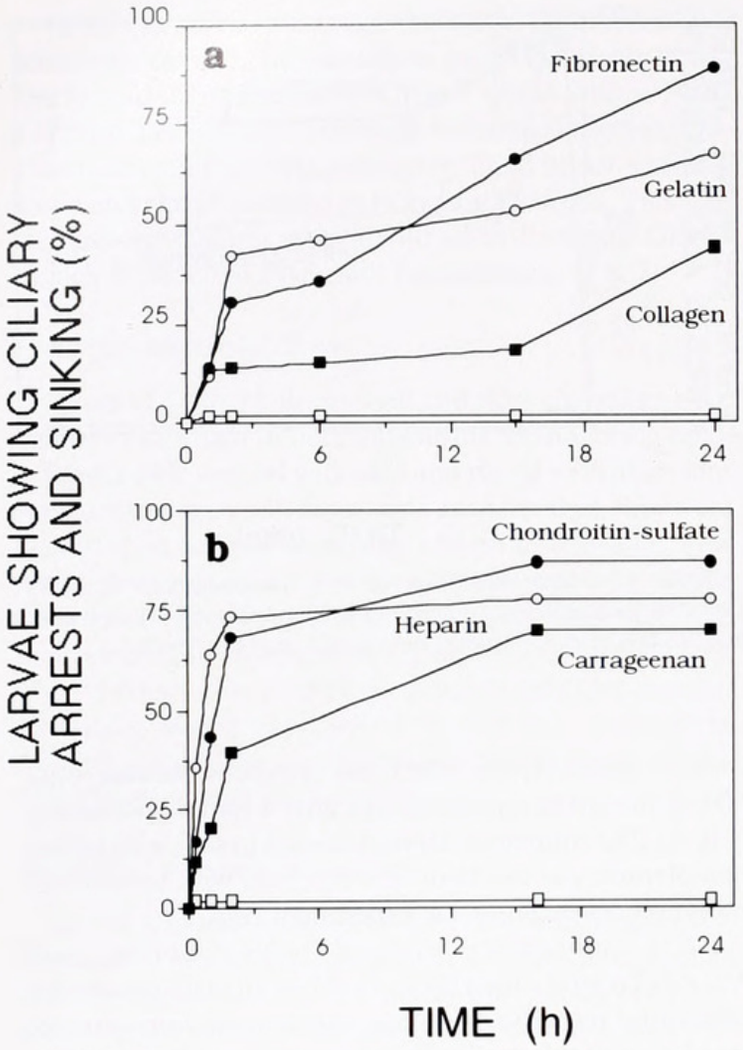

Figure 3. Mean percentage of early pre-competent larvae of *Concholepas concholepas* induced to sink by extracellular matrix (ECM) components and sulfated polysaccharides. (a) Mean percentage of *C. concholepas* larvae induced to sink by ECM constituents. ● = Fibronectin, ○ = gelatin, ■ = collagen, and □ = control. (b) Mean percentage of *C. concholepas* larvae induced to sink by sulfated polysaccharides. ■ = Carrageenan, ● = chondroitin sulfate, ○ = heparin, and □ = control.

10 m*M* NH_4Cl were washed and placed in normal fresh seawater to see if they would recover. Three hours later, all larvae were observed swimming normally (data not shown).

Chemotaxis to microbial films. Microbial films are well known as settlement inducers for a number of benthic marine invertebrates. We studied the attraction responses of pre-metamorphic *C. concholepas* larvae exposed to several such films. In the chemotaxis experiment in which glass plates were coated with different films, the larvae responded positively to *P. marinus*, multispecific, diatom, and bacterial films after 25 min of incubation; rates of attraction were 20%, 30%, 20%, and 20% respectively (Fig. 5a). At that time, no significant difference was observed among the different films (*G* test with 3 df). However, after 60 min of incubation, the respective rates of larval attraction increased to 40%, 60%, 60%, and 30%, and a significant preference was observed for the multispecific and the diatom films compared to the *P. marinus* and bacterial films ($P < 0.001$, *G* test with 1 df) (Fig. 5a). When *C. concholepas* shells were used as substrata, a positive response of larvae to *P. marinus*, multispecific, and diatoms films was observed after 25 min of incubation; rates of attraction were 30%, 40%, and 10%, respectively (Fig. 5b). At that time, the attraction to the multispecific and *P. marinus* films was significatively higher than to the diatom films ($P < 0.001$, *G* test with 1 df). At the end of the experiment, the larvae showed a clear and significant preference for the multispecific films over the other films, with an 80% rate of attraction ($P < 0.001$, *G* test with 1 df) (Fig. 5b). In the experiments with both glass and shell substrata, the larvae were not attracted to the sterile control at any time.

Larval activity in response to microbial films. The activity of *C. concholepas* larvae exposed to different microbial films was recorded. Larvae showing velar contraction were observed in the presence of all the films after 6 min of incubation (Fig. 6a). The percentage of larvae presenting this behavior was, however, significatively higher for the bacterial films at 4, 6, and 8 min of incubation with 20%, 30%, and 30%, respectively ($P < 0.001$, *G* test with 1 df). At the end of the experiment, the response to the bacterial film decreased, and no significant difference was observed among the bacterial, multispecific, and diatom treatments (Fig. 6a). On the other hand, the ciliar movement of larvae increased only in the presence of the multispecific and the *P. marinus* films (Fig. 6b). However, the effect of the former was higher after 8 min of incubation. At the end of the ex-

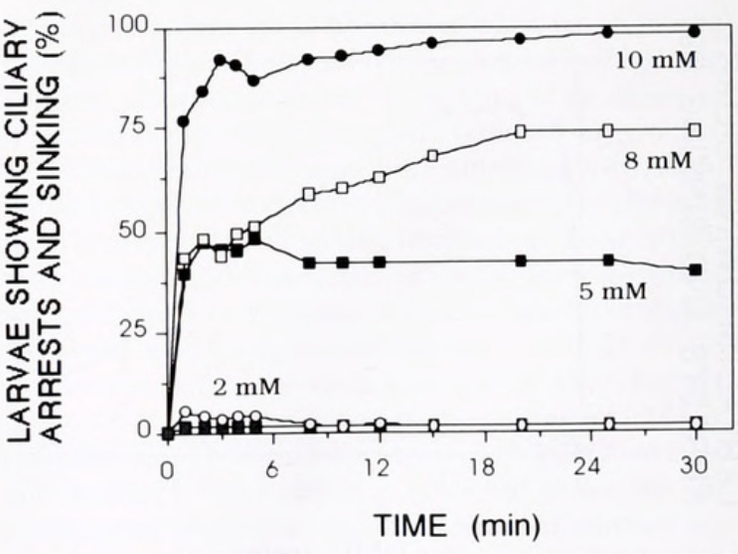

Figure 4. Mean percentage of early pre-competent larvae of *Concholepas concholepas* induced to sink by different concentrations of NH_4Cl. ● = 10 m*M*, □ = 8 m*M*, ■ = 5 m*M*, ○ = 2 m*M*, and □ = control.

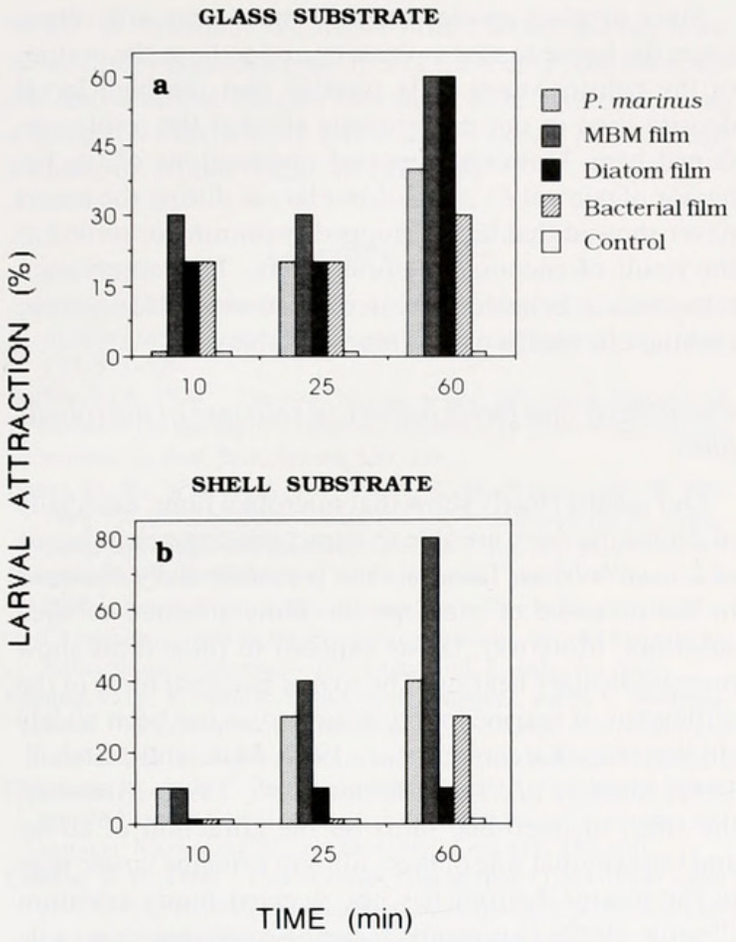

Figure 5. Mean percentage of attraction of pre-metamorphic *Concholepas concholepas* larvae to different microbial films. Microbial films prepared on glass (a) and *C. concholepas* shells (b).

periment, larvae exposed to the bacterial films exhibited an increased ciliar movement, but this response was significantly less than that observed for the other two treatments ($P < 0.001$, G test with 1 df) (Fig. 6b).

Discussion

Mucus and hemolymph

Early pre-competent larvae of *Concholepas concholepas* stopped swimming, sank, and remained inactive on the bottom of the wells when exposed to both mucus and hemolymph of adult individuals. Traces of mobile animals (*e.g.*, mucus) can influence the settlement of sessile animals (*e.g.*, barnacles) (Johnson and Strathmann, 1989), and conspecific mucus induces larval settlement in the abalone *Haliotis rufescens* (Slattery, 1992). Structural factors such as glycoproteins and growth factors as well as bacteria associated with mucus have been suggested as possible morphogens involved in triggering the larval settlement response in gastropods (Slattery, 1992; Cantillana and Inestrosa, 1993). Recently, a heparin-binding growth factor, which shows properties similar to those of fibroblast growth factors (FGF), has been identified in the foot of *C. concholepas* (Cantillana and Inestrosa, 1993). The binding of basic FGF to high-affinity receptors requires the presence of an ECM component (*i.e.*, heparan sulfate proteoglycans) (Yayon *et al.*, 1991). Therefore, it is possible that growth factors in the mucus of mollusc species could be interacting with ECM molecules (mainly sulfated polysaccharides) and then with high-affinity growth factor receptors. On the other hand, it has been hypothesized that lectins (*i.e.*, sugar-binding proteins or glycoproteins of non-immune origin that agglutinate cells or precipitate glycoconjugates) may be involved in the settlement and metamorphosis of marine invertebrate larvae (Maki and Mitchell, 1985). Lectins have been reported in the mucus of different fish species (Kamiya and Shimizu, 1980; Kamiya *et al.*, 1988) as well as in the hemolymph of a number of marine invertebrates such as starfish (Kamiya *et al.*, 1992), barnacles (Kamiya *et al.*, 1987), and isopods (Kaim-Malka, 1993). Therefore, lectins present in the mucus and hemolymph may be another factor mediating the larval settlement of *C. concholepas* and other species. The effect of heated hemolymph was clearly lower than that observed with unheated hemolymph during most of the incubation period. Kamiya *et al.* (1992) found that the hemagglutinating activity of lectins was heat labile in

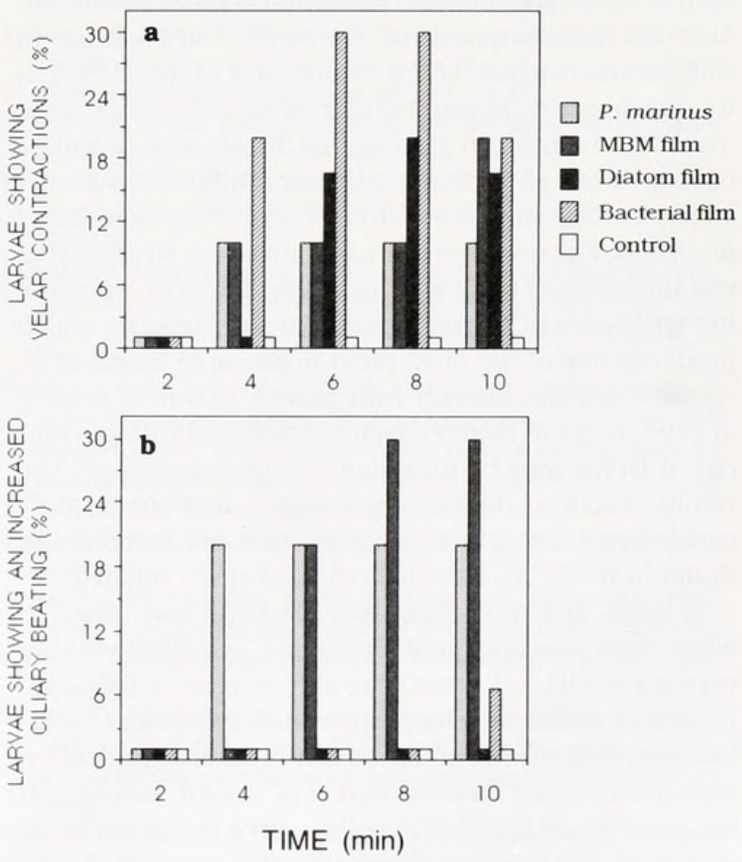

Figure 6. Mean percentage of pre-metamorphic *Concholepas concholepas* larvae showing velar contractions and an increased ciliary beating in response to microbial films. (a) Mean percentage of *C. concholepas* larvae showing velar contractions. (b) Mean percentage of *C. concholepas* larvae showing an increased ciliary beating.

the hemolymph of a starfish, decreasing or disappearing when exposed to high temperatures. A similar effect could have occurred in our case. Why the boiled hemolymph initially affects larvae in the same way that the unheated hemolymph does is not clear—maybe there are two types of cues or responses; in any case further studies are necessary to clarify this matter. The transient effect produced by both natural substances in larvae of *C. concholepas* may be related to some kind of habituation to the cue; alternatively, it may be due to a deficient larval response, given the presence of an immature signal-transduction mechanism.

ECM macromolecules, sulfated polysaccharides, and NH_4Cl

A positive and time-dependent behavioral response was observed in early pre-competent larvae of *C. concholepas* after exposure to ECM molecules and sulfated polysaccharides. In both cases larvae sank and remained attached to the substratum. The effect of sulfated molecules was faster than that of the other ECM components. Morse and Morse (1991) identified the molecule that is biologically active as a morphogen for a scleractinian coral larvae (*i.e., Agaricia humilis*) as a sulfated glycosaminoglycan. Moreover, they showed that some sulfated polysaccharides such as κ- carrageenan, fucoidan, and keratan sulfates induce the metamorphosis of *A. humilis,* but chondroitin sulfates and heparin do not (Morse and Morse, 1991). It was previously demonstrated that larvae of *C. concholepas* incubated in artificial seawater in the absence of sulfate or in presence of a specific sulfation inhibitor show a decrease in their mobility (Urrea *et al.,* 1992; Brandan *et al.,* 1995). The results presented in this work strongly suggest that sulfated polysaccharides play a role in aspects of the settlement of *C. concholepas* larvae. Heparin, which produced one of the most rapid response in larvae of *C. concholepas*, can interact with growth factors (Yayon *et al.,* 1991). In this context, factors associated with the mucus of larvae may be important in this interaction. The results described in this paper suggest that larvae of *C. concholepas* are able to interact with macromolecules found in the ECM, especially those that are sulfated.

A dose- and time-dependent response was observed when early pre-competent larvae of *C. concholepas* were exposed to NH_4^+. Larvae were able to recover their mobility after an extensive washing with fresh seawater. NH_4^+ has been described as an important inducer of settlement behavior in oyster larvae (Coon *et al.,* 1990). Likewise, it has been found that NH_4^+ is the active inductive factor associated with bacterial supernatants (Bonar *et al.,* 1990). Following in this vein, marine zones rich in dissolved organic matter would represent important areas where high settlement of benthic marine invertebrates may occur (Morse, 1990).

Since in many species repeated encounters with others cause the larvae to stop swimming and settle to the bottom of the culture vessel, it is possible that the high larval density used in our experiments affected the results obtained here. However, repeated observations of the behavior of control *C. concholepas* larvae during the assays never showed that larvae stopped swimming or settled as the result of encounters among them. This observation represents a behavioral pattern of remarkable interest, making our results all the more notable.

Chemotaxis and larval activity in response to microbial films

Our results clearly show that microbial films, especially multispecific ones, are able to attract pre-competent larvae of *C. concholepas*. This response is preferentially observed in the presence of multispecific films attached to shell substrata. Moreover, larvae exposed to these films show increased ciliary beating. The role of bacterial films in the settlement of marine invertebrate larvae has been widely studied (*e.g.,* Kirchman *et al.,* 1982; Maki and Mitchell, 1985; Maki *et al.,* 1989; Bonar *et al.,* 1990). However, the effect of microbial films on the attraction of larvae and the eventual role of these films in bringing larvae near to the marine bottom has not received much attention (Pawlik, 1992). Our results suggest a quite important role for microbial films in attracting larvae of *Concholepas concholepas*. This attraction could favor the approach of larvae to the chemical metamorphic inducers on the marine bottom. The higher response of larvae to multispecific films on shell than on glass was perhaps due to a better attachment of the bacteria to an irregular surface. Also it is possible that the films produced on the shell were either more numerous or had a different composition than those on the glass.

In this paper we have provided evidence that pre-competent larvae of *Concholepas concholepas* exposed to different natural and artificial cues exhibit behaviors (*i.e.,* ciliary arrests, contraction of the velar lobes, and retraction of the velum into the shell) similar to those described for competent veliger larvae of other gastropod species during metamorphosis. Likewise, they showed different degrees of attraction to different microbial films isolated from a native recruitment zone of this species. This information on natural and artificial metamorphic inducers of *C. concholepas* larvae may be of paramount importance in developing successful methods for culturing this overexploited species.

Acknowledgments

This work was supported by FONDECYT Grants 3502/89, 0651/91, and 19406/94 to Dr. N. C. Inestrosa and 0997/92 to Dr. C. Riquelme, and by IFS Grant 1407-3F

to Dr. E. Brandan. We thank Prof. Gerald Boalch from Citadelhill Plymouth Labs, U.K., for identifying microalgae and diatoms. During this study S. R. Rodríguez was a Research Fellow from DIUC. He is now a Fellow from Fundación Andes (Dept. of Ecology).

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